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**Posters**


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**P 1**
**CLINICAL, HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF BREAST CANCERS PRESENTING DURING PREGNANCY OR LACTATION**

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**Introduction:**

As a rare condition, pregnancy associated breast cancers are of the most challenging medical issues. The poor prognosis and disappointing nature of these kinds of cancers have made them an area of research interest in the past years. There been controversy regarding the independent role of pregnancy alone in making such poor outcomes. We conducted this descriptive cases series study to determine the different aspects of this kind of breast cancer.

**Method and Materials:**

All the charts and medical records of breast cancer patients presenting in Cancer Institute of Imam Khomeini General Hospital were reviewed to find the patient who were pregnant or had a childbirth 6 months before the diagnosis of breast cancer. The patients were evaluated regarding the presenting complaint, diagnosis method, tumor histopathologic pattern and immunohistochemical studies like Estrogen and Progesterone receptors positivity and determining HER-2/neu (c-erb-B2) and P53. Immunohistochemical studies performed using pathologic blocks if IHC data was insufficient for the study purposes.

**Results:**

We found 10 patients, 7 of which were pregnant, 2 were lactating and one was in the postpartum period but not lactating. Mean age was 32 (27-38) and the mean weight was 70.9(63-81). The mean gestational age was 17.4 weeks (6-35) and the mean number of previous pregnancies was 1.8 (1-3). Seventy percent of patients had a positive history of breast-feeding with a mean of 20 months. Eight patients (80%) presented with a breast mass and two with nipple bloody discharge. One patient was in the Stage 1 (10%), 6 patients in Stage 2 and 3 in the stage 3. The most common histopathologic pattern has been Invasive ductal Carcinoma (7 Patients, 70%) followed by Invasive lobular (1 Patients, 10%) and Mucinous (1 Patients, 10%) and pure DIC (1 Patients ,10%).

50% of patients were ER positive , 62.5 % PR positive and 57.1 % were positive for both. P53 and HER-2/neu has been positive in 50% and 33% of patients, respectively.

**Conclusion:**

Most of the obtained results were comparable with previous studies. Because of the controversial nature of the disease, further studies for comparison between such patients and non-pregnancy associated breast cancer patients are needed considering the different confounding factors. Because of determination of most important confounding factors, Data of current study can be used as a base for further cohort studies.

**P 2**
**TNM STAGING AND CLASSIFICATION OF BREAST CANCER (FAMILIAL AND NON-FAMILIAL) IN JORDANIAN FEMALES**

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**Introduction:** Cancer becomes one of the most common diseases in our lives today, and breast cancer is the women's worst enemy.

**Purpose:** Staging or morphology of breast tumor has important implications for treatment and prognosis and it would be of interest to determine the relation between various morphological types and age of patient as well as the frequency of each stage among familial and non-familial breast cancer.

**Results:** Out of ninety-nine breast cancer females enrolled in this study, forty three were familial females and fifty six were non-familial females. Breast cancer cases were staged depending on the combination of TNM category into in situ, early invasive, advanced invasive and metastatic.

One female breast cancer Jordanian females was diagnosed with in situ cancer, fifty cases were in early stages of invasive cancer. The remaining was diagnosed with advanced invasive (29 cases) and metastatic (19 cases) breast cancer.

Stage 2b was the most common stage of early invasive cases and represented 48% of the staged cases. On the other hand, among cases diagnosed with advanced invasive breast cancer, stage 3a was the most common stage and represented 89.6% of cases. Interestingly, all cases of stage 3a belonged to TNM stages of T2N2M0 and T3N1M0. The tumor size in all cases of Jordanian females diagnosed with advanced invasive breast cancer exceeded the 2 cm size.

The incidence of non familial breast cancer was slightly higher (56.4%) than that of familial type (43.4%) among studied Jordanian females. Sixty two percent of cases diagnosed as early invasive belonged to familial breast cancer type. In contrast, most (72% and 84%) of advanced invasive and metastatic breast cancer cases, respectively were of non familial type.

Young females below 40 years were diagnosed with in situ (5.2%), early invasive (26.3%), advanced invasive (31.6%) and metastatic (36.8%) breast cancer. Among females older than 40 years, frequency of early invasive stage was higher (56.3%) than that of advanced invasive (28.7%) and metastatic (15.0%). Among age of 51-60 years, cases of early invasive represented 63.9% of cases.

**Conclusion:** Non-familial breast cancer is more common than familial breast cancer, most breast cancer females were diagnosed at early invasive stage. Non familial breast cancer were more frequent at lower stage, while familial breast cancer were more frequent at higher stage.

**P 3**
**PAGET DISEASE (PD) OF THE NIPPLE MAY BE DERIVED FROM LOBULAR CARCINOMA IN SITU (LCIS).**

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PD occurs in about 1-4% of patients with breast cancer and is characterized by the presence of neoplastic "glandular" cells within the epidermis of the nipple-areola complex. PD is usually associated with poorly differentiated ductal carcinoma in situ (DCIS) located in the lactiferous ducts, with or without invasive ductal adenocarcinoma (IDC). DCIS and PD show similar morphological features and an identical immunocytochemical (ICC) profile: both are positive for low molecular weight cytokeratins (CK), HER-2/neu and carcino-embryonic antigen (CEA). Therefore, PD is regarded as a high grade DCIS that is present in the epidermis. The intra-epidermal location of PD cells may be explained by epidermotropic migration of cells of the underlying DCIS, facilitated by the HER-2/neu receptor.

In our lab, 11 of the 570 mastectomies received between 1/8/2001 and 31/12/2004, showed PD. 8/11 cases were associated with poorly differentiated DCIS and IDC. However, in 3/11 cases LCIS and invasive lobular carcinoma (ILC) were present. In all 3 cases, LCIS extended into the

lactiferous ducts and PD consisted of single cells only. Cells of ILC, LCIS as well as PD expressed CK 8/18, epithelial membrane antigen (EMA) and CEA, but were consistently negative for epithelial cadherin (E-cadherin) and HER-2/neu. Expression of CK8/18, EMA and CEA suggests glandular differentiation, while absence of E-cadherin typically points towards a lobular phenotype. However, in PD cells the absence of staining for E-cadherin was difficult to corroborate, since they are sprinkled as single cells in between E-cadherin positive keratinocytes. The strongest evidence against an origin from DCIS in these 3 cases lies in the absence of HER-2/neu overexpression by PD cells.

In conclusion, morphologic as well as ICC findings indicate that in these 3 cases PD is not derived from DCIS, but from LCIS. To our knowledge, only one such case has been previously reported by Rosen et al, 2002. As a consequence, the mechanism by which PD cells end up in the epidermis has to be re-evaluated, since it is obvious that in PD derived from LCIS, there is no role for HER-2/neu as an facilitator of intra-epidermal migration of adenocarcinoma in situ cells.

**P 4**  
**PROGNOSTIC FACTORS OF NODE METASTASES IN PT1 BREAST CANCER**

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**Introduction:** The axillary lymph node status of patients with newly diagnosed breast cancer remains the most important prognostic information available at the moment. The incidence of metastases in axillary lymph node (MAL) is related with the neoplasia's diameter. In breast cancer < cm1 in diameter, it has been observed a low incidence of MAL, because only a minority of patients presents with such node metastases at diagnosis. It may be possible to forecast the MAL by prognostic factors variation other than axillary lymph node dissection.

**Purpose:** We reviewed our database to find which factors are associated with MAL.

**Methods:** We studied 500 consecutive cases of breast cancer < or equal 2 cm in diameter (pT1) from 1999 to 2004 and we evaluated immunohistochemistry expression of the estrogenic and progesteric receptors and prognostic factors pS2, Ki67, bcl2, p53 e c-erbB2 (neu). We considered positive the nuclear (ER, PgR, p53), cytoplasmatic (pS2 e bcl2) and complete of membrane (neu) expression in over than 10% cells. Proliferative Index was calculated with immunohistochemistry expression of Ki67. These dates are related with MAL, grade and istotype of the tumor, diameter of the neoplasia, patient's age, multifocale tumoral nodes. The statistical analysis was performed with c2 test and a multiple regression logistic model.

**Results:** Significant statistical results were observed as like as MAL and age (p<0,01), MAL and tumoral grade (p<0,001), MAL and tumoral size (p<0,001), MAL and P.I. (p<0,001) and MAL and p53 expression (p<0,01). We did not observed statistical correlation between MAL and tumoral type, multifocal tumor, ormonal receptors expression, PS2, bcl-2 and c-erbB2. Cut off was 6.5 with sensivity of 77,2% and specificity of 50%. ROC-curve result was 0,69.

**Conclusion:** We found that age inferior to 50 years, high grade, diameter superior to cm 1, elevated ki-67 and expression of oncogene p-53 are all factors associated with lymph node metastases

**P 5**  
**SENTINEL LYMPH NODE IN BREAST CANCER: IS IT POSSIBLE A STANDARD EXAMINATION?**

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**Introduction:** Sentinel lymph node (SLN) biopsy allows enhanced pathology through serial sections and immunohistochemical analysis of the retrieved node, with detection of micrometastases (MICRO) and isolated tumor cells (ITC) not otherwise recognized and this technique is usually applied in most of surgical oncology centers.

**Purpose:** We present our experience with an effective protocol for analysis of SLN in breast cancer.

**Methods:** We studied 416 consecutive patients (median age 63 years) with primary breast cancer (median diameter 1.5 cm) who underwent SLN biopsy from March 2001 to December 2004 in two different hospital. After surgery, SLN's were step sectioned. First section obtained was called "Level 0" and routinely processed. If first section was negative, at least six additional couples of sections were obtained from each SLN at 100 micron distance (Level I, II and III) and were analysed with conventional staining and immunohistochemistry (IHC-MNF 116). SLN metastases were classified according to TNM 2002 and called MICRO when less than 2mm in diameter and ITC when less than 0,2 mm in diameter.

**Results:** With conventional analysis, SLN was positive al Level 0 in 25% of cases (106/416). Further metastases or ITC was discovered in 14,5 cases (61/416): 8,9% at Level I, 4% at Level II and 1,6% at level III, of which ITC were 62% (38/61), MICRO were 36% (22/61) and 2% were macrometastases (1/61). Our protocol discovered MICRO or ITC in 13% of patients with tumors less than 2 cm in diameter (T1) and in 15% of those more than 2 cm in diameter (T2) (p=0.7). Evidence of MICRO and ITC at level III was 1,3% in T1 patients and 3,8% in T2.

**Conclusions:** SLN step analysis is most useful to discover MICRO and ITC in a good percentage of cases. This technique allows to study more accurately women with breast cancer. Our protocol seems best possible because in our experience the incidence of MICRO and ITC at Level III is very low, especially in T2 tumors. Currently, it doesn't known which is prognostic relevance of MICRO and ITC

**P 6**  
**FINE NEEDLE ASPIRATION CYTOLOGY OF MAMMARY CARCINOMA WITH OSTEOCLAST-LIKE GIANT CELLS. A REPORT OF NINE CASES**

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**Introduction:** Carcinoma with osteoclast-like giant cells (OCGC) is an uncommon neoplasm characterized by giant cells, prominent vascularization, haemorrhage and areas of cribriform epithelial growth with moderate atypia. Multinucleated giant cells have been described in several other breast lesions raising an interesting differential diagnosis, mainly with benign disorders. Due to its rarity few cases have been described cytologically.

**Purpose of the study:** To determine if mammary carcinoma with OCGC shows cytologic features characteristic enough to

permit a specific preoperative diagnosis using fine needle aspiration.

**Methods:** We retrospectively reviewed 13 fine needle aspiration samples from 9 patients with this variant of carcinoma. Nine corresponded to breast tumours and four to axillary, liver, subcutaneous and mediastinal metastatic lesions. The expression of CD68 by giant cells was evaluated immunocytochemically in six cases. All patients had a complete pathologic study of the breast neoplasm.

**Results:** Smears showed a double component of epithelial and giant cells. Epithelial clusters were predominantly of intermediate size with irregular contours. Most were cohesive but others showed cellular dissociation with scarce to moderate cellular pleomorphism. Giant cells had a well defined, deeply stained cytoplasm and round to elongated morphology. Two metastatic cases were devoid of them. Hemosiderin-laden macrophages were common in smears from breast tumors. In the six cases tested CD68 was expressed in multinucleated giant cells. Except for one case that was diagnosed as suspicious of malignancy all the cytologic diagnoses were of carcinoma. There were no false negative diagnosis.

**Conclusion:** Cytologic features of mammary carcinoma with osteoclast-like giant cells correlate closely with the histologic ones. Most cases are clearly recognizable as malignant but in others cytologic atypia may be minimal mimicking a benign lesion. In difficult cases the presence of hemosiderin-laden macrophages and the histiocytic nature of the MGC are helpful diagnostic features.

**P 7**  
**PATHOLOGY EVENTS (MALIGNANT OR NOT) IN PATIENTS WITH BREAST CANCER TREATED WITH TAMOXIFEN WITH OR WITHOUT CHEMOTHERAPY**

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The benefit from prolonged use of tamoxifen in reducing the incidence of breast carcinoma is considered sufficiently great to justify its use as a chemopreventive agent despite the risk of undesirable side effects.

**AIMS:** To determine the spectrum of pathological findings in breast cancer patients treated with tamoxifen.

**METHODS:** The study population comprised 109 breast cancer patients, out of 1009 diagnosed with the disease in our laboratory over a period of five years (1997-2001). The above patients were treated with tamoxifen and presented with secondary pathological findings, other than metastatic disease. The data were collected retrospectively and included 73 patients with D&C and hysterectomy/oophorectomy specimens for tamoxifen-associated gynaecological symptoms and 36 patients with other than gynaecological pathology.

**RESULTS:** Of the 73 patients who underwent D&C or surgery for gynaecological symptoms, 28 had normal endometrium (16 atrophic), 15 simple hyperplasias, 30 hyperplastic endometrial polyps, 5 endometrial carcinomas, 5 endometrial sarcomas, 18 leiomyomas, 8 adenomyomatosis, 6 ovarian cysts, 2 ovarian endometriosis, 1 ovarian adenocarcinoma, 6 ovarian stromal tumors, 1 ovarian teratoma, 2 Brenner tumors, 3 CINIII and 1 squamous carcinoma of the cervix.

In 12 patients, a second malignancy, other than gynaecological, was diagnosed: 3 in the contralateral breast, 2 renal cell carcinomas, 3 thyroid carcinomas, 1 urothelial carcinoma, 1 adenocarcinoma of the colon, 1 Hodgkin's disease and 1 multiple myeloma.

In 9 patients there were diagnosed 3 adenomas of the colon, 2 neurofibromas and 1 GIST.

In the remaining 15 patients, the specimens concerned gallbladders with lithiasis or chronic cholecystitis.

**CONCLUSIONS:** In our study 67% of the women treated with tamoxifen presented with gynaecological-related pathology concerning the endometrium and the ovaries, 16,4% of which were malignant in nature. In 11% malignant disease in other organs was diagnosed. Interestingly 13,7% of the patients showed biliary tract-related problems, probably due to tamoxifen-producing cholestasis.

**P 8**  
**PLEOMORPHIC LOBULAR NEOPLASIA (PLN) OF THE BREAST NEEDS TO BE EXCISED AND BEHAVES LIKE A PRE-INVASIVE ENTITY: AN HISTOLOGIC AND PHENOTYPIC STUDY FROM A SERIE OF 15 NEEDLE CORE BIOPSIES (NCB) AND THEIR SUBSEQUENT SURGICAL SPECIMENS**  
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**Introduction:** Lobular neoplasia is considered to be a marker of cancer risk, but its appropriate management is still controversial. The increasing use of percutaneous NCB for non palpable breast lesions, has highlighted evolving entities such as PLN.

**Purpose:** Our goal is to emphasize this pleomorphic variant, whose presentation differs from the classical form of lobular neoplasia and whose recognition has clinical relevance.

**Methods:** Fifteen PLN were primarily diagnosed from fourteen 8 or 11 Gauge (G) Mammotome® procedures, using stereotactic mammography and one 14 G biopsy, using ultrasound guidance. All PLN, but one, were mammographically detectable, because of microcalcifications. Subsequent surgical specimens were obtained in 13 cases, during which 7 sentinel lymph node procedures or 3 axillary dissection were performed. An immunohistochemical study for estrogen and progesteron receptors (ER, PR), c-erbB2, E-Cadherin and high molecular weight cytokeratin 34 beta E-12 (HMW-CK), was realized on NCB and excision specimens.

**Results:** Among the 15 PLN cases, 5 were associated with an invasive lobular carcinoma (ILC) and 3 with a microinvasive lobular carcinoma. Seven invasive or microinvasive cases were already identified on NCB. One ILC was identified on the surgical resection alone. ILC and microinvasive carcinoma were always closely located to the PLN areas, both on biopsies and surgical specimens. Morphologically, PLN cases were composed of pleomorphic, dyshesive cells with comedo-like, apocrine and signet ring features. Comedo-like patterns with central necrosis were associated with microcalcifications. PLN presented the following phenotype: ER 13+/15, RP 5+/15, CerbB2 3+/15, E-cadherin 0+/15, HMW-CK 15+/15. PLN and their invasive/microinvasive counterparts shared a similar phenotype. Two metastatic axillary involvement were noted.

**Conclusions:** These clinical, histological and phenotypic data support the evidence that PLN looks more like a preinvasive lesion than a marker of increased risk. Thus, percutaneous biopsies with PLN should be evaluated diligently for the presence of an invasive or microinvasive lobular component and require a subsequent surgical excision. Pathologists, as well as physicians involved in the management of breast disease, should be aware of this mammographically detectable entity, which has distinct histologic patterns and behaviour.

**P 9**

## PROGNOSTIC FACTORS IN PRIMARY MUCINOUS BREAST CARCINOMAS

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### Background

Pure and mixed mucinous breast carcinomas (MCs) are separate morphological entities, with different biological potential and prognosis. The recent WHO classification of breast carcinomas suggests that 100%, and not the generally used 90% cut-off value, of mucinous component must be present for a diagnosis of pure MC.

### Aims of the study

To analyse clinical, cytological and histological features of MCs of the breast and to determine their prognostic significance.

### Material and methods

Altogether 98 patients with primary breast MCs were included in the study. A mucinous component of 90% has been routinely required for pure MCs at our institution. Clinical (age of patient, tumour size, regional lymph nodes status, distant metastases, treatment), cytological (amount of mucin, sample cellularity, nuclear pleomorphism, cytoplasmic features, presence of capillaries), histological (grade, amount of mucin) parameters, and DNA ploidy were assessed and their influence on survival was analysed.

### Results

Among 98 breast MCs, 64 were classified as pure and 34 as mixed. Regional lymph node metastases were found in 5 of 60 pure MCs and in 21 of 30 mixed MCs. Distant metastases were present in 5 of 64 pure MCs and in 17 of 34 mixed MCs. Overall 5 and 10 year survival for pure MCs was 89.0% and 83.0%, and 78.9% and 46.5% for mixed MC. The difference in survival was statistically significant ( $p=0.004$ ).

Univariate analysis revealed that histological type, axillary lymph node status, tumour size and cytologically assessed nuclear pleomorphism had a statistically significant impact on survival ( $p<0.05$ ). The histological type and size of tumour influenced survival of patients without axillary lymph node metastases. Axillary lymph node status and tumour size were found by multivariate analysis to be independent prognostic factors.

The 5 and 10 year survival was 93.7% for MCs with 100% of mucinous component, but dropped to 85.3% and 65.1% for MCs containing about 90% of mucinous component ( $p>0.05$ ).

### Conclusions

Regional lymph node status is the most important independent prognostic factor for breast MCs, followed by tumour size.

The present study supports the recent recommendations by WHO classification of breast carcinomas that 100% and not 90% of mucinous component is required for pure MCs. Since the component other than mucinous has a great influence on the survival of patients with MCs of the breast, a meticulous sampling of the tumour is necessary.

## P 10

### METASTATIC INVOLVEMENT IN INTERPECTORAL (ROTTER'S) LYMPH NODES RELATED TO LOCATION, SIZE AND GRADE OF BREAST CANCER

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**Aims and background:** The study was aimed at analyzing metastatic involvement in interpectoral (Rotter's) lymph nodes related to site, size and grade in primary breast cancer.

**Methods:** The study includes 210 female patients undergoing surgery for breast cancer at the University Hospital for Tumors, Zagreb, Croatia from November 2001 to March 2004. In addition to the standard surgical procedure,

interpectoral (Rotter's) lymph nodes were removed in all of the patients. Metastatic involvement of interpectoral lymph nodes was confirmed by histological and immunohistochemical examinations.

**Results:** Rotter's lymph nodes were identified in 66,2% of the patients, with metastatic involvement revealed in 18,5% of the Rotter's nodes. Metastatic involvement of Rotter's nodes in patients with negative and positive axillary lymph nodes was 2,8% and 34,6%, respectively. Considering location of the tumor in patients with metastatic involvement of Rotter's nodes, it was shown that tumors located in the upper breast quadrants were more prone to metastasis to Rotter's nodes, demonstrating a significant positive correlation between tumor location within the breast and positive Rotter's lymph nodes ( $r=0.93$ ,  $p=0.02$ ). As regards tumor size, Rotter's nodes were identified in 15%, 20% and 60% of stage T1 (<2 cm), T2 (2-5 cm) and T3 tumors (>5 cm), respectively with a significant positive correlation ( $r=0.759$ ,  $p=0.09$ ). Also considering tumor grade it was shown that metastatic involvement of Rotter's lymph nodes was 3,8%, 17,6% and 31,6% of histological grade I,II and III, respectively with a significant positive correlation ( $r=0.993$ ,  $p=0.08$ ).

**Conclusions:** The results show that almost one-fifth of breast cancer patients, or even one-third of them with positive axillary lymph nodes, are discharged with positive interpectoral lymph nodes that remain undiagnosed and non-extirpated. As the interpectoral nodes can be surgically removed without additional mutilation, the exploration of Rotter's lymph nodes should be introduced into routine clinical practice. In upper breast quadrants tumors were more prone to metastase in to Rotter's nodes, as well as higher tumor size and grade.

**Key words:** breast cancer, interpectoral (Rotter's) lymph nodes

## P 11

### OUTCOME OF PURE AND ATYPICAL MEDULLARY CARCINOMAS OF THE BREAST

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Pure medullary carcinoma (PMC) of the breast is a rare neoplasm. Using strict morphologic diagnostic criteria, it usually displays a favourable outcome. Atypical medullary carcinoma (AMC) of the breast has some medullary features and its prognostic significance is controversial. In this study, we aimed to compare our PMC and AMC cases, using clinicopathological findings and to reach a conclusion, concerning the diagnostic criteria of breast carcinomas with medullary features. For this reason, we retrieved 19 cases of PMC and 106 cases of AMC from almost 4000 breast carcinomas that were diagnosed at our department between years of 1988 and 2004. Of these, 7 PMC and 48 AMC cases with available follow-up were compared. The following data, including patient age, menopausal status, tumor size, grade, stage, lymph node status, estrogen and progesterone receptor status, existence of distant metastasis, disease free survival (DFS) and overall survival (OS) were recorded as well. The median follow-up time was 55 (range 8-156) months for all the cases ( $n=55$ ), 96 (range 13-145) months for PMC cases ( $n=7$ ) and 48 (range 8-156) months for AMC cases ( $n=48$ ). All of the PMC cases were lymph node-negative, while 20 AMC cases showed lymph node metastasis. None of the PMC patients has died, while 8 patients with AMC had died during the follow-up. No recurrence was noted, except for a patient

who developed a new primary PMC at the contralateral breast 7 years after the diagnosis, among the PMC cases. Cumulative survival rates of the AMC cases was 69.7 % at sixty-five months with Kaplan Meier analysis (SE: 0.091). There was no significant difference between the survival curves of two groups by log-rank test with Kaplan Meier analysis (log-rank: 1.86, df: 1, p: 0.172). We concluded that a favourable outcome can be expected for PMCs and some AMCs. Also, a redefinition of morphologic diagnostic criteria should be made for breast carcinomas with medullary features.

#### P 12

### PANNICULITIS-LIKE INVASIVE CARCINOMA OF THE BREAST

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**Introduction:** In the boundary between the invasive mammary carcinoma and the adipose tissue, isolated or groups of infiltrating tumor cells are often blended with fat cells. This pattern is responsible for an echogenic rim surrounding the hypoechoic mass of invasive breast carcinoma observed by ultrasound. We termed this pattern panniculitis-like infiltration (PLI) because this pattern resembles inflammation of the adipose tissue. Invasive lobular carcinomas are occasionally composed predominantly of PLI without a significant desmoplastic reaction, resulting in a spider web-like structure. However, the clinicopathological significance of such pattern in non-lobular invasive carcinomas are not known. **Methods:** We reviewed all the histological slides of 155 consecutive invasive carcinomas of the breast and classified the degree of PLI into three degrees; PLI-1 (<10%), PLI-2 (10-50%) and PLI-3 (>50%) according to the extent of this pattern in invasive area. The relationships between PLI and histological types, grade, steroid receptors, lymph node status were examined. **Results:** We found that the number of cases in invasive mammary carcinoma with PLI-1, PLI-2 and PLI-3 were 113 (73%), 30 (19%) and 12 (8%), respectively. Invasive mammary carcinoma with PLI-3 was composed of 9 panniculitis-like invasive ductal carcinomas (PLIDC) and 3 panniculitis-like invasive lobular carcinomas (PLILC). Remarkably, two PLIDC were composed purely (>90%) of this pattern and presented echogenic masses without hypoechoic cores in fatty breast of the elderly women. Pure PLIDC and PLILC may be overlooked unless the examiners keep this unusual ultrasound pattern of invasive carcinoma in mind. Mammographically, most PLIDC presented distortion or spiculated masses less dense than common invasive ductal carcinoma. Most (11/12) PLIDC and PLILC showed estrogen-receptor positive and HER2 negative immuno-phenotypes ( $P < 0.05$ ). In spite of relatively small size by palpation, the half cases had positive axillary lymph nodes. Definite diagnosis by aspiration cytology was unsuccessful in five cases. This is due to hypocellularity and frequent co-existence of benign ductal elements in the cytological specimens. Core needle biopsy may be a more reliable tool for pre-operative diagnosis of PLIDC and PLILC. **Conclusions:** Panniculitis-like invasive carcinoma is a new clinicopathological entity with unique histopathological and ultrasound features.

#### P 13

### ACCURACY OF TYPING AND GRADING MAMMARY LESIONS ON CORE BIOPSIES AND COMPARISON WITH THE EXCISIONAL SPECIMENS.

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**INTRODUCTION.** Needle core biopsy of palpable lesion (NCB; 14 gauge) and stereotactic vacuum assisted biopsy

(SVAB; 11 gauge) of non-palpable mass are the major preoperative methods of breast diagnosis. The UKNHS Breast Screening Project recommends the use of a core biopsy reporting form that recognize five diagnostic categories (B1-B5). Purpose of this study was to evaluate the incidence and the predictive value of the two categories: B4 (suspicious of malignancy) and B3 (lesion of uncertain malignant potential) in lesions diagnosed by NCB and SVAB and to assess the accuracy of the two methods in typing and grading infiltrating and in situ breast carcinomas.

**MATERIALS AND METHODS.** 2170 core biopsies (893 NCB and 1277 SVAB) were performed between January 2000 and February 2005. In 879 cases, the correlation between biopsy results and subsequent surgical excision was available.

**RESULTS.** The frequency of B3 diagnosis was 8 % (72 cases) in NCB and 9 % (115 cases) in SVAB. The B4 category was reported in 7 % (62 cases) of NCB and in 3 % (38 cases) of SVAB. At definitive histology, 24 % of B3 (NCB: 32 cases, SVAB: 12 cases) and 87 % of B4 (NCB: 54 cases, SVAB: 33 cases) showed invasive carcinomas or ductal carcinoma in situ. Type and grade of the invasive and in situ carcinoma, were determined in both biopsy and definitive excision and the results compared. For histological type, 12 % of NCB and 3 % of SVAB were changed to a different category. The agreement with overall grade was of 85 % with SVAB and of 62 % with NCB. On 94 cases that differed, 98 % differed by one grade and 63 % were upgraded.

**CONCLUSIONS.** SVAB has been shown to have a greater accuracy in diagnosis, mainly because of the increased quality and quantity of tissue obtained. However, the incidence of B3 category is comparable with SVAB and NCB methods. This behaviour could be explained considering that the B3 category often refers to atypical intraductal epithelial proliferation, most frequently associated to clustered microcalcifications sampled by SVAB. Grading based on NCB is not as accurate as SVAB, and its evaluation should be delayed until the surgical specimen examination.

#### P 14

### AUTOMATIC CLASSIFICATION OF CLUSTERED MICROCALCIFICATIONS IN BREAST CANCER

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**Introduction:** Breast cancer is a significant public health problem worldwide. Plenty of mammographic screening programs resulted in the detection of breast carcinomas at early stage. The presence of microcalcifications in mammogram not only correlates with breast cancer in many cases, but also sometimes is the only early sign of this disease. Nowadays, Computer Aided Diagnosis (CAD) systems may help radiologists detecting and classifying clustered microcalcifications, which is vital for proper treatment.

**Objectives:** The objective of this work was to evaluate the performance of the computerized classification method for automatically detected clusters of microcalcifications.

**Materials and Methods:** In our research we used 106 images from Digital Database for Screening Mammography (DDSM), among which 67 presented malignant and 39 benign cases. Our experiments were carried out in two steps: automatic detection and automatic classification. For the detection, previously developed morphological method has been used. Before the classification task was conducted, the whole set of mammograms was divided into two groups: training and testing sets, including the same number of images. Only images from training set were used to train the

Support Vector Machine (SVM) classifier. The performance of the classifier has been tested on images from testing set. Results: Automatic classification of clustered microcalcifications in mammograms is a final stage of CAD system which helps the radiologist in diagnosis. According to ROC analysis the presented algorithms yielded  $Az=95,23\%$ . Conclusions: The SVM classifier used in experiments gave very good results and will be used in further research on complex CAD system.

#### P 15

##### **BILATERAL BREAST CARCINOMA – THE HISTOLOGICAL PATTERN**

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Histological grade and tumor biology remain important predictors of clinical behaviour of breast carcinomas. The aim of this study was to compare the histopathological and genetical / BRCA-1 mutation/ patterns of breast carcinomas in women who underwent a surgery due to invasive carcinomas and in which the bilateral invasive carcinoma presence was ascertained.

From 1995 to 2004 year 542 women underwent mastectomy in our Military Medical Institute. In 13 patients /2.4%/ a bilateral invasive carcinoma was diagnosed. There were 9 synchronous and 3 metachronous breast carcinoma patients. In order to estimate the estrogen /ER/ and progesterone /PR/ receptors status and an overexpression of HER2 in primary tumors immunohistochemical examinations were performed. Furthermore, these receptors rates/expression was evaluated in metastases to regional lymph nodes and in remote metastases. In 10 patients the presence of the invasive ductal carcinoma was ascertained in one breast at least; in 6 cases the ductal carcinoma was bilateral. The tubular carcinoma was diagnosed in 4 patients / in 1 woman it was bilateral/. The lobular carcinoma was diagnosed in 3 cases /in 2 cases it was bilaterally/.

There was no difference found in the status of ER, PR in patients with bilateral tumors of the identical histopathological pattern. No correlation of receptor's status was stated in all other cases. In metastases the ER and PR status corresponded with the receptor's status of one of primary tumors. BRCA-1 mutation analysis confirmed its presence in 1.4% of 427 examined breast carcinoma cases but none woman with bilateral cancer carried such mutation. Compare to unilateral cancers the bilateral one was mostly present in women under the 40 year of age. Thus, also the other genetical risk factors should be searched in order to predict the development of bilateral breast cancer. Our findings indicate that synchronous bilateral breast carcinoma showed a higher frequency of invasive ductal carcinoma. Also they indicate problems in estimating the TNM status of bilateral cancers, especially concerning metastases into regional lymphnodes. The examination of receptors rates and HER2 overexpression of both tumors in bilateral breast carcinomas and their metastases is required and their analysis remains an important predictor of the clinical behaviour of breast carcinomas.

#### P 16

##### **PANNICULITIS-LIKE BREAST CANCER**

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Background: In the boundary between the invasive mammary carcinoma and the adipose tissue, isolated or groups of infiltrating tumor cells are often blended with fat cells. This pattern is responsible for an echogenic rim surrounding the hypoechoic mass of invasive breast carcinoma observed by ultrasound. We termed this pattern panniculitis-like

infiltration (PLI) because this pattern resembles inflammation of the adipose tissue. Invasive lobular carcinomas (ILC) are occasionally composed predominantly of PLI without a significant desmoplastic reaction, resulting in a peculiar spider'

s web-like structure. However, the frequency and clinicopathological significance of such lace-like pattern in invasive ductal carcinomas (IDC) is unknown. Material and Methods: We reviewed all the histological slides of

155 consecutive invasive carcinomas of the breast and classified the degree of ! PLI into three degrees; PLI-1 (<10%), PLI-2 (10-50%) and PLI-3 (>50%) according to the extent of this pattern in invasive area. The relation of PLI to histological types, grade, hormone receptor and lymph node status were examined. Results: We found that the number of cases in invasive mammary carcinoma with PLI-1, PLI-2 and PLI-3 were 113 (73%), 30 (19%) and 12 (8%), respectively. Invasive mammary carcinoma with PLI-3 was composed of

9 IDC and 3 ILC. Remarkably, two IDC with PLI-3 were composed solely (>90%) of this pattern and presented pure echogenic masses without hypoechoic cores in fatty breast of the elderly women. Pure panniculitis-like IDC and ILC may be overlooked unless the examiners keep this unusual ultrasound pattern of invasive carcinoma in mind. Mammographically, most panniculitis-like IDC presented distortion or spiculated masses less dense than common IDC. Most

(11/12) invasive carcinomas with PLI-3 showed estrogen-receptor positive and! HER2 negative immuno-phenotypes ( $P<0.05$ ). In spite of relatively small size by palpation, the half cases showed positive axillary lymph nodes metastasis. Definite diagnosis by aspiration cytology was unsuccessful in five cases. This was due to hypocellularity and frequent co-existence of benign ductal elements in the cytological specimens. Core needle biopsy may be a more reliable tool for pre-operative diagnosis of invasive carcinomas of PLI-3. Conclusions: Predominant panniculitis-like infiltration is associated with unique histopathological and ultrasound features and occurs in a subset of IDC as well as ILC.

#### P 17

##### **PROGNOSTIC VALUE OF PLASMOCYTOID DENDRITIC CELLS INFILTRATE IN POOR PROGNOSTIC BREAST CARCINOMAS.**

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Lymph node status is the most important prognostic factor to select high-risk patients for chemotherapy. However, the classical prognostic factors did not explain the 30% 10-year survival of patients with a large axillary involvement (> 8N+) without chemotherapy. Our purpose was to determine the prognostic impact of dendritic cells (DC) for this group of patients.

In a retrospective series of 248 patients with > 8 N+ treated at the IGR between 1972 to 1983 without chemotherapy, a group of 37 patients without relapse within 15 years was matched to 37 patients who relapsed and died within 15 years. The matching was done on number of N+, grade, estrogen receptor status, year of diagnosis, age. Immunohistochemistry staining was used to determine the infiltration of immature DC (CD1a, Immunotech), mature DC (DC-lamp and p55, Schering Plough), plasmocytoid DC (CD 123, Schering Plough). The staining was assessed using a semi-quantitative grading system and consider negative for absent or low infiltrate and positive for intermediate and high infiltrate.

In long terms survivors, 16%, 21%, 37% of tumors contained CD1a, p55, DC-lamp cells. These infiltrates did not

significantly differ from those of matched control tumors. A significant higher infiltrate of plasmocytoid DC (pDC) (CD 123+) was observed in long survivors patients (38%) compared to control group (13%) ( $p = 0.006$ ). Plasmocytoid DC were localized in the stroma of the tumor. The presence of pDC was not correlated to number of N+, grade and estrogen receptor status.

These results allow us to hypothesize a protective role of plasmocytoid DC infiltrate in this group of high-risk tumors. pDC may produce interferon type I who promote their differentiation into antigen presenting tumor cells and may also hamper tumor cell growth.

Further studies are thus needed to determine the role of pDC and their potential therapeutic use.

#### P 18

##### **SENTINEL LYMPH NODE IN BREAST CARCINOMA: THE EXPERIENCE OF DEPARTMENT OF PATHOLOGICAL ANATOMY - BARI**

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**Introduction:** Assessment of axillary lymph node status in breast cancer is a collaborative and multidisciplinary work between surgeons and pathologists. Sentinel lymph node (SLN) biopsy can truthfully predict the axillary nodal status in clinically node-negative breast cancer patients. The pathological examinations of SLN typically involves the evaluation of multiple tissue levels and/or keratin in immunohistology.

**Purpose:** of our study was to estimate the usefulness of SLN examination by evaluation of multiple-level sectioning in SLN and to assess the value of SLN as the only method in nodal staging in breast cancer patients.

**Material and Methods:** From January 1999 to December 2004, 253 patients underwent SLN procedure for breast cancer. The mean age was 56 (range 30-82). The tumors were: 27 Tis (10%), 64 T1b (25%), 105 T1c (41%) and 57 T2 no larger than 3 cm.

A mean number of 1.4 nodes were examined per case for a total of 354 lymph nodes.

SLN was completely and serially examined after wax embedding and E-E staining. Cytokeratin were performed only in case of dubious metastasis. The mean number of histologic section for each SLN was 19,2.

One hundred seventy six of patients also underwent axillary dissection.

**Results:** SLN revealed in 55 (21.7%) cases metastatic deposits: 38 (69%) macrometastases (> 2 mm) and 17 (31%) micrometastases (<= 2 mm). In 27 (49%) cases SLN was the only metastatic node

In 198 cases SLN was metastasis free; of these patients 121 underwent also axillary dissection; metastases in non sentinel node (NSLN) were detected in 9 of them. The false-negative rate (FNR) was 8%. In five of these cases only one other node was involved, in one case 2 nodes and in three cases 3 nodes. In two cases there were micrometastases.

The comparison between these cases and the other ones, did not show any relevant differences.

Micrometastases in SLN were associated with axillary involvement in 4 (23.5%) cases: in 3 cases one only other node was involved, in one case four nodes.

Macrometastases were associated with axillary involvement in 23 cases (60.5%).

No cases of in situ carcinoma showed metastatic involvement of SLN.

**Conclusions** Our results confirm the importance of this method to avoid unnecessary axillary dissection in node negative patients and of the extension of metastases in the SLN in relation to the different rate of axillary involvement.

#### P 19

##### **CORRELATIONS BETWEEN NUCLEAR PLEOMORPHISM AND INTRATUMORAL STROMAL COMPONENTS IN INVASIVE DUCTAL CARCINOMA OF THE BREAST**

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**Introduction, Aims:** The authors made a preliminary assesment of possible correlations between the degree of nuclear pleomorphism and the amount of fibrillary components and capillaries of intratumoral stroma in 20 cases with invasive ductal carcinoma of the breast.

**Methods:** The samples were fixed in buffered formalin and included in paraffin wax. Stromal components were marked silver staining (Gömöri) and antibodies for CD34 Classs I. Five fields with no necrosis were selected randomly using x10 objective, for each case, from the most representative slide for the tumoral pattern. The selected tumoral areas were aquired using a Nikon DN100 digital camera and a LuciaNet 1.16.2 soft. The quantitative determinations were performed, after image calibration, with analySIS Pro 3.2 soft. The 100 selected fields were subdivided in three groups following the degree of nuclear pleomorphism: group I (mild atypia), group II (moderate atypia), group III (severe atypia). The studied parameters for each group were: area occupied by stromal fibrillary elements, area occupied by malignant cells and capillary densities related to tumoral area, to malignant cells area and to stromal fibrillary components.

**Results.** The group with moderate nuclear atypia on the field area was the most numerous. Stromal fibrillary component ranged between 25,85% in group II, with a good Confidence Level (95%) of 2,93, and 34,9% in group III but with a Confidence Level (95%) of 9,07 (group III). Vascular density related to whole tumoral area was higher in groups with intermediate and severe pleomorphism but with a better Confidence Level (95%) of 5 for group II only. Vascular density related to epithelial malignant cells had an increasing trend from group I to group III, but the latter had a small number of analysed fields and a large range of values, reflected in a Confidence Level (95%) value of 13,23. Vascular density related to stromal fibrillary components had high mean values in the second group (193/mm<sup>2</sup>) but with large intervals of range in all three groups and especially in the third.

**Conclusions.** Our preliminary data showed that severe nuclear atypia seems to be present in tumoral areas with an increased amount of stromal fibrillary components No significant correlation was found between the vascular density and the degree of nuclear atypia, despite the relative increasing trend of vascular density related to tumoral parenchyma from areas with mild to those with severe atypia.

#### P 20

##### **CORRELATIONS BETWEEN TUBULE FORMATION AND INTRATUMORAL STROMAL COMPONENTS IN DUCTAL INVASIVE CARCINOMA OF THE BREAST**

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**Introduction, Aims.** The authors made a preliminary assessment of possible correlations between the amount of fibrillary components and capillary network of intratumoral stroma and the degree of glandular formation in 20 cases of invasive ductal carcinoma of the breast.

**Material and Methods.** The samples were fixed in buffered formalin and included in paraffin wax. Stromal components were marked silver staining (Gömöri) and antibodies for CD34 Class I. Five fields with no necrosis were selected randomly using x10 objective, for each case, from the most representative slide for the tumoral pattern. The selected tumoral areas were acquired using a Nikon DN100 digital camera and a LuciaNet 1.16.2 soft. The quantitative determinations were performed, after image calibration, with analySIS Pro 3.2 soft. The 100 selected fields were subdivided in three groups following tubule formation: group I (score 1), group II (score 2), group III (score 3).

The studied parameters for each group were: area occupied by stromal fibrillary elements, area occupied by malignant cells and capillary densities related to tumoral area, to malignant cells area and to stromal fibrillary components.

**Results.** The group with less than 10% tubule formation (score 3) on the field area was the most numerous, followed by the group with score 2. Fibrillary component represented between 27,6% (group II) and 30% (group III), with a Confidence Level (95%) between 4,52 (group I) and 3,66 (group III). Vascular density related to whole tumoral area ranged between 39/mm<sup>2</sup> (group I) and 43/mm<sup>2</sup> (group II) with a Confidence Level (95%) between 8,72 (group I) and 4,68 (group III). Vascular density related to epithelial malignant cells was higher (around 66/mm<sup>2</sup>) in groups II and III, with a Confidence Level (95%) between 8,18 (group II) and 7,1 (group III). Vascular density related to stromal fibrillary components had its highest value in third group (191/mm<sup>2</sup>) but with large intervals of range in all three groups.

**Conclusions.** These preliminary data showed no significant variations of the intratumoral stroma and capillaries amounts in different areas of ductal invasive breast carcinomas and also no significant correlation between stromal components variations and the degree of tubule formation.

**P 21**  
**FINE NEEDLE ASPIRATION CYTOLOGY AND CORE NEEDLE BIOPSY BY MAMMOTOME IN THE EVALUATION OF NON PALPABLE BREAST LESIONS.**

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**Introduction:** Radiologically dubious breast lesions, often requires a second diagnostic step, generally consisting in a FNAC (Fine Needle Aspiration Cytology) or in a core biopsy.

**Purpose:** -to evaluate the distribution of lesions in C1-C5 and B1-B5 categories and the meaning of them in respect to the final diagnosis -to compare the quality assurance of the two methods.

**Material and Methods:** During the last 5 years 1519 patients underwent FNAC under stereotactic or ultrasound guidance (needle 23-24 gauge) and 504 patients had a core needle biopsy by Mammotome (11 gauge). All patients had non palpable breast lesions.

Cytological smears were classified as: C1: 433(28.5%), C2: 590(38.8%), C3: 100 (6.5%), C4: 131(8.6%), C5: 265(17.4%); Mammotome biopsies as: B1: 3(0.6%), B2: 279(55.2%), B3: 36(7.3%), B4: 6(1.2%), B5: 180(36%).

**Results:** histology was available in 365 cases of cytologies: 46 C1 (10.6%), 36 C2 (6%), 25 C3 (25%), 61 C4 (46%), 147 C5 (55%).

C1 were malignant in 29 (60%), benign in 16 (33%); one case (2%) was diagnosed as atypia.

C2 resulted malignant (false negative) in 19 (52.7%) and benign in 17 (47.2%).

Of C3 lesions 12 (48%) resulted benign and 13 (52%) malignant.

C4 were malignant in 43 cases (70%), benign in 17 (28%); one case was atypia.

C5 were malignant in 144 cases (98%), atypical in 2 cases (1.37%) and benign (false positive) in one case (0.6%).

183 cases of core biopsy had definitive histology: B1: No case had surgery. Of B2: two were benign one was a cancer (liponecrosis on the Mammotome). Of B3: 15 were benign and 2 malignant.

Among B4 cases (4) having surgery: three were atypical, one was a DCIS. A definitive histology was available in 159 of B5 cases. Diagnosis was confirmed in 151 cases (95 in situ and 56 invasive), 7 cases did not show tumor any more and one case (0.6%) was atypical duct hyperplasia.

Sensibility was 71% for FNAC and 97.8% for core biopsy. Specificity was 96.7% for FNAC and 99.6% for core biopsy. PPV of cytology was 99.2 (C5), NPV was 96.8%. PPV of microbiopsy was 99.4% (B5), NPV was 99.6%.

**Conclusions:** "C" and "B" categories showed a different distribution of lesions with a different meaning in relation to the final diagnosis.

Core biopsy presented a higher specificity, sensibility, PPV and NPV than FNAC; it, in spite of its high rate of inadequate samples, probably due to the small dimension of the lesions, can be still considered a valid approach for breast lesions.

**P 22**  
**STEROID AND HER2 RECEPTORS' STATUS IN BREAST CARCINOMA – 4493 CASES FROM POPULATION-BASED STUDY IN POLAND**

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The study is a part of national HER2 screening project in Poland, which started in 2002 and continues. Until April 2004, 11 557 cases of invasive breast carcinoma cases were included.

Breast cancer diagnostics, aside from well-established prognostic and predictive factors (e.g. histological type, histological grade, pTNM, lymph node status) employs the assessment of other parameters in order to make pathological report sufficient for oncological management. These parameters are estrogen (ER), progesterone (PGR) and HER2 status.

The aim of the study was the description of dependency between HER2 and estrogen/progesterone status.

**Materials and methods:** The study was performed on 4493 non-selected cases of breast cancer. The data originated from 14 oncological centers in Poland and were collected between April 2003 and March 2004. The estrogen, progesterone and HER2 receptor status were evaluated immunohistochemically (IHC).

HER2 IHC results were classed into 4 groups (DAKO criteria system: 0, 1+, 2+, 3+). The cases classed as 0, 1+ and 2+ were considered to have negative HER2 status. The remaining cases (3+) were clinically HER2-positive.

Steroid receptors positive cases were grouped by presence of 10% or more positively stained cancer nuclei, regardless of strength of the stain. ER/PGR status was stated positive, when the presence of ER/PGR receptors was detected. A mixed group consisted of cases, in which one steroid hormone was present.

Results: The group of cases described by IHC as "0", "1+" and "2+" showed the presence of at least on steroid receptor expression in more than two thirds of all cases (71%, 80,1% and 79,1%, respectively). Among the tumors exhibiting very strong HER2 receptor expression (3+), 48,2% were steroid receptors negative.

Discussion: The results indicate the difference between the expression of estrogen and progesterone receptors and HER2 status. Results suggest necessity of independent evaluation of both steroid receptors and HER2 status. The probability of existence of positive steroid receptor status in clinically HER2-positive group is much lower, than in clinically HER2-negative groups (0, 1+, 2+).

### P 23

#### THE USE OF FISH METHOD IN HER2 EVALUATION ALGORITHM IN BREAST CANCER

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There are two main techniques of evaluation of HER2 status: immunohistochemistry (IHC) for the protein expression and fluorescence in situ hybridisation (FISH) for amplification of HER2 gene.

During the year 2004, in our Department of Pathology, 1409 consecutive cases of invasive breast carcinoma were diagnosed and included in the study. Among them 365 were described by IHC as 2+. According to the algorithm of the diagnostic procedure, those cases were additionally examined with FISH method.

The aim of the study was to compare the results obtained by IHC and FISH methods in determination of HER2 status in breast cancer.

Materials and Methods: The material originated from 414 patients operated in Institute of Oncology in Warsaw. FISH method was performed in every 2+ (365) case and, in randomly selected cancers exhibiting 0/1+ (14 cases) and 3+ (35 cases) IHC stain. IHC was performed with DAKO HercepTest. The results were classed into 4 groups, accordingly to the four-tier DAKO criteria system (0,1+, 2+, 3+). FISH evaluation was performed with PathVysion (Abbott) reagents. The results were divided into three groups: NA - no amplification, LA - low amplification and HA - high amplification. The number of copies of chromosome 17 was also assessed.

Over 90% of cases described by IHC as 3+ exhibited amplification of HER2/neu gene. The majority of cases classed by IHC as 2+ were negative by FISH (74,5%) or showed no amplification of HER2/neu gene combined with aneuploidy of chromosome 17 (6,6%). Almost one fifth of IHC 2+ tumours presented gene amplification (18,9%). All cases described by IHC as 0/1+ were HER2-negative by FISH.

IHC is well-established method of assessing HER2 status in breast cancer. Nonetheless, group of cases described as 2+ should be additionally examined using FISH. The results obtained by the latter method are more reliable. With FISH method twenty percent of clinically negative 2+ group are potential beneficiaries from Herceptin therapy.

### P 24

#### EVALUATION OF C-ERB-B2/HER-2/NEU EXPRESSION IN INFILTRATING BREAST CARCINOMA IN A GREEK POPULATION COHORT OF 1100 CASES BY IMMUNOHISTOCHEMISTRY

#### AND CHROMOGENIC IN SITU HYBRIDIZATION. EARLY DATA OF THE GREEK MULTICENTER STUDY.

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OBJECTIVE: To evaluate the expression of c-erb-B2/HER2/neu in infiltrating breast carcinomas in Greek patients by immunohistochemistry, to compare the reactivity against HER2 of three different antibodies and to correlate HER2 expression levels to its gene amplification by CISH.

MATERIALS AND METHODS: In this study, 1100 specimens of infiltrating breast carcinoma (ductal and lobular) were analysed immunohistochemically, using three different antibodies against HER2 , AO485 (DAKO), TAB-250 (Zymed) and CB11 (Biogenex). Immunostaining was evaluated as membranous, and the intensity was graded 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong). Cases been evaluated as 1+ and 2+ in IHC were analysed by CISH (Zymed kit) to determine the presence or not of HER2 gene amplification. Gene amplification was graded as negative (2-5 gene copies/nucleus), low amplification (6-10 gene copies/nucleus) and high amplification (>10 gene copies/nucleus).

#### RESULTS AND CONCLUSIONS:

1. By using the commonly used AO485 antibody, 22.81% of the tumors were 3+, 16.50% showed 2+ and 27.66% exhibited 1+ HER2 reactivity.
2. There was discordance in evaluating HER2 expression among the three different antibodies, especially in the cases that have been graded as 1+ and 2+.
3. Concerning CISH, among 1+ IHC cases, 92.59% had no gene amplification,4.93% displayed low gene amplification and 2.47% high gene amplification. On the other hand, 2+ cases showed low gene amplification in 11.69%, high gene amplification in 11.69% and no amplification in 76.62% of the cases. We conclude that CISH is a highly accurate method in evaluating HER2 gene status, more sensitive than IHC and should be used not only in 2+ IHC cases but also in 1+ IHC ones.

### P 25

#### ASSESSMENT OF HER2 STATUS IN BREAST CANCER BY FLUORESCENCE IN SITU HYBRIDISATION

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Introduction

The aim of this study was to analyse the presence of gene amplification and/or overexpression of the HER-2/neu (c-erbB-2) proto-oncogene in invasive breast carcinomas. Furthermore, to ascertain the ability of our laboratory to create specific for HER-2/neu probe and test it versus the commercially available assay (Abbott <sup>®</sup>C Vysis, Downers Grove, IL).

#### Materials and Methods

Forty cases of invasive breast carcinomas were evaluated by immunohistochemistry (IHC). Interpreted 2+ borderline reactions were considered inconclusive and referred to FISH analysis (Guidelines of HER-2/neu, 2003). The amplification of the HER-2/neu gene was determined by dual-colour FISH using the c-erbB-2 specific probe (SpectrumOrange) and the chromosome 17 centromere specific probe (SpectrumGreen) from the commercially available kit (Abbott <sup>®</sup>C Vysis). FISH probe, generated by our team, from BAC clone, specific for HER-2/neu gene, was also tested and both, the Vysis assay versus ours were compared in order to evaluate their efficacy.

#### Results

According to the scoring criteria, HER-2/neu to chromosome 17 ratio < 2 signifies non amplification of the HER-2/neu gene, whereas HER-2/CEP17  $\geq 2$  is considered amplified.

From the studied cases 31 of 40 (77.5%), showed no amplification of the c-erbB-2 gene and 4 (10%) demonstrated amplified signals after FISH examination. The test failed in the remaining five cases (12.5%) because the intensity of the hybridization signals was too faint to be counted. Both the generated probe of HER-2/neu gene and the commercially available probe (Abbott <sup>®</sup>C Vysis) showed the same results with the same intensity and hybridization efficacy in FISH analysis.

#### Discussion

Despite the tendency of IHC interpreted 2+ cases to present HER-2/neu amplification, a high percentage of non-amplified cases (77.5%) was observed. Although, a possible failure in IHC cannot be excluded due to non-specific staining and the subjective nature of interpretation that led to misclassification, further number of new cases will allow statistical analysis to be performed for final conclusions.

#### P 26

### THE ASSOCIATION OF HER-2/NEU OVEREXPRESSION IN RELATION WITH P53 NUCLEAR ACCUMULATION, HORMONAL RECEPTOR STATUS AND COMMON CLINICOPATHOLOGICAL PROGNOSTIC PARAMETERS IN A SERIES OF EGYPTIAN WOMEN WITH INASIVE DUCTAL CARCINOMA

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Introduction: HER2/neu protein has garnered a great deal of interest in the popular media. However, it has long been known amongst pathologists and oncologists for its potential role as a tumor and prognostic marker. This protein exists on the surface of epithelial cells and functions in the normal cell as a receptor for a cellular growth factor. Aim of the study: is to determine the association of HER-2/neu and p53 as well as hormonal receptor status with conventional common pathologic parameters in a series of Egyptian women with invasive ductal carcinoma of breast. Methods: expression of HER-2/neu and p53 was examined by immunohistochemistry in samples of breast tissue from 50 patients with invasive ductal carcinoma and their significance for prognosis was analyzed. Results: Of the 50 carcinoma tissue samples, 26% were positive for HER2 over-expression. In the present study

there was a high significant association between HER-2 over expression and the accumulation of nuclear p53 (p=0.0017) also there was an inverse significant association with ER (p=0.0001) and PR status (p=0.0003). There was a significant association between HER-2 over expression and the increased size of tumor (p=0.0115) as well as the high grade tumors (p=0.0001). Meanwhile no significant association was there as regard number of the invaded lymph nodes, grade of tumor necrosis and menopausal status. Conclusion: The study had indicated that Her-2/neu may be a powerful predictor of poor prognosis as it's over expression was strongly directly correlated with p53 nuclear accumulation, tumor size, tumor grade and inversely correlated with hormonal receptors status, of breast carcinoma. Meanwhile; no significant associated for number of invaded lymph nodes and the menopausal status.

Key words: Immunohistochemistry, Her-2/neu, p53, hormone receptors, clinicopathological prognostic parameters & breast cancer

#### P 27

### COMMON EXPRESSION OF EGFR (HER-1) IN METAPLASTIC BREAST CARCINOMAS: POTENTIAL RELEVANCE TO ADJUVANT THERAPY WITH EGFR-INHIBITORS?

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Metaplastic carcinomas (MCs) of the breast rarely express steroid receptors and Her-2, which minimizes the options for adjuvant therapy in patients with advanced disease.

We immunohistochemically assessed the expression of steroid receptors and 4 members of the EGFR/Her-family (EGFR/Her-1, Her-2, Her-3 and Her-4) in a collective of 20 MCs (8 MCs with heterologous elements, 7 spindle cell MCs, 4 carcinosarcomas and 1 matrix producing carcinoma). 14 out of 20 MCs (70%) showed positive reactions for EGFR (Her-1). Among these cases, 1+, 2+ and 3+ reactivity was observed in 2, 4 and 8 cases, respectively. Her-2 was only present in one MC with 1+ reactivity. Her-3 (1+ reactivity), Her-4 (2+ reactivity) and Androgen receptors (2+ reactivity) were also expressed by one tumor. Estrogen and Progesterone receptors (3+ reactivity each) were only detected in the epithelial component of 2 carcinosarcoma-type MCs.

Compared to collectives of not otherwise specified (NOS) breast carcinomas investigated in previous studies, the MCs in our study expressed EGFR considerably more frequently. Therefore, we conclude that they represent a promising subject for future studies (especially molecular analyses to detect specific mutations in EGFR-exons) on EGFR expression in breast carcinomas. Androgen receptor targeted therapy or anti-Her 2 therapy do not seem to be an option for adjuvant treatment of MCs according to our results, but patients suffering from this aggressive variant of breast carcinoma might benefit from treatment with protein kinase inhibitors such as gefitinib or cetuximab.

#### P 28

### PROX-1, A LYMPHATIC-ENDOTHELIUM ASSOCIATED TRANSCRIPTION FACTOR, IS OVEREXPRESSED BY BREAST CANCER CELLS

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**Introduction:** The study of lymphatic vessels and lymphatic-derived tumours is frequently complex, due to common characteristics of morphological features between blood and lymphatic endothelial cells, as well as to the lack of specific lymphatic endothelial markers. Prox-1, a homeobox gene cloned by homology with the Drosophila gene prospero, that codifies a nuclear transcription factor that plays a major role during embryonic lymphangiogenesis and is supposed to be a useful marker to differentiate lymphatic from blood endothelial cells. **Purpose:** We studied a series of breast carcinomas in order to observe the specificity of this marker in lymphatic vessels. **Methods:** Tissue-array from formalin-fixed and paraffin-embedded samples of 116 invasive ductal carcinomas recovered from the Pathology files of IPATIMUP, Porto, Portugal were immunostained with a primary antibody raised against Prox-1 (diluted 1:133; Research Diagnostics, Inc., Flanders, NJ, USA). **Results:** Prox-1 immunostaining highlighted the lymphatic vessels, and was also detected in the cytoplasm and nuclei of neoplastic cells in 94.8% (110/116) of the cases. **Conclusion:** Our results suggest that invasive breast carcinoma cells can express Prox-1. Further studies are needed to understand the real meaning of this finding.

#### P 29

##### UNUSUAL CYTOPLASMIC IMMUNOPPOSITIVITY FOR MIB1 IN BREAST CARCINOMA

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##### INTRODUCTION:

Tumour cell proliferation can be assessed by a large number of antibodies: Ki-67, PCNA, KiS1... MIB1, the most commonly used, is a monoclonal antibody raised against the recombinant part of the Ki67 antigen. It reacts with cells in the late G1, S, G2 and M phases of the cell cycle and only stains nuclei with various intensity.

We report herein 2 cases of breast carcinoma with cytoplasmic immunoreactivity for MIB1.

##### POPULATION:

One occurred in a 62 year-old women, a 12 cm invasive ductal carcinoma, histoprognostic SBR grade 3. The second occurred in a 54 year-old women, a 23mm invasive ductal carcinoma, histoprognostic SBR grade 1. None of these patients received preoperative treatment.

Immunohistochemistry was done with MIB1 (Dako) after antigen retrieval (water bath) with LSAB kit and DAB revelation. The test was repeated at different times and with different blocks of the fixed tumour (Formaline, AFA) with the same results. The others immunohistochemistry (RE, RP, HER) didn't shown abnormal staining (nuclear staining for RE and RP, cytoplasmic staining for HER).

##### DISCUSSION:

In 1995, Hirokawa and al, first reported the cytoplasmic immunoreactivity for MIB1 in hyalinizing trabecular adenoma of the thyroid. Recently, two reports showed this unusual staining in atypical pleomorphic adenoma of the salivary gland and sclerosing haemangioma of the lung. To the best of our knowledge, this immunoreactivity hasn't been reported in breast tumours. Significance of this unusual staining is still unknown. There was no correlation between the properties of the cells stained and the mitotic count which suggests a cross-reaction of this immunostaining.

##### CONCLUSION:

Pathologists should be aware of this unusual MIB1 cytoplasmic staining. This pattern of reactivity of MIB1 in

breast carcinoma may be a result of cross-reaction of the antibody.

#### P 30

##### IGF-II MRNA EXPRESSION IN BREAST CANCER (BC) TISSUES AND CLINICAL OUTCOME.

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Insuline like growth factor II (IGF II) is an important regulator of neoplastic growth and it is generally expressed in stroma of BC tissues. Estrogen and Progesterone receptor (ER, PR) are considered a good prognostic predictors in BC. The aim of the study was to evaluate the impact of IGF II mRNA expression in clinical outcome in a large series of BC. The study group included 75 women (mean age +/- SD= 53.3+/-15.6 yr) submitted to radical mastectomy for ductal infiltrating breast carcinoma. The BC specimens was assessed for IGF II mRNA using in situ hybridization method and ER and PR by immunohistochemistry. Five years clinical follow-up was available in 65/79 BC (82.3%) and 46/65 (70.8%) were still alive and relapse free. ER+ was found in 39/65 (60%), PR + in 30/65 (46.2%) and stromal IGF II mRNA expression (IGF II +) in 33/65 (50.8%). 22/65 (33.8%) BC were IGF II+ ER+ and 19/65 (29.2%) IGF II+ PR+. No relationship was found between ER, PR, IGF II separately examined and clinical outcome. The better 5 yr survival was found in ER+ IGF II+ (16/22: 72.7%) and IGF II+ PR+ BC (14/19: 73.7%) and in contrast, the worse survival was found in IGF II+ ER- (6/11: 54.5%) and IGF II+ PR- (5/11: 45.5%) groups. (p=0.006, p=0.02, respectively). These data indicate that stromal IGF II may be considered a new important predictive factor in BC and suggest that this growth factor may have a role in both differentiation or proliferation of BC cells

#### P 31

##### CORRELATION BETWEEN CYCLOOXYGENASE-2 EXPRESSION AND CELL PROLIFERATION IN INVASIVE DUCTAL BREAST CANCER

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**INTRODUCTION:** Cyclooxygenase-2 (COX-2) is overexpressed in breast cancer and may have a role in tumor development and progression. There has been inconsistency in the literature regarding the precise significance of this. Some studies have found no clinicopathological relevance at all, while others have concluded Cox-2 expression is an important biomarker in invasive disease, correlating with poor prognosis features. The aim of this study was to determine the relationships between Cox-2 expression and cell proliferation in breast cancer, and to correlate the expression of this enzyme with classic clinicopathological parameters.

**MATERIAL AND METHODS:** We retrospectively analyzed 150 breast tumors in paraffin embedded tissue and from medical records, obtained clinicopathological data. Regarding to tumor grade we had three groups of patients and each group consisted of 50 patients. Cox-2 expression was investigated by immunohistochemistry using monoclonal antibody. Immunohistochemistry was done following protocol on DAKO TechMate Horizon automated immunostainer. S-phase fraction (SPF) determined by flow cytometry used as a marker of cell proliferation.

**RESULTS:** Cox-2 expression was detected in 86% of all tumors studied. The preliminary results have shown that Cox-2 protein expression significantly correlates only with tumor

grade but not with SPF. We have found statistically significant difference in expression of Cox-2 in different grade of tumors ( $p=0.02$ ). Comparing different grade with SPF we found statistically significant higher SPF in poorly differentiated tumors ( $p<0.001$ ).

**CONCLUSIONS:** We have confirmed that Cox-2 expression does occur in invasive breast ductal cancer and is associated with tumor grade. It remains to be investigated whether treatment with selective inhibitors of Cox-2 may be an additional therapeutic option for patients with breast cancer.

### P 32

#### **IMMUNOHISTOCHEMICAL ANALYSIS OF RET AND ITS LIGAND GDNF IN 139 CASES OF BREAST CANCER**

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**Introduction:** The RET proto-oncogene tyrosine kinase receptor is activated by a ligand complex comprising glial cell line-derived neurotrophic factor (GDNF) and GDNF family receptor alpha 1 (GFR $\alpha$ 1). They are expressed in multiple organs during development and in some types of human tumors in adults. In this study we evaluated the expression of RET, and GDNF in different types of breast cancer.

**Material and Methods:** RET, expression was analyzed in samples of formalin-fixed tissues from tumoral zone, from 139 patients with different types of breast cancer: 109 IDC; 5 IC; 7 ILC; 6 mucinous carcinomas; 4 medullary carcinomas; 3 papillary carcinomas 2 tubular carcinomas and others (3) using immunohistochemical methods.

**Results:** A total of 98/137 (71.6%) of breast tumors showed cytoplasmic immunoreactivity for RET, and 130/138 (71.6%) for GDNF. By statistical analysis based on 111 months follow up showed that RET expression was associated with less tendency to the regional lymph nodes metastases ( $p<0.0001$ ); less tendency to relapse ( $p<0.004$ ); increase of overall survival ( $p<0.0004$ ); There was no statistically significant correlation of RET or GDNF expression with the histological grade, microscopic type, size or stage. Those findings were not supported by multivariate analysis.

**Conclusions:** We found that RET and its GDNF/GFR $\alpha$ 1 ligand complex were expressed in normal breast tissues and in a high percentage of different types of breast tumors using immunohistochemical methods. The presence of RET did not show clear association with any particular light microscopic growth pattern or special tumor subtype, but interestingly, this expression was associated with less tendency to the lymph nodes metastases and increase of overall survival. In summary RET determination by immunohistochemical analysis could be used as a prognostic factor in breast cancer.

### P 33

#### **PROGNOSTIC VALUE OF MIB1 PROLIFERATION INDEX AND C-ERBB2 IN PATIENTS WITH STAGE II BREAST CANCER AND LYMPH NODE INVOLVEMENT**

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**INTRODUCTION:** Breast cancer is the first leading cause of cancer in women from Spain, and its incidence rates have

increased in the recent last years. Study of prognostic value of different factors in breast cancer patients with lymph node involvement can identify subgroups of patients with high risk for more effective clinical management. C-erbB2 that encodes HER2/ Neu protein (p185HER2), and MIB1 proliferation index are useful parameters to assess this issue.

**PURPOSE:** The goal of this study was to evaluate if the prognostic role of c-erbB2 and MIB-1 proliferation index, p53, S-phase, were dependent or independently useful to characterise more aggressive breast tumors.

**PATIENTS AND METHODS:** 87 positive-node infiltrating breast cancer patients classified as stage II (T2N1). C-erbB2, p53, MIB-1 proliferation index, estrogen (ER) and progesterone receptors (PR) were determined immunohistochemically by assigning a score with an analysis image software. C-erbB2 membrane expression was graded negative, indeterminate, 1+, 2+, 3+ when  $\geq 5\%$ , 20%-30%, 30%-50%, and more than 50% of cells were stained, respectively. Comparisons in distributions were analyzed by ANOVA and Pearson tests, using SPSS. Differences in disease-free and overall survival were evaluated by using the log-rank test.

**RESULTS:** 48.3% of the patients resulted to be negative for c-erbB2, whereas 12.6%, 18.4%, 15% and 5.7% of the patients were indeterminate, 1+, 2+ and 3+, respectively. C-erbB2 immunostaining was correlated to node involvement and number of positive nodes ( $P=0.022$ ,  $P=0.023$ , respectively), tumor size ( $P=0.037$ ) and PR ( $P=0.03$ ). There are no correlations between c-erbB2 immunostaining, age, ploidy, MIB-1 and ER. The median follow-up time was 90 months. Disease-free survival is inversely correlated to c-erbB2 immunostaining ( $P=0.027$ ), number of positive nodes ( $P=0.037$ ) and tumor size ( $P=0.042$ ), being nearly significant for MIB1 ( $p=0.09$ ). Predictive value for c-erbB2 was confirmed with a relative risk of 2.5 times in multivariate analysis.

**CONCLUSIONS:** 1. Higher proliferation index is a bad prognosis factor in patients with infiltrating breast cancer patients, stage II tumors and lymph node involvement. 2. C-erbB2 oncogene is an indicator of bad prognosis in these patients. 3. Patients that were negative for proliferation index and C-erb-B2 presented longer overall survival.

### P 34

#### **PLATELET-DERIVED GROWTH FACTOR RECEPTOR-ALPHA (PDGFR-ALPHA) EXPRESSION IN INVASIVE BREAST CARCINOMAS**

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**Introduction:** Receptor tyrosine kinases have been extensively studied due to their frequently abnormal activation in the development and progression of many human cancers. Platelet-derived growth factor receptors (PDGFRs) are receptors with intrinsic tyrosine kinase activity that regulate several functions in normal cells and are widely expressed in a variety of malignancies. After the demonstration that GISTs without c-kit mutations harbour PDGFR-alpha activating mutations, and that it is also a therapeutic target for imatinib mesylate, the interest for this receptor increased considerably. Since breast cancer is one of the most frequent neoplasia in women worldwide, and there are only one study reporting PDGFR-alpha expression in breast carcinomas, the aim of this work was to investigate the potential meaning of PDGFR-alpha expression in invasive mammary carcinomas.

**Methods:** We used immunohistochemistry to detect PDGFR-alpha overexpression on a series of 181 formalin-fixed paraffin-embedded invasive ductal breast carcinomas and associated its expression with known prognostic factors. We also performed PCR-SSCP and direct sequencing in order to screen for PDGFR-alpha gene mutations.

**Results:** PDGFR-alpha expression was demonstrated in 39.2% of the breast carcinomas and showed an association with lymph node metastasis ( $p=0.0079$ ), HER-2 expression ( $p=0.0265$ ) and Bcl2 expression ( $p=0.0121$ ). It was also found a correlation with PDGF-A ligand expression ( $p=0.0194$ ). Screening for mutations revealed the presence of the 2500 A>G and 2529 T>A alterations in exon 18, the 2472 C>T (exon 18) and 1701 G>A (exon 12) missense mutations, and also the presence of the intronic insertion IVS17-50insA at exon 18 in all sequenced cases. These mutations did not correlate with PDGFR-alpha expression.

**Conclusions:** PDGFR-alpha is expressed in invasive breast carcinomas and is associated with biological aggressiveness factors. The mutations found did not show any correlation with PDGFR-alpha expression.

### P 35

#### **CA-125 IS A USEFUL TUMOUR MARKER IN BRCA2 ASSOCIATED BREAST CANCER AND IN POORLY DIFFERENTIATED SPORADIC TUMOURS.**

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CA-125 is a useful tumour marker for ovarian cancer. As such the marker is not specific for ovarian cancer. A number of other tumours diseases and conditions have been found to be related to elevated plasma levels of CA-125. In breast cancer overall 5-80% (median 30%) have been found to be positive for CA-125.

In twenty one consecutive patients with BRCA2 germ line mutations the breast tumours were stained for Ca-125 immunoreactivity. 8/21 BRCA2 tumours showed moderate to strong immunostaining and in the sporadic tumours 8/17 showed similar immunostaining. 5/8 BRCA2 tumours were classified as histological grade 3 tumours while all of the the tumours that showed positive immunoreactivity for CA-125 were classified as histological grade 3 tumours. Two of the BRCA2 patients had a recurrence and the tumour recurrence correlated with an elevation of plasma Ca-125. The elevation occurred before metastatic symptoms. Further one of the patients progressed twice while first responding favourably to endocrine therapy and later to chemotherapy with the tumour status strongly correlated with Ca-125 levels.

Immunohistochemistry and serum assay were used to detect CA-125.

Tumour cells displaying Ca-125 were demonstrated in eight of twenty one BRCA2 tumours. Six of these tumors were ductal carcinomas and two were lobular carcinomas. Five of these eight tumors expressed both estrogen and progesterone receptor immunoreactivity. Three men with breast cancer were included and none of them expressed CA-125 immunoreactivity in their breast tumours.

In the sporadic tumours Ca-125 immunoreactivity were demonstrated in eight of seventeen tumours. All of these tumors were ductal carcinomas and the tumours were poorly differentiated and classified as histological grade 3 tumours. Six of these eight tumors were negative for both estrogen and progesterone receptor immunoreactivity.

The data demonstrate that Ca-125 is a useful early tumour marker in a subset of breast cancer patients associated with BRCA2 mutations and possibly also in other high grade tumours in the breast.

This knowledge is of importance as Ca-125 is used to screen for ovarian tumours in BRCA1/2 patients. The finding that Ca-125 is an early marker of breast tumours in a subset of

BRCA2 cases lends hope that this marker also could be used in therapeutic settings.

### P 36

#### **ESTROGEN RECEPTOR (ER) NEGATIVE INVASIVE BREAST CARCINOMAS FREQUENTLY EXPRESS MYOEPITHELIAL MARKERS**

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**Introduction:** Luminal, basal and myoepithelial cell differentiation have been reported in ductal breast cancer (BC), using several markers, including cytokeratins (CKs) 5, 14, 8, 18, actin, S100, and vimentin. Luminal type BCs are associated with ER and PgR positivity and a good prognosis. Few studies documented basal and myoepithelial markers expression in relationship to p53 positivity and tumor grade. The aim of our study was to investigate the expression of basal (CK5 and CK14) and luminal (CK8 and CK18) cytokeratins in high-grade p53+, ER- and PR- BCs, and to correlate them with the expression of myoepithelial markers (a-smooth muscle actin, vimentin and S100).

**Design:** Formalin-fixed, paraffin-embedded sections from 60 selected p53+, ER-, PgR-, c-erb-B2-negative BCs, including 56 NOS invasive ductal and 4 medullary carcinomas, were immunostained for CK14 (Novocastra), CK5 (Novocastra), CK8 (Dako), CK18 (Novocastra), a-smooth muscle actin (Sigma), S100 (Dako) and vimentin (Dako).

**Results:** All cases were G3, ER-, PR-, c-erb-B2-negative, p53-positive BCs. The mean age was 59 years ranging from 29 to 87. Twenty of sixty cases (33%) had lymph node metastases (15 N1 and 5 N2). The most common morphological features were the presence of large sheets of cells sometimes with spindle appearance, festoon-like pattern, pushing margins and occasional large central acellular zones, represented by foci of tissue infarction and deposits of collagenous or hyaline-like material. The proliferative index (Ki-67) and p53 expression were high in all cases (mean percentage of 63% and 81% respectively). Forty-four of 60 (73%) BCs were positive for S100, 31/60 (52%) for vimentin, 5/60 (8%) for a-smooth muscle actin, 43/60 (72%) for CK5, 20/60 (33%) for CK14, 55/60 (92%) for CK8 and 31/60 (52%) for CK18. Coexpression of basal and luminal CKs was seen in 43 cases (72%); 39 of these cases were positive for at least a myoepithelial marker.

**Conclusions:** Our study suggests that a subset of high grade ER/PR/c-erb-B2- and p53+ BCs frequently displays an intermediate luminal/basal phenotype (immunoreactive both for basal and luminal CKs), often coexpressing myoepithelial markers. This particular phenotype could be the result of a peculiar pathway of breast carcinogenesis of great clinical relevance because of the high rate of lymph node metastasis. The results suggest that p53+, ER-, BCs should be investigated for the expression of basal and myoepithelial markers.

### P 37

#### **PROGNOSTIC SIGNIFICANCE OF TOPOISOMERASE II ALPHA IN BREAST CANCER PATIENTS**

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**Introduction:** There are many studies that show tumors overexpressing TOPO II have better response to anthracyclines. In our study we aimed to show the expression

of this enzyme at high and low risk breast cancer patients and the effect of this activity on prognosis of this disease.

**Material and Methods:** Thirty-seven operable breast cancer patients including 21 high risk and 16 low risk who were followed at Erciyes University Medical Oncology clinic between March 1999 and June 2003 were enrolled into this study. High risk patients received adjuvant chemotherapy consisting taxane and anthracycline and radiotherapy, 3 of the low risk patients received no adjuvant therapy, 4 adjuvant hormonal therapy, 9 adjuvant CT. Only one patient received RT in this group. TOPO II alpha expression was categorized in 4 groups according to cell numbers showing nuclear staining, 35% and higher expression (3+ and 4+ nuclear staining) was accepted as positive and others negative.

**Results:** Median age of all patients was 42 years (range 23-78). Histopathologically 32 patients (86.5%) were diagnosed as IDC. Two patients were stage I, 15 stage II, 20 stage III. Median follow-up time was 32.5 months (range 18-64). TOPO II alpha expression was positive in 45.9% of the patients. Ten patients (58.8%) among 17 patients showing TOPO II expression had also HER-2 expression. There was statistically significant relationship between TOPO II alpha expression and tumor grade ( $p=0.015$ ), diameter ( $p=0.019$ ), lymph node positivity ( $p=0.001$ ), increased number of lymph nodes ( $p=0.033$ ) and age ( $p=0.039$ ). There was statistically significant relationship between disease free survival (DFS) and lymph node positivity and increased number of lymph nodes ( $p=0.04$  and  $0.03$ , respectively), however no significant relation was detected between TOPO II alpha expression and DFS ( $P=0.14$ ). **Discussion:** TOPO II alpha expression seemed to be related to the aggressive behavior of the tumor in this study, however there is a need to confirm these findings in larger studies. High expression of TOPO II alpha and HER-2 in this group may be due to many factors, and this may also show that tumor behavior and anthracycline response may be different in our patients compared to other group of patients.

#### **P 38 EXPRESSION OF CD10 IN STROMAL FIBROBLASTS IN INVASIVE BREAST CANCER**

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**Aim:** To explore presence and prognostic value of CD10 in stromal fibroblasts in invasive breast cancer and its correlation with other basic histopathological parameters, hormonal status (Estrogen receptor, ER and Progesterone receptor, PR), bcl-2 and cyclin D1.

**Material and methods:** Medical records of 46 patients, diagnosed in 1998, from Central Database of Institute of Oncology, Clinical Center University of Sarajevo were analysed. The median follow-up was 67 months (range: 4-103 months). Routine histopathological evaluation was performed for 46 formalin fixed and paraffin embedded tissue samples. For immunohistochemical staining we used monoclonal antibodies for ER, PR, bcl-2, cyclin D1 and CD10.

**Results:** Twenty three (50%) out of 46 samples were negative for CD10; 9 (19.6%) showed expression 1, 8 (17.4%) expression 2 and 6 (13%) expression 3. Expression of CD10 inversely correlated only with ER ( $p=0.05$ ) and no significant correlation was found with other parameters analysed in our study. Patients with higher CD10 expression had shorter overall survival but not relapse-free survival ( $p=0.006$ ). Cox regression analysis for overall survival (OS) showed that lymph node status, tumor size, age, bcl-2 and CD10 expressions were independent prognostic factors ( $p=0.009$ ,  $0.029$ ,  $0.019$ ,  $0.034$  and  $0.049$ , respectively).

**Conclusion.** Expression of CD10 is associated with worse disease outcome in invasive breast cancer.

**Key words:** Breast cancer, CD10, stromal fibroblasts, prognostic factors.

#### **P 39 HIF-1 ALPHA AND CA IX : TWO PROGNOSTIC FACTORS IN INVASIVE BREAST CARCINOMA**

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**Introduction :** Hypoxia inducible factor 1 (HIF-1) is a key regulator of oxygen homeostasis and controls the expression of several genes involved in various aspects of cancer progression (metabolic adaptation, angiogenesis and cell survival). Among those genes, ca9 (carbonic anhydrase 9) plays a role in extra-cellular pH regulation. HIF-1 $\alpha$ , one of the 2 subunits of HIF-1 is stabilized by hypoxia.

We retrospectively studied the impact of HIF-1 $\alpha$  and CA IX expressions on the outcome of patients with breast cancer.

**Methods :** Immunohistochemical stainings of HIF-1 $\alpha$  and CA IX were evaluated in an unselected cohort of 132 patients with invasive breast carcinoma. The follow-up was more than 10 years. We examined overall survival, distant metastasis-free and disease-free survivals by univariate and multivariate analyses. Correlations to clinicopathological parameters (tumour size, nodal status, histological grade, hormonal receptors and carcinoma in situ associated with the invasive tumour) were performed using Chi-square test. The level of significance was 5% ( $P<0.05$ ).

**Results:** No significant correlation exists between tumour size or nodal status and the expression of HIF-1 $\alpha$  or CA IX. HIF-1 $\alpha$  or CA IX staining is however statistically associated with the histological grade ( $P<0.0001$  et  $P=0.0004$ , respectively). Both markers are in general negative in low grade tumours and HIF-1 $\alpha$  is positive in high grade tumours. HIF-1 $\alpha$  and CA IX are significantly positive when hormonal receptors are lost, mainly estrogen receptors ( $P<0.0001$ ). HIF-1 $\alpha$  is also expressed in tumours associated with carcinoma in situ ( $P=0.0022$ ).

Expression of HIF-1 $\alpha$  or CA IX is correlated to worse overall survival ( $P=0.0005$  and  $0.048$ , respectively), distant metastasis-free survival ( $P=0.0001$  and  $0.0066$ ) and disease-free survival ( $P<0.0001$  and  $P=0.0129$ ). Stromal CA IX expression is correlated with worse overall survival ( $P=0.0014$ ). Multivariate analysis, regarding overall survival and including all parameters correlated with HIF-1 $\alpha$ , shows that the grade is the most significant factor. HIF-1 $\alpha$  positive immunoreactivity is more significant than CA IX's.

**Conclusion :** This preliminary study confirms that HIF-1 $\alpha$  and CA IX are poor prognostic factors in breast cancer, but mainly related with the grade in our hands. One larger study is ongoing to confirm these results and evaluate the impact of these markers in therapeutic response to establish a putative predictive role.

#### **P 40 MICROSATELLITE INSTABILITY AND LOSS OF HETEROZYGOSITY IN BREAST CARCINOMA**

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Previous studies have shown that younger women exhibit more aggressive pathologic features of breast cancer in comparison with older women. Young age could be an independent predictor of adverse prognosis. In order to evaluate any existing differences in the molecular progression of breast cancer between younger and older women, Microsatellite instability (MSI) and loss of heterozygosity (LOH) was investigated in paired tumour and normal tissue DNA from 32 younger (less than 40 years old) and 32 older (more than 50 years old) breast cancer patients for 12 simple repeated primer sets.

MSI was observed at a single locus in 5 (15.6%) of younger patients and 8 (25.0%) of older patients. LOH was noted at a single locus in 7 (21.8%) and at multiple loci in 22 (68.7%) of 32 younger patients. In older patients, LOH was revealed at a single locus in 9 (28.1%) and at multiple loci in 15 (46.9%). Greatest frequency of LOH was at loci UT5320 (37.5%), D8S321 (34.4%), D9S242 (31.3%), and D19S394 (31.3%) in younger patients and L17686 (34.4%) and D19S394 (28.1%) in older patients. LOHs at D19S394 and L17686 were highly identified in both age groups. LOHs at D9S242 and D8S321 were significantly higher in the carcinoma of younger women. LOH status was unrelated to clinical stage, nodal status, tumour size, histologic grade or estrogen receptor (ER) status. LOH at D8S321 was associated with tumor size and LOH at UT5320 was associated with histologic grade and ER status. Amplification of CyclinD1 was relatively higher and was associated with histologic grade, nodal status and clinical stage in the younger women, indicating its prognostic significance.

These results indicate that the pattern of chromosomal alterations are not exactly same, especially at D9S242 and D8S321, in the carcinomas in the two age groups and suggest that the molecular pathogenesis of the carcinomas is not similar.

**P 41**  
**EVALUATION OF CYCLIND1 EXPRESSION IN DUCTAL CARCINOMA OF BREAST AND ITS CORRELATION WITH CLINICOPATHOLOGIC ASPECTS**

**Introduction:**

Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma death in women. CyclinD1 is a key cell cycle regulatory protein with demonstrated oncogenic activity in a variety of malignancy including breast cancer. We analyzed expression of cyclinD1 in ductal carcinoma of breast and its correlation with clinicopathologic parameters such as age, lymph node status, tumor size and grade.

**Materials& Method**

Formalin fixed tissue samples from 54 patients diagnosed as ductal carcinoma of breast were analyzed by Immunohistochemistry. We designed a Scoring system based on intensity and percentage of staining cells. Then we performed a statistical analysis to investigate the relationship between clinicopathologic parameters and cyclinD1 expression.

**Result:**

CyclinD1 expression was observed in all of cases with cytoplasmic pattern. We found positive significant correlation between intensity of staining and number of involved lymph nodes ( $P=0.013$ ). Also we observed positive significant correlation between percentage of positive cells and tumor size ( $P=0.016$ ). No relationship was seen between tumor grade and cyclinD1 expression ( $P>0.05$ ). Also no relationship was seen between patient's age and cyclinD1 expression ( $P>0.05$ ). In additional studies we found positive correlation between tumor grade and number of involved lymph nodes ( $P=0.01$ ).

**Conclusion:**

According to our finding we conclude evaluation of cyclinD1 expression could be useful for evaluation of lymph node status, tumor size and thus prognosis.

**Keyword:**

Cyclin D1 , Ductal carcinoma , Grade , Immunohistochemistry.

**P 42**  
**EXPRESSION OF THE INSULIN RECEPTOR SUBSTRATE 1 IN PRIMARY TUMORS AND LYMPH NODE METASTASES IN BREAST CANCER: CORRELATIONS WITH ERÁ, KI-67, BCL-XL AND BAX PROTEINS.**

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Insulin receptor substrate 1 (IRS-1) transmits signals from insulin-like growth factor I receptor (IGF-IR) and insulin receptor (IR) and it has been associated with pathogenesis of cancer disease. IRS-1 down-regulation was suggested with breast cancer progression, but simultaneous assessment of IRS-1 expression in primary and metastatic breast cancer have never been reported before. Consequently, we examined by immunohistochemistry IRS-1 expression in 109 samples of primary breast cancer and in 42 matched pairs of primary and metastatic tumors. Additionally, we correlated IRS-1 expression with selected clinicopathological features, including the ERÁ status and proliferation marker Ki-67 as well as with antiapoptotic Bcl-xL and proapoptotic Bax proteins. We found positive, cytoplasmic IRS-1 immunostaining in 69.7% and 76.2% of studied primary and metastatic tumors, respectively. Both IRS-1-positive and IRS-1-negative primary tumors were found to produce IRS-1-positive as well as IRS-1-negative metastases. IRS-1 expression in primary tumors correlated with poorly differentiated (G3) breast cancer ( $p<0.005$ ) and with lymph node involvement ( $p<0.05$ ). In primary tumors as well as in lymph node metastases, IRS-1 expression did not significantly correlate with ERÁ and Ki-67 expression. However, in the subgroup of ERÁ-positive primary tumors IRS-1 expression positively correlated with Ki-67 ( $p<0.02$ ,  $r=0.351$ ). In primary tumors, we found a significant positive correlation between IRS-1 and Bcl-xL ( $p=0.001$ ,  $r=0.315$ ) as well as between IRS-1 and Bax expression ( $p<0.0001$ ,  $r=0.346$ ). Additionally, in lymph node metastases we also observed positive correlation between IRS-1 and Bcl-xL ( $p=0.004$ ,  $r=0.447$ ) as well as between IRS-1 and Bax expression ( $p=0.037$ ,  $r=0.356$ ). Thus, our results could suggest that IRS-1 might affect turnover of cancer cells and breast cancer progression through activation of mitogenesis and participation in the regulation of the balance between anti- and proapoptotic pathways. Moreover, we suppose that knowledge about heterogeneity between primary and metastatic tumor might help to understand mechanisms of breast cancer progression as well as lead to the development of more effective anti-cancer drugs.

**P 43**  
**PROLACTIN RECEPTOR EXPRESSION IN BENIGN AND MALIGNANT BREAST LESIONS OF MALE AND FEMALE PATIENTS**

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**Background:** Despite the well established function of prolactin (PRL) in normal breast development, the role of this

pituitary hormone in breast cancer pathogenesis is still controversial. PRL activity is dependent on the activation of a transmembrane protein, the PRL receptor (PRLR). The expression of PRLR is common in female breast carcinoma (FBC), but, to our knowledge, no studies were reported in male breast carcinoma (MBC).

**Objective:** Evaluate and compare PRLR expression in male and female breast carcinoma, as well as in gynaecomastia and benign female breast lesions.

**Methods:** PRLR expression was detected immunohistochemically, using a monoclonal antibody (clone B6.2), in formalin-fixed paraffin-embedded tissue of 30 cases of gynaecomastia, 30 cases of benign female breast lesions (including ductal hyperplasia, fibroadenoma, sclerosing adenosis, papilloma and radial scar), 30 cases of MBC and 30 cases of FBC (all invasive ductal carcinomas NOS). The whole series was also assessed for estrogen receptors (ER), progesterone receptors (PR) and androgen receptors (AR). A cut-off of 10% was used as positivity criteria. Histological type was evaluated according to the WHO classification. Tumour differentiation and pathological stage were assessed using the Elston & Ellis grading system and TNM-UICC system, respectively. Statistical analysis was performed with Fisher's Exact Test.

**Results:** PRLR positivity was seen in 20% of gynaecomastia cases, in 20% of benign female breast lesions, in 60% of MBC and in 50% of FBC. PRLR expression was significantly higher in MBC than in gynaecomastia ( $p=0,003$ ). Likewise, it was significantly higher in FBC than in benign female breast pathology ( $p=0,029$ ). No statistically significant differences between gynaecomastia and benign breast lesions or between MBC and FBC were found ( $p=1,000$  and  $p=0,604$ , respectively). There was no statistically significant correlation between PRLR expression and ER, PR, AR, pTNM, histological grade or patients' age.

**Conclusions:** The results indicate that: 1) there is no difference in PRLR expression between male and female benign breast lesions; 2) there is no difference in PRLR expression between MBC and FBC; 3) PRLR is significantly more expressed in invasive carcinoma than in benign male and female breast lesions, suggesting a role of PRL in breast carcinogenesis.

#### P 44

##### **THE BRMS1 (BREAST METASTASIS SUPPRESSOR GENE 1) GENE IS RELATED TO TUMOR PROGRESSION IN HUMAN BREAST CANCER**

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The BRMS1 (Breast Cancer Metastasis Suppressor Gene 1) gene has been identified by differential display, using mRNA extracted from the metastatic cell line of human mammary carcinoma (MDA-MB-435) and from the parental cell line stably transfected with chromosome 11. Experimental evidences show that BRMS1 reduces the metastatic ability, without affecting tumorigenicity, of both human and murine breast cancer cells in vivo metastasis assays. Preliminary studies into the mechanism of the observed metastasis suppression suggest that BRMS1 is a protein involved in a complex that represses gene expression, and that it is associated with regulation of cell-cell communication.

So far, BRMS1 gene involvement in human cancer has not been demonstrated. Aim of this study is to determine if

BRMS1 might play a role in tumor progression by using an in vivo system (paired samples from human primary breast tumors and local metastasis) and to verify the correlation between BRMS1 expression and cell proliferation in vitro (human breast carcinoma cell line Hs578T).

BRMS1 mRNA expression was quantified by TaqMan assay in peritumoral, carcinoma tissues and in lymph-nodal metastasis of breast cancer patients with at least 10 years follow-up and in Hs578T cell line. Total cellular RNA has been extracted from laser-microdissected cells isolated from fresh frozen tissues.

The BRMS1 mRNA is expressed in all tissues analyzed. BRMS1 expression in carcinoma cells is generally higher than in matching normal cell populations (10/14 cases;  $p=0,0005$ ), it decreases then in lymph-nodal carcinoma cells (9/15 cases;  $p=0,001$ ). The BRMS1 mRNA was then quantified in 47 breast cancers from selected patients with a good (free from disease and/or alive) or a poor (recurrence and/or breast cancer mortality) outcome. Univariate (Log-Rank test:  $p=0,007$ ) and multivariate analysis (Cox regression: HR=4,0;  $p=0,054$ ) have shown that BRMS1 higher expression is associated with a shorter disease free survival and overall survival of breast cancer patients. BRMS1 higher expression was not related to cell proliferation rate of tumor cells as shown by an in vivo (breast carcinoma tissues) and in vitro system (Hs578T).

This study is the first and a preliminary evidence of BRMS1 involvement in human breast carcinoma. The BRMS1 expression decreases during tumor progression. However our data suggest that BRMS1 higher expression in primary breast carcinoma is correlated to a poor prognosis.

#### P 45

##### **EXPRESSION OF MISMATCH REPAIR PROTEINS IN SPORADIC AND FAMILIAL INVASIVE AND IN SITU BREAST CARCINOMA**

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**Introduction:** Microsatellite instability appears to be one of the various genetic alterations found in breast tumors, however the results differ considerably in various studies concerning sporadic as well as familial breast cancer. It is not clear yet whether defects in the mismatch repair system accompany this instability.

**Aim:** The purpose of this study is to explore the expression of two of the proteins encoded by the DNA mismatch repair genes, namely MLH1 and MSH2, in sporadic and familial in situ and invasive breast carcinoma.

**Materials and Methods:** MLH1 and MSH2 expression was monitored immunohistochemically using the monoclonal antibodies MLH1 and MSH2 (BIOCARE MED-Menarini Diagnostics) in 60 sporadic breast carcinomas (20 in situ and 40 invasive of various types and grades) and in 30 familial breast carcinomas (ductal and lobular type) defined based on published criteria. In the familial cases we examined concomitantly the invasive and the in situ component.

**Results:** Nuclear staining for MLH1 and MSH2 was observed in all cases of sporadic in situ carcinoma examined. In only one case of DCIS, papillary type the staining for MLH1 was stronger than that of the adjacent normal breast tissue while the staining for MSH2 was similar. Immunostaining for MLH1 and MSH2 revealed preservation of expression in all cases of sporadic invasive carcinoma. Regarding MLH1 only one papillary carcinoma exhibited decrease in percentage and intensity of staining. Interestingly, 6 out of 8 papillary carcinomas displayed increase in MSH2 staining intensity compared to normal tissue. In contrast, in familial breast

carcinomas we found loss of expression of MLH1 in 8/30 (26%) and of MSH2 in 4/30 (13%) of the cases examined. Three carcinomas (10%) were negative for both MLH1 and MSH2. Staining for both antibodies was observed in all cases in the adjacent in situ carcinoma, however a clear degradation in the expression was noted in the in situ foci of MLH1 and MSH2 negative cases. Although not statistically significant, there was an association between loss of expression of MLH1 and MSH2, negative estrogen receptor status and higher grade of the tumor.

**Conclusions:** Our data suggest that the DNA MMR pathway may be involved in the development of familial breast cancer. Regarding sporadic neoplasms, the increased expression of MSH2 in papillary carcinomas can be another clue of their unique differentiation profile. Further molecular studies are necessary for definite conclusions.

#### P 46

##### **HORMONE RECEPTORS AND ONCOGENE EXPRESSION IN APOCRINE CARCINOMAS**

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**Introduction:** Apocrine carcinomas are 1%-2% of breast carcinomas. 98% of apocrine lesions have been reported as estrogen and progesterone receptors negative. Androgen receptor expression was found to be high rates in this rare type of tumors comparing to more common types of the breast as ductal carcinoma or lobular carcinoma.

**Purpose of the study:** The main aim of the study was to search the GCDFP-15, estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), p53 and c-erbB-2 immunoeexpressions in apocrine carcinomas of the breast.

**Methods:** In Marmara University Hospital, Department of Pathology we have diagnosed 13 (2.47%) cases from 526 breast biopsies as apocrine carcinoma of the breast. In thirteen cases, we determined ER, PR, AR, GCDFP-15, c-erbB-2 and p53 immunoeexpression with Sr.ABC/HRP.

**Result:** All of the cases were GCDFP-15 positive. ER was positive in five cases (38.5%), PR was positive in four cases (30.8%), AR was positive in eight cases (61.5 %), c-erbB-2 was positive in eleven cases (84.6%), and p53 was positive in five cases (38.5%).

**Conclusion:** High rates of AR and c-erbB-2 expression brings the discussion of specific treatment protocols in apocrine carcinomas rather than the classical treatment protocol of the breast carcinomas.

#### P 47

##### **LYMPHATIC INVASION AND METASTASIS IN BREAST CANCER IN THE ABSENCE OF INTRATUMORAL LYMPHATICS**

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**Introduction:** Lymph node metastasis is a frequent complication in breast cancer. However, the extent to which this depends on lymphangiogenesis or on invasion of pre-existing lymphatic vessels remains controversial.

**Purpose:** To address this issue, we investigated lymphatic vasculature in 60 primary breast cancer patients.

**Experimental Design:** We used immunostaining for the specific lymphatic endothelium marker podoplanin. We examined whether intratumoral lymphatics were present in our tissue samples, and quantified peritumoral lymphatic density. Furthermore, we recorded the presence of peritumoral lymphatic invasion, and addressed the association of all the above parameters with various clinicopathological features.

**Results:** We did not observe any intratumoral lymphatic vessels. Podoplanin positive lymphatic vessels were confined to tumor periphery, and their density was generally low. Higher peritumoral lymphatic density was associated with lower differentiation (x2 p=0.019) and higher incidence of peritumoral lymphatic invasion (x2 p=0.002). We observed a trend towards a correlation of higher peritumoral lymphatic density with the presence of lymph node metastasis at diagnosis, but the observed association was not formally significant (Mann Whitney p=0.07).

**Conclusion:** The above results are in accordance with previous studies, and suggest that breast carcinomas preferentially invade and metastasize via pre-existing peritumoral lymphatics rather than promoting lymphangiogenesis.

#### P 48

##### **PURE MUCINOUS CARCINOMAS OF THE BREAST: PROGNOSTIC INTEREST OF DNA-FLOW CYTOMETRY.**

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Pure mucinous carcinomas (PMCs) of the breast are considered as a quite specific entity which represents less than 2% of all breast cancers and are said to have a favorable prognosis. There is a lack of investigation concerning DNA-ploidy and S-Phase fraction (SPF) as prognostic factors for this histologic subtype. This study included 69 patients with pure mucinous carcinomas where DNA flow cytometry was performed on fresh or frozen samples in this purpose.

A tumor size higher than 2 cm was observed in 56.5% of cases. A lymph node involvement concerned only 12% of all patients and just 3% when tumor size was lower than 2 cm. Hormone receptors were negative in only 16% of cases. Aneuploidy concerned 26% of tumors which is a lower rate comparing with invasive ductal carcinomas. A high SPF was observed in only 7.5% of cases.

A higher tumor size (> 2cm) was predictive (p=0.03) of shorter disease-free survival (DFS) and overall survival (OS). No correlation was found between nodal involvement, hormone receptor status, S phase fraction and DFS or OS. DFS was shorter for patients with aneuploid tumors compared to diploid ones (p=0.048). A prognostic classification based on tumor size and DNA-ploidy was obtained (p<10<sup>-3</sup> for OS and DFS): a bad prognosis concerned six patients with large (>2cm) aneuploid tumours whose 5-year DFS rate was 16.7%.

**Conclusion:** the good prognosis of PMCs has to be questioned and this study discriminates a bad prognosis group of patients taking into account tumour size and DNA-ploidy.

#### P 49

##### **ALTERNATIVE SPLICING OF CYCLIN D1 TRANSCRIPTS HAVE DIFFERENT EFFECTS ON CELL CYCLE REGULATION IN BREAST CANCERS**

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#### Introduction

The cyclin D1 (CCND1) plays a key role in early G1 phase progression of cell cycle. The amplification and/or overexpression of CCND1 gene have been detected in different types of cancer, such as esophagus, stomach, liver, lung, urinary bladder, head and neck, and breast. It has been reported that a A/G single nucleotide polymorphism (SNP) at the intron 1-exon 1 junction (G870A) of CCND1 gene, and the A-allele has higher risk for cancer generally. This SNP can produce two transcripts (a and b) by alternative splicing, and they may have different functions in cell cycle regulation.

#### Purpose of Study

In this study, we analyze this G870A SNP of CCND1 gene, the expression levels of CCND1 transcripts a and b, and correlate with cell proliferative index in breast cancers, to evaluate their functions in cell cycle proliferation.

#### Materials and Methods

The study population consisted of 97 sporadic infiltrating ductal carcinoma of breast who were treated at Tri-Service General Hospital between September 1997 and March 2002. All patients were diagnosed histologically with specimens both fresh and paraffin-embedded. Clinical and histologic information were obtained from patient charts and pathologic reports. Tumor stage was assigned according to the tumor-node-metastasis (TNM) staging system. Pathologic grade of tumors was determined according to the Modified Bloom-Richardson histologic grading criteria. The SNP of G870A was analyzed by PCR-RFLP method from fresh tissue extracted genomic DNA. The expression levels of transcripts a and b were determined by RT-PCR method. The proliferative index of cancer cells was evaluated by immunohistochemistry of Ki-67 (A0047, Dako, Denmark). One-Way ANOVA and Independent-Samples T tests were applied for statistic analysis.

#### Summary of Results

The A-allele of CCND1 G870A is more prevalent in breast cancer than the G-allele. Compared with GG or AG+GG, the AA genotype has higher proliferative index. Compared with GG, the AA genotype has higher levels of transcript b. The levels of proliferative index have no significant correlation with the expression levels of transcripts a and b. Higher levels of transcript a have higher tumor grade.

#### Conclusions

The A-allele of CCND1 G870A is a risk factor for breast cancers. The genotypes of G870A have effects on differential expression of CCND1 transcripts in breast cancer, and this differential expression is grade-related but not proliferation-related.

#### P 50

##### EXPRESSION OF TOPOISOMERASE II $\alpha$ IN BREAST CANCER. CORRELATION WITH CERBB2 AND OTHER PREDICTIVE FACTORS

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Top2a is a fairly new molecular marker, involved in prediction of response to anthracycline therapy in breast cancer. Prior studies described a possible correlation between Top2a and Her2 neu. Aim of this study was to assess the

expression of Top2a related to commonly used markers in breast cancer.

Indirect triserial ABC method was performed on formalin fixed paraffin embedded tissue from serial file biopsies of 142 patients using the following antibodies: TOP2A, CerbB2, ER, PGR, and PCNA. The cases with strong expression for CerbB2 were confirmed by FISH.

From 110 cases that presented low or negative expression of CerbB2, 20 were positive for Top2A (18.2%); from 20 cases with moderate expression of CerbB2, 9 were positive for Top2A (45%); from 11 cases with strong expression of CerbB2, 2 were positive for Top2A (18.2 %). After removal of 92 cases with no expression of both markers, using multiple regressive analysis, a negative correlation between Top2A and CerbB2 was found ( $r=-0.43$ ) with a high statistical significance ( $p=0.001$ ). A very low correlation with no statistical significance was found between Top2a and PCNA ( $r=0.2$ ,  $p=0.09$ ) and a direct positive correlation between PGR and CerbB2 was found ( $r=0.97$ ) with statistical significance ( $p=0.02$ ). A low negative correlation with statistical significance was found between ER, PGR and TOP2A ( $r=-0.2$  and  $-0.3$ ,  $p=0.006$ ).

In conclusion overexpression of CerbB2 associates high levels of Top2A but they show a significant reverse proportional correlation, and TOP2A expression proved to be independent to hormonal receptors and proliferating index.

#### P 51

##### FASCIN AND CAVEOLIN EXPRESSION ARE ASSOCIATED WITH THE BASAL-LIKE SUBTYPE OF INVASIVE BREAST CARCINOMA.

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INTRODUCTION: Using cDNA expression profiling, previous studies classified breast carcinomas into different groups: the "luminal" (estrogen receptor positive), the "normal breast-like", the "HER2 positive", and the "basal-like" subtypes. The latter two groups have also been associated with poor outcomes. Basal-like tumours are typically negative for estrogen receptor (ER), progesterone receptors (PR) and HER2, but positive for basal cell (myoepithelial) markers, such as cytokeratins 5/6 (CK5/6) and Epidermal Growth Factor Receptor (EGFR). However, basal-like tumours remain poorly characterized. Caveolin and fascin proteins are expressed by breast myoepithelial cells. Furthermore, caveolin was identified as a tumor suppressor gene, whereas fascin has been involved in cell-to-matrix interactions, and tumour invasion.

AIMS: To study caveolin and fascin expression in breast tumors, and to assess whether these makers are associated with the basal-like phenotype.

MATERIALS AND METHODS: We studied caveolin and fascin expression in a tissue microarray (TMA) including 226 node-negative invasive breast carcinomas. In addition, we evaluated ER, PR, HER2, CK5/6, EGFR, p53, and ki-67 expression.

RESULTS: Overall, 21 (9.3%) and 56 (24.8%) breast carcinomas expressed caveolin and fascin, respectively. The basal-like phenotype (RE-/HER2-/CK5+ and/or EGFR+) was found in 28 (12.4%) carcinomas. Interestingly, 25% (7/28) of tumours with the basal-like phenotype, but only 7.1% (14/197) of non-basal tumours expressed caveolin. Likewise,

fascin was more frequently expressed in basal-like tumours (14/27, 52%) than in non-basal carcinomas (42/196, 21.4%). Therefore, a direct statistically significant correlation was found between caveolin and fascin expression and the basal-phenotype ( $p=0.007$  and  $p=0.001$  for caveolin and fascin, respectively).

**CONCLUSION:** These data indicate that caveolin and fascin expression is associated to breast cancers with basal phenotype and thus, they could serve to identify more accurately this subgroup of tumours.

## P 52

### **THE EFFECT OF PHYSICAL CONDITIONS AND SLIDE AGING ON STAINING QUALITY OF BREAST CARCINOMAS FOR C-ERBB-2, ESTROGEN (ER) AND PROGESTERON (PR) RECEPTORS**

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Fixation and tissue processing conditions effect the staining quality and may create difficulties on interpretation of immunostaining for receptors (ER, PR) and c-erbB-2 in breast carcinomas. There are also evidences about the negative effects of tissue section's age (slide age) and storage temperature on the staining intensity of these prognostic markers. Today slide aging is becoming more important, because of the need for storage of hundreds of tissue microarray sections for future planned studies concerning immunohistochemistry (IHC) or in-situ hybridisation methods. Our aim in this study was to examine the effects of age and the storage conditions on immunostaining quality of ER, PR, and c-erbB-2 on breast carcinoma cases.

#### **MATERIALS AND METHODS:**

Tissue cores obtained from 14 donor paraffin blocks of different breast carcinomas previously scored for receptors and c-erbB-2, were re-blocked in an order to prepare the tissue array block. Four micron sections of the tissue array block were stored for 36, 24, 12 weeks in 4 different conditions: 1- Room temperature dry, 2- Room temperature humid, 3- Room temperature sealed by paraffin, 4- at 40C. The slides were stained with primary antibodies against ER (NCL-ER-6F11, 1/50), PR (NCL-PGR, 1/600) and c-erbB-2 (Neomarkers e2-4001+3B5, 1/700) by using Ventana Benchmark autostainer on the same session. The results of the staining intensity of all groups were compared with newly cut paraffin section. Friedman test was used for statistical analysis.

#### **CONCLUSIONS**

We examined that the staining intensity of immunostaining for breast cancer prognostic markers were decreased when the paraffin slides are stored in humid conditions.

Sealing the slides by paraffin does not have any conservative effect on staining quality.

The staining quality of the slides decrease by the length of the storage period.

Keeping the slides at 40C can decrease the negative effect of slide aging on immunostaining.

## P 53

### **HORMONE RECEPTORS AND ONCOGENE EXPRESSION IN APOCRINE CARCINOMAS**

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**Introduction:** Apocrine carcinomas are 1%-2% of breast carcinomas. 98% of apocrine lesions have been reported as

oestrogen and progesteron receptors negative. Androgen receptor expression was found to be high rates in this rare type of tumors comparing to more common types of the breast as ductal carcinoma or lobular carcinoma.

**Purpose of the study:** The main aim of the study was to search the GCDFF-15, oestrogen receptor (ER), progesteron receptor (PR), androgen receptor (AR), p53 and c-erbB-2 immunoeexpressions in apocrine carcinomas of the breast.

**Methods:** In Marmara University Hospital, Department of Pathology we have diagnosed 13 (2.47%) cases from 526 breast biopsies as apocrine carcinoma of the breast. In thirteen cases, we determined ER, PR, AR, GCDFF-15, c-erbB-2 and p53 immunoeexpression with Sr.ABC/HRP.

**Result:** All of the cases were GCDFF-15 positive. ER was positive in five cases (38.5%), PR was positive in four cases (30.8%), AR was positive in eight cases (61.5 %), c-erbB-2 was positive in eleven cases (84.6%), and p53 was positive in five cases (38.5%).

**Conclusion:** High rates of AR and c-erbB-2 expression brings the discussion of spesific treatment protocols in apocrine carcinomas rather than the classical treatment protocol of the breast carcinomas.

## P 54

### **P53, C-ERBB-2, OSTROGEN AND PROGESTERON RECEPTOR EXPRESSION: CORRELATION WITH THE HISTOPATHOLOGICAL PROGNOSTIC PARAMETERS IN DUCTAL CARCINOMAS OF THE BREAST**

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Until today many prognostic factors have been studied on ductal carcinomas of the breast in order to find out the clinical and therapeutic implications.

The aim of this study was to search the p53, c-erbB-2, oestrogen receptor (ER) and progesteron receptor (PR) expression in ductal carcinomas of the breast and correlation with the well known histopathological prognostic parameters.

#### **Material-Method:**

55 cases of invasive ductal carcinomas were retrieved from the archives of Marmara University Hospital, Department of Pathology. Immunohistochemical detection was performed by the StreptAvidin-Biotin complex method on 5 µm sections from archival representative paraffin embedded blocks.

**Results:** The cut of values for p53, ER and PR were 10%. The cut of value for c-erbB-2 was considered as positive when we have revealed ++ and/ or +++ spesific membranous staining.

31% of the cases had immunoeexpression with c-erbB-2. 41% of them were positive with p53. 53% of the cases were also positive with ER/PR, 65% of the same cases were advanced stages as T2, T3 or T4, 88% were either grade 2 or 3 and 47% of them had lymph node metastasis .

35% of the invasive ductal carcinoma cases were positive with p53. p53 positive cases demonstrated 68% ER/PR positivity, 79% were advanced stages, 89% were either grade 2 or 3, and 42% had lymph node metastasis.

#### **Discussion:**

In the literature, c-erbB-2 protein overexpression is shown to be independently associated with poor prognosis in women with node-positive breast cancer. In this recent study immunoeexpression results of c-erbB-2 have concordance with the literature as

c-erbB-2 has a statistically significant correlation with the tumor grade, stage and lymph node metastasis. Although

ER/PR positivity are known to be as good prognostic parameters we have revealed ER/PR positivity of 53% of the c-erbB-2 positive cases. ER/PR positivity have also been observed in p53 positive cases. p53 positivity have also been revealed in advance stages and high grades.

Conclusion: Results of our study is concordance with the literature except the high immunorexpression ratios of the ER/PR. This result could be attributable to the geographic, social differences with Western Europe.

#### P 55

##### **CELL CYCLE CONTROL RELATED PROTEINS (P53, P21, AND RB) AND TRANSFORMING GROWTH FACTOR-BETA (TGF-BETA) IN 'IN SITU' AND INFILTRATING HUMAN BREAST CANCER: AN IMMUNOHISTOCHEMICAL STUDY.**

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INTRODUCTION: It has been reported that several regulators of the cell cycle –including mtp53, p21, pRB, and TGF-beta are altered in many malignant tumors.

PURPOSE: The aim of this study was to investigate the expression patterns of p53 (mutated form), p21, Rb, pRb and TGF-beta in order to elucidate the possible roles of this cell cycle regulatory system in breast cancer.

METHODS AND METHODS: A comparative study of the products of the cell cycle control genes p53 (mutated form), p21, Rb (non-phosphorylated and phosphorylated form) and TGF-beta was performed by immunohistochemistry and Western blot, in 17 benign breast disorders and 62 breast cancer (17 'in situ' and 45 infiltrating tumors) samples.

RESULTS: For these proteins studied, the relative numbers of positively stained cells were higher in 'in situ' carcinoma than in benign breast diseases. In infiltrating breast tumors the relative numbers of positively stained cells were even higher than in 'in situ' tumor, except for the percentage of pRb immunostained cells, which decreased slightly in infiltrative tumors. For the other proteins, the percentages of positively stained cases were similar to those found in 'in situ' tumors. In the three groups of patients, TGF-beta immunoreaction appeared in the cytoplasm while immunoreactions to p53, p21, Rb and pRb were always found in the nucleus, except for p21 in 'in situ' tumors, which showed cytoplasmic immunoreaction to this protein.

CONCLUSIONS: Present results suggest that accumulation of mutated p53, cytoplasmic p21, and pRb in breast gland epithelium might be a crucial point in the development of 'in situ' adenocarcinoma. In the infiltrating tumors, the expression of p21 in the nuclei and the decrease in pRb expression suggest an insufficient attempt to hinder cell proliferation.

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#### P 56

##### **INTERFERON-GAMMA AND ITS FUNCTIONAL RECEPTORS IN IN SITU AND INFILTRATING HUMAN BREAST CANCER: AN IMMUNOHISTOCHEMICAL STUDY.**

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INTRODUCTION: Interferons are a group of proteins that trigger multiple responses including prevention of viral replication, inhibition of cell growth, and modulation of cell

differentiation. In different mammary carcinoma cell lines IFNgamma induces growth arrest at mid-G1. At the present there are no in vivo studies in human breast.

PURPOSE: The aim of this study was to investigate the expression patterns of IFNgamma and its two receptors (IFNgamma-Ralpha and IFNgamma-Rbeta) in order to elucidate its role in the different types of human breast cancer (in situ and infiltrative).

METHODS AND METHODS: A comparative study of IFNgamma and its two receptors (IFNgamma-Ralpha and IFNgamma-Rbeta) was performed by immunohistochemistry and Western blot, in 17 benign breast disorders and 62 breast cancer (17 in situ and 45 infiltrating tumors) samples.

RESULTS: For IFNgamma, IFNgamma-Ralpha and IFNgamma-Rbeta, the relative numbers of positively stained cells were higher in benign breast than in carcinoma. In in situ tumors the relative numbers of positively stained cells were even higher than in infiltrating breast. In the three groups of patients, IFNgamma and IFNgamma-Ralpha immunoreactions appeared in the cytoplasm while IFNgamma-Rbeta also was found in the nucleus.

CONCLUSIONS: The decreased expression of IFNgamma and its receptors in cancer could be regarded as an attempt, although insufficient, to inhibit the uncontrolled cell proliferation. The mechanism causing the antiproliferative effect could be related to the other factors variation as wild p53 or Rb.

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#### P 57

##### **THE STATUS OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR), GRADE AND AXILLARY LYMPH NODES INVOLVEMENT IN PRIMARY BREAST CANCER**

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Introduction: The breast cancer is the most common worldwide malignancy of women. The number of prognostic factors in breast cancer has grown recently. A conceptual framework is now emerging that outlines how certain principles of EGFR may be employed in the future management of patients with Breast cancer.

In this study, we want to show the status of EGFR and its correlation with grade and axillary lymph nodes involvement in primary breast cancer.

Material and Methods: In a cross-sectional double-blind study, 57 paraffin blocks from patients with breast cancer from pathology department of shaheed Mostafa Khomeini hospital, since 2000-2003 were obtained and from each one, 3 micron sections were provided. One of the tumoral section and one of the lymph node section were stained by H & E. We determined size and type of tumor, number of lymph nodes and grade according to Natingham modification of Bloom-Richardson criteria. We studied EGFR on the other section by IHC. We had 10-paraffin blocks without breast cancer.

Result: Our study showed that 89.5% were positive and 10.5% were negative for EGFR. The most patients were Grade III (43.9%) and the lowest patients were Grade I (22.8%). 66.7% had axillary lymph nodes involvement. In this study, we didn't find significant correlation between EGFR and axillary lymph nodes involvement.

Conclusion: The high expression of EGFR in patients with breast cancer is important to note as a prognostic factor in the world and Iran, but further investigions with more specimens and follow-up are required to clarify the exact role of EGFR in prognosis of breast cancer.

**P 58****ANDROGEN RECEPTORS ARE FREQUENTLY EXPRESSED IN MAMMARY AND EXTRAMAMMARY PAGET'S DISEASE.**

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**Aims:** Mammary paget's disease (MPD) and Extramammary paget's disease (EMPD) are rare intraepithelial neoplasms. MPD is almost exclusively associated with underlying invasive breast carcinoma (IDC) or high grade Carcinoma in situ, DCIS (ductal intraepithelial neoplasia, DIN). EMPD arises in areas rich in apocrine glands and is thought to have apocrine gland origin. The aim of the study was to investigate the presence of estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR) and Her2/neu in a large number of cases.

**Methods:** We investigated 81 cases of MPD (58 cases) and EMPD (23 cases). Formalin fixed and paraffin-embedded tissues were analyzed using antibodies against AR, PR, ER and Her2/neu according to standardized guidelines.

**Result:** In MPD, positive immunoreactions for Her2/neu, AR, and ER were observed in 56 of 58 (97%), 51 of 58 (88%), and 6 of 58 (10%) cases, respectively. All cases of MPD were negative for PR. MPD showed a co-expression of Her2/neu and AR in 51 out of 58 cases (88%). In EMPD, positive immunoreactions for AR, Her2/neu, and ER were observed in 18 of 23 (78%), 12 of 23 (52%), and 1 of 23 (4%) cases, respectively. All cases of EMPD were negative for PR. EMPD showed a co-expression of AR and Her2/neu in 12 out of 23 cases (52%).

**Conclusion:** In contrast to ER and PR, AR and Her2/neu are commonly expressed in MPD and EMPD. The immunohistochemical determination of AR in paget's disease could lead to the development of a new adjuvant therapy, particularly in patients with recurrent disease.

**P 59****CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMISTRY FEATURES ASSOCIATED TO THE BASAL PHENOTYPE OF INVASIVE BREAST CARCINOMAS**

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In the normal breast, the majority of luminal cells express the cytokeratins 7, 8, 18 and 19, while the basal myoepithelial cells express the cytokeratins 5, 14, 15 and 17. However, there is a subset of normal mammary luminal cells that are positive for CK5/6 and negative for CKs 8/18/19. These cells have the capacity to differentiate toward a glandular phenotype (CKs 8/18/19 positive) with intermediate populations that are positive both for CK5 and CKs 8/18/19. Considering that all of these cells can undergo malignant change, the breast carcinomas can be classified as expressing a basal (CK5+ CKs 8/18/19- or CK5+ CKs 8/18/19+) or a luminal phenotype (CK5- CKs 8/18/19+). This work aims to study the immunoprofile of CK-5 positive breast carcinomas and its relationship with pathologic features of clinical significance. Immunohistochemistry with the antibodies CK5, CK8/18, p63, BRCA1, estrogen receptor, progesterone receptor, p53, c-erbB-2, and Ki67 were performed in 102 formalin-fixed paraffin-embedded samples of invasive ductal carcinomas. The clinical data were collected from the medical files. Conventional clinical features were evaluated, including age, menstrual status, pathological grading, tumor size and lymph

node metastasis. Focal expression of CK5 was seen in 18.6% of carcinomas. CK5 expression was significantly correlated with younger age, high tumor grade, positive nodal status, negativity for hormonal receptors, and high proliferative rate. There was a strong relationship between CK5 and p63 expression in breast carcinomas indicating that p63, like CK5, may be considered a marker for the basal phenotype of breast cancer. There was also a strong relationship between reduced expression of BRCA1 with both p63 and CK5 expression as well as there was an inverse correlation between p63 and CK8/18 expression suggesting that loss of p63 expression is required for the transition between the basal to luminal phenotype of breast carcinomas. Since p63 is thought to be a marker of stem cells and may act as an oncogene our data reinforce that BRCA1 acts as stem cell regulator.

**P 60****PROGNOSTIC SIGNIFICANCE OF P63-POSITIVE NEOPLASTIC CELLS IN INVASIVE BREAST CARCINOMAS**

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P63 is a p53 homologue gene located on chromosome 3q27-29. P63 protein is highly expressed in embryonic ectoderm and in the nuclei of basal cells of many epithelial tissues in the adult including skin, oral epithelium, prostate and urothelium. In the normal mammary gland p63 is considered to be a myoepithelial cell marker but p63-positive neoplastic cells may be found in up to 11% of invasive breast carcinomas. The prognostic significance of this finding, however, is still unknown. This work aims to study the overall survival and the disease-free survival associated with p63-positive breast carcinomas. Immunohistochemistry with the antibody p63 (clone 4A4) was performed in 100 formalin-fixed paraffin-embedded samples of invasive ductal carcinomas diagnosed between 1992 and 1995. Clinical data concerning the outcome of the patients were retrieved from the medical files. Sixteen of 100 carcinomas showed rare and scattered neoplastic cells stained for p63 and were interpreted to be p63-positive. The median age of patients (n=100) included in this study was 54.2 years (range 25-85 years). Median follow-up was 6.5 years (range 1-10 years). The average overall survival of patients whose tumors were positive and negative for p63 was 6.5 and 6.9 years, respectively (p=.8134). The average disease-free survival of patients whose tumors were positive and negative for p63 was 4 and 5.8 years, respectively, with the difference being statistically significant (p=.0474). These data indicate that p63 might be used in routine practice to identify patients at high risk of precocious recurrence or metastasis.

**P 61****IMMUNOPROFILE OF P63-POSITIVE INVASIVE BREAST CARCINOMAS**

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P63 is a p53 homologue gene that is essential for the maintenance of a stem cell population in several epithelial tissues. P63 expression is associated with several malignancies including squamous cell carcinomas of the head and neck, primary cutaneous adnexal neoplasms, lung carcinomas, endometrial carcinomas, esophageal squamous cell carcinomas, transitional cell carcinomas of the urinary tract, and papillary thyroid carcinomas. These data indicate

that p63 may act as an oncogene in the genesis of these tumors. In breast carcinomas, p63 expression correlates with several indicators of aggressiveness, including advanced pathologic stage, tumor size, lymph node metastasis, high histological grade, and negativity for estrogen receptors. Very little is known about the molecular profile of p63-positive carcinomas. Thus, this study was carried out to study the expression of several key regulators of the cell cycle including oncogenes and apoptosis-related proteins in p63-positive carcinomas. Immunohistochemistry with the antibodies BAG-1, Bcl-2, c-erbB-2, c-erbB-3, Checkpoint Kinase 2 (CHK2), Cyclin D1, Epidermal Growth Factor Receptor (EGFR), Telomerase (hTERT), Osteopontin (OPN), p16, p21, and p27 was performed in 100 formalin-fixed paraffin-embedded samples of invasive ductal carcinomas. Association between p63 expression and other markers was determined by the Fisher exact test. Sixteen of 100 carcinomas expressed p63-positive neoplastic cells. P63 correlated only with the expression of hTERT. The transcriptional activation of telomerase is considered to be a crucial step in the development of malignancy and is responsible for the continued growth and immortalization of cancer cells. In breast carcinomas hTERT is associated with aggressiveness and a poor clinical outcome. It is frequently activated in early breast carcinogenesis and this activity is associated with nodal metastases and cellular proliferation. These data suggest that differential regulation of h-TERT in p63-positive breast carcinomas may contribute to the clinically more aggressive behavior of these neoplasms.

#### P 62

##### **BRCA1 EXPRESSION IN SPORADIC BREAST CARCINOMAS: A CLINICOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY**

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Although mutations in BRCA1 are rare in non familiar breast carcinomas, it is common the lost of expression of the protein codified by this gene probably due to epigenetic changes. The prognostic importance of this finding is not well established. This work aims to analyze the immunohistochemistry expression of BRCA1 in sporadic breast carcinomas and to compare the results with clinical and histopathologic data of prognostic significance.

Immunohistochemistry with the antibodies BRCA1, estrogen receptor, progesterone receptor, p53, c-erbB-2, and Ki67 was performed in 102 formalin-fixed paraffin-embedded samples of not otherwise specified invasive ductal carcinomas. BRCA1 expression was graded as follow: 0/+ (negative or positivity in less than 25% of neoplastic cells), ++ (positivity between 25 to 50% of neoplastic cells), and +++ (positivity in more than 50% of neoplastic cells). Conventional clinical features were evaluated, including age, menstrual status, pathological grading, tumor size and lymph node metastasis. The clinical data were retrieved from medical files. Association between BRCA1 expression and other pathologic variables was determined by the Fisher exact test (2 groups) or  $\chi^2$  test (3 or more groups). In normal epithelium, BRCA1 showed only nuclear staining, while the carcinomas showed either nuclear or cytoplasmatic staining. The histological analysis of BRCA1 showed that 25.5% of carcinomas were graded as 0/+, 25.5% were graded as ++, and 49% were graded as +++. Carcinomas with reduced expression of BRCA1 (0/+) were poorly differentiated, with high proliferative rate, negative for hormonal receptors, and positive for c-erbB-2. There was not correlation between BRCA1 expression with age, menstrual status, tumoral size, lymph nodes status, pathologic stage, and p53 expression. The mean survival and disease-free survival of patients which

tumors expressed less and more of 25% of neoplastic cells stained for BRCA1 were 4.2 and 3.9 ( $p < 0,001$ ); and 7.8 and 6.3 ( $p = 0,0095$ ) years respectively. In conclusion, BRCA1 may be considered a useful marker in identification of sporadic carcinomas of poor prognosis that is in high risk for precocious recurrence.

#### P 63

##### **THE RELATIONSHIP BETWEEN CYTOKERATIN 5, CYTOKERATINS 8/18, P63 AND MATRIX METALLOPROTEINASE-19 IN MAMMARY CARCINOMAS INDUCED BY 7,12 DIMETHYLBENZ[A]ANTHRACENE IN MICE FED WITH FAT DIET**

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Human Breast carcinomas usually express cytokeratins 8/18 (CK8/18) but a reduced expression of CK8/18 may be found in up to 40% of poorly differentiated tumors. CK5 is a 58 kD protein found in many non-keratinizing stratified squamous epithelia. There is a subset of human breast carcinomas that express CK5. P63, unlike its homologue p53, is not a classical tumor suppressor gene, being essential for the maintenance of a stem cell population in several epithelial tissues. The expression of CK5 and p63 in experimentally induced mammary tumors is still unknown. Unlike most matrix metalloproteinases, MMP-19 is downregulated during malignant transformation and histologic dedifferentiation. This study was carried out to verify the expression of p63 and cytokeratin 5 (CK5), as well as their relationship with cytokeratins 8/18 (CK8/18) and metalloproteinase 19 (MMP19), in DMBA-induced mammary tumors in mice fed a diet enriched with linoleic acid. Sixty 8-week-old virgin female balb-c mice were given by gavage 1 mg of 7,12 dimethylbenz[a]anthracene (DMBA) dissolved in 1 ml of corn oil once a week for 6 weeks. Linoleic acid was supplied as 15 g of corn oil for each 100 g of standard diet. When tumors reached 1 cm in size, euthanasia was performed and mammary tissue was pooled for analysis. Formalin-fixed paraffin-embedded tumors were submitted to immunohistochemical study with CK5, p63, CK8/18 and MMP19. Sixteen animals developed poorly differentiated adenosquamous carcinomas which were diffusely positive for CK5 and p63 and focally positive for CK8/18 and MMP19. Like p63, CK5 stained the poorly differentiated areas as well as the foci of squamous differentiation, suggesting that they may be used together in immunohistochemical panels to characterize squamous differentiation in poorly differentiated carcinomas. The focal positivity for CK8/18 may be attributed to suppressed gene expression or accelerated protein degradation. MMP19 expression indicates that progression towards an invasive phenotype and neoplastic dedifferentiation led to the disappearance of MMP-19 from tumor cells. In conclusion, the addition of linoleic acid to the diet of mice given DMBA induces mammary carcinomas with immunoprofile similar to the poorly differentiated human carcinomas suggesting that a fat diet may be involved in the pathogenesis of these neoplasms.

#### P 64

##### **BREAST TUMOR RESEMBLING THE TALL CELL VARIANT OF PAPILLARY THYROID CARCINOMA** CAMESELLE-TEIJEIRO José1, ABDULKADER Ihab1, RUIZ-PONTE Clara2, REYES-SANTÍAS Rosa1, BARREIRO Francisco3, SOBRINHO-SIMÕES Manuel4

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The breast tumor resembling the tall cell variant of papillary thyroid carcinoma (BTRPTC) is a very unusual mammary carcinoma whose histologic and predominant nuclear features mimic a papillary thyroid carcinoma. We report a case of BTRPTC with cytologic, pathologic and molecular analysis. A 64-year-old woman presented with a palpable nodule in the right breast. Fine needle aspiration of breast mass disclosed abundant cellularity with isolated cells, sheets and papillary formations of epithelial cells with nuclear grooves. The tumor measured 5.2 cm. Histologically, the neoplastic cells were arranged in a solid to papillary architecture, with areas showing a follicular or cribriform pattern of growth. The neoplastic nests and papillae were made up of columnar to cuboidal cells with eosinophilic cytoplasm. In addition, the neoplastic cells showed numerous nuclear grooves and occasional eosinophilic pseudo-inclusions. Tumor cells were positive for cytokeratins (CAM5.2 and AE1/AE3), estrogen receptor-alpha and -beta, progesterone receptor, androgen receptor, CEA, and bcl-2. Thyroglobulin, thyroperoxidase, calcitonin, TTF-1, chromogranin, synaptophysin, vimentin, and galectin-3 were consistently negative. Because of the peculiar nuclear appearance of the cells of the BTRPTC, a previous study for RET/PTC rearrangements had been reported in the literature with negative results. Due to the fact that RET/PTC rearrangements and BRAF mutations are mutually exclusive etiopathogenetic events in papillary thyroid carcinomas we also searched for BRAF mutations, but no mutations were found. The awareness of the existence of mammary tumors that histologically mimic the tall cell variant of papillary thyroid carcinoma will avoid unnecessary clinical investigations. This work was supported by research grant (PGIDE 99PXI 90201B) sponsored by Xunta de Galicia, Spain.

#### P 65

#### PRIMARY ANGIOSARCOMA OF THE BREAST: CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS ON 4 CASES OZLUK Yasemin(\*), TUZLALI Sitki(\*), YAVUZ Ekrem(\*), DERIN Duygu(\*\*), ASOGLU Oktar(\*\*\*), ILHAN Ridvan(\*), Iplikci A(\*)

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Primary angiosarcoma of the breast (PAB) is an unusual neoplasm with an adverse outcome. The incidence of its association with pregnancy is reported as 6% of PAB.

The aim of this study is to detect the incidence, the hormonal status and the prognostic indicators of PAB.

The archive material of our department was reviewed to identify clinicopathological features of PAB. Immunohistochemical analyzes were performed for antibodies CD31, CD34, ER, PR and Ki-67. During a 17-year period 4 cases of PAB were established out of almost 5000 breast biopsies. All patients clinically presented with a palpable mass. Two patients were at the end of their first trimester in pregnancy. The mean patient age at diagnosis was 40.8 years (29-56). All patients underwent simple mastectomy after an initial diagnosis on either excisional or incisional biopsy. None of the patients had received prior radiotherapy. Grossly, all tumors had hemorrhagic cystic spaces with solid, white coloured areas and ill-defined borders. The mean tumor size

was 8.37 cm (2.5-12). One case revealed two masses of PAB, 9 cm and 1.3 cm in diameter. Two of the tumors were Grade I, one of the tumors was Grade II and one was Grade III, histologically. Immunohistochemistry revealed positivity for CD31 in all tumors and CD34 in 3. The mean score of Ki-67 index was 28.6% (2-54.6%). The tumor without CD34 immunoreactivity was Grade III and exhibited the highest Ki-67 index (54.6%). Two patients with Grade I tumor and with the lowest Ki-67 index were alive with no evidence of disease 7-8 years after surgery. One of the pregnant patients, having two foci of Grade II tumor, is still alive after 1 year follow-up with distant metastasis 5 months after surgery and has been receiving radiotherapy and chemotherapy. The patient with Grade III tumor died with disseminated disease one year after initial diagnosis.

Since, it was previously stated that grading is the most important prognostic indicator, we suggest that Ki-67 proliferation index may also be used for the same purpose. We also propose that at least two endothelial markers must be used in differential diagnosis, especially in poorly differentiated tumors in which expression of endothelial markers may be lost. No evidence of an association with hormonal status could be drawn, therefore, the occurrence with pregnancy should be related to the age of incidence. These statements need further investigations on large series of PAB.

#### P 66

#### LYMPHOEPITELIOMA-LIKE CARCINOMA OF THE BREAST: IS IT A DISTINCT ENTITY?

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#### INTRODUCTION:

Lymphoepitelioma (LE), a tumor originally described within the nasopharynx, is an undifferentiated carcinoma with prominent lymphoid infiltration and with variable expression of the Epstein-Barr virus (EBV) genome within the tumor cells. There are only 13 cases of lymphoepitelioma involving the breast described in the literature, none of them associated with EBV. It is considered to be a less aggressive type of breast carcinoma.

#### MATERIALS AND METHODS:

A set of 30 breast cancer with prominent lymphoid interstitial infiltration were examined and selected from a total of 1226 reviewed biopsies. There were excluded from the study cases following the criteria for medullary carcinoma, cases with syncytial growing patterns higher than 75%, papillary areas, glands or carcinoma in situ, and cases with morphological features not consistent with LE.

The presence of EBV was subsequently analysed in 14 histologically confirmed cases of LE by immunohistochemistry (LMP-1), in situ hybridisation (EBERs) and PCR (LMP-1 terminal region and EBNA5). Morphological and complementary data (hormonal receptors, proliferation index, p53, and Her-2) were also evaluated.

#### RESULTS:

None of the cases were associated with EBV, 11 of them were N+, and all of them showed histological findings suggestive of high grade tumors (GIII), high proliferative activity (>25%), negative hormonal receptors (13) and high p53 expression (10). Two cases were HER 2 (+++).

#### CONCLUSIONS:

There is no evidence to consider LE as a distinct entity of infiltrating breast carcinoma.

#### P 67

#### NON-HODGKIN'S LYMPHOMAS OF THE BREAST

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**Introduction:** Primary non-Hodgkin's lymphoma of the breast is a rare disease, constituting less than 1% of malignant mammary neoplasms. Lymphomas secondarily involving the breast are also uncommon. A lymphoma is considered primary if the breast is the first or major site of presentation, without concurrent nodal disease except for the involvement of ipsilateral axillary nodes and with no previous history of extramammary lymphoma.

**Materials and Methods:** We report 5 cases of breast lymphoma, 3 primary and 2 secondary. All patients were female, ranging in age from 42 to 85 years. Of the 3 primary cases, one was classified as extranodal marginal zone B-cell lymphoma of MALT type, one as diffuse large B-cell lymphoma arising in a pre-existing maltoma and one as Burkitt lymphoma. In the other 2 cases, breast was secondarily involved by a MALT lymphoma of salivary gland and a nodal diffuse large B-cell lymphoma.

**Discussion:** Primary non-Hodgkin's lymphomas of the breast are uncommon. Two distinct clinicopathologic types have been described. One type occurs in pregnant or lactating young women as massive bilateral breast swelling and corresponds to a Burkitt lymphoma. The second and larger type usually affects postmenopausal women and presents with clinical features identical to those of carcinoma of the breast. This latter type, histologically, is usually a diffuse large B-cell lymphoma.

Differentiation must be made from benign lymphocytic infiltrations and non lymphoid neoplasms of the breast.

Primary lymphomas of the breast behave in a way similar to lymphomas of corresponding type and stage in other sites. Therapeutic management is controversial and consists of various combinations of surgery, radiation therapy and chemotherapy.

#### P 68

##### **DIABETIC MASTOPATHY: REPORT OF FIVES CASES**

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**Introduction:** The diabetic mastopathy (D.M) is a benign disease of the breast parenchyma but may clinically simulate a breast carcinoma. This mastopathy usually occurs in women who suffered from insulin-dependent diabetes mellitus for some years.

**Aims:** To describe the clinical, radiological, histological features of this benign disease. M.D is little known by clinician, radiologist and pathologist.

**Materials and methods:** Five cases of M.D were examined during 7-year period from 1997-2003. We analysed the clinicopathological and immunohistochemical findings of the lobular lymphocytic infiltrate. Immunohistochemical profile (CD20 and UCHL-1) was analysed using avidin-biotin-immunoperoxidase technique.

**Results:** All cases were female. Three cases had type 1 insulin-dependant diabetes and one case had type 2 insulin-independent diabetes. In one case the type of diabetes was unknown. The mean age at presentation was 40.8 years (33-57). The mean duration of diabetes was 18.25 years (12-24). In one case the patient was treated 6 years ago for a medullary carcinoma of the homolateral breast (T1N0M0). All the palpable lesions were dense at mammography. Macroscopically, the mean size was 5.7 cm (2.5-8). The parenchyma was found to be a white and fibrotic plaque indistinctly separate from surrounding normal breast tissue. Histologically, the lesion was characterized by the association of three elements: a lobular lymphocytic infiltrate, perivascular lymphocytic infiltrate of variable intensity, often organized in lymphoid nodules and stroma fibrosis with

myofibroblast hyperplasia. The lymphocytic infiltrate was predominantly of B phenotype in 2 cases, predominantly of T phenotype in 2 cases and equally B and T phenotype in 1 case.

**Conclusion:** Our epidemiological, clinical and radiological findings are in agreement with published data. The diagnosis of M.D is based on the confrontation of the clinical context, radiological and characteristic histological features.

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##### **GYNAECOMASTIA IN HIV-INFECTED MEN ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY: A CASE REPORT AND REVIEW OF THE LITERATURE**

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An estimated 610.000 individuals were infected with HIV/AIDS in Brazil in 2001. The rate of reported AIDS cases increased from 10.6 per 100.000 in 1992 to a high of 18.7 per 100.000 in 1998. Brazil has experienced a stabilizing trend with rates of 16.5, 16.4, and 14.8 per 100.000 in 1999, 2000, and 2001, respectively. Since 1998, the death rate from AIDS has stabilized at 6.3 per 100.000. This tendency is attributed to Brazil's guarantee of access to free antiretroviral drugs since 1996. HIV-infected patients who are treated with highly active antiretroviral therapy (HAART) can develop breast enlargement due to benign and malignant diseases. Benign disorders comprise gynaecomastia (GM), pseudoangiomatous stromal hyperplasia and lipomastia. We describe a case of HAART-induced left-sided gynaecomastia in a 42-year-old HIV-infected man treated with lamivudine and efavirenz for 2 years and discuss the pathogenesis of these lesions according to the literature. Physical examination showed a nontender, freely mobile nodular mass in the periareolar area of left breast measuring 6.5x5.0 cm. The patient underwent a complete excision of the lesion. The pathological examination revealed gynaecomastia with extensive pseudoangiomatous stromal hyperplasia. The patient developed GM of the contralateral breast after 7-month follow-up period. Gynaecomastia is not uncommon in HIV-infected men under HAART, especially in those taking efavirenz and didanosine. The role of antiretroviral therapy on GM development is still discussed.

#### P 70

##### **SOLITARY FIBROUS TUMOR OF THE MALE BREAST: A CASE REPORT**

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Solitary fibrous tumor is currently recognized as a benign soft tissue neoplasm that may occur in almost every site in the body. The breast is rarely involved. We describe a case of this rare lesion with the clinicopathological and immunohistochemical findings. A 64-year-old white man presented a left breast mass that grew in a 18-month period. He denied any steroid abuse and did not disclose any sign or symptom of endocrinopathy. Physical examination showed a large, firm, round, well circumscribed tumor measuring 14x12 cm, replacing the entire left breast. Contralateral breast was normal. The patient underwent a simple mastectomy. The gross appearance was that of a well circumscribed but unencapsulated solid nodule. On cut section, the tumor was pale white to grayish. Histological examination showed a spindle cell tumor with pushing borders, which was

composed of bland spindle cells, haphazardly arranged and admixed with collagen fibers. There were hypercellular areas alternated with hypocellular fibrotic areas. Mitosis, necrosis and haemorrhages were not seen. Immunohistochemistry revealed that the tumor cells were diffusely positive for vimentin, CD34 while myogenic markers (Desmin [D33],  $\alpha$ -smooth muscle actin [1A4]), cytokeratin AE1/AE3 and S-100 protein were negative. The pathological diagnosis was Solitary Fibrous Tumor of the breast on the basis of the gross, histological and immunohistochemical findings. No recurrence or any other clinical problems have been noted in the 6-month follow-up period. The structural and immunohistochemical characteristics of these tumors are similar to those of a myofibroblastoma. Some investigators attempted to distinguish between the two entities and the diagnosis of solitary fibrous tumor is reserved for lesions actin-negative and CD34-positive.

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##### **CLINICOPATHOLOGIC FEATURES OF PHYLLODES TUMORS**

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Phyllodes tumor represents a type of fibroepithelial breast tumor, previously named cystosarcoma phyllodes or periductal stromal tumor. The purpose of our study was the evaluation of the variability of clinicopathological features and its correlation with prognosis in this type of breast tumor. We present 6 cases of phyllodes tumors, diagnosed in patients of 34-59 years old. Fine needle aspiration cytology (FNAC) or needle core biopsies were applied in 3 cases, followed by intraoperative examinations, in 2 cases. Fragments from the excisional biopsies were stained with usual stainings. Immunohistochemistry for vimentin, actin and desmin was applied. The clinical aspect was that of a firm, palpable, solitary, unilateral breast tumor, larger than 4 cm diameter (maximum 8.5 cm) and in only one case the tumors were multifocal, with diameter of 1 and 2 cm respectively, and history of rapid growth. Mammography revealed a rounded, lobulated, opaque mass, sharply defined in 4 cases and with indistinct borders in 2 cases. Cytology aspect was that typical of a fibroepithelial tumor, with excess bipolar stromal cells and fragments in the background. Gross pathology revealed nonencapsulated, well circumscribed external surface of nodules in 4 cases and a less circumscribed surface in 2 cases. Sections presented firm, bulging gray tissues, with papillary aspects. In 2 cases, small foci of necrosis and hemorrhage were observed. The main histologic hallmark was represented by an exaggerated structure of intracanalicular fibroadenoma (rising also issues of differential diagnosis) with increased cellularity, with epithelial-lined clefts and variable degree of epithelial hyperplasia. Three cases were diagnosed as benign, exhibiting well defined border, only few mitoses, modest cellular overgrowth and slight cytologic pleomorphism, with a correlation between the degree of epithelial proliferation and appearance of the stroma. 1 case presented invasive aspect, in the form of secondary fibroepithelial stromal overgrowth. One case presented the aspect of low-grade malignant phyllodes tumor, exhibiting microscopically invasive border, less than 5 mitoses/ 10 hpf, and moderate stromal cellularity. Immunohistochemistry expressed vimentin positivity, actin and desmin variable positivity. In one case a local recurrence was registered, corresponding to the high grade of the initial tumor.

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##### **EFFECTS OF ESTROGEN THERAPY IN POST-MENOPAUSAL WOMEN BREAST TISSUE**

##### **MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION.**

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Introduction: Estrogen replacement therapy, isolated or combined with progestin has been prescribed widely in the past decade. However, many studies associate hormone replacement therapy (HRT) over 5 years with increased risk of breast cancer. Despite this important evidence, not much is known about the mechanisms by which hormones act on normal breast epithelium.

Objective: To analyze breast tissue of postmenopausal women before and after 6 months of continuous HRT with combined estrogen-progestin (0.625 mg of conjugated equine estrogens associated with 2.5 mg of medroxyprogesterone acetate).

Methods: All patients were evaluated before and considered clinically eligible to the HRT. Samples were obtained from the upper outer left quadrant, through a percutaneous large-core breast biopsy. All microscopic variables were evaluated by two independent observers. Morphology assessment included epithelial density and nuclear volume on hematoxylin-eosin stained slides. Morphometry was graphically analyzed with the use of IMAGELAB 2000 software, after image capture by the VIDCAP 32 system. Immunohistochemical reactions used a standard ABC method and included epithelial proliferative activity (Ki-67 index), expression of the estrogen and progestin receptors, and angiogenesis (CD-34).

Results: After 6 months of estrogen-progestin HRT, there was a significant increase in nuclear volume in late postmenopausal women. There was no difference in epithelial density, neither in Ki-67 labeling index and angiogenesis. Estrogen and progestin receptors expression did not change with the HRT.

Conclusions: Estrogen-progestin HRT during 6 months induced an enhanced nuclear volume of breast epithelial cells, suggesting an increase in their metabolic activity. However, it is this finding was observed only in late postmenopausal women. Other factors such as the exposure length, the type of hormone utilized, the timing of therapy initiation, the treatment regimen used and individual characteristics must be further evaluated.

#### P 73

##### **MYOEPIHELIAL CARCINOMA ARISING IN AN ADENOMYOEPITHELIOMA OF THE BREAST: A CASE REPORT WITH IMMUNOHISTOCHEMICAL AND MUTATIONAL ANALYSIS**

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Adenomyoepithelioma of the breast is an uncommon tumor characterized by biphasic proliferation of both epithelial and myoepithelial cells. It was first recognized by Hamperl in 1970, and approximately 140 cases of adenomyoepithelioma in breast have been reported in English literature. Although most of adenomyoepithelioma has been considered to be benign, they can progress a malignant change and give rise to metastases in rare instances. Malignant transformation may involve epithelial, myoepithelial or both cellular elements of an adenomyoepithelioma. We report a 69-year-old female with a 2.5 cm mass in her right breast. Histological examination revealed a highly cellular neoplasm, consisting of a predominantly spindle cell proliferation admixed with scattered glandular epithelial lined spaces. The spindle cell component, which was surrounding the latter, proliferated in broad sheets, strands or multinodular fashion, and was separated by hyalinized collagenous stroma of varying thickness. Immunohistochemically, the spindle cells of the

tumor were diffusely positive for S-100,  $\alpha$ -SMA, GFAP (Glial fibrillary acidic protein), p63, and cytokeratin (CK) 14, whereas the glandular epithelial cells were strongly positive for EMA, CK19. Both glandular and myoepithelial cells were consistently negative for estrogen receptor (ER), progesterone receptor (PgR) and HER2/neu. These results confirmed biphasic epithelial and myoepithelial character of the tumor. On the basis of overall architecture, the tumor was classified as the spindle cell type of adenomyoepithelioma, and diagnosed as myoepithelial carcinoma arising in an adenomyoepithelioma because of cytological atypia, high mitotic rate, and local invasion of the myoepithelial component. MIB1 index was high (>70%) in myoepithelial cells. Immunohistochemical study also revealed strong expression of P53 and phosphorylated extracellular signal-regulated kinase (ERK) 1/2 in myoepithelial carcinoma cells, but not glandular epithelial component. We hypothesized that myoepithelial cells in this tumor might possess p53 gene mutation. Laser capture microdissection (LCM) technique and mutational analysis revealed point mutation of p53 gene (T->C transversion at codon 176) in myoepithelial cells but not in glandular epithelial component and adjacent normal ductal epithelium. To our knowledge, this is the first report using mutational analysis on a malignant adenomyoepithelioma of the breast.

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##### MYOEPITHELIAL LESIONS OF THE BREAST- THE DIAGNOSTIC PROBLEMS

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**Introduction** The myoepithelial cells are an integral part of the normal histology of the mammary glands. Lesions with striking overgrowth of myoepithelial cells are rare. Histologically and cytologically they may disclose different patterns, and some additional features may result in diagnostic errors.

**Aim** We report two cases of adenomyoepithelioma ( tubular variant and spindle cell variant) and one case of adenomyoepithelial adenosis.

**Material and methods** We analysed their histologic, cytologic, immunohistochemical features and their differential diagnosis. The tumors were characterized by a bicellular pattern of gland-forming epithelial cells and proliferative myoepithelial cells. They showed foci of monotonous growth of myoepithelial cells devoid of glands with low mitotic rate ( 1-2 figures/10HPF) or middle mitotic rate ( 3-6 figures/10 HPF). Immunohistochemically myoepithelial cells were reactive with SMA, Actin, Calponin, but negative for Desmin. The cytologic features of adenomyoepitheliomas characterized by hypercellularity and clusters of regular polygonal cells with abundant pale cytoplasm ( tubular variant) or sheets of spindle cells ( spindle cell variant). The nuclei were round to oval with dispersed chromatin and small nucleoli.

**Conclusion** Failure to recognize adenomyoepithelioma or adenomyoepithelial adenosis may lead to an inappropriate diagnosis such as fibroadenoma, sclerosing adenosis, tubular adenoma, and even invasive carcinoma. Immunohistochemical examination is needed to distinguish epithelial cell proliferation from myoepithelial cell proliferation.

#### P 75

##### ADENOMYOEPITHELIOMA OF THE BREAST

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**Introduction:** Adenomyoepithelioma (AME) of the breast is a very rare low-grade malignant biphasic tumor, morphologically and immunohistochemically identical to epithelial-myoeplithelial cell carcinoma of salivary gland.

**Materials and methods:** We report 10 cases of adenomyoepithelioma of the breast. All patients were female, ranging in age from 27 to 87 years (average 58 years). One patient had a history of chronic lymphocytic leukemia, two patients had coexistent ductal carcinoma and another one had fibroadenomas in both breasts.

All tumors presented as palpable nodules, ranging from 1-7 cm in maximum diameter and were located in the peripheral portion of the breast (8 cases) or near the areola (2 cases). Patients were treated by local excision. The excised tumors had the typical macroscopical, histological and immunohistochemical features of adenomyoepithelioma.

**Discussion:** Breast and salivary gland tumors can share morphological features, but often differ in incidence and clinical behavior. Breast tumors showing pure myoepithelial or epimyoeplithelial differentiation are rare.

AME of the breast is a biphasic tumor, with a distinctive architectural growth pattern (tubular, papillary or solid), that is composed of two cell types, epithelial and myoepithelial. It usually affects adult female patients and presents as a palpable nodule. Tumors are treated by local excision.

AMEs of the breast are currently classified as low grade malignant tumors that may recur and rarely metastasize, but their histoprognostic factors are not yet defined.

The prognosis of adenomyoepithelioma is usually good. Local recurrences can occur when the primary tumor has a tubular growth pattern and has been incompletely excised. Tumors consisting mainly of myoepithelial cells and/or with increased mitotic activity (> 2/10 H.P.F) can behave in a malignant fashion. Lung is the most frequent site of distant metastases.

As regards our patients, all are well, without recurrences and distant metastases.

#### P 76

##### EVALUATION OF FREQUENCY OF EPSTEIN-BARR VIRUS (EBV) IN VARIOUS HISTOLOGICAL SUBTYPES OF HODGKIN'S DISEASE

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**Introduction:** The incidence of Hodgkin's disease (HD) shows marked heterogeneity according to age, gender, race, geographical state, socioeconomic position and histological subtypes. Recently, multiple studies in different countries applied by newer technologies such as Immunohistochemistry (IHC) and polymerase chain reaction (PCR), to being more familiar with the pathogenesis of this neoplasm. The Epstein-Barr virus (EBV) is detected with high incidence in HD cases, approximately 40-50% in developed countries and much more (up to 95% of cases) in developing countries. There is evidences that mixed cellularity (MC) Hodgkin's disease is more likely to be EBV-associated which is against association of nodular sclerosis (NS) subtype.

**Purpose of the study:** With regard to the geographical location of Iran and absence of similar documented research in our knowledge, it is need to perform studies like this.

**Methods & Materials:** This study was done by IHC method with antibody against latent membrane protein-1 (LMP1) antigen of EBV for assessment of relationship between EBV infection and parameters such as age, gender and histological subtype. We collected 30 paraffin section samples of HD and positive cytoplasmic reactivity of Reed-Steinberg (RS) cells was evaluated.

Results: From 30 cases that surveyed in this study only 2 cases were not immunoreactive for EBV marker which both was NS subtype in two adult male. We confirm frequency of 93% EBV associated HD in our cases and also confirm the above histological subtype distribution and that childhood cases are more likely to be EBV-associated than adult cases. There is possible female predominant of EBV associated HD. This survey as a pilot study needs further studies with more cases for distinct confirmation.

Conclusion:

It seems EBV is a strong etiologic factor especially in developing countries like Iran and in childhood cases.

#### P 77

##### **EXPRESSION OF T-CELL MARKERS IN CLASSICAL HODGKIN LYMPHOMA IS RARE: A TISSUE MICROARRAY STUDY**

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Abstract (265 words)

Introduction and purpose of the study: In most instances, Hodgkin and Reed-Sternberg cells (HRSC) of classical Hodgkin lymphoma (cHL) are of B-cell origin. Nevertheless, expression of T-cell related antigens on HRSC has been observed in some cases, pointing to a possible T-cell origin. We comprehensively analyzed the expression of a panel of T-cell antigens in a large series of classical Hodgkin lymphoma (cHL) cases under standardized conditions using the tissue microarray (TMA) technique.

Methods: A TMA containing 330 cHL cases was used. Expression of the T-cell antigens CD2, CD3, CD4, CD5, CD7 and CD8 was analyzed immunohistochemically. A case was considered positive if >20% of HRSC demonstrated specific staining. The HRSC of T-cell marker positive cases were microdissected (75 to 120 HRSC per case) and analyzed by a multiplex PCR for clonal immunoglobulin heavy chain- and T-cell receptor gamma (TCRg) gene rearrangements.

Results: Twelve cases (5%) expressed at least one T-cell marker in the following order: CD2 in 10 cases, CD4 in 5 cases, CD3 in 2 cases and CD5 and CD8 in 1 case each. There was no CD7-positive case. The expression of more than one T-cell antigen was seen in 5 cases (2%). In the positive cases, a mean fraction of 40% of the HRSC (range 20-100%) expressed the T-cell markers analyzed. In two cases (<1%) a clonal TCRg gene rearrangement was demonstrated.

Conclusions: The expression of T-cell antigens in HRSC of cHL is rare (5%) and in most cases due to aberrant expression of these markers on HRSC of B-cell origin. Only isolated cHL cases may be true clonal T-cell proliferations.

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##### **SENSORY NEUROPATHY REVEALING A HODGKIN'S LYMPHOMA.**

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Association of a sensory neuropathy with a Hodgkin's lymphoma is rare.

Causes of a such neuropathy might vary. They are either due to the Hodgkin's lymphoma itself, infiltrating the peripheral

nervous tissue, to opportunistic infections, viral in particular such as the HIV, to neurotoxic effects of chemotherapy or to a systemic reason leading to a paraneoplastic syndrome.

Hereby, we report a case in which a young woman aged 30 years presented progressive worsening gait troubles for 8 months with pain and lower limbs heaviness, a fall of 15 kg weight and a pruritus on trunk and hands.

Bilateral upper and lower limbs reflexes were abolished, with tactile and vibratory hypoaesthesia. EMG confirmed the sensory neurogenic fit.

Chest and abdominal scanner showed large mediastinal and intra and retroperitoneal adenopathies. It also revealed the presence of numerous micro-nodules in lung, liver and spleen. Biopsy of a right upon-clavicle node which appeared during hospitalization concluded to a scleronodular form of Hodgkin's lymphoma.

Medullary and sural nerve biopsy did not show any tumoral cells infiltration.

As far as etiopathogeny is concerned, the retained hypothesis is the one of a systemic origin

#### P 79

##### **OSSEOUS PRESENTATION OF HODGKIN'S DISEASE: A CASE REPORT AND REVIEW OF THE LITERATURE**

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Although bone involvement in the later stages of Hodgkin's disease is an expected phenomenon, bone involvement in early stages of the disease are very rare. About forty-nine cases of Hodgkin's disease presented with bone involvement were reported in the literature. We reported a 14 years old boy initially evaluated with the pain at the left ilium localization. Although all the radiological examinations were targeting an osseous anomaly, pathologic evaluations of the pelvic lymphadenopathies determined the disease. We discussed the possible differential diseases and reviewed the literature for the osseous presentation of Hodgkin's disease.

#### P 80

##### **EPSTEIN-BARR VIRUS INDUCED B-CELL PROLIFERATION OF HODGKIN AND REED-STERNBERG CELL GENO- AND PHENOTYPE MAY DEVELOP IN PERIPHERAL T-CELL LYMPHOMAS**

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Introduction. Peripheral T-cell lymphoma (PTCL) can display not only a morphological, but also an immunophenotypical overlap with classical Hodgkin lymphoma (cHL) as PTCL may contain CD30 and CD15 as well as T-marker positive Hodgkin-Reed-Sternberg (HRS) cells of cHL type in a mixed inflammatory background of eosinophils, lymphocytes, histiocytes and plasma cells. The distinction between PTCL and cHL is further complicated by the rare variant of PTCL resembling cHL because of the presence of HRS type of cells which are constantly Epstein-Barr virus (EBV) and CD30 positive. Single cell molecular analysis of these cells indicated B-cell genotype, but lack of monoclonality.

Purpose of the study. To clarify the nature of the scattered HRS-like cells, without the aspecific inflammatory background of cHL, in PTCLs.

Methods. Three cases of PTCLs with scattered giant cells of HRS type, resembling classical cHL because of the inconspicuous to moderate atypia of the PTCL cells, are described. Immunohistochemistry, in situ hybridization for EBV encoded RNA (EBER) and immunoglobulin (Ig) light chains, antigen receptor gene rearrangement studies on whole tissue as well as sequencing and mutational analysis of the FRI-J region on DNA obtained from EBER+, flow sorted nuclei were performed.

Results. Monoclonal rearrangement of the T-cell receptor gamma gene in whole tissue DNA extract as well as constant EBV association (EBER+, LMP-1+) of the HRS cells could be proved in all three cases. In one tumor, the HRS cells exhibited the CD15-, CD20+, CD30+, CD45+ phenotype and polyclonal Ig heavy chain (IgH) gene rearrangement in flow sorted, EBER+ nuclear preparation. In the other two lesions, however, the HRS type cells exhibited the CD15-/+ , CD20-/+ , CD30+, CD45- phenotype, lacked any light chain mRNA expression and showed clonal IgH gene rearrangement. In one of these two cases the IgH-V gene mutation and Ig gene associated transcription factor (Oct2, BOB.1/OBF.1) expression patterns corresponded to those of HRS cells in cHL.

Conclusion. EBV induced B-cell proliferation in a PTCL related immunosuppression may range from a polyclonal activation state even to HRS cells being indistinguishable in pheno- and genotype from those in cHL of B-cell origin and these latter cells might be the neoplastic precursor cells for a pathomorphologically overt cHL.

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##### **TITLE: IMMUNOHISTOCHEMICAL MARKERS CAN BE OF PREDICTIVE VALUE IN HODGKIN'S LYMPHOMA RESPONSE TO TREATMENT. A PRELIMINARY STUDY OF 23 CASES.**

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Hodgkin's lymphoma (HL) belong to the most curable tumor diseases in adults and about 80% of patients can be cured with modern treatment strategies. However, some HL are primary refractory to usual treatments and only clinical factors are considered as relevant for prognosis. In the current study we evaluate immunohistochemical markers of apoptosis, proliferation and of the environmental non neoplastic cells in HL in order to predict their prognostic value in treatment response. A retrospective study was performed on pre-treatment biopsy specimen of 23 patients presenting with HL, 8 with primary refractory HL and 15 responding well to chemotherapy and free of disease for 2 or 3 years. These patients ranged in age from 23 to 52 years old (median= 36.5 years) for refractory HL and from 21 to 75 years old (median = 37.9 years) for responding patients. All refractory HL, except one for whom histological type could not be precised (too small biopsy), had a nodular sclerosis (NS) histological type. The histological type was NS in 13 responding patients and mixed cellularity for the 2 others. The semi-quantitative immunohistochemical study used CD30, bcl2, p53, Ki67, TiA1 and c-kit antibodies. The results were statistically evaluated using a Wilcoxon sum rank test. CD30 and Ki67 were strongly expressed on Hodgkin (Hg) and Reed-Sternberg (RS) cells whatever patients were refractory or responded well to chemotherapy. By contrast, p53 and bcl2 had a significantly higher expression on Hg or RS cells in refractory patients (median = 90% & 60%) compared to

responding HL (median = 42.5% & 8%) (p=0.0001 & p=0.026 respectively). The cytotoxic marker TiA1 stained a significantly higher number of small lymphocytes in refractory HL (median=53 per high power field (hpf)) compared to responding patients (median=24 per hpf) (p= 0.0003). C-kit antibody was negative in Hg or RS cells but stained significantly more mastocytes in refractory HL (median=13 per hpf) comparing to responding HL (median=3.8 per hpf) (p= 0.001). These results indicate that some immunohistochemical markers may be useful for predicting the response to therapy of HL. A high expression of bcl2 and p53 in refractory HL supports the notion that an intact apoptosis cascade is essential for cell killing effect of chemotherapy. The increasing of TiA1 and c-kit positive cells raises the importance of the environmental non-neoplastic cells in HL. A larger study is mandatory to confirm these preliminary results.

#### P 82

##### **EPSTEIN-BARR VIRUS (EBV) AND HODGKIN'S DISEASE IN A MOROCCAN CENTRE**

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The Epstein-Barr virus (EBV) has been implicated as a contributing factor in the development of Hodgkin's disease (HD). Western cases of Hodgkin's disease have shown the presence of EBV in tumoral cells in approximately 50%. The presence of EBV varied according to the histological subtype. Mixed cellularity HD is more like to be EBV-positive compared with nodular sclerosis. The purpose of our study is to analyse the prevalence of EBV in this disease in a Moroccan population using immunohistochemical detection of latent membrane protein LMP-1.

We studied a total of 62 cases of classical Hodgkin's disease, diagnosed between January 2004 and December 2004. Of 10 patients (16 %) less than 15 years of age (childhood), 5 had a nodular sclerosis subtype, 4 mixed cellularity, and one unclassified. Of the 52 from patients aged 15 years and above, 30 (58%)

had nodular sclerosis, 20 (38,50 %) mixed cellularity, and 2 unclassified. 11 patients were 50 years and above (5 sclerosis nodular, 5 mixed cellularity and 1 unclassified). All the cases were tested for CD30 and CD15. 95% were CD30 positive and 83% were CD15 positive.

Positive immunostaining for LMP1 was demonstrated in 39 / 62 cases (63 %) respectively 50% in sclerosis nodular and 47 % in mixed cellularity . In the younger age group, 7 of the 10 cases (4 mixed cellularity, 2 nodular sclerosis) showed the presence of EBV (70 %). In the older age group, EBV was detected (61,50%) in the following proportion: 33% nodular sclerosis, 29 % mixed cellularity.

The results of the current study showed that nodular sclerosis HD is more frequent ( 53 %) than mixed cellularity (42%), without bimodal distribution. The peak of incidence was between 15 and 50 years. The findings suggest also, a strong association of EBV with Hodgkin's disease in Moroccans (39/62, 63%), especially in children when it reach 70%. In this study there was no evidence of predominance association of EBV in mixed cellularity HD. Our results must be confirmed by in situ hybridization.

#### P 83

##### **B-CELL DIFFERENTIATION IMMUNOPHENOTYPES IN CLASSICAL HODGKIN LYMPHOMAS**

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The bcl6/CD10/MUM1/CD138 B-cell differentiation immunophenotypes were analyzed in 101 cases of classical Hodgkin lymphomas (cHL) in order to gain further insight in their histogenesis.

Three major bcl6/CD10/MUM1/CD138 immunophenotypes were distinguished on the basis of the immunohistochemical positivity of Hodgkin and Reed-Sternberg (H/RS) cells: a) the late germinal center (GC)/early post GC B-cell-like immunophenotype (bcl6-/CD10-/MUM1+/CD138-); 59/101 cases (59 %), b) the post GC B-cell-like immunophenotype (bcl6-/CD10-/MUM1+/CD138+); 24/101 cases (24 %) and c) the indeterminate immunophenotype 18/101 cases (18 %) (bcl6+/CD10-/MUM1+/CD138-: 14 cases and bcl6+/CD10-/MUM1+/CD138+: 4 cases).

The above findings indicate that H/RS cells in most cHL display bcl6/CD10/MUM1/CD138 immunophenotypes consistent with late GC/early post GC or post GC B-cell differentiation. In addition, H/RS cells in a small fraction of cHL display indeterminate bcl6/CD10/MUM1/CD138 immunophenotypes which are characterized by simultaneous expression of GC, late GC/early post GC and post GC B-cell differentiation proteins. These immunophenotypes do not correspond to the differentiation immunophenotypes of normal B-cells and their identification in a part of cHL suggests that the differentiation process of H/RS cells is not complete in a fraction of these cells and/or is still ongoing at the time of observation.

**P 84**  
**PROLIFERATION PROFILE OF CLASSICAL HODGKIN'S LYMPHOMAS. INCREASED EXPRESSION OF THE PROTEIN CYCLIN D2 IN HODGKIN AND REED-STERNBERG CELLS.**

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There is accumulating evidence that Hodgkin and Reed-Sternberg cells of classical Hodgkin's lymphomas display multiple and concurrent alterations in different pathways and checkpoints of the cell cycle. However, the expression of cyclin D2 and its relation to other major cell cycle proteins has not been analyzed in classical Hodgkin's lymphomas.

The aim of the present study was to assess expression of cyclin D2, Ki67, cyclin A, cyclin B1, cyclin D1, cyclin D3, cyclin E, p53, Rb, p16 and p27 proteins in order to gain further insight in the proliferation profile of classical Hodgkin's lymphomas.

Overexpression of cyclin D2 in Hodgkin and Reed-Sternberg cells was detected in 64/89 (72%) cases of classical Hodgkin's lymphomas. This finding, in view of recent in vitro data showing that constitutive activation of nuclear factor (NF)-kB could upregulate cyclin D2 expression in part via signal transducer and activator of transcription (STAT)-5a, suggests that induction of cyclin D2 expression may support the proliferation of Hodgkin and Reed-Sternberg cells. In addition, the present study showed that 1) increased p27 expression status was significantly correlated with higher levels of cyclin A expression ( $p=0.048$ ) and 2) increased p53 expression status was significantly correlated with higher levels of cyclin A ( $p<0.001$ ) and cyclin B1 ( $p=0.040$ )

expression. The association between increased p27 and p53 expression status and higher expression levels of G2/M cyclins suggests that the impairment of the growth inhibitory activity of the p27 and p53 tumor suppressor pathways may promote the proliferation of Hodgkin and Reed-Sternberg cells.

**P 85**  
**RECTAL EPSTEIN BARR VIRUS POSITIVE HODGKIN LYMPHOMA IN A PATIENT WITH CROHN'S DISEASE**

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Primary gastrointestinal Hodgkin lymphomas arising in the setting of Crohn's disease (CD) are rare. Only ten cases have so far been reported. A strong association with Epstein Barr virus (EBV) infection, similar to that found in post-transplantation immunosuppression-related lymphoproliferative disorders, has been suggested.

We present a 35-year-old man with ileocolonic Crohn's disease diagnosed at the age of 27, several months after an operation for a colorectal adenocarcinoma. Patient was on maintenance treatment with immunosuppressants and additionally he received two doses of infliximab. Seven years after adenocarcinoma diagnosis, the patient presented with anal pain and diarrhea. At that time digital examination revealed a small rectal area, which was thick in digital contact leaving blood traces at palpation. Rectosigmoidoscopy and subsequent biopsies from this area located 5 cm above the anal ring were compatible with rectal Hodgkin lymphoma. Histopathological examination revealed extensive ulcerations of the mucosa and diffuse infiltration composed by a mixed inflammatory cell population and numerous Hodgkin and Reed-Sternberg cells. The tumor cells showed the immunophenotype of classical Hodgkin lymphoma (CD15+, CD30+, CD45-, CD20-, CD3-, EMA-). In situ hybridization study with EBV RNA 1/2 (EBER 1/2) probes and immunohistochemistry for the latent membrane protein-1 (LMP-1) showed that almost all tumor cells carried EBV infection. No peripheral lymph nodes were noticed and CT examination showed no evidence of visceral lymph node enlargement or hepatosplenic lesions. Anal ultrasonography showed a hypo-echogenous lesion (1.5X2 cm<sup>2</sup>) extending to the one third of the whole rectal wall perimeter. Perirectal fat was invaded. Bone marrow biopsy was normal. Infliximab administration was discontinued and patient underwent chemotherapy. After a eighteen-month follow up, the patient does well with no evidence of secondary lesions or further local progression.

We present herein a primary rectal EBV positive Hodgkin lymphoma metachronous to a colorectal adenocarcinoma in a Crohn's disease patient who was on maintenance treatment with immunosuppressants. It is suggested that immunosuppressive drugs may facilitate the emergence of a EBV-positive neoplastic clone, but this scenario still remain only theoretical.

**P 86**  
**THE COMPARISON OF IMMUNOHISTOCHEMICAL EXPRESSION OF CD34, VEGF AND CD44 IN LYMPH NODE AND BONE MARROW INFILTRATES BY HODGKIN'S LYMPHOMA**

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**Introduction:** Bone marrow (BM) infiltration is present in 5-15% of patients with Hodgkin's lymphoma. It is usually associated with prominent fibrosis and proliferation of blood vessels. Limited information exists about the significance of neoangiogenesis and expression of "homing receptor" CD44 in Hodgkin's lymphoma.

**Aims:** In this study we analyzed immunohistochemical expression of CD34, VEGF and CD44 in lymph node (LN) and BM infiltrate by Hodgkin's lymphoma.

**Methods:** We identified 40 patients (29 male and 11 female, median age 45 years) with BM infiltration by Hodgkin's lymphoma. The most frequent histological type was nodular sclerosis (28/40). Slides from paraffin embedded LN and BM biopsy of the same patient were stained with haematoxylin&eosin and reticulin fibers (Gordon-Sweet). Microvessels were visualized by immunohistochemical staining for CD34. We counted the number of vessels per 400x high power field (hpf) in the area of most dense vascularization. All samples were further analyzed for the expression of VEGF and CD44 in neoplastic Hodgkin-Reed-Sternberg (HRS) cells. The intensity of staining was graded as weak, moderate and strong.

**Results:** In majority of patients (31/40) we found marked increase of microvessels density, which correlated with the number of VEGF-positive HRS cells. Expression of CD34 was almost twice lower in BM than in LN in majority of patients. We observed that mean vessels count per hpf in LN was 12.0, but in BM this count was 7.5/hpf. The intensity of VEGF expression in HRS cells was usually strong in LN and moderate in BM infiltrate of the same patient. Immunohistochemical expression of CD44 both in LN and BM was high in HRS cells in majority of patients (35/40), but in this group of 35 cases, in 4 cases HRS cells lost CD44 expression in BM infiltrate.

**Conclusion:** Our findings suggest that neoangiogenesis in BM infiltrates by Hodgkin's lymphoma was extensively increased. Therefore, the density of microvessels and expression of VEGF may provide useful prognostic information. The results of CD44 expression might suggest causal involvement of this marker in the progression of Hodgkin's lymphoma, especially nodular sclerosis type.

#### P 87

##### **EXPRESSION OF NITRIC OXIDE SYNTHASES IN CLASSICAL HODGKIN LYMPHOMA: ROLE ON APOPTOSIS DEREGLATION AND INFLUENCE ON CLINICAL OUTCOME**

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**Introduction:** Hodgkin Lymphoma (HL) is a monoclonal neoplasm in which the neoplastic cells, named Hodgkin-Reed&Sternberg (H-RS) cells, bear somatic mutations in immunoglobulin genes, inactivating immunoglobulin production. Unlike normal B lymphocytes, which suffer apoptosis when failing to produce immunoglobulins, H-RS cells, by mechanisms not fully understood, are capable of escaping apoptosis. Nitric oxide (NO), a molecule involved in

multiple steps of carcinogenesis, is produced by a family of enzymes known as the nitric oxide synthases (NOS), which have been shown to be overexpressed in many human tumors. NOS expression and NO production in HL have not been described yet. **Objective:** This study analyzed the role of NOS and apoptosis-related proteins expression in HL, to test the hypothesis that NOS expression and NO production are involved in the defective apoptotic program of HL. The role of EBV on NOS expression and NO production was also studied. Finally we investigated the influence of NOS expression on the clinical outcome of HL's patients. **Material and methods:** 171 cases of HL were retrieved from the archives of the Departamento de Patologia do Hospital do Câncer A C Camargo. Relapse biopsies and HIV-associated HL were excluded. Immunostains with antibodies against NOS1, NOS2, nitrotyrosine, bcl-2, bax, p53, fas, fasL and LMP1 were performed. Cases were also tested for EBV status using in situ hybridization for EBER-1. **Results:** NOS1 and NOS 2 expression were detected in 42.1% and 55% of the cases, respectively. Nitrotyrosine expression was observed in 18.7% of the cases. Bcl-2, bax, p53, fas, fasL and LMP1 expression were positive in 35.1%, 44.4%, 44.4%, 93.6%, 5.8% e 49.7% of the cases, respectively. EBER-1 was detected in 46.8% of the cases. Significant associations were established between bax and p53 (p=0.036), bax and fasL (p=0.046), NOS1 and p53 (p=0.006), bax and NOS2 (p=0.022); and between bcl-2 e NOS2 (p=0.034). An inverse correlation was observed between EBV (LMP-1) and NOS2 (p=0.018), and between EBV and bcl-2 (LMP-1, p=0.002; EBER, p=0.006). NOS2 expression was associated with a shorter overall survival (p=0.0098). It also predicted a poor prognosis in cases treated with MOPP/ABVD (p=0.0003). **Conclusions:** H-RS cells express NOS and produce NO, which appear to play a role in the defective apoptotic program of HL. NOS2 may be involved in bcl-2 overexpression in EBV-negative HL. NOS2 expression is associated with a poor outcome in HL.

#### P 88

##### **FREQUENT ASSOCIATION OF BOB-1 AND MUM-1 IN EBV INFECTED EGYPTIAN CLASSIC HODGKIN LYMPHOMA**

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**Background:** Previous studies concerning the expression of Bob-1, MUM-1 and Bmi-1 in classic Hodgkin lymphoma (CHL), confirmed the expression of Bmi-1 and MUM-1 in Hodgkin/Reed-Sternberg (H/RS) cells, while almost all the researchers agree on the lack of Bob-1 expression pointing out to a defect in transcription machinery associated with lack of immunoglobulin gene expression. **Objectives:** To study the expression of Bob-1, MUM1 and Bmi-1 proteins in Egyptian CHL and to correlate this expression with EBV viral load. **Methods:** Paraffin sections of randomly selected 24 CHL cases were included: 3 lymphocyte rich (LR), 6 mixed cellularity (MC), 13 nodular sclerosis (NS) and 2 lymphocyte depletion (LD). **Results:** All the cases except five (two MC and three NS) were positive for EBV by in-situ hybridization and immunostaining for LMP-1. Unexpectedly, strong to moderate nuclear Bob-1 expression was observed in 94% of cases ranging from 25-100% of the neoplastic cells. Moderate MUM-1 staining, observed in 94% of cases, was nuclear, nucleolar and cytoplasmic. The percentage of MUM-1+ cells ranged from 25-75%. Mild to moderate nuclear bmi-1 positivity was observed in 56% of cases; few to 25% of bmi-1+ cells were identified. There was a frequent co-expression of Bob-1/MUM-1 in all the cases (p<0.005). This expression parallels the EBV positive H/RS cells. **Conclusion:** These findings concerning the expression of Bob-1, MUM-1 and Bmi-1 in the Egyptian population are different from the Western ones denoting different genetic and environmental factors. Does this difference should be taken into

consideration in the treatment strategy? A point under consideration.

#### P 89

##### **CLINICOPATHOLOGIC PROFILE OF HODGKIN DISEASE IN A MOROCCAN CENTRE. ABOUT 269 CASES**

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Epidemiological distribution of Hodgkin disease (HD) show in developing countries two peaks occurrence, one in childhood for boys and another in older adults. The subtype of HD that occurs most often in this countries is cellularity mixed. In developing countries there is a bimodal age specific incidence rate with early peak in adulthood and another in advanced age.

In order to define epidemiologic and histoprognosis aspects of the Hodgkin disease, we conduct a retrospective study on 269 cases recorded at the department of Pathology, Ibn Rochd University Hospital, Casablanca, between 1997 and 2001. The mean age of our patients is 40 [04-84 years]. The sex-ratio is 1,5. Incidence according to age finds a peak between 17 and 35 years, without bimodal repartition. Actually, 74,3% of our patients are less than 40, 17% only are under 14. The predominant histologic type is the sclerosinodular (SN) ( 70,5%) versus 26,8% for the cellularity mixed subtype (CM) . The SN subtype is predominant without regard of age (< 14 years, 14-40 years and >40 years). The bone marrow biopsy showed invasion in 30% . The immunohistochemistry study was realised in 80 cases (29,7%). CD15 was positive in 84,4 % and CD 30 in 93,7% . Stage III et IV constitute 64,43%. The rate of complete remission is 69,5%. This rate is 89% for the under 40 years old and only 64,4% for the more 40 years old.

Factors that influence on the complete remission rate are : the advanced age (the rate of complete remission of the patients <40 ans is 89% versus 64,4 % for patients under 40 years old; the histologic subtype, but we find that complete remission in mixed cellularity subtype is higher than sclerosis nodular subtype.

The absence of bimodal distribution according to age, predominance of the SN subtype and the diffuse stages are the main characteristics of our study.

#### P 90

##### **NODULAR LIVER INVOLVEMENT IN A PATIENT WITH MULTIPLE MYELOMA**

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Multiple myeloma (MM) is a malignant plasma cell disorder characterized by the production of a monoclonal immunoglobulin. Recurrent infections, anemia, osteolytic lesions and renal insufficiency are the typical clinical manifestations. Liver involvement in the form of solitary or multiple nodules is extremely rare.

We report the case of a 42-year-old male, who presented in our hospital due to pathologic fracture of his right femur. From the hematologic examination, bone marrow aspiration and biopsy, the diagnosis of IgG-k MM was made. The patient was treated with oral melphalan and prednisone. Three years later, at the time of reevaluation, it was found pancytopenia, multiple lytic bone lesions, diffuse osteopenia, renal function impairment, but normal liver function. Bone

marrow biopsy showed an hypocellular marrow and infiltration by plasma cells (30%). He was treated with VAD regimen for only one cycle, because of severe cardiac complications, and pulse dexamethasone. Whole blood cell counts and renal function improved, but he developed progressive impairment of liver biochemical parameters. A CT scan revealed multiple hypodense liver lesions. A fine needle CT-guided biopsy of a liver nodule was performed to rule out metastasis of another origin. Histological examination showed infiltration of the liver by plasma cells which were positive for CD138 and k-light chain. A bone marrow biopsy at the same time showed plasma cell infiltration (60%). The patient underwent treatment with proteasome inhibitor bortezomib, showing improvement of renal function but progressive hepatic failure with portal hypertension and ascites.

We present this case because liver involvement by MM in the form of multiple nodules is extremely rare in living patients. Conclusively, liver infiltration should be suspected in a case of nodular liver lesions in patients with known MM.

#### P 91

##### **MYELOYDYSPLASTIC LESIONS IN THE BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA AT FIRST DIAGNOSIS.**

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**INTRODUCTION:** The myelodysplastic lesions (MDL) in the bone marrow (BM) of patients with multiple myeloma (MM) that are more widely known are the ones related to the therapeutic treatment. Studies on MDL as a result of the toxicity that myeloma cells may have on the haemopoietic BM and are present at first diagnosis have not until now been reported.

**PATIENTS AND METHODS:** We investigated the presence of MDL in BM biopsies (H+E stains) as well as in BM and peripheral blood imprints (May Gruenwald Giemsa stains) of 80 patients with newly diagnosed MM. Each case was evaluated by two observers. The presence of MDL was statistically correlated with the percentage of infiltration for each case (paired t-test) and the survival of the patients (Spearman's and Kruskal-Wallis's tests).

**RESULTS:** MDL were present in 35% of the patients and they were similar to those observed in myelodysplastic syndromes, but of less severity. They concerned mainly the red cell line (33,75%), secondarily the granulocytic line (10%) and less often the megakaryocytic line (6,25%), while MDL from two of the three lines were found in 10% of the cases and from all the three lines in 3,75% of the cases. Statistical analysis brought up a significant correlation between the presence of MDL and the percentage of MM infiltration ( $p < 0,01$ ), as well as between MDL and patients' survival ( $p < 0,01$ ).

**CONCLUSIONS:** We demonstrate the prognostic value and therefore the diagnostic significance of the presence of MDL in the BM of patients with MM. Due to the absence of other relevant data in the current literature more research should be performed.

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##### **BONE MARROW FIBROSIS IN PATIENTS WITH MULTIPLE MYELOMA**

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Bone marrow fibrosis is a reactive process that results from an increased stimulation of fibroblast proliferation and collagen synthesis. Multiple myeloma (MM) is a malignant neoplasm characterised by the increase of clonal plasma cells in bone marrow. The levels of cytokines, which are responsible for fibrosis, are raised in patients with MM as compared to healthy patients. Histopathological examination of bone marrow by bone marrow trephine biopsy (BMT) makes possibility for evaluation of the degree of reticulin fibrosis (an increasing number of argentophylic fibres) and collagen fibrosis (an increasing number of collagen fibres). BMT is also used for the assessment of bone marrow involvement by plasma cells. The aim of the study was to determine the degree of bone marrow fibrosis in patient with multiple myeloma. The specimens were obtained by BMT from 82 patients with multiple myeloma, as initial examinations prior to any treatment, from the postero-superior iliac spine using the Jamshidi needle. Bone marrow tissue was carried out in Oxford solution and paraffin embedding. Hematoxylin and eosin, Gomori's, AZAN staining was applied. The degree of bone marrow fibrosis using Kundel criteria with Bauermeister modification was determined. The morphometric tests were done using the image analysis (DP 12) for evaluation the percentage of plasma cells infiltration. Nodular and diffuse patterns of infiltration in bone marrow by plasma cells were observed in 67 patients. In the most of patients the marrow was infiltrated in diffuse pattern. The infiltration was accompanied by an increase in the number of reticulin fibres. The frequency of reticulin fibrosis was positively correlated with advanced clinical stage according to the classification of Durie and Salmon. Collagen fibrosis was found in 13 patients. BMT is a useful method for assessing the degree of myelofibrosis in MM. It has prognostic value, because when the collagen fibrosis is present, only partial regression after treatment is possible.

#### P 93

##### **PLASMA BLASTIC MICROLYMPHOMA ASSOCIATED WITH A MULTICENTRIC CASTLEMAN DISEASE IN A HIV+ PATIENT**

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Multicentric Castleman's disease (MCM) in HIV+ is associated with human herpes virus 8 (HHV8) infection. Most of the patients present a mixed histological pattern: hyaline-vascular variant and plasma cell variant. HHV8 infected cells are plasmablasts localized within the mantle zone or organized in small sheets of large plasmablastic cells or in a microlymphoma toward aggressive NHL.

Aim of the study: evaluation of HHV8 antibody in a plasma cell variant of MCM in a HIV+ 34 year's old man who presented cervical polyadenopathy with night sweats, fever and inflammatory biological abnormalities.

Material and methods: immunostaining were performed on formal fixed, paraffin embedded 3microns sections on a Dakostainer with CD3(1/75), CD20(1/150), CD68(1/500), CD138 (1/50), CD15(1/200), CD30(1/200), EMA(1/50), HHV1 et 2 (2/2500), kappa (1/15000), lambda (1/15000) Dako; CMV(1/300) Biosoft and HHV8(1/25) Novocastra.

Results: histological evaluation of one of these adenopathy revealed atrophic lymphoid follicles with small germinal centers and peripheral onion lymphoid arrangement, hyaline vascular proliferation, mixed with regular plasma cells sheets in the interfollicular zone. In small foci, the peripheral sinus exhibits small sheets of large lymphoid cells with atypical nuclei and prominent nucleoli. These atypical cells were HHV8+ whereas plasma cells were negatives. These large cells were also CD138+. They didn't exhibit any

immunostaining with CD20, CD3, CD30, CD15 and EMA antibodies. The diagnosis of a plasmablastic micro lymphoma associated with a MCM was retained in presence of a restricted monotypic phenotype IgM $\lambda$ .

Conclusion: In case of lymphadenopathy in a HIV+, the HHV8 staining is helpful to detect plasmablastic microlymphoma and even aggressive NHL.

#### P 94

##### **IN VIVO WNT-SIGNALING ANTAGONIST SFRP3 EXPRESSION IN MULTIPLE MYELOMA: ANOTHER POTENTIAL PATHWAY IN MYELOMA BONE DISEASE.**

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Multiple myeloma (MM) is commonly associated with lytic bone lesions. Although the mechanisms of increased osteoclast activity are partially understood, comparatively little is known about the mechanisms that lead to the observed decrease in osteoblast number and activity.

Wnt glycoproteins comprise a family of extracellular signaling ligands that play essential roles in cell proliferation and differentiation during normal developmental processes. Recently, secreted frizzled-related proteins (sFRPs) have been identified as possible modulators of the Wnt signal transduction pathway, based on their ability to bind and sequester the Wnt ligands. The sFRP3 glycoprotein suppress osteoblast cell number, acting as a decoy receptor for Wnt proteins that promote osteoblast proliferation.

Methods: Using a double-immunostaining (CD138/sFRP3) on tissue microarrays (TMA), we analysed plasma cells (PCs) sFRP3 expression in 59 fixed paraffin-embedded bone marrow samples (BMS) from patients with newly diagnosed MM (according to International Myeloma Working Group criteria (2002)) versus 43 control normal BMS.

All BMS were Bouin's (alcohol-based) fixed, decalcified in EDTA and paraffin embedded. The TMA consisted of 2 cores (2mm in diameter) from each BMS.

The percentage of CD138/sFRP3-positive PCs was determined by counting all PCs in normal samples to 400 PCs in MM samples in areas with the impression of highest expression ("hot spot") at low magnification.

Results: Depending on sample, the percentage of positive PCs varied from 0% to 100%.

We found consistent, uniform cytoplasmic sFRP3 expression for an average of 80% of the PCs (mean: 90%) in MM samples, versus 19% (mean: 16.4%) of the PCs in normal BMS (p-value < 0.0001).

If we considered as positive for sFRP3 expression, BMS with more than 50% of PCs sFRP3/CD138+, 88.1% of MM samples were positive as opposed to 11.6 % of normal samples (p<0.001).

Conclusion: Using bone marrow TMA for the first time, we observed, by double-immunostaining, significant differences in the relative expression of sFRP3 between PCs of newly diagnosed MM and normal BMS. The production and secretion by myeloma cells of sFRP3, which is an inhibitor of osteoblast proliferation, is a new potential pathway in myeloma bone disease. We propose that sFRP3 inhibits the proliferation of osteoblasts and shift the balance between osteoblasts and osteoclasts, thereby diminishing bone formation and enhancing the development of lytic lesions.

#### P 95

##### **PLASMA CELL MORPHOLOGY AND PROLIFERATION FRACTION IN MULTIPLE**

## MYELOMA ASSESSED IN TREPHINE BIOPSIES: RELATION TO CLINICAL STAGE

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Multiple myeloma (MM) is a haematologic malignancy, built of terminally differentiated B-cells with morphological features of plasma cells and their predecessors. Morphological presentation of the tumour is well known, but little data exist on cellular architecture assessed in paraffin sections and related to clinical stage and proliferation rate.

The OBJECTIVE of the study was to evaluate cellular composition and proliferation fraction of the tumour in different clinical stages.

The study was based on 62 trephine biopsies (TB), containing myeloma clone. TB were taken from patients (aged 47-78) for diagnostic and follow-up purposes with informed consent. 9 patients were in Durie stage I, 24 – in stage II and 29 in stage III. Routinely HE and Romanovsky-Giemsa stains were performed, as well as immunostaining for CD138 and Ki-67 (Dako Cytomation, Denmark). CD138 immunoreactivity contributed greatly to recognition of abnormal or immature plasma cells and in some cases increased the total myeloma cell count up to 30% as compared to HE stain alone. The cell sub-classification protocol, proposed by J.E.Goasguen et al. (1999) for cytological purposes, was applied to histological sections. It appeared possible to put over 85% of myeloma cells into four groups - plasmablastic, proplasmacytic I and II and plasma cell using "positive selection" methodology. Statistically significant increase in plasmablastic cells was documented in patients in stage III of the disease in comparison with those in stage I (14% against 2%), accompanied by concomitant decrease in mature plasma cells (30% against 68%). Proliferation index was 0,9 in stage I and 9,5 in stage III. Values in stage III showed substantial variation, entirely because of 3 cases included in the group with predominantly blastic morphology. These cases taken separately showed plasmablastic cell count 39% and proliferation index of 40%. Interestingly, plasmablastic cell count performed in aspirate smears showed mean of 22%.

WE CONCLUDE that blastic cell count and proliferation rate assessed in bone marrow TBs correlate with clinical stage of MM. In bone marrow sections plasmablastic cell count is higher than reported in smears - possibly due to differential ability of mature and immature bone marrow cells to get aspirated.

### P 96

#### CD20 ABERRANT EXPRESSION OF MULTIPLE MYELOMA

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**Introduction** It is well known that reactive and neoplastic plasma cells may express various hematopoietic and non-hematopoietic antigens. However, there are few reports that correlate the aberrant immunophenotype of multiple myeloma (MM) with the survival of patients. The aim of this study was to investigate whether the expression of particular B-cell antigens by myeloma cells is suggestive of a more aggressive phenotype.

**Material and method** A total of 102 routinely processed bone marrow biopsy specimens from untreated MM patients were immunostained with anti-LCA (CD45), L26 (CD20), anti-CD79a and anti-syndecan-1 (CD138) monoclonal antibodies using Envision detection kit.

**Results** All the specimens showed a strong positive immunostaining for CD138 in almost 100% of neoplastic

cells. CD20- and CD79a-expression (>5% of myeloma cells) was detected in 24.5% and 43.1% of cases, respectively. The great majority to 100% of the myeloma cells were reacted with anti-CD20 and anti-CD79a antibodies in 14 (13.7%) and 15 (14.7%) cases, respectively. Overall survival of patients was not associated with CD20 and/or CD79a expression by myeloma cells ( $p = 0.30$ ). A significant percentage of CD45 positive neoplastic plasma cells was observed in only two specimens.

**Conclusions** Syndecan-1 appears to be a reliable marker for plasma cells. A positive CD20-immunostaining does not exclude the possibility of a plasma cell tumor, especially in the case of an extramedullary neoplasm (e.g. plasmacytoma). CD20 and/or CD79a expression by myeloma cells does not seem to be of significant value in predicting survival. Myeloma cases with a strong CD20 expression may respond to treatment with anti-CD20 monoclonal antibody, in combination with the standard therapy.

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#### EXPRESSION OF PI3K/PDEN/AKT-HIF-1 SIGNALING PATHWAY PROTEINS, THEIR RELATION WITH CELL PROLIFERATION AND CLINICAL OUTCOME IN PATIENTS WITH MULTIPLE MYELOMA (MM)

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The factors related with the expansion of the malignant clone in MM, are enhanced proliferation and resistance to apoptosis. Recent studies have focused on different related pathways stimulating proliferation or suppressing apoptosis. The phosphatidylinositol-3-OH kinase (PI3K) signal pathway integrates receptor tyrosine kinase signaling which could be the major candidate regulating the apoptotic cascade related to the survival and proliferation of myeloma cells. One mediator of PI(3)K signaling is the Akt/protein kinase B, which phosphorylates multiple downstream effectors that ultimately regulate a variety of cell functions including glucose metabolism, cell proliferation and survival. mTOR and HIF-1 are among these mediators. And tumor suppressor protein PTEN is one of major inhibitors of Akt phosphorylation.

In this study we aim to examine the expressions of the phosphorylated proteins PI3K/PTEN/Akt, related downstream proteins mTOR, HIF-1 on bone marrow biopsy specimens of MM patients and to analyze their relation between the proliferative activity and survival.

#### PATIENTS AND METHODS:

Fourty seven myeloma patients diagnosed in our department between 2002-2004, aged 54(33-77), whose bone marrow biopsy specimens could be retrieved were included in this study. All patients were treated with VAD or MP as first line treatment.

Immunohistochemical staining was performed using monoclonal antibodies against phospho- PTEN (Cell Signaling Tech), phospho-Akt (Cell Signaling Tech), phospho-mTOR (Cell Signaling Tech), HIF1 alpha (abcam) and Ki67 (Labvision) with Zymed ABC Px Kit manually or Ventana Benchmark automated immunostainer for secondary visualisation. Negative control was present in each experiment.

#### CONCLUSIONS:

Loss of PTEN expression (10 of 35 patients) was correlated with high proliferative activity examined by Ki67 staining ( $p=0.014$ , Mann-Whitney U test).

Akt activation was seen in 20% of the cases which was not correlated with the survival.

There were no correlations between survival, proliferation status of the myeloma cells and expressions of the proteins involved in PI3K/PTEN/Akt, HIF-1 pathway.

The cases having loss of PTEN expression by immunohistochemistry did not show increased Akt expression. It seems that Akt activation on the way of proliferation may be related to the other stimulating factors rather than the negative control of PTEN.

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##### **EXPRESSION PROFILES OF CYCLIN A, D1, D2, D3 AND CYCLIN DEPENDENT KINASE INHIBITORS (CDKI) P16 AND P21 IN PATIENTS WITH MYELOMA ON BONE MARROW SPECIMENS**

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#### INTRODUCTION

Cyclins are expressed during specific phases of the cell cycle and are controlled by cell cycle dependent kinase inhibitors (CDKI). p16 and p21 control Cyclin D and Cyclin A, respectively. The sequential role of Cyclin A and p21 has not been determined yet. We aimed to analyze the cyclin and CDKI's in marrow plasma cells by immunohistochemistry.

#### PATIENTS and METHODS

Fourty five myeloma patients diagnosed in our department between 2002-2004, aged 54(33-77), M/F:21/13 whose bone marrow biopsy specimens could be retrieved were included in the study. All patients were treated with VAD or MP as first line treatment.

Immunohistochemical staining was performed using monoclonal antibodies: Cyclins A, D1, p16, p21(LabVision), Cyclin D3(DAKO), Cyclin D2 (Santa Cruz) and Ki67 (Labvision) with Zymed ABC Px Kit manually or Ventana Benchmark automated immunostainer for secondary visualisation. Negative control was present in each experiment.

#### CONCLUSION

We could not find any correlation between the expression profiles of Cyclins A, D1, D2, D3, p16, p21, Ki67 and survival. But there was an inverse correlation between p16 and cyclin A expressions as expected.

Cyclins and their inhibitors can be expressed in different combinations by plasma cells. A decreased expression of cyclin A or D and a relevant CDKI's increased expression was evaluated as a low proliferative situation (P min).

P min was observed 5/45 of the patients, resulted with 80% overall response and 100% survival at 5 years.

On the contrary P max patients (high Cyclin and low CDKI expressing patients) were observed more frequently(19/45). 10/19 of these patients responded and performed 57% Survival. Pmax and P interm patients responded (7/17) and survived similarly.

Prognostic factors such as age, B2MG and CRP were similar between Pmin, P max or P interm groups thus did not influence the response and survival effect.

Cyclin-CDKI patterns are associated with bone disease: P max patients had bone lesions more frequently (11/14) whereas P min patients had none (p=0.008). The role of bone disease as a prognostic factor has been shown before by other investigators. This association supports the prognostic role of cell cycle regulators and inhibitors.

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##### **MUC1 EXPRESSING PLASMA CELLS IN BONE MARROW EXHIBITING INVOLVEMENT BY PLASMA CELL MYELOMA**

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Introduction: MUC1 represents a mucin molecule expressed in various epithelial tissues and neoplasms as well as normal haematopoietic cell lines. Additionally, its presence could be demonstrated in diffuse large B cell lymphomas as well as plasma cell myelomas. However, a thorough analysis of the clinical as well as pathological importance of MUC1 positive plasma cells in bone marrow involved by plasma cell myeloma was not performed up to now.

Purpose: In the present study, we tried to evaluate the expression of MUC1 in a larger series of bone marrow specimens with plasma cell myeloma involvement in order to evaluate possible correlations with histopathological parameters as well as survival data.

Material and methods: Trephine biopsy specimens with involvement by plasma cell myelomas derived from 105 patients were investigated immunohistochemically visualizing syndecan-1 (CD138) and MUC1 positive myeloma cells. Initially, the percentage of CD138 positive plasma cells was determined. Additionally, MUC1-specific monoclonal antibodies (mabs), anti-epithelial membrane antigen (EMA) as well as HMFG-2 were applied. The rate of MUC1 positive plasma cells was evaluated semiquantitatively. Possible correlations with pathological variables (grade, fibre content, pattern of infiltration) as well as survival were analysed statistically applying chi square tests as well as survival analyses.

Results: About 55 % of the cases showed EMA expression and 47 % HMFG-2 immunoreactivity in more than 5 % of the plasma cells in the bone marrow. In cases exhibiting a so-called packed marrow, EMA immunoreactivity was reduced. However, MUC1 positivity as assessed semiquantitatively did not show a statistically significant correlation with the cytologic grade of differentiation or the fibre content, respectively. Correlations with survival probability could also not be observed.

Conclusions: About 50 % of the cases with plasma cell myeloma involvement of the bone marrow exhibited an expression of MUC1 in plasma cells. This observation may be of special interest with regard to recently published data. For example, enrichment of MUC1-specific T cells or MUC1 vaccination may represent new aspects in the therapy of plasma cell myeloma and MUC1 expression in plasma cell myeloma may be regulated by interleukin 7. However, MUC1 expression by bone marrow-infiltrating plasma cells did not correlate with most histomorphological parameters or patients' prognosis according to our results

#### P 100

##### **EXTRANODAL LYMPHOMA : A CLINICOPATHOLOGIC STUDY OF 87 CASES**

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#### Introduction:

Primitive extranodal lymphomas (PENL) constitute 25 % of whole non Hodgkin lymphomas. They are defined as localised lymphomas which appear in extranodal tissue with or without invasion of regional nodes.

#### Objectives:

The purpose of this study is to specify the clinicopathologic and immunohistochemical characteristics of the principal locations of extranodal lymphomas and to discuss their prognosis.

#### Patients and methods:

Our study is retrospective relating to 87 cases of PENL diagnosed in the period between January 1995 and December 2000 at the Pathologic Department of Habib Bourguiba Hospital ( Sfax -Tunisia ). This study is based on the

clinicopathologic and immunohistochemical findings. All cases were analysed and reclassified using the latest WHO (2000) classification.

#### Results:

The mean age of patients was 45 years (extreme: 3 and 84 years). There was a male prevalence with a ratio of 1,7. Primitive lymphomas of the digestive tract (PLDT) were the most common (44 cases : 50,6%) followed by Waldeyer's ring lymphoma (17 cases : 19,5%), cutaneous lymphomas ( 9 cases : 10,5 %), lymphomas of the central nervous system (6 cases: 6,9 %), centrofacial lymphomas (5 cases : 5.8 %), osseous lymphomas (2 cases : 2,3 %), splenic lymphoma, thymic, orbital, and salivary glands lymphoma (one case for each location : 1,1 %). The association of a digestive lymphoma to a lymphoma of the ring of Waldeyer were observed among 4 patients (9 % of PLDT). The most common histologic type was diffuse large B-cell lymphoma (56 cases : 64,4 %) followed by MALT- lymphoma (10 cases : 11,6%). Treatment policies adopted as well as the outcome were documented for each location

#### Conclusion:

The PENL represent a very heterogeneous group of neoplasms . They are not always easily identified on routinely stained sections and their diagnosis need immunohistochemical analysis and even molecular biology. Their outcome is variable depending on the site and the histological type.

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##### **ABSENCE OF MICROSATELLITE INSTABILITY IN GASTRIC LYMPHOMA : AN IMMUNOHISTOCHEMICAL STUDY OF HMLH1 AND HMSH2 PROTEIN EXPRESSION**

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Defective DNA mismatch repair results from genetic or epigenetic alterations that most frequently inactivate the genes hMLH1 and hMSH2. This pathway is known to be involved in the pathogenesis of a subset of solid neoplasm, most prominently encountered in hereditary non polyposis colorectal cancer (HNPCC) syndrome, characterized by microsatellite instability (MSI). However its role in lymphomagenesis is not well established. In gastric lymphoma of mucosa-associated lymphoid tissue (MALT) type, contradictory results concerning the prevalence of MSI have been reported in few studies using molecular testing.

The aim of this study was to identify tumours with a mutator phenotype by detection of abnormal MMR gene protein expression in a large series of gastric lymphoma.

Immunohistochemistry was performed in 111 low grade MALT lymphomas and in 44 diffuse large B cell lymphomas using two monoclonal mouse antibodies that react against hMLH1 and hMSH2 protein. In all cases with contributive immunohistochemistry (80), tumours showed nuclear expression of both proteins.

These results suggest that defects in DNA mismatch repair, due to inactivation of hMLH1 and hMSH2, do not seem to contribute significantly to the molecular pathogenesis of gastric MALT lymphoma.

#### P 102

##### **CASTLEMAN'S DISEASE OF THE HEAD OF THE PANCREAS: UNUSUAL ASSOCIATION WITH ADENOMATOUS HYPERPLASIA OF THE PAPANILLA VATERI**

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**INTRODUCTION:** Castleman's disease has been described in a variety of anatomical sites including the peripancreatic retroperitoneum. We present a case of its unusual intrapancreatic presentation associated with simultaneous hyperplasia of the papilla Vateri.

**A CASE PRESENTATION:** A 54 years old female patient suffered back pain, abdominal discomfort and short interval intermittent jaundice. The ultrasound finding suggested cystic lesion and after CT scan examination pancreatic mass was diagnosed in the head of the pancreas. There were no other significant or conclusive laboratory findings. Intraoperative surgical examination found well circumscribed tumor occupying the posterior part of the head of the pancreas and peripancreatic fat in the proximity of obstructive enlargement of the papilla Vateri. Frozen section biopsy was misinterpreted as non-representative peripancreatic lymph node and Whipple's cephalic pancreatico-duodenectomy was performed.

**METHODS AND RESULTS:** Gross examination revealed well circumscribed mass measuring 64 mm in greatest diameter in the head of the pancreas with small part extruding to peripancreatic fat. Histologically, nodular architecture of lymphoid infiltrate was composed of follicle-like structures with hyalinised and vascularised centres and onion skin-like distribution of surrounding small lymphoid cells. Immunostaining showed B-cell phenotype (CD20+, CD5-, CD10-, CD43-) with CD21+ rich dendritic cell networks, focally mantle zone-like areas (focal expression of CD10+ and bcl-6+) and a lack of bcl-2 immunorexpression in germinal centres, but CD3+/CD5+ positive small T lymphocytes among polymorphous interfollicular lymphoid infiltrate. PCR analysis with Biomed 2 primers (FR1, FR2, FR3) showed a polyclonal pattern. In addition, slight fibrotic ampullary stenosis and adenomatous hyperplasia of the papilla Vateri were found, not accompanied with epithelial atypia or simultaneous myomatous hypertrophy. In 14 months follow-up there was no relapse or any signs of the disease.

**CONCLUSION:** Very unusual anatomical presentation of Castleman's disease with profound ingrowth of the lymphoid proliferation within the head of the pancreas associated with obstructive adenomatous hyperplasia of the papilla Vateri was initially misunderstood as pancreatic cancer. This broadens differential diagnosis of obstructive periampullary and pancreatic lesions.

#### P 103

##### **CHEMOKINE RECEPTOR EXPRESSION IN NONGASTRIC MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT)-TYPE LYMPHOMAS**

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**Introduction:** Chemokine receptors mediate the migration and activation of lymphocytes through binding of their ligands. Several recent studies have revealed important contributions of chemokine receptors and their ligands to the development and progression/dissemination of hematopoietic neoplasms. By promoting tumor dissemination CXCR3 has recently been

identified as a major contributor in the pathogenesis of mucosa-associated lymphoid tissue (=MALT) lymphomas. Little is known about the chemokine receptor expression profile of nongastric MALT lymphomas.

**Purpose:** The aim of our study was to identify the expression pattern of all 17 currently known and characterized chemokine receptors in nongastric MALT-lymphomas in comparison to normal, surrounding tissue.

**Material and Methods:** 10 extragastric MALT lymphomas of the parotid gland were selected from the archives of the Department of Pathology, Medical University Graz. As control specimens 5 pooled non-malignant parotid glands were included.

Makrodissected tissue containing >80% lymphoma cells from paraffin embedded MALT lymphomas of the salivary gland were processed for RNA isolation. Semiquantitative Real Time PCR was performed on a GeneAmp® 5700 Sequence Detector. Peripheral blood from healthy donors served as calibrator, due to the broad expression of all chemokine receptors investigated. Expression of 17 chemokine receptors was determined in triplicate and the number of cycles was compared to the reference gene HPRT and peripheral blood, based on calculations of 2-delta-deltaCT.

**Results:** Compared to normal tissue we demonstrated de novo expression of CCR4 and CCR8.

As opposed to non-malignant control tissues, receptor upregulation occurred in CCR5, CCR6, CCR7, CCR9, CXCR3, CXCR4, CXCR5 and XCR1. Equal expression could be observed in CCR1, CCR2, CCR3, CXCR1, CXCR2 and CX3CR1, whereas the chemokine receptor CCR10 was down-regulated. In the CC receptor family the most pronounced upregulation could be attributed to the family members CCR6 (57,6 times) and CCR7 (31,8 times), whereas CXCR4 (20 times) was highest expressed in the CXC receptor family group.

**Conclusion:** Hence, our data support a model of the etiopathogenesis of extragastric MALT lymphomas involving a complex interplay of multiple interacting signals mediated by chemokine receptors, where differentially regulated members of the CXC as well as CC receptor family might play a crucial role in the dissemination/homing of malignant B-cells.

#### **P 104 INDUCIBLE NITRIC OXIDE SYNTHASE AND APOPTOSIS IN HUMAN B CELL LYMPHOMAS**

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**Purpose:** Nitric oxide synthases (NOS) are isoenzymes that catalyse the synthesis of nitric oxide (NO). NO plays both pathological and physiological roles depending on its rate of synthesis and concentration in cellular source and microenvironment.

Apoptosis is an important biological factor in lymphomas.

This study evaluates expression of inducible nitric oxide synthase (iNOS) in human lymphomas and its relation with apoptosis.

**Material and Methods:** This study comprised 46 cases of B-cell lymphoma. The lymphomas were classified as 3 mantle cell, 5 marginal zone, 4 follicular, 2 Burkitt, 25 diffuse large cell, 2 anaplastic large cell, 3 lymphoblastic, 2 lymphoplasmacytic according to WHO classification of lymphoid neoplasms.

Hematoxylin eosin slides of the cases were reviewed and immunoperoxidase technique was performed iNOS and Caspase monoclonal antibodies to selected sections of each

case. Antigen staining was carried out with iNOS and Caspase proteins and Ultravision Polyvalent, Rabbit, HRP-AEC kit ( Neomarkers- Biogen USA).

For the evaluation of iNOS and Caspase, tumor areas with a high density of expression were chosen. Positive stained cells were counted in 5 different areas at a magnification X40 by an Olympus BX51 microscope in each case.

The iNOS and Caspase expressions were independently recorded by two pathologists and the results were averaged.

**Results:** Our study revealed that there is a positive relation between iNOS expression and apoptosis (p= 0.032 spearman correlation). This relation might be promising new agents in therapeutic protocols.

#### **P 105 SYNCHRONOUS PRESENTATION OF NON- HODGKIN'S LYMPHOMA AND MALIGNANT MELANOMA**

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The association of malignant melanoma (MM) with different neoplasias is uncommon but not exceptional. Over the past 2 decades, both MM and non-Hodgkin's lymphoma (NHL) incidence rates, have increased substantially. Several epidemiological studies have suggested an association between MM and NHL and an interaction between genetic and environmental factors in the pathogenesis of these tumors.

We report the case of a 72-year-old man who presented in our hospital due to enlarged, not painful cervical, axillary and inguinal lymph nodes, persisted for 6 months. On physical examination it was found peripheral lymphadenopathy with no hepatosplenomegaly. Hematological examination was normal. CT scan of the chest and abdomen revealed generalized lymphadenopathy, with no hepatosplenomegaly. An inguinal lymph node was excised and histological examination showed morphological and immunohistochemical features of B-cell small lymphocytic lymphoma (SLL). At the same time, a melanocytic lesion on the frontal region of the head was excised due to recent enlargement and change of the colour. Histological examination of the lesion showed features of superficial spreading MM, Clark's level IV and Breslow's depth of invasion 2,5 cm, for which he received no further treatment. The patient received 10 courses of systemic chemotherapy with mabthera, 1 course with fludarabine, decadron and mitoxantron for SLL, and remained in partial remission. One year later he presented with neurological signs due to brain metastasis, and multiple skin nodules on the tract. Histological examination of an excised skin nodule showed the features of MM. One month later he died due to complications of CNS involvement.

In the present study we report a rare case of synchronous presentation of NHL and MM. Although it is rare, we should suspect this association and make the correct diagnosis using the appropriate immunohistochemical stains.

#### **P 106 MORPHOIMMUNOHISTOCHEMICAL DIFFERENTIAL DIAGNOSTICS OF "GREY ZONE" LARGE LYMPHOMAS.**

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**INTRODUCTION:** Overlap between Hodgkin's lymphoma and large-cell lymphomas creates the difficult task for morphological diagnostics. At the last time "grey zone" is

complicated for the account of T-cell/histiocyte-rich B-cell lymphoma (TCRBCL), anaplastic variant of diffuse B-cell large lymphoma (DBCL), primary mediastinal B-cell lymphoma (PMBL), anaplastic large-cell lymphoma. Purpose of study was to investigate group of "grey zone" lymphomas.

**MATERIALS AND METHODS.** We have studied 143 lymph nodes biopsies of "grey zone" lymphomas using immunohistochemical method with wide panel of monoclonal antibodies: CD3, CD4, CD8, CD10, CD15, CD20, CD21, CD23, CD30, CD43, CD45, CD57, Granzyme B, ALK, EMA, bcl-2, bcl-6.

**RESULTS:** We have diagnosed 21 cases of TCRBCL (m:f 0,7:1,0; mediana 47 years, range 16-67). Diffuse growth was marked in 20 cases and nodular growth in 1 case, that demands differential diagnostics with nodular lymphoid-predominant of Hodgkin's lymphoma. PMBL was diagnosed in 35 pts (m:f 1,0:3,5, mediana 31,5 years, range 16-62) with immunophenotype of neoplastic cells: CD45-positive and CD20-positive (all cases), CD30 (75%), CD23 (48%), bcl-6 (33%) and all cases were IgM-negative. 2 cases of anaplastic variant DBCL were revealed. ALCL was diagnosed in 39 pts (mediana 22 years, m:f 1,0:1,0; range 4-79).

ALK-positive cases were observed in 58% (till 40 years) but any case in 40 years and more senior. The others cases were referred to Hodgkin's lymphoma (mixed cellularity, NS II subtypes).

**CONCLUSION:** "Grey zone" lymphomas may be diagnosed only by using wide panel of antibodies. ALK-negative with 0-immunophenotype cases of ALCL are generated

#### P 107

#### **CYTOTOXICITY OF THE SMALL MOLECULE BCL-2 ANTAGONIST, HA14-1, AND SELECTED ANTICANCER DRUGS IN FOLLICULAR LYMPHOMA B-CELLS.**

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The Bcl-2 overexpression is a hallmark of follicular lymphoma (FL). Since patients with FL often suffer from progressively resistant to chemotherapy refractory disease, the development of new regimens is required. In this study we analyzed for the first time the effects of a Bcl-2 antagonist, HA14-1, alone and in combination with antineoplastic agents commonly used against follicular lymphoma, in human FL cell lines with t(14;18). The cell lines were established from patient samples sent for PAD analysis and earlier analysed in detail. All cells were sensitive to HA14-1 induced cytotoxicity (the LC50s of 4.5 to 12.6 µM) and apoptosis. Moreover, HA14-1 enhanced dexamethasone and doxorubicin mediated (in schedule independent and dependent manner, respectively) cytotoxicity and apoptosis, but antagonized with vincristine, in all FL cells. Together these findings warrant further in vivo studies and provide conceptual basis for therapeutic intervention in follicular lymphoma.

#### P 108

#### **TUMORS OF HEMATOPOIETIC AND LYMPHOID TISSUES IN CHERNOBYL CLEAN-UP WORKERS (1996-2004)**

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The role of ionizing radiation as a causative factor of various biological types of leukemias and lymphomas after Chernobyl is still controversial. Different predictive models on the

incidence of hematopoietic malignancies in Ukrainian Chernobyl clean-up workers have been suggested up to present. Nevertheless, the actual data are still lacking mainly because of the inconsistency in the primary findings preventing the precise diagnosis of hematopoietic malignancies.

The aim of the study was to characterize in details the major forms and cytological variants of oncohematological diseases in Chernobyl clean-up workers exposed in 1986-1987 to radiation at a dose within the range of 7.5-25 cGy.

The data on 200 consecutive cases of malignant diseases of hematopoietic and lymphoid tissues in Chernobyl clean-up workers diagnosed in Reference Laboratory of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology in 1996-2004 on the basis of up-to-date techniques of cytomorphology and immunophenotyping in accordance with novel WHO classification have been summarized. The specimens were analyzed using immunocytochemical techniques (PAP, APAAP, ABC) and panel of monoclonal antibodies to differentiation antigens of leukocytes employed for immunophenotyping of leukemic cells. The patterns of the main forms of hematopoietic malignancies in Chernobyl clean-up workers have been compared with that in general population.

Practically all the main forms of malignant diseases of hematopoietic and lymphoid tissues were revealed in the group of Chernobyl clean-up workers under study. The comparison of relative distribution of the particular forms of hematopoietic malignancies in the patients diagnosed among Chernobyl clean-up workers demonstrates increasing multiple myeloma rate and the tendency to increasing non-Hodgkin's lymphoma and CML rates as compared to general population. The peculiar feature of AML in clean-up workers under study consists in the development of leukemia on the background of preceding myelodysplastic syndromes in 50% of all AML cases studied. 4 cases of large granular lymphocyte leukemia were recorded among clean-up workers. Strongly alkaline phosphatase-positive villous cells identified in bone marrow stroma in clean-up workers suffering from malignant as well as non-malignant diseases of hematopoietic and lymphoid tissues seem to represent a response to incorporation of the osteotropic heavy metals including radionuclides.

#### P 109

#### **EBV ASSOCIATED EXTRA-NODAL NK CELL LYMPHOMA NASAL TYPE OF THE BREAST IN BEHCET DISEASE: A CASE REPORT**

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Primary non Hodgkin lymphoma of the breast is uncommon. Most are of B-cell phenotype, with only rare cases showing a T-cell phenotype, NK-cell lymphomas are extremely rare, only 2 cases are reported so far in the literature. The association of Behcet's disease with malignancies has rarely been reported. Only 6 cases of lymphoma have been reported, of whom one is a nasal NK-cell lymphoma.

We report a case of NK-cell lymphoma extranodal nasal type of the breast

It is a 20 year-old woman followed up for Behcet disease under immunosuppressive treatment for 4 years. She presented with a mammary nodule of 3 cm. The biopsy of the lesion revealed the infiltration of medium to large sized lymphoma cells with necrosis and angiocentric pattern. The cells were positive for cytoplasmic CD3, CD56, CD8, TIA1 and Granzyme B, negative for surface CD3. Analysis of T-cell receptor fails to show clonal rearrangement. EBV studies show clonal episomal integration of EBV (EBER-1 positive). She underwent chemotherapy and radiotherapy and achieved complete remission.

To our Knowledge this case report is the first to describe NK-cell lymphoma of breast arising in Behcet disease.

#### P 110

### REAL-TIME QUANTITATIVE PCR DETECTION OF FUSION GENE ALK/NPM IN PATIENTS WITH ALCL: RESIDUAL DISEASE MONITORING AND A CORRELATION WITH THE DISEASE STATUS

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**Introduction:** Anaplastic large cell lymphoma (ALCL) represents a heterogeneous group of malignant lymphoproliferative diseases. Most of the cases are of T cell origin. ALCL is frequently associated with t(2;5)(p23;q35) coding for NPM/ALK fusion gene - anaplastic lymphoma kinase (ALK) gene is fused with nucleophosmin (NPM) gene. The NPM/ALK chimeric gene encodes a constitutively activated tyrosin kinase that has been shown to have potent oncogenic properties. The NPM/ALK fusion protein is found in up to 75% of pediatric ALCL.

**Purpose:** To monitor residual disease using real-time quantitative reverse-transcription PCR (RQ-RT-PCR) of NPM/ALK fusion gene.

**Methods:** RQ-RT-PCR is a recently developed technique for nuclear acid quantification. We developed an assay using RQ-RT-PCR for the quantitative assessment of minimal residual disease (MRD) in childhood ALCL by using a hydrolysis probe for quantification of NPM/ALK and hybridization probes for quantification of housekeeping gene (Beta-2 microglobulin gene), which was used as internal cDNA quality and quantity control. We constructed plasmid standards with NPM/ALK fusion gene and Beta-2 microglobulin gene. Normalized expression of NPM/ALK (NPM/ALKn) was determined as a ratio between NPM/ALK and Beta-2 microglobulin levels assessed by RQ-RT-PCR.

**Results:** We analysed NPM/ALK expression levels at diagnosis and/or relapses (cryopreserved and/or paraffin-embedded tissues) from affected lymph nodes and residual samples during the treatment in a group of 6 patients (4 – 19 years). All of them presented hyperexpression of NPM/ALKn (median 17321 copies) suitable for the MRD detection. Combined analysis of NPM/ALKn levels, qualitative RT-PCR NPM/ALK (1st vs 2nd round positivity) and the course of disease in 49 residual samples (bone marrow, peripheral blood) showed a clear correlation.

**Conclusion:** RQ-RT-PCR-based analysis of NPM/ALK expression is a promising and rapid approach for monitoring MRD in patients with ALCL. The impact of NPM/ALK various levels found during monitoring the residual disease on the treatment and prognosis needs further clarification.

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#### P 111

### MOLECULAR CHARACTERIZATION OF COMPOSITE MANTLE CELL AND FOLLICULAR LYMPHOMA

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**Introduction.** Composite lymphomas are rare associations of two distinct lymphoma types at the same anatomical sites. Reporting of such cases is important since they pose major biologic, diagnostic and therapeutic dilemmas. Purpose of the study. We describe the third reported case of Mantle Cell and Follicular lymphoma. Methods. We performed accurate immunohistochemical, FISH and molecular studies in order to verify molecular hallmarks of the two components and to define the mono- versus bi-clonal nature of this neoplasm. We used both manual and LASER-capture microdissection combined to multiple molecular approaches for clonality determination, including detection of heavy and light chain recombination, as well as presence of kappa /Kde recombination. Results. By immunohistochemistry and FISH we confirmed the presence of two distinct lymphoma types characterised by specific translocations (namely, t(11;14) and t(14;18)), while by molecular techniques we demonstrated two distinct and not clonally related cell populations.

**Conclusions.** Composite lymphomas are a diagnostic and pathogenetic dilemma, that should be investigated using multiple combined approaches. Care should be taken in the definition of mono- vs bi-clonality. The light chain approach, and particularly the kappa /Kde recombination detection, proved very useful for solving the clonality issue.

#### P 112

### CUTANEOUS T-CELL LYMPHOMA, FLOW CYTOMETRIC IMMUNOPHENOTYPING AND ASSESSMENT OF CYTOKINE PRODUCTION

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**Background:** The diagnosis of cutaneous T-cell lymphoma (CTCL), a tumor predominantly of T-helper cell origin (Th, CD4+), may be facilitated by flow cytometry (FC). If the precise immunophenotype of tumor cells is known, it is possible to study the cytokine production, which may help in explanation of interrelationships between neoplastic and tumor infiltrating cells (TIL). It is known, that Th1 and Th2 cytokines have distinct roles in proliferation and elimination of tumor cells. Th1 cells producing e.g. INF gamma or IL2 may activate cytotoxic T lymphocytes (CTL), but Th2 cells producing e.g. IL4 may inhibit antitumor CTL.

**Purpose:** The purpose of this study was to assess a contribution of FC to the diagnosis of CTCL and elucidate the relationships between TIL/CTL and tumor cells in CTCL. We presumed that tumor cells might produce the Th2 cytokines and thus inhibit TIL/CTL.

**Patients and methods:** 20 skin biopsies from 17 patients with the clinical suspicion of primary CTCL were examined. The suspension of lymphocytes for FC was prepared by enzymatic digestion of skin biopsy. After an immunophenotyping with a standard panel of antibodies the spontaneous and induced (by ionomycin and PMA i.e. phorbol 12-myristate 13-acetate) production of IL2, INF gamma and IL4 by tumor lymphocytes was examined.

**Results:** The suspension of lymphocytes was obtained in 70% of the cases. In this group the aberrant immunophenotype of T-cells was found in 70% and the findings were conclusive for T-cell lymphoma. Tumor cells produced IL2 only. No production of IL4 or INF gamma even after activation by ionomycin and PMA was showed.

**Conclusion:** If the lymphocytic infiltrate of the skin is not dense (as assessed from a frozen section) and only sporadic tumor cells are present, obtaining a representative sample is problematic. In such cases FC does not seem to be a diagnostic method of choice. Inconclusive results of FC may be caused by an absence of aberrant immunophenotype on

tumor cells and/or because of “masking” of the tumor cells by prevailing populations of TIL.

Furthermore, our results did not confirm our hypothesis, that tumor cells produce Th2 cytokine. Tumor cells produced mainly IL2. It is possible, that they may locally activate themselves by autocrine loop and may contribute to the tumor cell proliferation.

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#### P 113

##### **IMMUNOHISTOCHEMICAL DETECTION OF STAT1 AND TRAF1 IN MEDIASTINAL LARGE B-CELL LYMPHOMA**

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**Background:** Mediastinal large B-cell lymphoma (MLBCL) is a subtype of diffuse large B-cell lymphoma (DLBCL) defined by its clinicopathological features. Recent studies on the gene expression profile identified a molecular link between MLBCL and classical Hodgkin's lymphoma (cHL). These two tumors share similar pathogenetic mechanisms different from those of most non-mediastinal DLBCLs, with a decreased expression of B-cell receptor signalling cascade components, with an increased expression of cytokine pathways components, and an activation of NF-kappaB pathway. The tumor cells were shown to express among other proteins STAT1 (signal transducer and activator of transcription-1) and TRAF1 (tumor necrosis factor receptor-associated factor-1).

**Purpose of the study:** The aim of our study was to analyze the immunophenotype of MLBCL focusing on STAT1 and TRAF1 expression and to compare it with cHL and non-mediastinal DLBCL.

**Patients and methods:** Formalin fixed and paraffin-embedded tissue from 27 cases of MLBCL with a classical immunophenotype (18 females and 9 males, age 21 - 64, average 33 y, median 29 y) and a set of mediastinal nodular sclerotic subtype of cHL and non-mediastinal DLBCL was investigated. The expression of STAT1 (BD Biosciences) and TRAF1 (Santa Cruz) was evaluated by a standard immunohistochemical technique.

**Results:** The expression of STAT1 and TRAF1 was detected in most MLBCL tumor cells in all cases, the positivity was strong, cytoplasmic. Tumor cells of cHL were also strongly positive. The cells of DLBCL also expressed both proteins, but the positivity was in most cases moderate. Small T cells revealed a weak cytoplasmic positivity and stromal cells were negative.

**Conclusion:** Our study confirms that MLBCL and mediastinal cHL are characterized by a strong expression of STAT1 and TRAF1. However, the expression of these proteins does not discriminate reliably between MLBCL and non-mediastinal DLBCL which are positive as well, though the positivity of the latter group is weaker.

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#### P 114

##### **BONE MARROW BIOPSY FINDINGS IN T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA (LGL): REPORT OF 4 CASES**

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**Introduction:** T cell large granular lymphocytic leukemia (T-LGL) is a rare disease, with indolent clinical course, often associated with autoimmune conditions (rheumatoid arthritis), hematological disorders (myelodysplastic syndromes, aplastic anemia) or neoplasias (hairy cell leukemia, myeloma). Diagnosis can be made on marrow aspirate and peripheral blood by cytological, immunophenotypical and molecular findings (assessment of T cell receptor/TCR rearrangement). However, presenting features can overlap with other low grade lymphomas and a bone marrow biopsy (BMB) is performed.

**Purpose of the study:** to describe the clinico-pathologic findings in 4 cases of T-cell LGL.

**Patients and methods:** 4 patients (M : F= 3:1; mean age: 56 years, range 48-70) presented cytopenia (particularly, neutropenia), lymphocytosis (4/4) and hepatosplenomegaly (2/4); in all cases, the clinical picture had been present relatively unchanged for more than 1 year before BMB (range: 1-24 years); one patient showed associated pulmonary hypertension. Paraffin embedded BMBs were used for morphological and immunophenotypical studies; flow cytometry and molecular assessment of the TCR rearrangement were performed on peripheral and/or marrow blood.

**Results:** in all cases, the hemopoietic matrix was partially (20-40%) replaced by an interstitial lymphoid population of medium-sized cells, with clear cytoplasm. By immunostains, underscoring a constant sinusoidal involvement, these cells had a T (CD2+, CD3+, CD5+, CD7+, CD20-) activated cytotoxic phenotype (CD8+, CD4-, CD57+, CD56-, TIA1+, granzyme B+, perforin+); they were TCR alpha-beta+, gamma-delta- by flow cytometry and carried a monoclonal TCR rearrangement by molecular analysis. A decreased leuco-erythroblastic ratio and a mild diffuse fibrosis were seen. In 1 patient, sequential BMBs, after therapy, at 1 and 2 years from diagnosis, showed overlapping morphofunctional features, with a mild decrease of lymphoid infiltration.

**Conclusion:** T-cell LGL is a rare diagnosis in hematopathologist's practice; its distinction from other lymphoproliferative diseases both of B (hairy cell leukemia, splenic marginal zone lymphoma) and T cell origin (hepatosplenic T cell lymphoma) is of paramount importance, as these entities carry a worse prognosis and require specific or more aggressive therapy. In our cases, the final diagnosis could be reached only by combining different techniques, thus underlining the need of a multidisciplinary approach.

#### P 115

##### **RETROPERITONEAL FOLLICULAR DENDRITIC CELL SARCOMA**

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Follicular dendritic cell sarcoma (FDCS) is an extremely rare neoplasm with only a few reported cases in the literature reviewed, arising from antigen-presenting immune accessory cells in nodal and extranodal sites. It is considered to be an intermediate grade malignancy with a significant recurrent and metastatic potential. We report a case of excisional biopsy of a retroperitoneal mass performed in a 33 years old woman. The tumor tissue was gray to tan, 5cm in the greatest dimension. On microscopic examination, the prominent histological feature was the presence of sheets, fascicles and trabeculae formed by spindle to ovoid cell with indistinct cell borders, oval nuclei, dispersed chromatin and inconspicuous

nucleoli. There were also scattered multinucleated cell forms and marked necrosis. Striking lymphocyte and plasma cell infiltrations with increased focal vascularity were also present. The neoplastic cells showed positive reaction to vimentin, CD21, CD23 and negative to CD1a, HMB-45 and cytokeratins, an immunoprofile consistent with FDCL. There is an overlap of the immunoprofile among various dendritic cell lesions, which makes the differential diagnosis complicated. The combination of CD21 and CD23 markers is found primarily in FDCL, thus it is of great importance in the diagnostic distinction.

#### P 116

##### **COMPARISON OF FASCIN TO DF3/MUC-1 EXPRESSION IN RELATIONSHIP TO TCR- $\beta$ CLONALITY, NPM/ALK CHIMERIC PROTEIN EXPRESSION AND 2P23 REARRANGEMENT FINDINGS IN ANAPLASTIC LARGE CELL LYMPHOMAS**

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**Introduction.** Fascin is expressed in almost all cases of Hodgkin disease (except lymphocyte predominance type) and in a high percentage in anaplastic large cell lymphomas (ALCLs) and peripheral T cell lymphomas. DF3/Muc-1 mucin-like glycoprotein is aberrantly overexpressed in most adenocarcinomas and in a variety of hematopoietic cell malignancies as T and B-cell lymphomas and myelomas. It is also frequently expressed in ALCL. The aim of this study is to investigate the expression of fascin and DF3/Muc-1 in ALCL in comparison with other markers used in these neoplasms. **Material and Methods.** A total of 52 cases of ALCL (46 of T-cell and 6 null-cell type) from various sites (39 systemic ALCL, 10 primary cutaneous [C-ALCL] and 3 HIV-related ALCL) were analyzed for fascin and DF3/Muc-1 expression by immunohistochemistry (IHC). Molecular assessments PCR and RT-PCR) and cytogenetic investigation (FISH) were performed in 42 and 43 cases respectively. Tissue sections from 11 ALCL and tissue microarray blocks containing 41 cases of ALCL were used for IHC and FISH. Chi-Square ( $\chi^2$ ) tests of significance were performed using the statistical software SPSS 12.0.

**Results.** Fascin was expressed in the cytoplasm of tumor cells in 41/52 (78,8%) cases; 34 systemic and 7 C-ALCL, ( $p < 0,001$ ); 37 of T-cell and 4 of null-cell phenotype. Fourteen out of 15 (93,3%) ALK+ and 27/37 (72,9%) ALK-tumors, as well as all ALCL bearing ALK rearrangements were positive for fascin. The majority of ALCL positive for TCR- $\beta$  stained for fascin (20/21) (95,2%). Cytoplasmic and membranous expression of DF3/Muc-1 was detected in 18/52 (34,6%) of ALCL cases; 15 systemic ALCL and 3 C-ALCL; 15 of T-cell and 3 of null-cell type. Muc-1 was positive in 60% ALK+ and in 24,3% ALK- tumors ( $p < 0,05$ ). Ten out of 21 (55,6%) cases with clonal rearrangement and 5/12(41,7%) cases with 2p23 region rearrangement showed Muc-1 expression. Seventeen out of 18 cases with Muc-1 expression were also positive for fascin ( $p < 0,05$ ).

Fascin and/or DF3/Muc-1 were detected 42 (80,8%) cases. The two antibodies were also expressed in the majority of systemic (34/39) or C-ALCL (8/10), ( $p < 0,001$ ) cases. None of the HIV-related ALCL cases was positive for fascin or DF3/Muc-1.

**Conclusion.** Fascin is widely expressed in ALCL, in contrast to DF3/Muc-1. Up-regulation of fascin protein and its clinical usefulness in these tumors have not yet been determined. It seems that this aberrant expression is more common finding in

ALK+ ALCL

#### P 117

##### **PROGNOSTIC SIGNIFICANCE OF THE APOPTOSIS-RELATED PROTEINS BAX, BAK, BCL-2, MCL-1, CASPASE 3, ACTIVATED CASPASE 3 AND CASPASE 9 IN ACUTE MYELOID LEUKEMIA**

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**Background:** Acute myeloid leukemia (AML) is an aggressive disease with generally poor prognosis. Common mechanisms of resistant disease are defects in apoptotic pathways. Apoptosis is regulated by i) mitochondrial-mediated and ii) receptor-mediated pathways. This study investigates the expression of mitochondrial related apoptotic genes bcl-2, bax, bak and mcl-1, and the apoptosis enzymes caspase-3 and caspase-9 in AML and their potential correlation with patients' outcome.

**Material and methods:** Paraffin-embedded sections of 40 bone marrow biopsies, from equal number of patients with newly diagnosed AML (34 de novo not otherwise categorised AML cases classified as follows: M0(n=1), M1(n=9), M2(n=8), M3(n=2), M4(n=10), M5(n=4), AML following MDS(n=6)) were analyzed immunohistochemically using antibodies against bcl-2 (Dako), bax (Dako), bak (Dako), mcl-1 (Neomarker), caspase 3 (Neomarker, epitope not determined), activated caspase 3 (Chemicon, cleaved p17 fragment) and caspase 9 (Neomarker, middle fragment of caspase molecule). Patients were followed up for 0-78 (mean 14.45) months.

**Results:** The mean indices for bax, bak, bcl-2, caspase 3 and caspase 9 were: 52.45 (15-100), 47.1 (11-90), 39.26 (5-88), 27.5 (0-95) and 39 (0-90) respectively. In all cases, activated caspase 3 was detected in a very small percentage of tumor cells (mean 2.9%-range 0-5). Mcl-1 was detected only in 4 cases. Bax/bcl-2 ratio mean index was 2.36 (0.26-9.00). A direct correlation was recorded between i) caspase 3 and caspase 9 expression ( $p < 0.01$ ) ii) bax, bak and caspase 3 expression ( $p < 0.05$  respectively) and iii) bax/bcl-2 ratio and caspase 3 expression ( $p < 0.05$ ). No significant relationship was observed between the expression of the above markers and disease free or total survival. Cases expressing bax/bcl-2 ratio  $> 1.5$  showed longer total survival compared with those expressing bax/bcl-2  $< 1.5$  but this was not statistically significant. Histologic subtyping showed no correlation with any of the markers studied.

**Conclusions:** Overexpression of bax and bak as well as increased bax/bcl-2 ratio suggest that the apoptotic pathway mediated by mitochondria seems to be activated in AML. However, failure of caspase 3 activation seems to exist and results in accumulation of uncleaved caspase-3 in leukemic cells. Whether this deficit is sufficient to block bax-induced cell death or apoptosis signal may be mediated by other pathways, remains to be evaluated.

#### P 118

##### **INTERDIGITATING DENDRITIC CELL SARCOMA OF THE TONSIL**

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Interdigitating dendritic cell sarcomas (IDCSs) derive from antigen-presenting cells of the lymph node paracortex and deep cortex, the splenic periarteriolar lymphoid sheaths and the interfollicular space of mucosa-associated lymphoid tissue. They are extremely rare, generally occur in adults with male predominance and usually show an aggressive course.

We report a case of a 55 years old male presented with a painless enlargement of the right tonsil, having no past medical history. A bilateral tonsilectomy was performed. The affected tonsil exhibited a polypoid mass measuring 15 X 15 X 6 mm. On light microscopy, the normal architecture of the tonsil had been replaced by a diffuse infiltration of large irregularly shaped histiocytoid cells with clumped chromatin and indistinct cell borders. Spindle shaped, polylobated and multinucleated forms were also present. The tumor cells were moderately to strongly immunoreactive for vimentin, S-100, CD1a and lysozyme. Other immunostains including cytokeratin, actin, desmin, HMB-45, B-cell markers and T-cell markers, CD30 and follicular dendritic cell markers including CD21 and CD35 were negative. Based on morphological and immunohistochemical profile a diagnosis of IDC was suggested. Ultrastructural study eliminated the minor possibilities of misdiagnosis. The IDCs exhibit significant overlap with other malignancies such as undifferentiated metastatic carcinoma, primary and metastatic sarcoma, malignant melanoma, anaplastic large cell lymphoma and histiocytic-dendritic cell proliferations. The phenotypic heterogeneity of the IDCs renders the combination of a thorough pathological and clinical investigation necessary for an accurate diagnosis.

#### P 119

##### **EXPRESSION OF SURVIVIN IN LEUKEMIC CELLS IN RESPONSE TO INITIAL PREDNISONE THERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA: RELATION TO OTHER PROGNOSTIC FACTORS.**

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**BACKGROUND:** Decreased apoptosis associated with overexpression of endogenous inhibitors of apoptosis, e.g. survivin (SUR) may be responsible for treatment failure in children with acute lymphoblastic leukemia (ALL). This study aimed to assess time resolved changes of SUR expression and its intracellular distribution (nucleus versus cytoplasm) in peripheral blood mononuclear cells, collected prior to and after 6 and 12 hours of prednisone administration as well as the relation between expression of SUR and other prognostic factors (age, risk factor group, peripheral blood leukemic cell count at the diagnosis and immunophenotype).

**METHODS:** The study comprised 26 children with de novo ALL. Cytospin preparations of mononuclear cells isolated on density gradient were stained with mouse anti-SUR antibody followed by goat anti-mouse antibody conjugated with APC. Cellular DNA was counterstained with PI/RNase A. SUR-associated long red fluorescence excited by HeNe (633nm) was measured simultaneously over the nucleus and the rim of cytoplasm (2 pixels width) by laser scanning cytometer (CompuCyte).

**RESULTS:** Expression of SUR measured: (1) over the nucleus and over cytoplasm before and after 6 and 12 h from prednisone administration was significantly higher in children in medium/high risk groups than in standard risk group ( $p < 0.005$ ), (2) over the nucleus before and 12 h after treatment was significantly higher in children with peripheral blood leukemic cell count more than 20 000/uL ( $p < 0.05$ ), (3) over the nucleus before treatment was higher in ALL-T than ALL-B ( $P < 0.05$ ).

**CONCLUSION:** The association of high expression of SUR and poor prognostic factors in ALL suggest that SUR may be

responsible for higher rates of treatment failures in children with ALL. Further studies are needed to confirm this preliminary observation.

#### P 120

##### **MANTLE CELL LYMPHOMA IN TAIWAN: A CLINICOPATHOLOGIC AND MOLECULAR STUDY OF 21 CASES EXCLUSIVELY IN MALE PATIENTS**

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**INTRODUCTION:** Mantle cell lymphoma (MCL) is a rare and aggressive B-cell lymphoma and has never been fully characterized in Taiwan.

**PURPOSE OF THE STUDY:** We conducted this multi-institutional study in an attempt to have a better understanding of MCL in Taiwan.

**METHODS:** Retrospective study with morphology, immunohistochemistry, and real time RCR for cyclin D1 mRNA using paraffin sections.

**SUMMARY OF RESULTS:** A total of 21 cases, all male patients, were identified with a median age at 61.0 (mean, 60.7; range, 41-75). The most common organs of involvement at diagnosis were lymph node (19 patients, 90.5%), bone marrow (15, 71.4%), spleen (8, 38.1%), and gastrointestinal tract (5, 23.8%). Four patients (19.0%) developed multiple lymphomatous polyposes including 3 at diagnosis and 1 at disease progression. Eighteen (85.7%) were at high-stage disease. The 1- and 5-yr survival rates were 77.8% and 16.7%, respectively. The most common growth pattern in non-marrow tissue was mixed nodular (mantle zone) and diffuse patterns (9/20, 45%); while in the marrow, interstitial pattern (12/13, 92.3%). Cytologically, 18 (85.7%) cases were of classic variant; and 3, blastoid. The tumor cells expressed CD20, Bcl2, and IgM (in 100% cases), cyclin D1 (95.2%), CD5 (85.7%), CD43 and IgD (61.9%), CD52 (60.0%), and Bcl6 (5%). No tumor expressed CD23. There was no statically significant impact of p21, p27, and p53 expression and Ki67 labeling index on survival. Real time PCR for cyclin D1 mRNA was performed on 11 cases. One case was not amplifiable; the single cyclin D1-negative case showed a low level of mRNA while the remaining 9 cyclin D1-positive cases showed extremely high levels.

**CONCLUSION:** We confirm that the usual morphologic variants and aberrant immunophenotype of MCL in the West occur in Taiwan and this disease carry a poor prognosis. Real time PCR for cyclin D1 mRNA is a useful adjunct for supporting the diagnosis of cyclin D1-negative MCLs.

#### P 121

##### **ANGIOGENESIS IN CHRONIC MYELOPROLIFERATIVE DISEASES DETECTED BY CD34 EXPRESSION.**

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Increased bone marrow angiogenesis estimated as bone marrow microvessel density (MVD) or as serum angiogenic factor levels and/or immunohistochemical expression of these

factors in bone marrow biopsy has been demonstrated in a variety of hematological disorders including chronic myeloproliferative diseases (MPDs).

The aim of this study was to investigate the MVD in 25 cases of myelofibrosis with myeloid metaplasia (MMM). MVD was estimated by CD34 immunohistochemical expression in bone marrow biopsies. A control group of 27 patients without bone marrow disease, eight cases of polycythemia vera (PV), 41 cases of essential thrombocythemia (ET) and nine cases of chronic myeloid leukemia (CML) were also studied. Moreover, in cases with MMM and cases with ET, MVD was correlated with clinical, laboratory, histological parameters and the outcome of the patients.

Our study confirmed a significantly higher degree of angiogenesis in MMM, PV, ET and CML compared with controls ( $P < 0.001$ ,  $P = 0.0007$ ,  $P < 0.001$  and  $P = 0.0008$ , respectively). Angiogenesis was higher in MMM than PV, ET and CML cases ( $P < 0.001$ ,  $P < 0.001$  and  $P = 0.008$ ). Increased angiogenesis was correlated with hypercatabolic symptoms in MMM patients ( $P = 0.009$ ). No correlation with other clinicopathological parameters or clinical outcome was found. In ET cases, increased angiogenesis was not correlated with clinical, laboratory, bone marrow histopathological parameters or clinical outcome.

Definitive conclusions regarding the prognostic value of increased angiogenesis may require additional follow-up and a larger group of patients.

#### P 122

##### **ASSOCIATION BETWEEN MEDIASTINAL GERM CELL TUMOR AND ACUTE MYELOID LEUKEMIA** CAMPARO P (1), DESANGLES F (2), MOSSAFA H (3), SOULEAU B (4), BRANQUET D (5)

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**Introduction :** Acute myeloid leukemia (AML) in the follow up of other malignant tumors are usually chemotherapy-induced and may present treatment specific chromosomal alterations. We report a case of AML occurring in the short follow up of a mediastinal germ cell tumor (MGCT) that was not therapy-induced, but cytogenetically proved to originate from the germ cell tumor.

**Observations :** A mediastinal tumor was discovered in an asymptomatic 24 years old man. Histologically, this tumor consisted of a mixed germ cell tumor associating seminoma and teratoma with some foci of myeloid differentiation. It was first treated by chemotherapy. Ten months later the remaining mediastinal tumor was partially removed by surgery while a myeloid leukemia occurred (35000 WBC). No Phi chromosome was observed on blood cell cultures (myelodysplastic syndrome MDS/MPS). Because of bad general condition, second chemotherapy was delayed and four months later the MDS/MPS transformed into AML (110 000 WBC with 28 % of blast cells). The patient died two months later.

**Discussion :** 60 cases of such an association between mediastinal germ cell tumor (MGCT) and AML have been described. The leukemia is usually of M7 type according FAB classification (our case). It occurs during the 5 years following the diagnosis of the germ cell tumor, a too short period for a chemotherapy-induced leukemia. The germ cell tumor is usually mixed with seminoma and yolk sac components.

The proof of a common ancestor between germ cell tumor and leukemia is made by cytogenetic analysis that shows the specific germ cell tumors  $i(p12)$  in both tumors, while others genetic alterations commonly observed in induced leukemia are absent ( $7q32-35$  or  $11q23$ ). Only ten of such cases have been fully documented.

**Conclusion :** Association between GCT and AML is rare. The link between the two tumors is proved by cytogenetic analysis. These patients show poor clinical outcome with death occurring in the year following the acutisation.

#### P 123

##### **B-CELL PROLYMPHOCYTIC "LEUKEMIA" (B-PLL): A HETEROGENEOUS DISORDER WITH EMPHASIS IN ITS CLINICAL, HISTOLOGICAL AND GENETIC CHARACTERISTICS**

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**Background:** B-PLL is an infrequent disease, defined by the WHO as an aggressive lymphoid neoplasia, characterized by splenomegaly without lymphadenopathy and peripheral lymphocytosis with  $> 55\%$  prolymphocytes.

We report 3 cases with morphologic and immunohistochemical features of B-PLL with unusual clinical and genotypic features shared by mantle cell lymphomas. Based on these findings we consider the wider term B-cell prolymphocytic leukemia/lymphoma more appropriate and discuss its use.

**Design:** Retrospective, descriptive.

**Results:** Patient 1: 51-year-old male with splenomegaly and cervical lymphadenopathy, lymphocytosis ( $>55\%$ ), infiltrated bone marrow.

**Patient 2:** 54 year-old female with fever and generalized lymphadenopathy. CBC without lymphocytosis, infiltrated bone marrow.

**Patient 3:** 59 year-old male with fever and generalized lymphadenopathy. CBC without lymphocytosis, bone marrow without infiltration.

Lymph nodes in all cases had prolymphocytic differentiation. All three patients had the next immunophenotype in lymph nodes: CD20+, IgM+, CD5-, CD23-, CD43-, cyclin D1-, with variable expression of Ki-67 (5-50%), and p53 (5-70%).

Genotyping studies using FISH probes showed  $t(2;8)(p12;q24)$  in patient 1. Patients 2 and 3 showed  $t(11;14)$  in 20% and 40% of the analyzed cells respectively.

**Discussion:** B-cell prolymphocytic lymphoproliferations constitute a heterogeneous group of disorders in which c-myc rearrangement can be present in very few cases. However, direct involvement of c-myc specifically affecting 8q24 has been proposed as an important factor in B-PLL pathogenesis, as detected in patient 1.

Patients 2 and 3 correspond to a B-cell prolymphocytic lymphoproliferation with isolated lymph node involvement without leukemic component. To our knowledge, this presentation is very uncommon, and is not part of the WHO classification scheme. Although it has been considered that some cases of B-PLL could represent a variant of mantle cell lymphoma, our cases were CD5 and cyclin D1 negative, partially ruling out this possibility. However, using FISH we documented  $t(11;14)$  in both patients. Our clinical and genetic data support the idea that these B-prolymphocytic disorders can be considered as B-cell prolymphocytic lymphoma/leukemia they shares a broad and still undefined genetic spectrum.

#### P 124

##### **CD56 EXPRESSION WITH OR WITHOUT CYTOTOXIC PHENOTYPE IN T-CELL NON-HODGKIN INTESINAL LYMPHOMAS**

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In the WHO classification two major neoplasms derived from mature NK/T cells are recognized; the nasal/nasal-type T/NK-cell lymphoma and the aggressive NK cell leukemia lymphoma. Recent papers have documented the expression of CD56 in non-Hodgkin T-cell lymphomas in a variety of anatomic locations, like cutaneous lymphomas, where CD56 expression has been associated with a poor prognosis. The aim of our study is to identify the cases of NH-T cell intestinal lymphoma in our institution, describe in detail their morphologic features and to classify them according to their CD56 expression.

Material and methods: Paraffin blocks from 9 cases of T-cell intestinal NHL were evaluated, by clinical, morphological, immunophenotypic (CD3, CD20, CD4, CD8, CD7, CD5, CD 56, granzyme, LMP-1, and EBER-ISH), and molecular biology analysis (TCRg and IgH by PCR) in most of them.

Results: We identified 67 intestinal lymphomas from 1980-2004; 46 of them were B-cell lineage and 21 of T-cell lineage. Nine cases had available material for immunohistochemical studies. Of these, 6 cases were NK-(CD56-); one with expression of cytotoxic granules (granzyme +) and two without it. Three cases were NK + (CD56+); one with expression of cytotoxic granules and 2 negative. A variable expression of CD4 and CD8 markers was found, showing no correlation with either CD56 or cytotoxic phenotype. Epstein-Barr virus presence was detected in all NK - (CD56-) cases and in none of NK + (CD56+) cases.

Discussion: We describe herein three unusual cases of intestinal large NK/like T-cell lymphomas with or without cytotoxic phenotype and without evidence of enteropathic damage. These cases had morphologic characteristics similar to those described in nasal and nasal-type NK-T cell lymphomas as angiocentric lesions and necrosis. Interestingly, EBV infection was not documented in our CD56+ cases. The particularly aggressive behavior of our cases was confirmed by clinical follow-up.

Conclusions: Our results demonstrated that T-cell intestinal NHL represent a heterogeneous group of neoplasms in which expression of CD56 can be present, with an apparent poor clinical prognosis. These cases could be considered as part of the group of NK-T cell nasal type lymphomas or as a particular group of CD56 + (cytotoxic or not) intestinal NHL. These items are open to future investigations with larger series of cases.

#### P 125

##### **CEREBRAL INVOLVEMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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Introduction: Central nervous system (CNS) involvement is an extremely rare complication of chronic lymphocytic leukemia (CLL). We reviewed 26 autopsy cases of CLL with special attention on pathological findings in CNS.

Patients: The study group consisted of 18 males and 8 females aged 45-75 years (average 62 years). The diagnosis of CLL was obtained on review of the hematoxylin and eosin-stained slides and immunophenotype data.

Results: Leukemic infiltration of CNS was evident clinically and grossly in two cases. The first patient was 53-years-old man with CLL of 3 years' duration, who developed a sensorimotoric aphasia with right-sided hemiplegia six weeks

prior to death. At autopsy, a large (6x6x4cm) ill-defined tumor mass was found in the temporo-occipital region of the left cerebral hemisphere with temporal herniation. Histologically, tumor tissue was very cellular, composed of large cells with vesicular nuclei, prominent nucleoli and occasionally giant and Reed Sternberg-like cells admixed with a small number of mature lymphocytes. Immunohistochemically, both types of malignant proliferation (small and large cells) stained positively for CD20 (pan-B marker). The diagnosis of cerebral diffuse large cell lymphoma in CLL (Richter syndrome) was obtained. The second patient was 70-years-old man with CLL of one year's duration, who developed a polyradiculoneuropathy one month prior to death. At autopsy, infiltration of spinal roots and cranial nerves as well as diffuse leptomeningeal dissemination ("leukemic meningitis") were found. Histologically, peri- and endoneural leukemic infiltration, leukostasis and perivascular leukemic infiltration (prevalent in the brainstem and spinal cord) and meningiosis were presented. Histopathological findings (without evidence of a mass lesion) in 2/26 CLL cases without neurological signs were in the form of petechial hemorrhages with leukostasis and perivascular leukemic infiltration, localized in the white matter of the cerebral hemispheres. In all other cases of CLL (22/26, 84,6%) cerebral leukemic infiltration was not present. Conclusions: The clinicopathological features of these cases indicate that, despite of rarity of CNS involvement in CLL, any neurological manifestation in CLL patients should be aware of the possibility of this complication.

#### P 126

##### **EXPRESSION LEVEL OF CASPASE-3 IN DIFFUSE LARGE B-CELL LYMPHOMA: CORRELATION WITH GERMINAL CENTRE EXPRESSION PROFILE.**

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Background: Abnormalities in programmed cell death frequently induce drug and/or radiation resistance in non-Hodgkin's lymphoma (NHL). Caspase-3 is an enzyme crucial to the apoptotic process and is activated in both major apoptotic pathways, including stress-induced pathway and a cell surface death receptor signalling pathway, leading to final execution of apoptosis. Recent study showed that the expression of caspase-3 in NHLs can reflect proper functioning of apoptotic pathways. Diffuse Large B-Cell Lymphomas -DLBCLs- are a group of tumours with heterogeneous clinical, morphological, immunophenotypic, cytogenetic and molecular features.

Purpose of the study: In order to elucidate whether good prognosis of germinal centre cell DLBCLs is related to well functioned caspase apoptotic pathway, we studied the expression of active form of caspase-3 (activated CCP32) in 46 cases of DLBCLs which were classified in four groups according to bcl-6 protein, CD10 and MUM-1 antigen expression: I) bcl-6 positive 16/46, II) bcl-6 positive, CD10 positive, germinal centre cell immunophenotype 14/46, III) bcl-6 positive, MUM-1 positive 10/46 and IV) bcl-6 negative 6/46. Chi-square was used as the statistical test.

Results: 1) Group I -bcl-6 positive- showed significantly higher expression of caspase-3 than the group IV -bcl-6 negative-. 2) Group II -bcl-6 positive, CD10 positive- showed significantly higher expression of caspase-3 than the groups I -bcl-6 positive-, and III -bcl-6 positive, MUM-1 positive-.

Conclusions: Our results indicate that DLBCLs with germinal cell immunophenotype maintain well functioning apoptotic

pathway which can explain good response to current chemotherapeutic agents.

#### P 127

##### **EXTRANODAL DETERMINATION OF ANAPLASTIC LARGE-CELL LYMPHOMA**

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T/null Anaplastic Large-Cell Lymphoma (ALCL) is a very rare Malignant Lymphoma (~5% incidence), frequently with extranodal determination. 45 cases of systemic ALCL diagnosed in our laboratories were studied, and a pathological and clinical study was performed. The 45 patients (M/F = 2.75, 66% T-cell ALCL and 33% null ALCL, 56.5% expressing ALK) presented extranodal disease in 42% of cases. Only in 3 cases the first presentation was extranodal (bone-1 case and digestive-2 cases). The most frequently sites were: bone (8 cases), cutaneous (6), bone marrow (4), digestive (4), pulmonary (4), hepatic (4), central nervous system (3). Four patients presented more than 3 extranodal determinations. The pediatric population presented particularly cutaneous and bone marrow infiltration. The phenotype of the malignant cells was more frequently T-cell (28% of the T-ALCL and 18% of the null-ALCL). More than 50% of the ALCL ALK negative presented extranodal disease (half of the cases with more than 2 localizations), and only 38% of the ALCL expressing ALK. The presence of extranodal disease in ALCL is an important characteristic of these malignancy and, sometime, pose problems of diagnosis and treatment.

#### P 128

##### **IMMUNOHISTOCHEMICAL ANALYSIS OF T-CELL RECEPTOR SIGNALING ACROSS THE SPECTRUM OF CELIAC DISEASE ASSOCIATED LYMPHOCYTIC PROLIFERATIONS**

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Background: Small bowel biopsies with increased intraepithelial lymphocytes (IELs) are commonly seen in patients with celiac disease (CD). In individuals with refractory CD (RCD) the IELs display an abnormal phenotype, persist despite gluten withdrawal and are often clonal, possibly representing cryptic enteropathy associated T-cell lymphomas (EATLs) in a subset. We evaluated for possible dysregulation of the T-cell receptor (TCR) associated signaling proteins as well as mediators of the NF-kappa B pathway across the spectrum of CD associated IEL proliferations to determine whether any differences exist between IEL expansions in active CD, RCD, and EATL. Material and methods: Immunohistochemical (and in a subset immunofluorescent) staining of duodenal biopsies from patients with active CD (n=2), RCD with polyclonal IELs

(n=2), RCD with clonal IELs (n=1), EATLs (n=4), normal individuals (n=2), and suitable controls was performed with antibodies against Zap-70, Bcl-10, CD25, c-REL, p65, and junB by immunohistochemistry. IELs were also phenotyped by staining for the pan-T-cell antigens, CD30, and TIA-1. Results: IELs in all cases were ZAP-70+, CD25-, c-REL-, p65-, and junB-; Bcl-10 staining was equivocal in a few cases. One EATL was CD4+ TIA-1-, while the remaining 3 were CD4- CD8- TIA-1+, 2 of these were also CD30+, CD25+, and Bcl-10+. All EATLs were ZAP-70+. None of the EATLs stained with c-REL and p65. Both the CD30+ EATLs showed intense nuclear junB staining while CD30- EATL showed only scattered junB+ cells.

Conclusions: We did not observe any differential expression of TCR associated proteins in CD associated IEL expansions. A lack of CD25 expression by the IELs suggests the role of cytokines besides IL-2 in cell survival and proliferation. No detectable elevation in proteins of the classical NF-kappa B pathway in EATLs suggests the possible role of other signaling pathways. Lastly, nuclear accumulation of junB in a subset of EATLs appears to be the consequence of CD30 activation, as has been demonstrated for other subsets of T-cell lymphomas.

#### P 129

##### **STUDY OF TRANSLOCATION T(11;18)(Q21;Q21)(API2/MLT1) AND EXPRESSION OF BCL10 IN PRIMARY CUTANEOUS MARGINAL ZONE B-CELL LYMPHOMAS**

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##### Background

Primary cutaneous marginal B-cell lymphoma (C-MZL), an usually benign low-growth fraction neoplasm, is probably the most frequent form of primary B-cell lymphoma of the skin. API2/MLT1 fusion transcripts due to (11;18)(q21;q21) and aberrant bcl10 nuclear expression have been described in some noncutaneous marginal zone lymphoma subtypes, but little is known about cutaneous-MZL genetic alterations. Our aim was to evaluate a possible implication of API2/MLT1 fusion transcripts and bcl10 expression in primary cutaneous-MZL.

##### Design

A total of 42 patients diagnosed with cutaneous-MZL on the basis of WHO/EORTC criteria were studied. Skin biopsies from every patient were routinely tested with a wide panel of monoclonal antibodies. Additionally, MLT1 fusion transcripts were performed in 21 instances.

##### Results

Aberrant nuclear bcl10 expression, demonstrated in 15 cases, was related to a more aggressive clinical behaviour. Translocation t(11;18)(q21;q21) was absent in all evaluated samples.

##### Conclusion

The API2/MLT1 fusion, strongly associated with extracutaneous MALT lymphomas, does not seem to play a role in cutaneous marginal zone lymphoma pathogenesis. However, bcl10 aberrant nuclear expression could be a significant prognostic factor in cutaneous marginal zone lymphoma, signalling a more aggressive clinical course and a higher risk of extracutaneous involvement.

#### P 130

##### **BCL 2 AND BAX INTERACTION IN PERIPHERAL BLOOD B – LYMPHOCYTES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA**

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Chronic lymphocytic leukemia (CLL) is neoplastic disease characterized by the accumulation of morphologically mature monoclonal CD 5+ B cells in the early phase (G0/G1) of the cell cycle. The accumulation of neoplastically transformed B lymphocytes (CLL cells) is primarily the consequence of the blockade of apoptosis in these cells. The Bcl 2 proteins are well-known modulators of this process. Some of these proteins are anti-apoptotic while other are pro-apoptotic. All contain at least one of the four conserved regions called Bcl 2 homology domains (BH1-BH4). Evidence indicates that Bcl 2 and Bax form homo- and heterodimers. The antiapoptotic effect of Bcl 2 protein is based on its possibility to bind Bax protein in the heterodimer form, and in that way blocks forming Bax/Bax proapoptotic homodimers. The ratio of Bcl 2 / Bax represents a cell autonomous rheostat which determinates the type of cell reaction to an apoptotic stimulus. We used the co – immunoprecipitation method to determine the level of interaction between these two proteins in CLL cells. Our study included the analysis of 20 peripheral blood specimens from 20 patients with CLL, and 20 peripheral blood specimens of healthy persons who represented the control group. Simultaneously we analysed the level of binding Bcl 2 protein to Bax (IP: Bcl 2 / WB: Bax) and reversed (IP:Bax / WB: Bcl 2). The intensity of Bcl 2 and Bax protein binding compared to the control samples of the peripheral blood of healthy persons, was increased in CLL cells. Results IP: Bax / WB: Bcl 2 showed a high level of »free« Bcl 2 protein which is not bound in the heterodimer form to Bax protein. Simultaneously IP: Bcl 2 / WB: Bax has shown that a higher quantity of Bax protein was bound in the heterodimer form to Bcl 2 protein, than the rest of the potential homodimer bound to bax protein. Further studies involving larger groups of patients are necessary to explore potential significance of Bcl 2 / Bax protein ratio determination as a prognostic parameter in CLL treatment.

### P 131

#### AGGRESSIVE SYSTEMIC MASTOCYTOSIS WITH PRIMARY PRESENTATION IN GASTROINTESTINAL TRACT. CASE REPORT

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#### Introduction

The WHO classifies mastocytic proliferations into: 1. Cutaneous mastocytosis. 2. Indolent systemic mastocytosis. 3. Systemic mastocytosis with associated other clonal hematological disease. 4. Aggressive systemic mastocytosis. 5. Mast cell leukemia. 6. Mast cell sarcoma. 7. Extracutaneous mastocytoma.

Aggressive systemic mastocytosis is characterized by clonal mast cell proliferation in skin, bones, lymph nodes and the gastrointestinal tract.

Our aim is to report a rare case of primary presentation of aggressive systemic mastocytosis in small and large bowel.

#### Materials and methods

A 46 years old woman suffered from watery diarrhea, vomiting, abdominal pain, weakness and weight loss for 6 months. No skin lesions were documented. Clinical and other investigations revealed ascites, massive edema of the legs, leucocytosis, anemia, hypoproteinemia and patchy erosive

lesions in the large and small bowel (endoscopically). Small and large bowel biopsies, cytological smear of ascitic fluid and a bone marrow biopsy were evaluated with routine staining (haematoxylin and eosin), histochemical stainings (PAS, Giemsa, Ziehl-Neelsen, Toluidin, Leder). Immunohistochemical stainings using antibodies directed against: S-100, CD1a, CD68, mast cell tryptase, CD117 (c-kit) and myeloperoxidase (MPO).

#### Results

Routine staining revealed an accumulation of large cells with pale cytoplasm in the lamina propria of duodenum and large bowel. PAS, Giemsa, Ziehl-Neelsen and Toluidin blue stainings performed for the identification of these cells were negative. The immunophenotype of the cells was S-100 (-), CD1a (-), MPO (-), CD 68 (+), c-KIT (CD117) (+) and mast cell tryptase (+) which is consistent with mast cells. Infiltrates of mast cells occupied 50% of bone marrow space with collagen fibrosis. Giemsa (+) violet granules and Leder (+) red granules were found in mastocytes. Cytological evaluation of ascitic fluid was negative.

#### Conclusions

A diagnosis of primary mastocytosis in a visceral localization is very difficult, because this presentation is unusual and it is really a diagnosis of exclusion.

The main clue for the diagnosis of mastocytosis is the detection of mast cell tryptase immunohistochemically. Immunoreactivity of mast cells for CD68, CD117, MPO and positivity for Giemsa and Leder stains histochemically may suggest the diagnosis but the results of these stainings may vary according to the localization and in malignant mast cell proliferations.

### P 132

#### CDK9/CYCLIN T1 EXPRESSION DURING NORMAL LYMPHOID DIFFERENTIATION AND MALIGNANT TRANSFORMATION

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Cdk9 is a member of the CDC2-like family of kinases. Its cyclin partners are members of the Cyclin T family (T1, T2a and T2b) and Cyclin K. Cdk9/Cyclin T1 complex is very important in controlling specific differentiative pathways of several cell types. Limited data are available regarding the expression of Cdk9/Cyclin T1 in haematopoietic and lymphoid tissues. We analyzed the expression of this complex in lymphoid tissue, in order to assess its role in B and T cell differentiation and lymphomagenesis. Cdk9/Cyclin T1 expression was found by immunohistochemistry in precursor B and T cells. In peripheral lymphoid tissues, Cdk9/Cyclin T1 were expressed in germinal center cells and scattered B and T cell blasts in interfollicular areas, while mantle cells, plasma cells and small resting T lymphocytes displayed no expression of either molecule. Therefore Cdk9/Cyclin T1 expression appears to be related to particular stages of lymphoid differentiation/activation.

To monitor the expression level of Cdk9/Cyclin T1 during normal lymphoid differentiation, we isolated different B and T cell populations by magnetic separation, based on their surface antigens, to check the expression levels of the complex at different stages of activation of B and T cells. After isolation, the expression levels of the Cdk9/Cyclin T1 complex was monitored by RT-PCR in Real Time. We observed that the expression level of Cdk9/Cyclin T1 seems to increase in activated cells, compared to un-stimulated ones. Differentiation is achieved through the direct involvement of proteins of the basic helix-loop-helix family, which are specifically activated during differentiation. Different members of this family are involved in different

differentiation systems. In lymphoid cells the E2A gene products, E12 and E47, are specifically activated during differentiation. We investigated whether Cdk9 was able to promote lymphoid differentiation by interacting with E12 and E47. Our results showed that Cdk9 interacts, with the E2A gene products, both in vitro and in vivo, thus suggesting an active role for the Cdk9/Cyclin T1 complex during lymphoid differentiation, through physical binding with E12 and E47. These preliminary results suggest that the Cdk9/Cyclin T1 complex may affect the activation and differentiation program of lymphoid cells. However, through which molecular mechanism Cdk9/Cyclin T1 complex is altered in malignant transformation still needs to be elucidated.

#### P 133

##### THE VASCULAR CHANGES IN LYMPHADENOPATHIES

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Disorders primarily affecting the lymph node vessels are certainly uncommon in comparison with those that primarily involve lymphoid tissue. The vascular changes are commonly manifest, either in ischaemic lesions in the nodal parenchyma, or in vascular proliferation and modify the histopathological appearances in both reactive and neoplastic lymphadenopathies.

**Aims:** We conducted a study to establish vascular lesions from lymph nodes with valuable signification in the positive or differential diagnosis of reactive, inflammatory and neoplastic lymphadenopathies.

**Material and methods:** Our study included lymph node biopsies from 1701 patients, using histological and immunohistochemical techniques.

**Results:** Histopathological aspects of 1701 specimens of lymph nodes evidenced: 568 (33.4%) reactive lymph nodes, 969 (57.2%) neoplastic lymphadenopathies; 164 (9.4%) were biopsies that didn't contain lymphoid tissue. The neoplastic lymphadenopathies were primary NHL (432 cases – 25.4%), HL (207 cases - 12.2%) and metastatic neoplasm (330 cases – 14.4%). In the reactive lymphadenopathy cases, we observed a variety of vascular lesions as: diffuse vasodilatation, vascular granulation tissue formation, vascularization of lymph node sinuses, capillary vascular proliferation, post-capillary venule proliferation, vasculitis and total or subtotal, segmental or focal infarction, vasoproliferation in reactive states (angio-immunoblastic lymphadenopathy) or marked proliferation of small blood vessels in Castleman's disease and angiomatosis/haemangiomas in axillary lymph nodes draining breast carcinomas. Vasoproliferation in malignant neoplasms was evident whether the lymph nodes were occupied by malignant lymphomas of metastatic tumor.

**Conclusions:** Vascular transformation of sinuses is a pattern of fibrosis formation in chronic lymphadenitis with atrophy of lymphoid tissue. Reactive proliferation of blood vessels is one of the central features of hyperimmune angiofollicular and plasmacytic polyadenopathy, and angio-immunoblastic lymphadenopathy. Primary vascular lesion of lymph node was infarction with various intensity degrees, mainly in NHL.

Capillary proliferation in primary malignant lymphadenopathies was present in Hodgkin's lymphoma, in lymphoplasmacytic neoplasms while in T cell NHL we saw a marked proliferation of post-capillary venules. In latter condition, differential diagnosis with hyperplasia and stimulation of the paracortical areas is needed.

#### P 134

##### EXPRESSION OF TELOMERASE COMPONENTS IN NEUTROPHILS.

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**Backgrounds and aims:** Human telomerase comprises an RNA subunit (hTR) and a catalytic protein subunit with reverse transcriptase activity (hTERT). Telomerase extends chromosome ends in compensation for the attrition of the telomeres during replication. Telomerase is normally expressed in germinal or stem cells during the adult life and during cellular immortalisation and tumorigenesis in pathology. Even though hTERT protein is a key component controlled the telomerase activity, some data indicate that hTERT protein expression level did not always correlate with telomerase activity. In this work, we explore the expression of hTR and hTERT protein in neutrophils, which are highly differentiated cells without hTERT mRNA expression and telomerase activity.

**Materials and methods:** We investigated the expression of hTR and hTERT protein, respectively by in situ hybridization with a S35-labeled specific probe and immunohistochemistry with two different antibodies, in neutrophils of the stroma in 10 cases of bladder carcinomas. hTERT protein expression was also studied by immunoelectronmicroscopy in neutrophils of non-neoplastic liver tissues with "surgical hepatitis" in 3 cases of partial hepatectomy.

**Results:** Like others non-tumoral cells, neutrophils expressed hTR. hTERT protein was strongly expressed in the cytoplasm of neutrophils with the 2 antibodies. The ultrastructural study showed that the gold particles were not associated with specific organelles but scattered in the cytosol. No significant nuclear labeling was observed in neutrophils.

**Conclusions:** This study demonstrates that hTERT protein is expressed in the cytoplasm of neutrophils. We hypothesize that complex qualitative changes, i.e. post-translation modification, compartmental protein sequestration could be of importance in the enzyme activity level control. The functional significance of cytoplasmic hTERT protein without telomerase activity remains to be elucidated.

#### P 135

##### ULTRASONIC DECALCIFICATION IS RAPID AND SUITABLE FOR FISH ANALYSIS

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##### Aim

In haematopathology, the requisite analyses on bone marrow biopsies are increasing; in addition to morphology and immunohistochemistry (IHC), molecular analysis (MA) like FISH is now demanded in order to improve diagnostic accuracy. Certain chromosomal alterations in particular, are known to be of prognostic or predictive value. In some circumstances, e.g. surgical biopsy, "packed marrow", only formalin fixed, bone-containing tissue is available for MA. Since the various decalcification procedures can impair DNA quality, there is an urgent need for standardized decalcification protocols allowing FISH analysis on these materials. Our aim was to set up a routinely applicable protocol to optimise the MA method whilst preserving morphology and IHC results.

##### Materials and Methods

Bone marrow specimens were fixed in 4% buffered formalin. The decalcification procedures were carried out in an EDTA solution (Usedecalc, Medite, Burgdorf, Germany) with or without the use of an ultrasonic decalcifier (USE33, Medite,

Burgdorf, Germany). Decalcification solutions containing either formic or acetic acid were compared. The decalcification times varied from one and a half hours up to 24 hours. Thereafter, the ultrasonic decalcified tissue was dehydrated, paraffin embedded and, depending on the sample size, again incubated in the EDTA solution. Samples were stained with H+E and Giemsa, and IHC for nuclear (Mib1, Glycophorin A) and cytoplasmic markers (CD20, CD3, CD61, MPO) was performed. Interphase FISH analysis with a double colour, double fusion probe (Abbott Molecular Diagnostics, Illinois, USA) specific for the translocation t(11;14) was applied according to the manufacturers instructions.

#### Results

Ultrasonic decalcification allows reduction of the decalcification time. Overall, the best results for morphology, IHC and FISH analysis were obtained by 3-5 hours of ultrasonic decalcification using an EDTA solution. Of interest, FISH analysis was successfully applied only on samples positive for Mib1 IHC, thus being predictive for DNA quality of the sample.

#### Conclusion

The combination of ultrasound and EDTA for decalcifying bone-containing tissue samples is a rapid and easy method resulting in an excellent morphology comparable to plastic embedded bone marrow trephines. Most importantly, the same protocol permits successful interphase FISH analysis. Current investigations will reveal whether this decalcification approach is also suitable for PCR studies.

#### P 136

##### **HEDGEHOG SIGNALLING IN B-CELL DIFFERENTIATION**

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The Hedgehog signalling pathway plays a crucial role in embryonic tissue development and differentiation and has also been shown to function in adult self-renewing tissues.

Apparently, the pathway is also involved in the development of the hematologic system. It has been published that the ligand Sonic hedgehog (Shh) is involved in development of primitive human hematopoietic cells and T-cells. Together with the receptors Patched (Ptch) and Smoothened (Smo) Shh also ensures survival of germinal center B-cells.

Recently published findings showed expression of Shh by follicular dendritic cells and expression of the pathway receptors Ptch and Smo by germinal center B-cells.

Lately, we could show that the Hedgehog signalling pathway is involved in differentiation of human B-cells. Especially the transcription factor Gli2 is expressed in B-cells of the bone marrow.

In the present study we examined the expression of members of the Hedgehog pathway in normal and malignant B-cell development. Formalin-fixed and paraffin-embedded tissues of normal bone marrow and secondary lymphatic tissues were immunohistochemically analyzed using antibodies against ligands, receptors and transcription factors involved in the Hedgehog pathway. Non-Hodgkin's lymphomas and B-cell leukemias were investigated as each disease entity shows an increased proliferation of specific stages of B-cell development.

Lymphatic tissues displaying Precursor B-lymphoblastic lymphoma/leukemia (n=5), Precursor B-cell acute lymphoblastic lymphoma/leukemia (n=5), B-cell chronic lymphatic leukemia/small lymphocytic lymphoma (n=5), B-cell prolymphocytic leukemia (n=5), Lymphoplasmacytic lymphoma (n=5), Plasma cell myeloma/Plasmacytoma (N=5),

Extra-nodal marginal zone B-cell lymphoma of MALT type (n=5), Follicular lymphoma (n=5), Diffuse large B-cell lymphoma (n=5), Burkitt's lymphoma (n=10), and Hodgkin's lymphomas (n=10) were examined. By using CD markers in double fluorescence stainings cells with an active pathway were assigned to their hematopoietic lineages.

The results showed pathway activity in several stages of B-cell development. CD79a positive B-cells showed strong expression of Ptch and Gli2. Additionally, only a small minority of plasma cells showed pathway activity. In conclusion, active Hedgehog signal transduction plays a role in the differentiation of B-lymphocytes.

#### P 137

##### **STUDY OF EC CELLS IN PATIENTS WITH PERNICIOUS ANEMIA**

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Enterochromaffin (EC) cells are the members of diffuse neuroendocrine system and are classified according to ultrastructure characteristics as EC1-intestinal type; EC2-duodenal type and ECn-gastric type. Gastric type EC cells are arginophilic and argentaffilic in 10-20%. They are localized in antral and corporal gastric mucosa. In pathologic conditions EC cells can change their number, morphology and cytochemical characteristics, so as a functional properties.

The aim of this study is to investigate presence, type and granularity EC cells in patients with pernicious anemia (PA).

During the period from 1977 to 2005 (Clinic of Hematology-Niš), 78 patients with PA were examined as well as 30 patients with dyspeptic syndrome (control group). Classical (HE) histological method was used for pathological evaluation of gastric mucosa. Grimelius (argyrophilic) and Masson's (argentaffilic) methods were used for the identification of EC cells.

Hyperplasia, metaplasia and hypergranularity of EC cells were found in corporal mucosa of PA patients. The number of EC cells was increased proportionally to the changing of gastric mucosa and the grade of intestinal metaplasia (22 to 43 cells per field). Sometimes, EC cells hyperplasia is of so high grade that resemble intramucous carcinoidosis. EC cells were transformed from close type to the open type: they become triangle shape and high amount of serotonin in them.

These results can confirm that new formed, intestinal type of EC cells secrete high quantity of serotonin increasing permeability and inducing the retrograde diffusion of H ions in gastric mucosa. In the way, H ions induce acidosis and inhibit cellular metabolism, leading to cytotoxicity of all attached cells. Serotonin stimulates synthesis of acid mucins and has an inhibition effect on HCL secretion.

Hyperplasia and metaplasia of EC cells could be important for the cancer genesis through serotonin effects.

#### P 138

##### **D2-40 - SELECTIVE MARKER OF LYMPHATIC ENDOTHELIUM AND A PUTATIVE FOLLICULAR DENDRITIC CELL MARKER.**

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Introduction: M2A antigen is an oncofetal antigen associated with germ cell neoplasia, present in testis on fetal gonocytes and re-expressed on carcinoma in situ (CIS) and germ cell tumours. It was shown that the antigen is an O-linked sialoglycoprotein containing a simple mucin-type carbohydrate epitope. A specific monoclonal antibody named D2-40 (IgG1) against the antigen was developed. It was then

observed that D2-40 is a selective marker of lymphatic endothelium.

**Purpose:** The aim of the study was to examine the expression of D2-40 in various haematopoietic and lymphatic tissues.

**Materials and methods:** The study was performed on 60 cases, distributed as followed: 34 lymph node samples, 14 spleens and 8 bone marrow specimens. Half of the lymph nodes were involved by metastatic carcinoma and half were involved by various lymphoma types – 1 small lymphocytic lymphoma, 2 mantle cell lymphomas, 2 marginal zone lymphomas, 4 follicular lymphomas, 4 diffuse large B cell lymphomas, 2 with peripheral T cell lymphoma and 3 lymph nodes with angioimmunoblastic T cell lymphoma. Most of the spleen specimens (8) were with reactive changes, 3 were affected by lymphoma. Bone marrow was predominantly with reactive changes and 2 with lymphoma infiltration. The expression of D2-40 was evaluated immunohistochemically. Except for that on several specimens double immunofluorescence with D2-40 and CD21 was performed.

**Results and discussion:** All metastatic lymph nodes showed specific D2-40 expression in the preserved lymphoid follicles. In all specimens positive reaction was observed in both lymphatic vessels and follicular dendritic cells. In lymphomas the staining intensity in the follicular dendritic cells was generally weaker. In the spleen no immunoreactivity was detected. The same pattern was observed in the bone marrow. Except for a specific marker for lymphatic endothelium M2A antigen, detected by D2-40 monoclonal antibody detects also a subset of follicular dendritic cells. Further investigations should be performed to clarify the function and significance of this marker in reactive conditions and lymphoproliferative diseases.

#### P 139

##### **FATAL NECROTIZING HERPES SIMPLEX VIRUS LYMPHADENITIS IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND SEVERE HYPOGAMMA GLOBULINEMIA**

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Necrotizing lymphadenitis is a rare manifestation of herpes simplex virus (HSV) infection. We report a 80-year-old patient with fever, retroperitoneal necrotic lymphadenopathy and thrombosis of the lower vena cava, revealing a lymphocytic leukemia and a necrotizing HSV lymphadenitis mimicking Richter's transformation. Lymph node biopsy revealed a diffuse necrosis, with an infarct like pattern. Necrosis contained karyorrhectic bodies and numerous cells with Cowdry A inclusions. HSV was detected within a lymph node biopsy by immunohistochemistry. HSV type II DNA was detected within blood and bone marrow samples by PCR. The patient had a severe hypogammaglobulinemia and normal T cell repertoire. No skin nor mucosal lesion were present. Clinical and biological symptoms improved rapidly with acyclovir and immunoglobulins, however the patient died with pulmonary embolism. HSV lymphadenitis may be misdiagnosed with Richter's transformation. Humoral immunity seems to be the main defense in human against severe HSV infections.

#### P 140

##### **APOPTOSIS AND PROLIFERATIVE ACTIVITY OF HUMAN TROPHOBLAST CELLS IN SPONTANEOUS ABORTIONS WITH NORMAL AND PATHOLOGIC KARYOTYPES**

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**Introduction.** Apoptosis and proliferation are interrelated processes, that supply normal implantation of an ovum and development of pregnancy. Disturbances of these processes in cases of spontaneous abortions (SA) may reflect the pathogenesis of pregnancy loss. From this point of view it is interest to compare the apoptotic and proliferation rates in SA caused by chromosomal aberrations and in SA with normal karyotype.

**Materials and methods.** Proliferation and apoptosis in villous and extravillous trophoblast was estimated in SA with pathologic karyotype (15 cases), SA with normal karyotype (15 cases) and in normal early pregnancy (20 cases of elective abortions). Proliferative activity (PA) was determined as the rate of Ki-67-positive cells. Estimation of apoptotic index (AI) was performed using the ApoTag Peroxidase Kit ("Biotech", USA).

**Results.** In normal early pregnancy specimens Ki-67 was strongly expressed in villous cytotrophoblast (VCT) and extravillous trophoblast (EVT). Syncytiotrophoblast (ST) was negative for proliferation marker. Signs of apoptosis were mainly observed in ST, syncytial knots, VCT, some villous stromal cells and in EVT. PA of VCT was low in both groups of SA as compared to control group. The significant differences in rate of apoptosis between SA with normal and pathologic karyotypes was revealed. The rate of villi with apoptotic VCT was significantly higher in SA with pathologic karyotype (26.20 %) than that in SA with normal karyotype (7.6%) and normal first trimester pregnancies (16.65 %). AI of EVT was also higher in group of SA with pathologic karyotype (7.67±3.31) as compared to SA with normal karyotype (2.78±2.56). Furthermore, SA with pathologic karyotype showed uncorrelation between AI and PA in EVT: the level of AI was the highest whereas PA was minimal. The highest frequency of apoptosis in combination with minimal proliferative activity of EVT in SA caused by chromosomal pathology indicates active invasion restriction of chorion with pathological karyotype.

#### P 141

##### **HUMAN PLACENTA AND FETAL IUGR AT 20-22 WEEKS OF GESTATION**

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It is reported that some adult diseases has intrauterine 'programming' mechanism. Disproportions and restrictions of placental and fetal growth could be the marker for the high risk group of newborns.

The aim of the present study was to identify which organs are principally affected in cases of fetal growth restriction (FGR) at 20-22 weeks of gestation.

**Methods:** 8 cases with fetal growth restriction (FGR) and high (0.41±0.04) placenta/fetal weight index (PFI) were compared with 10 controls (PFI= 0.26±;0.01) in cases of induced abortions for psychotherapeutic reasons at 20-22 weeks of gestation. Weights of placenta (PW) and fetus (FW), fetus length (FL), head (Ch), chest (Cch), abdominal (Ca) circumferences, fetal kidneys (KW), pancreas (Pn W), liver (LW) and heart (HW) as well as some indices were recorded. **Results:** FGR group had smaller parameters of FW (390±2.0 vs 610±2.6 g, p<0.001), FL (26±0.9 vs 30±0.6 cm, p<0.001), Cch (15±0.6 vs 18±0.4 cm, p<0.001), Ch (18±0.04 vs 22±0.4 cm, p<0.001), Ca (13±0.4 vs 15±0.3 cm, p<0.001). All FGR cases had decreased LW (16±0.2 vs 29±0.2 g, p<0.001); PnW (0.2±0.01 vs 0.4±0.07 g, p<0.001); KW (4±0.3 vs 6±0.2 g, p<0.001); HW (2.1±0.2 vs 3.4±0.2 g, p<0.001). PW was unchanged (160±12 vs 160±8 g, p>0.05).

Conclusions: these results suggest that the discrepancy between fetal and placental growth at 20-22 weeks of gestation is associated with fetal kidneys, pancreas, heart and liver growth retardation. Pathological alterations of organs could be a structural basis of adult pathologies.

#### P 142

##### **HISTOLOGIC AND PLACENTAL ABNORMALITIES CORRELATE WITH SEVERITY OF PREECLAMPSIA AND ITS COMPLICATIONS**

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We have previously reported that the frequency and severity of placental lesions in preeclampsia (PE) are gestational age-dependent. This analysis was undertaken to identify the clinical and placental factors that are associated with severity of PE and its complications

In years 1994-2004, 332 consecutive placentae from patients with PE with gestational age at delivery of 20 weeks or more were examined (7.9% of all placentae examined). 112 women had mild PE (Group 1), 172 severe PE (Group 2), 36 HELLP syndrome (Group 3), and 12 eclampsia (Group 4). Univariate contingency table analyses were carried out to assess associations between possible risk factors and PE groups. When cell frequencies were sufficiently large, ordinal and nominal factors were analyzed using Mantel-Haenszel and likelihood ratio Chi-square statistics, respectively. For small sample sizes, 2-by-2 tables were analyzed using Fisher's exact test.

Statistically significant differences were found in the following 8 of 14 maternal/fetal clinical risk factors and outcomes, and 7 of 27 gross and microscopic placental features in Groups 1-4, respectively: average gestational age at delivery (36,33,30,30 weeks), maternal diabetes mellitus (14,7,5,0%), abnormal fetal heart rate (26,24,6,8%), meconium-stained amniotic fluid (12,3,3,0%), fetal weight (2594,1822, 1456,1250 gm), one (7,6,5,4) and five minute (8,7,7,5) apgar scores, cesarean sections (45,64,83,67%), placental weight (439,311,274,265 gm), hyperplastic decidual arteriopathy (20,36,36,33%), decidual atherosclerosis (10,27,44,41%), villous infarctions (29,55,56,58%), maternal floor trophoblastic giant cells (18,40,44,25%), diffuse villous hypoxia (23,32,36,33%), and meconium staining on histology (29,15,16,8%).

The significant overlap of frequencies of various clinical and placental factors in mild and severe PE and its complications indicates that they represent a spectrum of essentially the same process. While the frequency of abnormal maternofetal factors in general decreased from Group 1-4, possibly in relation to increasing frequency of cesarean sections, the frequency of decidual vascular lesions and placental hypoxic changes, except for the histologic evidence of meconium staining, increased from Group 1-4. Therefore, placental examination is a useful tool for retrospective evaluation of PE confirming the important role of uteroplacental hypoxia in fetal distress in PE.

#### P 143

##### **LETHAL OSTEOCHONDRODYSPLASIA: A FETOPATHOLOGICAL STUDY OF 31 CASES**

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Introduction:

Skeletal dysplasia is a large heterogeneous group of diseases characterized by malformation, disproportionate growth and deformation of the skeleton or of individual bones or groups of bones. They are common birth defects. Their prevalence differs between populations and in various studies. Lethal osteochondrodysplasia is generally tracked down during the third or the fourth month of pregnancy, or during the antenatal ultrasonography.

Materials and methods:

Our study is retrospective and descriptive of 31 cases of lethal osteochondrodysplasia: 22 fetuses, 6 new-born and 3 dead-born.

Cases were brought from 6 hospitals of the center of Tunisia during a period of 13 years from 1991 to 2004. Foetopathological examination was done for all cases including autopsies, radiography of skeleton and histological examination of bone.

Results:

Mothers were aged between 20 and 39 years. Consanguinity was found in 6 cases. The disorder was revealed on antenatal ultrasonography in 24 cases and showed micromelia in all cases, narrow trunk in 5 cases and spina-bifida in 3 cases. Correlation between radiographics and fetopathological examination, identified 8 cases of achondrogenesis type I, 3 cases of achondrogenesis type II, 9 osteogenesis imperfecta, 4 thanatophoric dwarfism, 4 schneckenbecken dysplasia, 2 short-rib dysplasia with polydactyly (Majewski-type) and one case of asphyxiating thoracic dysplasia (Jeune).

Conclusions:

Severe ostéochondrodysplasia seems more frequent in the center of Tunisia. Correlation between radiographic and histological data must be undertaken for the management of these bone anomalies in order to make adequate genetic counselling.

#### P 144

##### **ISOLATED OLIGOMEGANEPHRONIC HYPOPLASIA OF THE FETAL KIDNEY: A RARE CAUSE OF FETAL ANAMNIOS**

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Oligomeganephronic hypoplasia of the kidney is a rare condition leading to anamnios.

We report the first case diagnosed in our department.

It was a 28 week - old- aged female fetus.

The pregnancy was interrupted for anamnios and fetal hypotrophy objectivated by ultrasonography.

External fetal examination showed a diffuse oedema with deformative sequence caused by the anamnios.

At the visceral examination, the kidneys were hypoplastic weighting 2g together.

The histological study confirms the hypoplastic feature of the kidneys with a reduced number of the nephrons, which are hyperptophic.

These signs support the diagnosis of the oligomeganephronic hypoplasia of the kidney.

Than, we discuss the morphological feature of this anomaly, the embryological and the genetic hypothesis.

#### P 145

##### **BNOS EXPRESSION IN EARLY ENDOUTERINE FETAL TATH: AN IMMUNOHISTOCHEMICAL STUDY ON PARAFFIN-EMBEDDED PLACENTAS**

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Nitric oxide (NO) is a free radical that modulates vasodilatation, smooth muscle relaxation, inhibition of platelet aggregation and functions as an immune system mediator; during pregnancy there are increased levels of NO, partly due to autocrine secretion by fetoplacental structures. It is the substrate of the nitric oxide synthase (NOS) enzyme, which is also present in endothelial cells from umbilical cord and placental resistance vessels, but not in term villi.

The aim of the present study was to evaluate the presence of the constitutive isoform of NOS (brain NOS or bNOS), mainly expressed in human nervous tissue, also in early pregnancy (first trimester) endometrium and placenta.

We examined 39 cases of early endouterine death and 5 control specimens of induced abortion. Formalin-fixed paraffin-embedded tissues were submitted to heat-mediated antigen retrieval and immunohistochemistry with anti bNOS monoclonal antibody.

bNOS was detectable in endometrial decidua (both glands and stromal cells) and villous syncytiotrophoblast. In particular, its expression was reduced in syncytiotrophoblast and increased in deciduas from pathological cases in comparison to controls. In conclusion, we demonstrated that immunohistochemistry with bNOS antibody can be performed also on paraffin-embedded material, by a proper antigen retrieval system. The difference between the two groups would suggest that early miscarriages may have a vascular pathogenesis.

#### P 146

##### CAUSES OF PERINATAL DEATH AT OSIJEK CLINICAL HOSPITAL, 1995-2004

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AIM. Perinatal mortality and the causes of perinatal death as determined by autopsy and by placental biopsy at the Osijek Clinical Hospital in the period 1995 – 2004 are presented.

METHODS. The data were collected at the Department of Pathology: the autopsy reports and the placental biopsy reports were reviewed. The causes of death were classified into 10 categories: intrauterine hypoxia and birth asphyxia, congenital malformations, infection, abnormal pulmonary function, intracranial hemorrhage, immaturity, other diseases, macerated fetus with no signs of asphyxia, tumors, and violent death. The asphyxia was later subdivided into 3 categories: failure of blood to become oxygenated in the placenta, obstruction to circulation through the cord, and of unknown cause. Descriptive statistics are presented.

RESULTS. There were 23.860 deliveries at the Osijek Clinical Hospital in the period 1995 – 2004. In this period, there were 540 children deaths, and the autopsy was performed in 534 cases (98.89%). Perinatal mortality values were: 21.31‰ (1995), 17.47‰ (1996), 12.73‰ (1997), 17.75‰ (1998), 13.23‰ (1999), 11.90‰ (2000), 7.97‰ (2001), 13.81‰ (2002), 13.35‰ (2003), and 10.38‰ (2004). The majority of deaths were in fetuses and infants who showed evidence of asphyxia (58.33%): 71.43% of those cases was attributed to the failure of blood to become oxygenated in the placenta, 9.21% to the obstruction to circulation through the cord, and the rest 19.36% were of unknown cause. Congenital malformations (11.49%) ranked second, abnormal pulmonary function (9.26%) third, both immaturity and macerated fetus (6.29% each) fourth, and

other conditions were far behind. In the group of 66 congenital malformation, the most common were multiple organs malformations (50%), followed by central nervous system malformations (16.67%), and heart and large blood vessel malformations (12.12%). In the early perinatal period, the most common causes of death were intrauterine hypoxia (53.62%), immaturity (14.01%), and macerated fetus with no signs of asphyxia (13.52%).

CONCLUSIONS. Whether death should be attributed to one cause or another is often subject to variable opinion because of the much overlap among groups. Most of the deaths attributed to asphyxia were explained by placental biopsy report. The placental biopsy report must be an integral part of a autopsy report in the cases of the perinatal death.

#### P 147

##### COMPLETE TRISOMY 9: PRESENTATION OF TWO AUTOPSY STUDIES

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Objective: To present the autopsy study of two cases of complete trisomy 9 (CT9). This is a very infrequent chromosomal abnormality, which in most cases results in spontaneous abortion during the first 3 months of the pregnancy. To our knowledge only about 30 cases have been reported previously.

Case 1: A 35-year-old gravida III woman was referred for amniocentesis at 20 weeks' gestation (wg) because ultrasonography showed a lumbosacral neural tube defect, malposition of hands and micrognathia. A genetic study revealed a karyotype of 47,XY,+9 in all the amniocytes. The pregnancy was terminated at 22 wg. The foetus body weight was 290 g.

Case 2: A 25-year-old primigravida woman was referred to our hospital for evaluation at 32 wg because of an ultrasonographic Dandy-Walker malformation suspicion. The patient underwent amniocentesis with the result of 47,XX,+9 in all the cells. A 1934 g stillborn infant was delivered at 37 wg after spontaneous labour.

Autopsy findings: Both foetuses presented multiple congenital anomalies. In both cases we observed similar craniofacial anomalies: bulbous nose, enopthalmus, small mouth, retromicrognathia, microglosia, a short and thick neck and low-set malformed ears; clenched hands with two oblique palm folds, dysplastic nails (small with triangular form and profound implantation), absence of intergluteous fold, bilobed right lung, agenesis of dorsal pancreas, hypoplastic thymus and hypoplastic adrenal glands. Additional findings: Case 1: Intrauterine growth restriction, microstomia, anterior rotation of shoulders, dorsal cyphosis, small penis, myelomeningocele lumbosacral with Arnold Chiari malformation, diaphragmatic elevation with lung hypoplasia, liver calcification, agenesis of gallbladder, polylobed spleen, horseshoe kidney, atrial left isomerism and atrial septal defect. Case 2: anteroflexed right ankle, club feet, sacral pit, palatal pit, type II truncus arteriosus, aortic right arch, calcification in myocardial vessels, portal venous anomaly (esplenic and inferior mesenteric veins with drainage directly at cava inferior vein), pyloureteral bilateral stenosis, three supernumerary spleens and Dandy Walker malformation.

Conclusion: Foetuses with CT9 have multiple anomalies and can be identified prenatally with sonographic studies. Some of these abnormalities have not previously been described. The CT9 diagnosis is always done through a genetic study, but some autopsy findings can suggest this diagnosis.

#### P 148

**THE SIGNIFICANCE OF COLLAGEN IV STEREO DYNAMICS IN METANEFROGENESIS. IMPLICATIONS IN PATHOLOGY**

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**Introduction.** The collagen type IV, an important extra cellular matrix element greatly contributes to the building of reticular nets from lamina basalis of the metanefros structures.

**Purpose of the study.** The purpose of the study is to establish the stereo and topographic dynamics of the type IV collagen evolution in definitive kidney genesis.

**Materials and methods.** Renal fragments from 3 human embryos, 2 fetuses and 3 new born have been prepared using microanatomical techniques. The visualisation of the extracellular matrix elements was achieved by means of PAS, Gomori and Van Giesons stains.

**Results.** The knowledge of the type IV collagen stereo and topographic dynamics is possible by its identification inside tubular and vascular nefron micro anatomic structures. Corroborating the data observed on the differentiating kidney sections we noticed a variable distribution of glycosaminoglicans and type IV collagen inside lamina basalis. The determinant factor of type IV stereo and topographic dynamics is the existence of the reciprocal induction phenomena between urethral bud derivates and metanefrogen mesenchyma derived structures. In pediatric pathology the type IV collagen intervenes in the initiation of the glomerulosclerosis processes.

**Conclusions.** The type IV collagen is cause and effect in metanefrogenesis processes. It determines the mesenchyma cells migration for the establishment of the nefron structures. Its stereodistribution has as effect the differentiation of the lamina basalis inside tubular and vascular nefron structures.

**P 149**  
**VASCULOGENESIS – A DETERMINANT FACTOR IN THE TIME AND SPACE EVOLUTION OF THE METANEFROS STRUCTURES. IMPLICATIONS IN PATHOLOGY**

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**Introduction.** The genesis and the evolution of the metanefros structures are multi factorial dependent. A determining factor of the renal glomerulus morphogenesis is represented by the phenotype transformation of the metanefrogen mesenchymal cells in order to achieve the sanguine capillaries.

**Purpose of the study.** Our purpose was to visualize the time and space apparition of glomerulus vascular structures from the metanefrogen mesenchyma and its integration in the forming stages of the glomerulus basal membrane.

**Materials and methods.** We used fragments from 3 human embryos (7 weeks old) and tissue fragments from 3 human fetuses (28 weeks old). The serried sections were stained using Hematoxilin Eosine, Van Gieson, Gomori and Giemsa methods.

**Results.** By analyzing the 300 serried sections through human embryos and the 200 serried sections through human fetus kidney we noted that the glomerulus structures genesis is based on the metanefros vasculogenetic mesenchyma. These elements appear between the proximal sinusoid vessels of the primordial contort tube. The process of the capillary genesis is determined by the presence of the primordial podocytes derived from the nefrogen mesenchyma and of the mezangiocytes from the vasculogen mesenchyma. The three newly formed structures establish contiguity relations for the formation of the plasma filtration structures.

**Conclusions.** The phenotype transformation of the metanefrogen mesenchymal cells and their integration inside the glomerulus structures represent the basis of the complex mechanisms of the renal glomerulus capillaries vasculogenesis.

**P 150**  
**FETAL THROMBOTIC VASCULOPATHY (FTV)**  
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**Occlusion of a large fetal artery on the chorionic plate or of a fetal stem artery create a pattern of clustered avascular villi which is called fetal thrombotic vasculopathy (FTV). We report 3 cases of FTV. The chorionic plate vessels were grossly enlarged, distended, and hard. One case was associated with the rupture into the amniotic cavity. Microscopically, the downstream villi were avascular with densely collagenized, hyalinized stroma, and the nuclei of the syncytiotrophoblast cells were clustered into syncytial knots. The larger fetal stem vessels distal to the thrombus underwent septation and fibromuscular sclerosis, characterized by medial hyperplasia and intimal proliferation with eventual luminal obliteration. FTV diagnosis by histopathologic examination is important as it may be correlated with thrombotic lesions in the newborn.**

**P 151**  
**OSTEOGENESIS IMPERFECTA TYPE 2: A FETAL CASE REPORT**

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**Introduction**

Osteogenesis Imperfecta, also known as brittle-bone disease, is a heritable disorder characterized by low bone mass, propensity to fracture, blue sclerae, hearing loss, loose joints, and growth deficiency, caused by a quantitative or qualitative defect in collagen synthesis. The underlying defect is a dominant negative mutation affecting COL1A1 or COL1A2 alleles, which encode the proA1 and proA2 chains of type I collagen, a protein of paramount importance for normal skin and bone development. On the basis of clinical presentation and bone architecture the disease is currently classified into seven types. We present a case of post-mortem diagnosis of osteogenesis imperfecta of a 22 weeks-old fetus with skeletal anomalies. The purposes of this paper are to describe this typical example of osteogenesis imperfecta and to value the importance of prenatal and post-mortem diagnosis in managing pregnancy and delivery.

**Methods**

On a female fetus with skeletal anomalies, the decision of pregnancy termination was based on ultrasound examination. A post-mortem radiographic study and autopsy were carried out. Placenta examination was also performed.

**Results**

The fetus presented short trunk, blue sclerae, small nose, micrognathia, hypertelorism, low set ears, scoliosis, arthrogyposis, and smallness for gestational age. Post-mortem radiographs showed mild angulation and shortening of long bones due to multiple intrauterine fractures, irregular callus formation, a barrel-shaped rib cage, and a poor ossification of the calvaria with a poor mineralization of the skull. Bone histology revealed a marked decreased in both cortical thickness and trabecular bone, and growth plate showed disorganization. In the fractured areas, the bony

spicules were disorderly arranged and associated with fibrosis and callus formation. Placenta histology showed the presence of hydropic villi and altered Hofbauer cells suggesting a genetic defect.

#### Conclusions

The wide spectra of clinical, imaging, grossly and histologic features of the case studied led us to make the diagnosis of osteogenesis imperfecta type II. The differential diagnosis was made with hypophosphatasia, achondrogenesis, thorax dystrophy, osteosarcoma and battered child syndrome. Osteogenesis imperfecta is one of the commonest skeletal disorders with an incidence about one in 10,000 and it is often silent cause of adult osteoporosis for that reason is very important prenatal diagnosis.

#### P 152

##### **FIBROBLASTIC POLYP OF THE COLON: CLINICOPATHOLOGIC ANALYSIS OF 10 CASES OF A NOVEL TYPE OF BENIGN COLORECTAL POLYP.**

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**Background:** Benign fibroblastic polyp (FP) is a recently described, histologically distinctive type of colonic mucosal polyp. Herein we present the clinical, histological, immunohistochemical and ultrastructural features of a series of FP with the goal of further characterizing this entity.

**Methods:** Ten cases of FP were identified. Clinical and histologic features were reviewed. Immunohistochemistry was performed on formalin fixed paraffin embedded tissue using antibodies against vimentin, actin, desmin, CD-68, cytokeratin AE1/3, S-100 protein, COX-2, c-Kit and CD34. Ultrastructural examination was performed in two cases.

**Results:** All patients were asymptomatic adults (seven women, three men) with ages ranging from 44 to 63 years (median 59). Polyps ranged in size from 0.2 to 0.4 cm. Eight were located in the sigmoid colon, one in the descending colon and one in the ascending colon. Five cases were associated with hyperplastic polyps and two with tubular adenomas. Histologically, FP were characterized by a proliferation of uniform, bland spindle cells with focal periglandular or perivascular concentric arrangement. Five cases also showed superficial bundles of spindle cells arranged parallel to the surface. Eight lesions contained serrated (hyperplastic) crypts admixed with the fibroblastic cells and represented mixed fibroblastic/hyperplastic polyps (or FP with serrated crypts). Immunohistochemically, the spindle cells reacted to vimentin only. Ultrastructural examination revealed features supportive of fibroblastic differentiation.

**Conclusions:** 1) FP is a distinctive type of benign colorectal mucosal polyp characterized by its distal colorectal location, small size, frequent association with hyperplastic polyps, distinct morphologic appearance and typical immunonegativity for markers of specific differentiation. 2) FP with serrated crypts (mixed fibroblastic/hyperplastic polyp) represents a frequent variant of this polyp. 3) Pathologists should recognize FP and discriminate it from other types of colorectal polyps.

#### P 153

##### **CLINICOPATHOLOGICAL STUDY ON CARCINOID TUMOR OF THE RECTUM - MAINLY ON COMPARISON OF CASES WITH METASTASIS AND THOSE WITHOUT METASTASIS**

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Epidemiologic studies in Japan have found that gastrointestinal carcinoid tumors most commonly occur in the rectum. An increase in the size of rectal carcinoid tumors is associated with metastasis to regional lymph nodes and distant organs. Invasion to the muscularis propria or deeper also is a risk factor for metastasis. However, malignancy criteria of rectal carcinoid tumors remain to be established. The aim of our study is to clarify the clinicopathological factor relevant to the malignant potential of this disease.

Cases of rectal carcinoid tumor were divided into group A (12 lesions in 12 cases with metastasis) and group B (15 lesions in 14 cases without metastasis), and a comparative study was carried out from the clinicopathological standpoint. Nine of twelve cases of group A had metastasized to the regional lymph nodes at the time of surgery, and four of these cases metastasized to the liver. In seven cases of group A, the tumor had involved the liver.

There were significantly more cases in group A who manifested large tumor size, central depression and/or ulcer formation, deep infiltration, positive vascular invasion, a moderately elevated mitotic index, a moderately elevated Ki-67 labeling index, and appearance of P53 positive cells. Histologically, approximately 80 percent of cases of both group A and B showed Soga's classification type B and mixed type (type A+B), and there was no distinct difference between the two groups. From these results it could be pointed out that the findings of tumor size (more than 1.0cm), depth of invasion (muscularis propria or beyond), central depression and/or ulceration, mitotic index (>2/10HPF), vascular invasion and Ki-67 labeling index (>2%) are useful predictive criteria in the assessment of the malignant nature of these neoplasms. In addition, as a result of multivariate analysis of the above factors it was found that only tumor size was an independent and significant factor. Thus, it was concluded that the most important malignancy criterion of this neoplasm is tumor size.

#### P 154

##### **CLINICOPATHOLOGIC CHANGES IN TATTOO OF COLONIC NEOPLASM WITH STERILE CARBON COMPOUND**

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**BACKGROUND:** Endoscopic marking of intestinal lesions, 'colonic tattooing', is used as a guide for the intraoperative identification of polypectomy sites and follow-up examinations. Many dyes, such as India ink and methylene blue, have been employed but may cause serious tissue injury and clinical complications, which must be recognised in pathological examination. Recently, a new sterile carbon compound (SPOT) has been used.

**AIMS:** To determine the histologic changes, safety and effectiveness of SPOT

**METHODS:** During the last year colonic tattooing with SPOT was performed in 20 cases of colonic polyps. Six of them turned out to have an infiltrating carcinoma in endoscopic polypectomy and underwent surgical resection. Clinical data and pathological findings were collected.

**RESULTS:** Six surgically resected specimens (5 sigmoid colon/ 1 rectum) were reviewed. The average age ranged from 52 to 86 years (mean, 70). The interval from endoscopic injection to pathologic examination varied from 1 to 48 days. No clinical complications occurred. The SPOT was clearly

observed on gross examination in all cases during surgery. Tattoo was observed in submucosa (3 cases), submucosa and muscularis propria (1 case) and transmural (2 cases). No mucosal lesion was detected. Carbon particles were localized mainly in macrophages of the submucosa (5 cases) or as free particles involving the wall (1 case). Inflammatory infiltrate was predominantly neutrophilic and eosinophilic in 4 cases, and lymphocytic and histiocytic in 2 cases. Giant cell reaction was not observed. Areas of submucosal edema (2 cases), necrosis (2 cases) and fibrosis (2 cases) were noted (4 of them with haemorrhagic focus). The vascular injury observed was: endothelial hyperplasia (1 case), neovascularization (4 cases) and fibrinoid necrosis (1 case).

CONCLUSIONS: SPOT is a safe and effective marker for use in colonoscopy when surgical resection is required. It is also useful for endoscopic follow-up of patients who have not undergone surgery. There was no evidence of short-term clinical or histological complications although histologic changes were observed. Fibrosis was detected in cases with greater interval between injection and surgery. To determine long-term safety of colonic tattooing with SPOT, a longer follow-up studies should be performed.

#### P 155

##### **MALIGNANCY AND OVERDIAGNOSIS OF MALIGNANCY IN PEUTZ JEGHERS POLYPOSIS**

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Peutz Jeghers (PJ) polyps are rare hamartomatous tumors of the gastrointestinal tract frequently associated with skin and mucosal pigmentation. Despite their benign nature there is a certain increased risk of progression to malignancy in some cases, justifying a sustained follow-up of the patients.

We present 6 cases of Peutz Jeghers polyposis (PJP) diagnosed in our hospital during the last year on gastrointestinal specimens obtained by endoscopy and by opened surgery. We analyzed different degrees of dysplastic changes, epithelial intussusception, association with other types of polypoid lesions and others various aspects that might be related with disease progression. Clinico-pathological correlations were made.

Two of these cases consisted in two women, mother and daughter; both of them were operated in another hospital for small bowel tumors with a subsequent diagnosis of adenocarcinoma. The daughter (28 years old) was referred to our hospital for endoscopic follow-up; a small polyp of the transverse large bowel was excised by colonoscopy with a histopathologic diagnosis of PJ polyp; a careful histopathologic reevaluation of the both specimens of enterectomy (slides and paraffin blocks) revealed overdiagnosis of cancer due to the epithelial cystic dilatation and pseudoinvasion in both patients.

The other 4 cases showed diagnostic changes of PJP and also various aspects of hyperplastic (1 case) and adenomatous polyps (3 cases), one of them with mild and moderate dysplastic changes.

When a PJ polyp is diagnosed, the possibility of pseudoinvasion should be kept in mind, in order to avoid malignancy overdiagnosis; also, due to the fact that the malignant transformation of a PJ polyp is still on debate (hamartoma – dysplasia – carcinoma progression versus malignant transformation of an adenomatous area of a hamartoma versus coincidental association of a digestive cancer due to genetic aberrations of PJP), all the other

associated microscopic aspects of the lesion should be carefully analyzed.

#### P 156

##### **ALTERATIONS IN THE EXPRESSION OF MUCIN ANTIGENS IN COLONIC ADENOMAS HARBORING INVASIVE CARCINOMAS**

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Alterations in the synthesis of mucin glycoproteins have been shown to be related to both neoplastic transformation and malignant progression. We studied the expression patterns of four gastrointestinal mucins in colonic adenomas harboring invasive carcinomas with the aim of establishing the alterations that correlate with malignant progression.

##### **MATERIAL METHODS**

Immunohistochemical staining for muc1, muc2, muc5AC, muc6 were performed on 44 cases of colorectal carcinomas arising in adenomas. For each mucin antibody, nonneoplastic mucosa, adenoma and carcinoma areas were evaluated for the percentage of epithelial cells with mucin expression and for the staining intensity semi quantitatively. Qualitative changes of mucin expression in different areas of the same tumor were also evaluated.

##### **RESULTS**

Adenomas and carcinomas had aberrant expression of mucin antigens which are not normally synthesized in colonic mucosa. When compared to normal mucosa both adenomas and carcinomas had highly significant alterations of all four mucin types ( $p < 0.01$ ). Although there is no correlation between the expression patterns of non-neoplastic mucosa and neoplasms, adenomas and carcinomas had a significant correlation ( $r = 0.6-0.7$ ). Intratumoral heterogeneity of mucin expression were detected in all the neoplastic components. Although there were differences of mucin distribution patterns in adenomas and coexisting carcinomas it did not reach statistical significance.

##### **COMMENTS**

The high correlation of alterations in mucin expression patterns of adenomas and invasive carcinomas may be useful for the detection of adenomas which are more likely to develop invasive carcinomas.

#### P 157

##### **ADENOCARCINOMA OF SIGMOID COLON WITH CHORIOCARCINOMATOUS DIFFERENTIATION.**

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Colorectal carcinomas presenting with choriocarcinomatous differentiation are exceedingly rare; only six prior cases have been reported. We report a new case of carcinoma with germ cell differentiation developed in de novo ulcerative colitis after renal transplantation in a 42-year-old woman. Clinically, the patient presented ulcerative colitis 8 years after renal transplantation, developed colon cancer with liver metastasis 2 years after diagnosis and died one month after operation. Histologically, the removed tumor was composed of two distinctive elements consisting of an adenocarcinoma and a choriocarcinoma. The metastatic foci in the liver were exclusively composed of choriocarcinoma. Identification as choriocarcinoma was made on the basis of typical histologic appearance, immunohistochemical demonstration of human chorionic gonadotropin (hCG) in the tumor cells and the high hCG level in patient's serum, unrelated to trophoblastic disease.

In this report, pathogenesis is briefly discussed and clinical conditions are reviewed.

#### P 158

### ATHOLOGICAL REPORTS OF COLORECTAL CANCER SPECIMENS ON A NATIONWIDE SCALE IN FRANCE: IMPACT OF A CONSENSUS CONFERENCE.

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The pathological assessment of surgical specimens is a crucial issue in the management of patients with colorectal cancer (CRC). Tumour stage and lymph node (LN) status are major prognostic factors, circumferential margin is a predictor of local recurrence in rectal cancer, and the presence of LN metastasis is the only criterion to indicate adjuvant chemotherapy. Several studies have demonstrated regional differences in practices in various countries including France. A consensus conference on colon cancer was held in France in 1998, with recommendations on the features that have to be included in pathological reports.

The aim of this study was to evaluate practices in France regarding the reporting of CRC, before and after the consensus conference. All the reports made by pathologists from 82 laboratories throughout the country from June to December 1997 and 2000 were collected, and the items recommended were systematically recorded. The mean number of reports per structure was 30 in 1997 (range 2-158) and 34 in 2000 (6-130). The stage of tumour invasion was present in 99.9% (1997) and 100% (2000) of reports. The longitudinal margins were specified in 94.1% (1997) and 97.1% (2000) of reports. In rectal cancer, the circumferential margin was present in 12.8% (1997) and 51.7% (2000) of reports ( $p < 0.001$ ). The number of sampled and metastatic LN was specified in 99.8% (1997) and 99.5% (2000) of reports ( $p < 0.05$ ). The pTNM stage was indicated in 19.2% and 80.9% (2000) of reports ( $p < 0.001$ ). The mean number of LN per report increased from 9.67 in 1997 to 12.1 in 2000 ( $p < 0.05$ ). The percentage of reports with less than 8 sampled LN decreased from 45.65% in 1997 to 26.04% in 2000 ( $p < 0.05$ ). In conclusion, this study suggests that practices have improved in France regarding the reporting of CRC after a consensus conference held in 1998. However, there are still reports with insufficient data, especially regarding the number of LN sampled and the circumferential margin in rectal cancer. These features may be improved by the use of standardized report forms.

#### P 159

### FEATURES OF COLORECTAL CANCERS ARISING ADJACENT TO SESSILE SERRATED ADENOMA

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**Background:** Sessile serrated adenoma (SSA) is a novel subtype of colorectal polyps. It has been shown to be temporally associated with microsatellite instable colorectal cancer, but direct evidence on its neoplastic nature and malignant conversion is not well established.

**Aims:** To find out the prevalence and features of colorectal cancers associated with SSAs.

**Materials and methods:** Altogether 466 colorectal cancer cases were included in the study. SSA component was sought immediately adjacent to cancer tissue as previously described.

**Results:** Six colorectal cancers associated with SSA were discovered, with 1.3% prevalence in this study. In addition, 29 other serrated adenocarcinomas were detected (prevalence 6.2%) Four of SSA-associated carcinomas were found from

female patients, four of them were located in the proximal colon, and DNA microsatellite instability was present in three cases. SSA component was characterized by a large slightly elevated tumor composed of hyperplastic-like epithelium. Cellular atypia was evident in all cases. Carcinomatous component consisted of serrated clear or slightly eosinophilic serrated epithelial proliferation with a great resemblance to the adenomatous component. Colorectal cancers associated with SSA were more often mucinous than conventional colorectal cancers (67% vs. 8%;  $P = 0.001$ ), but did not differ from other serrated adenocarcinomas (67% vs. 38%,  $P = 0.36$ ). However, they were less often well differentiated than other serrated adenocarcinomas (0% vs. 50%;  $P = 0.017$ ).

**Conclusions:** SSA is a rare precursor of colorectal cancer. It shares both clinical and morphological features with serrated adenoma and commonly represents MSI. As SSA has been proposed to be more frequent than serrated adenoma, lower frequency of SSA-associated cancers in this study could indicate less frequent malignant conversion rate for SSAs compared to serrated adenomas.

#### P 160

### IS THERE A ROLE OF INFLAMMATION IN SPORADIC COLORECTAL CANCER? A STUDY OF COMMON POLYMORPHISMS IN INFLAMMATORY RESPONSE-RELATED GENES

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Epidemiological observations suggest that environmental and host immunological factors could significantly contribute to the initiation and the progression of colorectal cancer (CRC). Genetic polymorphisms can play a role in determining how individuals respond to various environmental factors. Although rather limited, evidence exists linking common polymorphisms in immune system related genes and CRC tumorigenesis.

The aim of the study was to investigate the association between common single nucleotide polymorphisms (SNPs) in the inflammatory response related genes interleukine (IL)-6, IL-8, tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), peroxisome proliferators-activated receptor  $\alpha$  (PPAR $\alpha$ ), and intercellular adhesion molecule-1 (ICAM-1) and the risk of colorectal cancer in a group of Greek patients.

The study group consisted of 222 CRC patients and 200 healthy controls. Genotyping was performed using allele-specific PCR of PRC-RFLP and the results were confirmed by sequencing. We have studied the association of SNPs in the IL-6 (-174G>C), IL-8 (-251T>A), TNF $\alpha$  (-308G>A), ICAM-1 (R241G and K469E), and PPAR $\alpha$  (Pro12Ala) genes and the risk of CRC.

The IL-6 -174G, R241 and K469 alleles are associated with increased risk of CRC (OR = 1.927 [CI95%: 1.508 to 3.336], OR = 1.833 [CI95%: 1.235 to 2.719] and OR = 1.546 [CI95%: 1.174 to 2.034], respectively). The IL-8 and the TNF $\alpha$  polymorphisms had no effect. Whereas the PPAR $\alpha$  Ala12 genotype was associated with reduced risk of disease (OR = 0.588 [CI95%: 0.416 to 0.832]).

The association between common SNPs in immunologic response related genes with CRC is reported in the present study. Apart from shedding light on the mechanisms of malignancy initiation and progression, SNPs may improve appropriate screening for risk sub-population.

#### P 161

### MORPHOLOGICAL FEATURES OF HEREDITARY NON-POLYPOSIS COLORECTAL CARCINOMAS COMPARED TO OTHER EARLY ONSET AND

## SPORADIC COLORECTAL CARCINOMAS IN THE WESTERN CAPE, SOUTH AFRICA.

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### Background:

Families with hereditary non-polyposis colorectal carcinoma (HNPCC) are not uncommon along the West-Coast of South Africa. These patients present with early onset carcinomas, mostly colorectal, predominantly in the right colon. They may develop tumours of other organs, including uterus, breast, stomach and skin.

### Objectives:

1. To evaluate and compare the microscopic characteristics of three groups of colorectal carcinomas (HNPCC, early onset colorectal carcinomas and sporadic colorectal carcinomas).
2. To determine the features most characteristic of the HNPCC group in the South African population.

### Methods:

Coded sections of formalin-fixed paraffin-embedded tissue from patients with

- (1) sporadic colorectal carcinomas (58 cases)
- (2) early onset (<45 years) colorectal carcinomas (93 cases) and
- (3) HNPCC (30 cases) are evaluated and graded on the following features:

intratumoral lymphocytes (TL), stromal lymphocytes (SL), lymphoid aggregates in the vicinity of the tumour (LA), presence and type of necrosis (Ne), percentage of mucin (Mo = 0%, M1 = <25%, M2 = 25-50%, M3 = 50-75%, M4 = >75%) and histological grade based on the standard criteria of gland formation (G1 to G4).

### Results and conclusions:

Intratumoral lymphocytes and lymphoid aggregates are fairly sensitive markers for HNPCC, the former being more specific than the latter. Stromal lymphocytes are a third, even more sensitive marker for HNPCC, but less specific than the other two. The other parameters tested are of no help in the differential diagnosis except for presence of necrosis which is more typical of sporadic and early onset colorectal carcinoma than of HNPCC.

## P 162

### ONCOCYTIC MODIFICATIONS IN RECTAL ADENOCARCINOMA

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**Introduction.** Oncocytes are granular, eosinophilic cells, with mitochondria occupying more than 40% of the cytoplasm. Tumors composed mainly of oncocytes are distributed in many organs, although thyroid and kidney are the most frequent. Oncocytic modifications have been reported after radiotherapy in salivary glands. To date few data are available on oncocytic modifications in adenocarcinomas of the colon-rectum.

The purpose of the study was to evaluate whether oncocytic modifications are present before radiochemotherapy (RCT) and if oncocyte-rich rectal carcinomas are resistant to RCT.

**Materials and methods.** 17 cases of advanced rectal carcinoma, treated preoperatively and simultaneously by 5-fluorouracil (200-225 mg/m<sup>2</sup>) and 46 Gy in 23 fractions were studied. All patients were biopsied before RCT and underwent surgery within six weeks after RCT. Specimens were routinely processed, stained with hematoxylin and eosin (H&E) and immunohistochemistry was performed using anti-mitochondria antibody (clone 113-1; dilution 1:500; Biogenex). In two cases, both pre and post RCT tissues were examined at ultrastructural level.

**Results.** All tumors were adenocarcinomas. Pre-RCT features: oncocytic changes were difficult to find on H&E, while at immunohistochemistry, anti-mitochondria antibody stained 5 to 100% of neoplastic cells (mean 59%). Post RCT features: Oncocytic cells were detected in all 17 cases on H&E and constituted 10 to 100% of the residual neoplastic population (mean 61%). With immunohistochemistry, the anti-mitochondria antibody confirmed the oncocytic changes, highlighting a larger number of neoplastic cells (range from 5 to 100%, mean 73%). Ultrastructural examination revealed large and bizarre mitochondria in tumor cells in both pre and post RCT tissues.

**Conclusion.** The present data suggest that rectal carcinomas can be "mitochondria rich" tumors. After pre-operative RCT, residual neoplastic cells may acquire a definite oncocytic phenotype. It is likely that the mitochondria enhance neoplastic cell survival.

## P 163

### PATTERNS OF MORPHOLOGICAL FEATURES IN RECTAL CARCINOMA AFTER PREOPERATIVE RADIATION OR CHEMORADIATION AND CLINICAL OUTCOME.

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**Introduction** Pre-operative radiation (RT) and chemotherapy ( CRT) improve outcome in patients with rectal adenocarcinoma (ACA).

**Aim** Analysis of histological features in residual rectal carcinoma following preoperative RT or CRT and their prognostic significance.

**Materials and methods** Resection specimens from 54 patients with T1-T4 advanced rectal ACAs treated with RT and CRT before surgery were analyzed for the following features: tumor treatment response (pPR1, pPR2, pPR3), histological architecture structures

(cribriforme, tubular, papillary, cystic), degree of cytological alteration, extent of damaged tubular structures( 0-30%, 30-60%, >60%), inflammatory or histiocytic reaction in the tubular structures, microfoci of carcinoma, tumor necrosis, mucus degeneration of stroma, inflammatory or fibroinflammatory reaction of stroma, cytoplasmic eosinophilia, cytoplasmic basophilia, neoplastic or post-irradiation ulceration. The data obtained were statistically analyzed using ANOVA, Kruskal-Wallis method, median test, ch<sup>2</sup> test and Mann Whitney test.

**Results** There were no significant correlations between the histological architecture structures and other features.

There was correlation between 30% extent of damaged tubular structures and RT type ( 42-60Gy)  $p=0.022$ ; type of ulceration and microfoci of carcinoma ( $p=0.036$ ), sex (  $\chi^2$  test  $p=0.045$ ) and treatment response ( $\chi^2$  test  $p=0.0001$ ); cytoplasmic basophilia with the time of recurrence ( $p=0.024$ ); sex and time of recurrence onset of relapse?) ( $p=0.024$ ).

Conclusion In our study we characterized histomorphology of post-irradiated rectal ADAs and report morphological features associated with the time of recurrence.

#### P 164

##### PROGNOSTIC VALUE OF THE CLASSIFICATION OF FIBROTIC STROMA IN COLORECTAL CANCER

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#### Abstract:

##### Introduction:

Various factors influence prognosis of colorectal cancer including local extension, lymph node involvement, tumoral growth pattern and lymphocytic infiltration at the tumor margin. There is controversy about the role of amount of fibrotic stroma but there are a few studies on the type of stroma. The aim of this study is evaluating the prognostic value of classification of stroma by means of Astre and coller staging system.

##### Materials & Methods :

Ninety two cases of colorectal cancer were examined.we classified fibrotic stroma in front of tumor extension based on Uneo and colleague criteria to three category:typA,multiple fine and mature fiber were stratified ;typeB,broad hyalinized collagen(keloid-tyoe) were intermingled;type C,myxoid stroma.we exited cancers were superimposed on IBD and familial polyposis.Then the data were analyzed by chi-square test..

##### Results:

45% of cases had typeA stroma, 18% type B stroma. And 37% type C.

The prevalence of type A stroma in the various stages of tumor was such:A=100%,B1=68%,B2=26%,C1=60%,C2=33% and the prevalence of type C stroma in the various stages of tumor was such: A=0, B1=16%, B2=49%, C1=40%, C2=48%.

In order to identify amount of correlation we employed pearson index ( $R=0.43$ ).

##### Discussion:

The results showed a decreasing in type A stroma when the stage of disease increase (stage A=100%, stage B1-68%, stage B2= 27%, stage C1=60%, stage C2=33%) and increasing in type C(stage A=0, stage B1=16%, stage B2=49%, stage C1=40%, stage C2=48%).

The result was statically significant and classification of stromal pattern had independent prognostic value same as lymph node involvement, lymphocytic infiltration and growth pattern.

##### Keywords:

Colon cancer, fibrotic stroma, Collagen, myxoid stroma

#### P 165

##### RESPONSE TO PREOPERATIVE CHEMORADIOTHERAPY ON RECTAL ADENOCARCINOMA: HISTOPATHOLOGICAL EFFECTS AND RELATIONSHIP WITH LEVEL OF P53 AND PCNA EXPRESSION.

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INTRODUCTION: Adjuvant chemoradiotherapy delivered before surgery has been used to improve control and to reduce the likelihood of local recurrence in selected patients with rectal cancer. Although the status of p53 gene and the expression of Proliferating Cell Nuclear Antigen (PCNA) has been widely studied in these tumors, little is known about their value to predict responsiveness of rectal cancer to chemoradiation.

AIMS: To assess effects of preoperative chemoradiation in advanced rectal adenocarcinoma.

METHODS: We examined the histopathologic effects of chemoradiotherapy on 73 rectal tumors and correlated the efficacy of treatment with the level of p53 and PCNA proteins expression in pretreatment biopsies.

RESULTS: Three patients showed no primary tumor in the resection specimen. Another eight carcinomas showed a good response to chemoradiation with only small nests of viable tumor cells. Grouping these two categories, we considered that the tumor response had been total or subtotal in 11 cases (15%). Nuclear accumulation of p53 protein was detected in 53 (72%) specimens. Thirty five tumors showed a high PCNA index (48%). No obvious relationship seemed to exist between pretreatment p53 immunostaining and response to chemoradiotherapy. Tumors with high PCNA index were more likely to respond to chemoradiation: 8/35 (72%) versus 3/38 (43%)( $p=0.07$ ).

CONCLUSIONS: Knowledge of proliferative activity of rectal cancer, as determined by PCNA immunostaining, should be useful in predicting the likelihood of response to preoperative chemoradiation.

#### P 166

##### THE CHANGES OF THE TYPES OF COLONIC MUCINS IN THE BENIGN ,PREMALIGNANT AND MALIGNANT LESIONS

Dr.Ibrahim Ghada(M.B.CH.B,F.I.C.Pathology), supervised by professor dr .Alkaptan Ikbal (F.R.C.Path.,M.r.c.Path)

#### the aim of study

To clarify the changing pattern of colonic mucins in colonic tumours, and to evaluate the benefit of the staining methods in diagnosis and prognosis of colorectal lesions ,for early detection of premalignant changes and compare this mucin pattern with western researches.

#### materials and methods

95 biopsies were selected and stained with haematoxylin and eosin,PAS stain ,Alcian blue ph(2.5) and Shikata's modified orcein/alcian blue

#### Results

sulphated mucin is the predominant mucin in normal colon.Mucin pattern in adenoma is variable. Adenoma with mild dysplasia mucin pattern is similar to normal.,these with moderate to severe dysplasia and carcinoma in situ,mucin production is decreased or absent and if it is present it is amixture of sialo and sulphated mucin (sialomucin is more predominant)

in ulcerative colitis with dysplastic changes mucin also is vairiable according to the degree of dysplasia

ie in mild and moderate dysplasia mucin pattern is close to normal ( both types is present however sulphated mucin is predominant) while in Ulcerative colitis with severe dysplasia the mucin pattern is differ as the amount of mucin is decrease and pattern is change predominantly to sialomucin

In all carcinomas,reduction of mucin is evident,less marked in well differentiated adenocarcinoma in which three types of mucin is present(sialo ,sulphated and neutral mucin)

In Moderately differentiated carcinoma mucin is mainly (intraluminal) sialomucin. In poorly differentiated carcinomas, mucin is scanty or absent.

#### Conclusion

The study of mucin pattern of colon is rewarding in detection of early malignancy, classification of polyps, assessment of their malignant potential, (increased sialylation is important in transition from adenoma to carcinoma and for determining prognosis in patients with colorectal cancer

#### P 167

##### COLONIC PSEUDOCARCINOMA-CASE REPORT

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**Aims.** Misplaced epithelium within an adenoma (pseudocarcinoma) is a rather common findings but currently there are no clinical or endoscopic methods available in differentiate adenomas with epithelial misplacement from those with adenocarcinoma.

**Material and methods.** We present the case of a 64-year-old female presented with bright red blood per rectum. Previous medical history was not significant. Upon colonoscopy, two pedunculated polyps were identified in the lower sigmoid, which were excised endoscopically and submitted to pathology for evaluation. The tumors were excised, fixed in formaline, embedded in paraffin and the sections were stained with H-E, PAS-ALCIAN blue. We performed also immunohistochemical staining: with Cytokeratin, Vimentin, Collagen IV and p53.

**Results.** The gross appearance was similar for both polyps. The histological features were different. An adenomatous polyp, with tubulo-villous architecture and misplaced epithelium in the submucosa stalk, mucinous pools lined, represented one of the polyps by dysplastic epithelium similar to that seen at the surface of the polyp. The second polyp was represented by an adenomatous polyp with villous architecture and high-grade dysplasia, focally with irregular, variably sized crypts, without lamina propria, with irregular mucous pools containing floating malignant cells within the stalk of the polyp. The immunohistochemical results were different in the two cases.

**Conclusions.** The most common differential diagnosis of adenomas with epithelial misplacement are adenomas that contain invasive adenocarcinoma, but several other entities may on occasion, be confused with the former lesions, such as solitary rectal ulcer syndrome-associated polyps, Peutz Jeghers polyps, hyperplastic polyps. Unfortunately, the diagnosis it cannot be made just based on the histologic appearance.

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##### ANALYSIS OF CYCLOOXYGENASE-2 EXPRESSION IN COLON CANCER AND NORMAL COLON USING TISSUE MICROARRAY

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#### Introduction:

Recent studies have shown that cyclooxygenase (Cox-2) may be involved in colorectal carcinogenesis.

Cyclooxygenase-2 is an inducible enzyme involved in the response of cells to growth factors, tumour promoters, and cytokines that induce its expression. Given its role in synthesizing prostaglandins, Cox-2 is therefore of interest in studying immune response regulation. It is also induced by a wide variety of other stimuli and was initially identified as an immediate-early growth response gene.

**Aims:** To analyse expression of COX-2 in colorectal carcinomas, and to compare this expression between normal and tumoral colon cells in a series of 35 patients.

#### Material and Methods:

Tissue samples of primary sporadic colorectal carcinoma and adjacent normal mucosa samples from 35 patients were included in a Tissue Microarray.

Cox-2 expression was first evaluated in a Tissue Microarray with normal tissue samples (thyroid, placenta, tonsil, mammary gland, colon mucosa, appendix, spleen, liver, kidney and lung) in order to establish the best protocol, using Immunohistochemical staining in 3µm Tissue Microarray sections, with Cox-2 antibody from Neomarkers. This protocol was then applied to the tumoral and normal colon Tissue Microarrays.

The specimens were graded based on the intensity and extent of staining as follows:

1+: 30% positive cells

2+: 30%-60% positive cells

3+: 60%-100% positive cells

Also the homogeneity and heterogeneity of the staining were validated.

#### Results:

From a total of 35 patients included in the study, the expression of COX-2 was:

- Normal samples were (3+) homogeneous positively with a very strong level of positivity

- From 35 tumour samples, one sample was not evaluated

17 cases (50%) 3+ homogeneous and with strong intensity

3/34 cases (8.8%) 3+ homogenous and weak expression

5/34 cases (14.7%) 2+ homogenous and weak expression

2/34 cases (5.8%) 1+ homogenous and weak expression

5/34 cases 14.7% heterogeneous expression

2 cases were completely negative

#### Conclusions:

1.- The data presented in our study show that Tissue Microarrays are a useful tool for identifying expression profiles and expression changes of proteins, allowing comparisons between normal and tumoral regions.

2.-Cox-2 is expressed intensively and homogeneously in normal mucosa colon

3.- Colorectal cancer shows lower intensity in number of cells compared with their normal mucosa colon counterpart

4.- Some tumours show

#### P 169

##### CYCLOOXYGENASE-2 (COX-2) EXPRESSION IN POLYPS AND CARCINOMAS OF HEREDITARY NON-POLYPOSIS COLORECTAL CARCINOMA (HNPCC) AND SPORADIC COLORECTAL CARCINOMAS IN THE WESTERN CAPE, SOUTH AFRICA.

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#### Background:

COX-2 inhibitors are being used as chemoprevention in familial adenomatous polyposis. If COX-2 is also expressed in HNPCC there might be a role of COX-2 inhibitor treatment for patients with a known mismatch repair gene defect - either

to inhibit progression of tumours or to prevent the onset of carcinogenesis.

Objectives:

1. To evaluate COX-2 expression in colonic adenomas and carcinomas in patients with genetically proven HNPCC.
2. To compare COX-2 expression in HNPCC cases with that in sporadic tumours.

Methods:

Coded sections of formalin fixed paraffin embedded tissue from patients with known hMLH1 or hMSH2 mutations (6 polyps and 12 carcinomas) and sporadic tumours (5 polyps and 39 carcinomas) were immunohistochemically stained with an antibody to the COX-2 (Santa Cruz). Expression of COX-2 in the tumours was scored on both intensity and distribution and compared to the adjacent normal epithelium.

Results:

Normal colonic epithelium does not express COX-2. HNPCC: 1/6(17%) polyps and 5/12(42%) carcinomas express COX-2. Sporadic tumours: 3/5(60%) polyps and 26/39(67%) carcinomas express COX-2.

Conclusions:

COX-2 is generally more expressed in sporadic compared to HNPCC tumours. Furthermore COX-2 expression is more often expressed in infiltrating lesions compared to the expression in polyps. Chemoprevention is probably indicated in a selected number of HNPCC individuals.

#### P 170

##### **EPIDERMAL GROWTH FACTOR RECEPTOR STATUS IN PRIMARY VERSUS METASTATIC SITES OF COLORECTAL ADENOCARCINOMAS**

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The epidermal growth factor receptor (EGFR) is expressed in 40% to 80% of colorectal cancers. It is well known that EGFR expression in tumor cells is not early event and it changes in time in opposition to HER2 status. Data from literature have shown different EGFR status comparing primary and metastatic lesions. Relation between EGFR status and response to specific treatment is still under investigation.

The aim of our study was to compare EGFR status on primary colorectal tumors with corresponding metastases to determine whether assessment EGFR status in primary tumor is reliable for planning EGFR - targeted therapy.

Patients and Methods: EGFR expression was evaluated by immunohistochemistry in primary tumors and related metastases in 50 patients with colorectal adenocarcinomas. In 35 (70%) cases metastatic sites were lungs and in 15 (30%) – liver. EGFR status was evaluated as positive if the percentage of membrane stained tumor cells was  $> \text{ or } = 1\%$  of all tumor cells.

Results: 33 (66%) of 50 specimens from primary tumor were EGFR positive. In 9 (27%) specimens from related metastases EGFR status was negative. 3 (17%) specimens from metastatic sites were EGFR positive whereas corresponding primary tumor specimens were negative.

Conclusion: Our data suggest that due to differences in EGFR expression on primary and metastatic sites of colorectal adenocarcinomas, for planning EGFR - targeted therapy EGFR status should be evaluated on specimens taken from at the moment existing tumor focus - metastases or recurrent lesions.

#### P 171

##### **EXPRESSION OF EGFR IN COLON CARCINOMAS AND THEIR CORRELATION WITH**

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Tatyana.

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Background: Epidermal growth factor receptor (EGFR) is commonly overexpressed in a number of epithelial malignancies and is often associated with an aggressive phenotype advanced stages of cancer and a poor prognosis for the patient.

Aims: The expression of EGFR was analyzed in 50 cases of colon carcinoma, using immunohistochemical technique of paraffin sections. A correlation was made between the EGFR expression in primary tumour and the following clinicopathological parameters: histological type, histological grade and stage of the disease.

Methods: The patients had undergone curative surgical resection at the Oncology Centre in University Hospital - Plevna. EGFR expression was examined by immunohistochemistry using the DakoCytomation's EGFR PharmDx (Dako Cytomation) in paraffin-embedded colon tumors and graded as percentage of cells stained. The correlation statistical analysis was used.

Results: EGFR immunoreactivity was detected in 34 (68%) cases of colorectal carcinomas.

High levels of EGFR protein were observed in 10/50 (20 %) samples. Thirteen (26%) show moderate of EGFR expression in tumor cells and 11/50 (22 %) show slight level of EGFR expression. Sixteen of tumors were totally negative (32%).

Most of the cases showed complex and heterogeneous patterns of EGFR-expression.

No association was found between the expression of studied EGFR protein and histological grade and type of primary tumor. Similarly, no correlation was detected between EGFR immunostaining and stage of colorectal adenocarcinoma.

Conclusions: Prospective multicentric studies should be conducted to confirm overexpression of EGFR as a prognostic factor.

Key words: EGFR, colorectal adenocarcinoma

#### P 172

##### **HER 2/NEU EXPRESSION IN COLON CANCER AND ITS CORRELATION WITH CLINICOPATHOLOGIC VARIABLES**

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Abstract

Aims: Colon cancer is a prevalent human malignancy. HER2/neu is an important oncogene in breast cancer, but its prevalence and significance in colon cancer have been poorly documented.

Aim of our study is to determine the rate and pattern of HER2/neu expression in colon carcinoma by immunohistochemistry (IHC).

Material and Methods: Sixty-nine archival paraffin wax embedded colon carcinoma specimens were chosen. IHC for HER2/neu was performed. Clinicopathologic data and IHC results were analyzed.

Results : Most of the carcinoma cases were well differentiated and located in the left side. There is positive

HER2/neu staining in a high percent of cases (41 Cases ,59.4 % ) with both cytoplasmic ( 27cases.65.9%) and membranous-cytoplasmic ( 14cases,34.1%) staining . In higher stages , the rate of positive staining were decreased but there was higher rate of strong membranous staining .Also, in higher grades , the rate of HER2/neu staining was decreased (P=0.04) and there was more membranous staining .

There were no correlation between HER2/neu expression and age , sex, site and type of tumor .

Conclusion : The rate of HER2/neu expression in colon carcinoma is high. Because of more prominent membranous staining in higher stages and grades,the Herceptin therapy could be helpful in patients with lymph node or distant metastases.

Keywords: coloncarcinoma, HER2/neu, immunohistochemistry

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### IMMUNOHISTOCHEMICAL ASSESSMENT OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION IN PRIMARY COLORECTAL CARCINOMA AND THEIR RELATED METASTASES. A COMPARATIVE STUDY ON TISSUE SECTIONS AND TISSUE MICROARRAY

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Introduction: Recently approved new EGFR-targeted treatment (Cetuximab®) in metastatic colorectal carcinoma (CCR), requires an immunohistochemical assessment for patient eligibility. Controversial and limited data are available on the EGFR status in distant metastases.

Purpose: Because of clinical implications, we studied EGFR expression of primary CCR and their related metastases, to assess whether EGFR status was equivalent on both sites.

Methods: EGFR immunohistochemistry (IHC) on tissue sections was retrospectively assessed from primary tumors and related synchronous metastatic sites in 32 CCR patients (colon: 21; rectum: 11). Metastatic sites analyzed were liver (31) and ovary (1). IHC standardized assay was used (EGF-R pharm Dx, DakoCytomation) and EGFR positive status (EGFR +) was defined by a percentage of stained cells >1%, displaying a membranous pattern. In addition to tissue sections, EGFR was assessed on two triple core tissue microarrays (TMA), in order to find out if this high throughput technique was valuable for EGFR screening (diameter of the core: 0,6 mm, punched in the deepest region of the tumor invasion known to contain the strongest immunoreactivity).

Results: EGFR + tissue sections were observed in 84% of the primary cases (27/32) and 94% of the metastases (30/32). All patients displayed an EGFR + status either in the primary site (2/32), in the metastasis (5/32) or both (25/32). Discordant cases (i.e primary positive/ metastasis negative and inversely) were not statistically significant (p=0.453). Additionally, the number of EGFR + cells in the primary site was significantly correlated with that observed in their metastasis ( $r^2= 0.616$ ,  $p=0.0002$ ). EGFR status on TMA was significantly underestimated on both sites, when comparing with tissue sections (p=0.0001 and p=0.0015 for primitive and metastatic sites, respectively). Moreover, using the TMA technology, we failed

to find any correlation of EGFR expression between both sites.

Conclusion: Our results suggest that EGFR expression is frequent and equivalent on both primary and metastatic sites. Moreover, all patients exhibit an EGFR + status when combining EGFR status from the primary and from the metastatic site. Consequently, patient exclusion for Cetuximab® therapy should not be based on EGFR negativity from one single site. Our results also demonstrated that TMA is not a suitable tool for EGFR assessment in CCR, since it significantly underestimates its status.

#### P 174

### Â-CATENIN, E-CADHERIN AND CYCLIN D1 EXPRESSION AT THE INITIAL STAGES OF THE ADENOMA-CARCINOMA SEQUENCE

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Introduction and aims:

Most of the colorectal carcinomas originate from adenomatous polyps and this process is known as adenoma-carcinoma sequence. Â-Catenin play an important role at Wnt signaling pathway and E-cadherin-catenin complex is essential at the maintenance of normal tissue architecture. An alteration of any of the components of E-cadherin-catenin complex are believed to result in the loss of cell-cell adhesion and to contribute to carcinogenesis. This study aimed to determine expression and cellular distribution of E-cadherin, Â-catenin, and its target gene cyclin D1 and c-myc during tumor progression and malignant transformation in colon and to reveal its diagnostic or prognostic usefulness.

Methods:

Following histopathological examination, immunohistochemical staining of E-cadherin, Â-catenin, cyclin-D1 and c-myc were performed at each section of 60 tumor samples and 31 polyp samples taken from 61 patients diagnosed as colorectal carcinoma and/or adenomas.

Results:

At polyps, the frequent loss of membranous expression of E-cadherin was found at high dysplastic lesions however it was not significant (p: 0,07). Cytoplasmic and/or nuclear expression of Â-catenin and nuclear expression of cyclin D1 was correlated with the degree of dysplasia (p: 0,01, p: 0,003, respectively).

At the tumors; the membranous expression of E-cadherin was negatively correlated with lymph node metastasis (p:0,04). Nuclear cyclin D1 was expressed more at well differentiated tumors and at early stage (p:0,02, p:0,04, respectively). The increase of nuclear cyclin D1 positivity was correlated with cytoplasmic Â-catenin positivity (p:0,034). Tumors of less than 5 cm diameter have more cytoplasmic and/or nuclear Â-catenin positivity than tumors of more than 5 cm diameter (p: 0,039). No specific expression pattern were detected at c-myc expression related to adenoma-carcinoma sequence.

Conclusion:

This study demonstrated a significant loss of membranous expression of E-cadherin during tumor progression at colon carcinomas. We hypothesized that loss of membrane staining of E-cadherin might be an indicator of worse prognosis at colon carcinomas. We propose to use nuclear expression of Â-catenin as a marker of invasiveness in adenomas. As the overexpression of cyclin D1 is an early event in adenoma-carcinoma sequence, cyclin D1 may account for good prognostic parameter in colon carcinomas.

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### E-CADHERIN EXPRESSION PATTERN IN PRIMARY COLORECTAL CARCINOMAS AND THEIR METASTASIS REFLECT DISEASE OUTCOME

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**Aim:** We using immunohistochemistry to examine the levels of E-cadherin expression in primary tumours and their metastasis from a series of 42 colorectal cancer (CRC) patients. **Material and Methods:** E-cadherin expression was separately analysed in membrane and cytoplasmic location by calculating two indices (MI and CI) in a series of 42 CRC and their metastases. Univariate and multivariate survival analyses were used to assess the value of these two E-cadherin indices as predictors of both disease-free (DFS) and disease-specific (DSS) survival.

**Results:** E-Cadherin MI was significantly higher in primary tumours as compared to their metastasis ( $p=0.0001$ ), whereas CI did not show such a difference. Both MI and CI in the primary tumour were higher among patients who developed subsequent metastasis ( $p=0.022$  and  $p=0.007$ , respectively). Interestingly, both indices were higher in metastasis of the liver than in those at other anatomic sites ( $p=0.034$  and  $p=0.022$ ), respectively. In univariate analysis, CI in the primary tumour was a significant predictor of DFS ( $p=0.042$ ), and retained its predictive power also in multivariate Cox model, with a strong inverse correlation between CI and DFS ( $p=0.006$ ). In the multivariate model, MI in the primary tumour proved to be a significant independent predictor of DSS, higher indices being associated with more favourable outcome ( $p=0.016$ ).

**Conclusions:** The examining E-cadherin expression and distribution in colorectal tumours can be extremely valuable in predicting disease recurrence. The observation that aberrant cytoplasmic expression of E-cadherin can predict disease recurrence is obviously of great importance for both the patient and the clinician and affects significantly decisions concerning patient therapy and management.

#### P 176

##### **EXPRESSION OF E-CADHERIN IN BUDS AT THE INVASIVE FRONT OF COLORECTAL CANCER (CRC) IN ASSOCIATION WITH LYMPH NODE INVOLVEMENT.**

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##### **Introduction:**

Tumor budding refers to microscopic clusters of undifferentiated cancer cells just ahead of the invasive front of the carcinoma. It has been suggested that dysfunction E-cadherin-mediated cell adhesion is associated with tumour invasion and metastasis.

That why the aim of this study was to evaluate the relationship of tumour budding to chosen anatomoclinical features of colorectal cancer, and to E-cadherin expression in the main tumour mass, lymph node metastases and in the buds.

**Methods:** Tumors from 55 patients with colorectal cancer (CRC) were assessed by immunohistochemistry. Tissue sections were fixed in 10% buffered formaldehyde solution, embedded in paraffin and stained immunohistochemically with anti-human E-cadherin and  $\beta$ -catenin antibody.

##### **Results:**

It has been found that loss of membrane E-cadherin expression at the invasion front of primary tumour and tumour budding correlate with lymph node involvement. A different location of E-cadherin expression was also observed in buds. A statistically significant correlation between localization of E-cadherin expression in buds and presence of lymph node metastases ( $p<0.01$ ) was found. We also noted a statistically

significant correlation between changes at the site of E-cadherin expression in primary tumor, buds and localization of E-cadherin expression in lymph node metastasis.

**Conclusion:** These data suggest that different aggressiveness of tumors may depend of changes at the location of E-cadherin expression in buds at the invasion front of colorectal cancer.

#### P 177

##### **EXPRESSION OF E-CADHERIN, BETA-CATENIN AND CD44V6 IN COLON CANCER: CORRELATION WITH CANCER METASTASIS.**

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Adhesion molecules are closely involved in the development and growth of metastatic tumours. The aim of the present study was to investigate the level of expression and cellular distribution of E-cadherin, beta-catenin and CD44v6 and to assess the role of proteins in progression of colon carcinoma. Immunohistochemical analysis for E-cadherin, beta-catenin and CD44v6 was carried out on formalin-fixed paraffin-embedded sections of colon cancer tissues by streptavidin-biotin immunoperoxidase method. We examined the expression patterns of these molecules in 84 primary colon, 92 liver and 35 lymph node metastatic tumours, obtained from 84 patients and 45 non-metastatic colon carcinomas as control. The presence of membranous, cytoplasmic and mixed staining for E-cadherin were found in tumour tissues. Loss of normal membranous staining and reduced E-cadherin expression were detected more frequently in the primary tumour with liver metastasis (64/84, 76%), than in those without secondaries (14/45, 31%) ( $p=0.014$ ). In addition, the expression of E-cadherin had an inverse relationship with level of differentiation of the tumour. The expression level of E-cadherin was markedly decreased in the cancer cells of metastasis in lymph nodes and liver. Beta-catenin immunoreactivity was increased with cytoplasmic accumulation and nuclear translocation in more than 80% of the neoplasms with distant metastasis and in metastasis in lymph nodes and liver. Nuclear localization of beta-catenin was significantly higher in the invasive margin of some tumours in metastatic group (70/84, 83%) when compared with the localized one (15/45, 33%) ( $p=0.01$ ). No significant difference in beta-catenin expression was found between primary tumours and their lymph node and hepatic metastasis. There was no correlation between the expression of CD44v6 and lymph node involvement or liver metastasis of colon carcinoma. Expression of CD44v6 in metastasis showed no consistent relationship to that in the primary tumours. These results indicate that decreased expression of E-cadherin and nuclear accumulation of beta-catenin in colon cancer tissues are closely related to liver metastasis. There may be no association between CD44v6 expression and metastatic ability of colon carcinoma. In conclusion, our study suggest that both E-cadherin and beta-catenin play an important role in invasion and metastasis of colon cancer and may become a significant prognostic markers for tumour behaviour.

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##### **ASSOCIATION BETWEEN PRESENCE OF TUMOUR BUDDING AND EXPRESSION OF CATHEPSIN B AT THE INVASION FRONT OF COLORECTAL CANCER (CRC).**

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**AIM:** Recent studies concerning the prognostic factors in colorectal cancer (CrC) have paid attention to tumor budding

as a prognostic factor. Cathepsin B play an important role in cancer invasion and metastases by degrading extracellular matrix components and basement membrane. However, the correlation between cathepsin B and presence of tumor budding at the invasion front of colorectal cancer has not yet been presented. The aim of this study was to clarify the association between the expression of cathepsin B in main mass of tumor, buds, and lymph node metastases, and presence of tumor budding at the invasion front of CRC, and lymph node involvement.

**METHODS:** A standard avidin-biotin immuno-peroxidase method (ABCu-NCL) staining technique was performed on 4µm paraffin-embedded tissue section with a mouse anti-human cathepsin B monoclonal antibody (Novocastra/NCL-CATH-B).

**RESULTS:** A tumor budding was observed in 78.2% cases out of 55 colorectal adenocarcinomas. It has been observed that tumor budding at the invasion front was strongly associated with lymph node metastases. Positive immunohistochemical staining for cathepsin B was observed in 52.7% cases. Statistically significant correlation was found between expression of cathepsin B in main mass of tumor and lymph node involvement ( $p < 0.01$ ). The expression of cathepsin B in primary tumor was also associated with tumor budding and vascular invasion ( $p < 0.01$ )

#### P 179

##### **CERBB2 AND P53 OVEREXPRESSIONS ARE POOR PROGNOSTIC PREDICTORS IN PATIENTS WITH COLORECTAL CARCINOMA**

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**Aim and background:** To assess biological markers of primary colorectal carcinoma (CRC) that may improve clinical staging and provide useful information for the application of novel therapeutic strategies, we investigated the protein product of the c-erb B2/HER-2 oncogene and p53 expression.

**Method:** Formalin-fixed paraffin embedded histopathological samples from 41 patients with CRC treated radical surgery in different stage were analysed to determine HER-2 and p53 protein expression by immunohistochemistry using Avidin-Biotin complex. Spearman Rank correlation test and Kaplan Meier survival tests were used in statistical analyses.

**Results:** Follow-up period was 12-84 months (mean; 27.6 ms) in study group. It's found that there was Lymph node metastases (LN met) in 21 cases (51.2%), perineural invasion (PI) in 13 cases (31%). There was no any expression of HER-2 in 10 cases (24%). There was Her-2 expression (+): n=5, 12%; (++) : n=13, 31.7%; (+++) : n=13, 31.7%. Expression of Her-2 was correlated with poor prognosis ( $p < 0.05$ ). P53 expression was less than 15% in 21 cases, whereas it was 15% or higher in 20 cases. Overexpression of p53 was found poor prognostic indicator. Both markers were correlated with LD met.

**Conclusion:** Our study suggest that analysis of c-erb B2/HER2 and p53 expression may provide relevant prognostic information about CRC patients that should be added in our pathology reports.

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##### **CLINICAL SIGNIFICANCE OF P53, K-RAS, DCC GENE ALTERATIONS AND EXPRESSION OF P53, RAS P21 ONCOPROTEIN IN THE EARLY STAGE COLORECTAL CANCERS**

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Genetic alterations of p53, K-ras and DCC genes have a pivotal role in the colorectal colorectal tumorigenesis. Clinical significance of these genetic changes includes contradictory results. The aim of this study was to clarify the association between point mutation of K-ras oncogene codon 12/13, p53 aberrations, DCC loss of heterozygosity (LOH) and p53, ras p21 immunexpression with the patient outcome and tumor characteristics in the early stage colorectal cancer patients.

Fifty-three stage I-II colorectal cancer patients who underwent surgical treatment with no postoperative adjuvant therapy were included in the study. Follow-up data were available for 43 patients with a median follow up 76 months (range, 6-168). Mutations in exons 5-8 of the p53 gene and codon 12 and/or 13 of the K-ras gene were assayed by PCR-SSCP and then confirmed by DNA sequencing. DCC LOH was studied by PCR-RFLP. p53, using the monoclonal antibody DO7 and ras p21 expression were evaluated by using immunohistochemistry on paraffin-embedded tissue sections.

Mutations of the p53 and K-ras genes were found in 15 and 5 tumors, respectively. DCC LOH was found in 5 cases. The most common combination of these alterations was DCC and p53, whereas p53 and K-ras was extremely rare. Cases with mutation of guanine to thymine (Gà T) which occurred in K-ras codon 12 and DCC LOH were found to be more aggressive than other cases with codon 12 mutations and DCC wild-type phenotype. Fourteen patients developed recurrence, 7 of whom had tumors with p53 or K-ras mutations or DCC LOH. K-ras gene mutations were found mostly in presence of a mucinous component within the tumor ( $p = 0.06$ ).

Nuclear staining of p53 was found in 54 % of the cases. Overexpression of the ras p21 were detected in 76% of the tumors. Ras p21 overexpression did not correlate with any of the clinicopathological parameters examined. Many tumors with p53 overexpression were localized at the left site ( $p = 0.005$ ). The 5-year disease free and overall survival rates indicated that no significance exists between the patients with or without these genetic alterations.

In conclusion, although none of these genetic alterations showed a significant prognostic value, specific mutation of K-ras gene and DCC LOH phenotype might have a predictive prognostic implication in colorectal cancer. Furthermore, distinct genetic patterns might be involved in the tumorigenesis of the left and right colon and mucinous colorectal adenocarcinomas.

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##### **DNA FLOWCYTOMETRIC ANALYSIS AND CYTOGENETIC ABERRATIONS DETECTED BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH) OF COLORECTAL CARCINOMA**

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Colorectal cancer is one of the leading cancer sites in developed countries. It is also one with high mortality rates. Inspite of the utilization of numerous histopathologic and tumour markers, the clinical behaviour of colorectal cancer is highly variable with unpredictable prognosis. New prognostic markers and increased biologic understanding of the growth of the tumour are therefore needed which would help to identify patients at risk of recurrence and therefore aid in improving treatment strategies. This study was conducted on

45 cases of Egyptian colorectal carcinoma. Detection of DNA content and calculation of S-phase fraction (SPF) was performed by flowcytometry (FCM). Also, detection of numerical chromosomal aberrations of chromosomes 7, 17 and 18 using FISH technique was done. It was found that 56.7% of cases were aneuploid, 52.9% in the hypotetraploid range, 23.5% in the near diploid and 11.8% for each of triploid and tetraploid ranges. SPF had a mean of 15.59%, ranging from 3.3% to 37.8%. Aneuploidy correlated with tumour size, grade, lymph node (LN) state and there was a tendency for aneuploid cases to be of metastatic Dukes C/D and to be distally located. Cases with high SPF tended to be more in distal tumours, metastatic Dukes C/D and in cases with more than 3 LNs and correlated significantly with tumour size. Aneusomy for at least 1 of the investigated chromosomes were present in 63.3%, being 53.3%, 26.7% and 43.3% for chromosomes 7, 17 and 18 respectively. Statistically significant correlation existed between chromosomes 7, 17, 18 and LN deposits, chromosomes 7, 17 aneusomies and tumour size and grade with high tendency of metastatic Dukes C/D to harbour high number of aneusomies. Statistically significant correlation existed between ploidy as detected by FCM and chromosomal aneusomies as detected by FISH and between chromosomes 7, 17 and SPF. In conclusion, FCM is a rapid, sensitive and accurate method in evaluating thousands of nuclei for their DNA content and proliferative activity. Ploidy state correlated with tumour size, grade and number of LNs. Also, FISH is a more sensitive technique than classic cytogenetic ones, allowing rapid and straightforward result interpretation. The use of these techniques, thus can help in improving treatment strategies for patients with colorectal carcinoma.

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##### **DNA PLOIDY, S PHASE FRACTION, P53 EXPRESSION AND CHROMOSOME # 17 COPY NUMBER ANEUSOMY IN COLORECTAL CARCINOMA IN EGYPT.**

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#### INTRODUCTION:

In Egypt high proportion of colorectal carcinoma (CRC) occurs below the age of 35 years and are associated with advanced disease at presentation.

#### AIM OF THE STUDY:

DNA ploidy, S-phase fraction (SPF), p53 Immunohistochemistry (IH) and chromosome # 17 aneusomy studied in a series of 30 patients below 35 years undergoing resective surgery for primary operable CRC to investigate whether these alternations have any clinical value in predicting CRC patient's prognosis.

#### METHODS:

DNA ploidy & SPF performed on tumour frozen tissues by flow cytometer (FMC) FAC scan B-D, chromosome copy number changes using non-radioactive fluorescent Insitu hybridization (FISH) using centromer specific DNA probe (D17Z1) for chromosome # 17 and p53 over expression by IH (M3566 -Dako cytometry) on paraffin embedded tissues. The median follow up time was 36 months.

#### RESULTS:

The majority of cases were males (79%), distal colon in 46.6% with tumors > 5 cm in 70% of patients. DNA ploidy was associated with high Histologic grade (G3) ( $p < 0.05$ ), high SPF ( $> 15.6$ ) in 43%, lymph node metastases ( $P < 0.01$ ), advanced Duke's stage (C&D) ( $P < 0.01$ ), lymph node metastases ( $P < 0.01$ ) and distal tumours ( $P < 0.001$ ). FISH showed chromosome #17 aneusomy in 26.6%, (20% deletion and 6% gain) while p53 over expression by IH in 59.4%. No association detected between chromosome #17 aneusomy by FISH and p53 expression by IH.

#### CONCLUSIONS:

DNA aneuploidy, high SPF, p53 over expression are the hallmark of CRC in young Egyptian patients and are predictors of poor outcome.

#### P 183

##### **EXPRESSION OF FASCIN PROTEIN IN COLORECTAL CARCINOGENESIS; RELATIONSHIP TO DECREASED EXPRESSION OF E-CADHERIN PROTEIN**

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**Background:** Fascin is an actin-bundling protein that induces cell membrane protrusions and increased motility, and is highly expressed in various transformed cells, and in specialized normal cells including neuronal, endothelial and dendritic cells. **Methods:** We evaluated the expression of fascin by immunohistochemistry in normal colonic mucosa, tubular adenomas and colorectal carcinoma, and investigated its relationship with E-cadherin expression and the various clinicopathologic factors. **Results:** Overall, variable fascin immunoreactivity was detected in 0% of 13 normal colonic glandular epithelium, and weakly in 52.6% of 16 adenomas, and weak to strongly in 40.9% of 115 colonic adenocarcinomas under study. Fascin immunoreactivity was high in poorly differentiated group compared with well to moderately differentiated group ( $p < 0.01$ ). And right side colon cancer showed slightly increase of fascin expression than left side one. Interestingly, fascin expression was inversely correlated with E-cadherin expression ( $p < 0.01$ ). And there was no significant correlation with the tumor size, metastasis or pathologic staging. **Conclusions:** In the multistep pathogenesis of colonic adenocarcinoma, fascin overexpression seemed to be a late event, and was associated with down-regulation of E-cadherin protein.

#### P 184

##### **EXPRESSION OF TGF- $\beta$ 2 AND SMAD4 IN COLON CARCINOMAS**

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**INTRODUCTION:** TGF- $\beta$ 2, a potent natural antiproliferative agent, is believed to play an important role in suppressing tumorigenicity. This effect is mediated through Smad4, a tumour-suppressor gene, at chromosome 18q21.1, that affects gene transcription and controls cell growth.

**PURPOSE:** To study the expression of TGF- $\beta$ 2 and Smad 4 in colon carcinomas and to correlate them with pathological parameters and patient's survival.

**MATERIALS AND METHODS:** Formalin-fixed paraffin embedded tissue from 50 cases of colon carcinoma was stained by immunohistochemistry for TGF- $\beta$ 2 and Smad4 protein. Statistical analysis was performed using the Fisher's exact test, Kaplan-Meier method and log-rank test.

**RESULTS:** Smad4 nuclear and cytoplasmic staining was absent or reduced in 29/50 (58%) colorectal carcinomas, while in the remaining 21 (42%) Smad4 expression comparable with colonic mucosa was observed. A subset of poorly differentiated and mucinous carcinomas revealed normal Smad4 expression. TGF- $\beta$ 2 cytoplasmic staining expressed in all colon carcinomas and was overexpressed in 25/50 cases. We observed a tendency for a relationship between Smad4 and TGF- $\beta$ 2 in a subset of poorly differentiated and in advanced stage carcinomas. No statistical relationship between the above markers and survival was detected.

**CONCLUSIONS:** In poorly differentiated carcinomas the normal Smad4 protein expression may be linked to TGF- $\beta$ 2 overexpression, findings that may represent activation or dysregulation of the TGF- $\beta$ 2 signalling pathway. Inactivation of TGF- $\beta$ 2 gene occurs at an early stage of colorectal carcinogenesis, while inactivation of Smad4 is likely to be a late event.

#### P 185

##### **HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF SPORADIC POORLY DIFFERENTIATED COLORECTAL ADENOCARCINOMAS**

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Sporadic poorly differentiated adenocarcinoma of colon and rectum are unusual findings. Although, TNM or Dukes systems of stages are the most important in establishing the five-year survival rate, the prognostic depends also on the histological type of the tumor. Hence, the necessity of distinction between these carcinomas and other variants (neuroendocrine carcinoma, large cell carcinoma).

**Aims:** Our purpose was to analyze the histological and immunohistochemical pattern of sporadic poorly differentiated colon carcinomas, in order to find reliable additional criteria in differentiating them from other types.

**Material and methods:** We studied eight cases of sporadic poorly differentiated enteric type colon adenocarcinomas. Selection of these cases was made considering histological aspects. All tumors were routinely processed (paraffin embedded, HE, van Gieson and Alcian blue staining). Immunohistochemical labeling included antibodies to Cytokeratin cocktail, Chromogranin A and Neuron Specific Enolase and was considered to be positive if present in at least 10% of the tumor cells.

**Results:** Macroscopically, seven tumors were exophytic, large (mean size of 5.4 cm), ulcerated, infiltrating the subserosa of the colon. Only one tumor, localized in cecum, was 7 cm large, circumferential, infiltrating the colon wall and penetrating an ileal loop situated at about 30 cm from ileocecal valve. Microscopically, all tumors were poorly differentiated, enteric type adenocarcinomas: large atypical tumor cells disposed in irregular cords or nests, with reduced glandular formation (under 10% of tumor mass). Immunostaining for Cytokeratin cocktail was largely positive in all tumors. The intensity of immunoreaction was similar in areas with reduced differentiation, glandular formation and in adjacent mucosa (with or without dysplasia). On the contrary, immunoreactivity for Chromogranin A and Neuron Specific Enolase was negative in these areas for all studied cases. Localization of the tumor, age and sex of the patient had no influence on immunoreactivity intensity.

**Conclusions:** Chromogranin A and Neuron Specific Enolase are important immunohistochemical markers helpful in distinguishing between poorly differentiated colorectal adenocarcinomas (with no neuroendocrine differentiation) and neuroendocrine carcinomas, when histological aspects are similar.

#### P 186

##### **HYPOXIA INDUCIBLE FACTOR - 1 $\alpha$ INFLUENCES ANGIOGENESIS AND PROGNOSIS IN LOCALLY ADVANCED RECTAL CANCER**

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Hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) is a critical regulatory protein of cellular response to hypoxia and is closely related to the triggering of the angiogenic process through activation of the vascular endothelial factor (VEGF) gene. The disruption of apoptosis, which is mainly regulated by the bcl-2 anti-apoptotic gene and the death promoting p53, causes loss of programmed cell death and tumour progression. We investigated the relationship between hypoxia, angiogenesis and apoptosis and their prognostic impact in patients with advanced rectal cancer.

HIF-1 $\alpha$  tissue immunostaining, assessment of angiogenesis profile, quantitation of apoptosis (apoptotic index) and expression of p53 and bcl-2 were carried out in formalin-fixed paraffin-embedded tumour tissue samples retrieved from 92 patients with locally advanced rectal cancer (T3,4/N+).

HIF-1 $\alpha$  high reactivity was noted in 47.8% of the examined cases and its expression correlated significantly with lymph node metastasis ( $p < 0.001$ ), low rectal location ( $p = 0.016$ ), infiltrative growth pattern ( $p = 0.039$ ), absence of peritumoural lymphocytic infiltration ( $p = 0.037$ ), depth of invasion (pT4) ( $p = 0.04$ ) and vascular invasion ( $p = 0.001$ ). VEGF positive expression was detected in 44.6% of tumour samples. VEGF expression and microvessel density were associated with lymph node involvement ( $p < 0.001$ ). VEGF was found more frequently in larger in size and low-lying rectal carcinomas ( $p = 0.003$  and  $p = 0.029$ ). HIF-1 $\alpha$  expression was directly correlated with VEGF up-regulation ( $r = 0.64$ ,  $p < 0.001$ ) and MVD ( $r = 0.41$ ,  $p < 0.001$ ). VEGF expression was also closely interrelated with MVD ( $r = 0.75$ ,  $p < 0.001$ ). In univariate analysis advanced grade, low rectal location, infiltrative pattern of tumour growth, vascular invasion, positive lymph node status, HIF-1 $\alpha$  expression, VEGF upregulation and p53 accumulation were related to decreased disease-free survival. Regarding overall survival, all aforementioned variables, except for tumour location and p53 status, were proved to be statistically significant negative prognosticators. In multivariate analysis only high HIF-1 $\alpha$  reactivity and positive lymph node status were emerged as independent variables of adverse prognostic significance.

HIF-1 $\alpha$  and VEGF may play an important predictive and prognostic role in patients with locally advanced rectal cancer. HIF-1 $\alpha$  may serve as a prognostic marker of

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##### **MOLECULAR MARKERS PREDICTORS OF SURVIVAL AND METASTASIS IN COLORECTAL CANCER**

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Introduction: Colorectal cancer is the second most common neoplasia in men and women and the second most deathful cancer in the west world.

Up to date, the most important variable in the outcome from colorectal cancer is the stage of disease at diagnosis (Dukes or TNM stage). Nevertheless, those classifications are not helpful enough to predict the prognosis of patients, mainly when intermediate levels of disease appear (Dukes B and C stage). In order to solve this issue, it is very important to identify molecular markers clearly associated to the outcome of patients.

Material and methods: With this objective, we analysed 198 (52% Dukes B and 48% Dukes C stage) colorectal carcinomas selected from the archives of 3 different Spanish hospitals. All cases were removed between 1997 and 1998. After the surgery 47% were treated with adjuvant chemotherapy (5-FU) while 53% of patients did not receive any additional treatment. Sixty two out of the total amount of the patients have developed metastasis after surgery. After a 5-year follow-up period, 47% of patients had died and 53% were still alive.

Four tissue microarrays (TMA) were constructed with paraffin embedded tissue samples. Thirty molecular markers were examined by immunohistochemistry, including markers of apoptosis, cell cycle, proliferation, signalling, adhesion, repair genes and immunologic response. Conventional histopathological features of colorectal cancer were also evaluated.

Results: In the univariate analysis, most of molecular markers did not show any significant association with survival or metastasis. However, the over-expression of p21 and cyclin B1 were related with recurrent disease ( $p=0,028$  and  $p=0,039$ ), and the loss of expression of p16 was also linked to distance metastasis ( $p=0,007$ ).

In addition, the over expression of cyclin D1 was associated with shorter survival ( $p=0,037$ ).

However, in current study the presence of lymphovascular invasion was considered the most relevant risk factor for disease recurrence, distal metastasis and survival in general ( $p<0,0001$ ).

Conclusion: As a conclusion, this immunohistochemical study of B and C Dukes stage of colorectal carcinoma on TMA reveals that the expression of p21, p16, cyclin B1 and Cyclin D1 could be used as prognostic markers of metastasis or survival.

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##### PROGNOSTIC EVALUATION OF CD44 EXPRESSION IN CORRELATION WITH BCL-2 AND P53 IN COLORECTAL CANCER

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Colorectal cancer results from a series of genetic events which disorder the normal mechanisms controlling cell growth. The aim of this study was to investigate the expression of CD44 in colorectal carcinoma and examine its association with clinicopathological features, bcl-2, p53 and long term outcome. MATERIALS and METHODS: A series of 100 patients underwent surgical resection of primary colorectal carcinoma at the III Department of Surgery, Tzaneio Hospital of Piraeus between 1995 and 1999. Colorectal cancer cases in non-inherited polyposis in adenomatous polyposis and in ulcerative colitis were not included in this study. Colorectal cancer cases which death occurred in the immediate postoperative period were also excluded.

Paraffin embedded specimens from 61 patients with Dukes stage B and 39 patients with Dukes stage C colorectal

adenocarcinoma were assessed. We determined the expression of CD44, bcl-2, P53 with 5 year follow up.

RESULTS: In our study low CD44 expression (<10%) was present in 30%, moderate CD44 expression (10%-50%) in 30% and extensive CD44 expression (>50%) in 40% of cases. Expression of CD44 was unrelated to patient sex and age but was related to tumor differentiation ( $p=0,0295$ ), tumors stage ( $p=0,009$ ) and tumor site. No significant association was demonstrated between CD44 and bcl-2 status. However there was significant evidence of an association between CD44 and P53 status in the 66 cases in which P53 had previously been assessed. When entered into a multivariate analysis model, which also included bcl-2 and P53, CD44 staining emerged as prognostic indicator variable.

CONCLUSIONS: CD44 expression may be an indicator of poor prognosis in colorectal cancer patients.

#### P 189

##### PROGNOSTIC SIGNIFICANCE OF NUCLEOLAR ORGANIZER REGIONS (AGNORS) IN ADENOCARCINOMAS OF THE RECTUM

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The prognostic significance of silver-binding nucleolar organizer regions (AgNORs) was evaluated in tissue sections of biopsies from 45 primary adenocarcinomas of the rectum prior to their curative resection. AgNOR index was correlated with five-year survival rate and various morphological parameters of the primary tumors such as malignancy grade, tumor and lymph node stage. A significant correlation between five-year survival rate as well as pT categories and the mean AgNORs number per tumor cell was found. There was no significant relationship between AgNORs content and grade of malignancy or pN categories. The most valuable variable to predict a poor prognosis were pN, pT, and mean AgNORs index. The analysis of AgNORs may represent a useful pretherapeutic evaluation of the aggressiveness of the rectum adenocarcinoma.

#### P 190

##### RACK1 OVEREXPRESSION ASSOCIATES WITH HIGH GRADE AND LYMPH NODE METASTASES IN COLORECTAL CARCINOMAS

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Introduction: The receptor for activated C kinase 1 (RACK1) is a protein kinase C-mediated (PKC) signaling enzyme. RACK1 is an adaptor protein that contains many domains that mediate protein-protein interactions, anchors activated PKC isozymes and other signaling enzymes and regulates cell growth. The function and levels of expression of RACK1 may help elucidate signaling pathways leading to carcinogenesis and could result in the identification of novel therapeutic targets.

Purpose of the Study and Methods: RACK1 expression was evaluated in 116 colorectal carcinomas between 2001 and 2003 in a prospective study, to assess the clinical utility of the levels of expression of RACK1 protein. Tissue microarrays (TMAs) representative of normal mucosa and colorectal carcinoma of all cases were performed, including areas from

the centre and infiltrative margin of the tumor. When present, additional TMAs from metastatic lymph nodes or adenomas were done. In order to assure the representativity of the areas we included 3 cores of 1,2 mm of each selected zone. Positivity was scored using the Hscore (range from 0 to 300) resulting from multiplying the intensity (0 to 3) by the percentage of positive staining. RACK1 levels were determined by immunohistochemical staining, using a monoclonal antibody (BD610177). Results were correlated with clinical, histopathological parameters, and survival. In a subset of cases Western-blot was also performed.

Results: RACK1 overexpression was observed in 61'2% (71) cases. Overexpression of RACK1 correlated with poor differentiation (grade)  $p=0.036$  and presence of lymph node metastases ( $p=0.049$ ). No differences were observed between RACK1 expression and tumor localisation, stage or depth of tumor infiltration. Interestingly RACK1 expression in adenomas showed only 16'6% overexpression (2/12), compared to 70% in concomitant carcinomas ( $p=0.010$ ). No differences in RACK1 immunostaining were observed between the centre of the tumor and the infiltrative margin, neither between the primary tumor and metastatic lymph nodes ( $p=0.927$ ). Western-blot analysis confirmed the high levels of the protein. Disease-free survival was not related to RACK1 expression ( $p=0.356$ ).

Conclusions: We have found significant differences in expression of RACK1 in high grade and lymph node metastatic colorectal carcinomas. We propose that RACK1 expression can be a potential marker of worse prognosis in colon carcinoma and be used as a novel therapeutical marker.

#### P 191

##### SCREENING FOR ASSOCIATION OF HEREDITARY NON-POLYPOSIS COLORECTAL CANCER (HNPCC) WITH GENOMIC REARRANGEMENT IN HMSH2 OR HMLH1 GENES USING LEUKOCYTE-DERIVED GENOMIC DNA FROM PATIENTS

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##### Background & Objectives:

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant disease due to germline mutations of human mismatch repair genes (MMR), mainly hMLH1 and hMSH2. Colorectal cancers associated with the HNPCC syndrome usually present in younger patients, show loss of mismatch repair (MMR) gene expression, and exhibit microsatellite instability (MSI). About 12% of sporadic colorectal cancers also show MMR loss and MSI.

##### Methods & Materials:

To evaluate the status of genetic lesions in hMLH1 and hMSH2 MMR genes in the peripheral lymphocytes of the patients and find out a prognostic criteria for detection of those who are at high risk to develop the disease in future, 25 individuals diagnosed with HNPCC included in this study.

A DNA sample isolated from peripheral blood leukocytes obtained from each of these probands was examined for genomic rearrangement using the multiplex ligation-dependent probe amplification (MLPA) method. The probe mixture included probes for each of the 19 exons of the hMLH1 gene as well as for each of the 16 exons of the hMSH2 gene.

##### Results:

Seventy percent of the individuals (18/25) were shown to have a genomic rearrangement resulting in the deletion of one or more exons of one of these two genes. Family cancer histories predictive of a high risk of HNPCC significantly associate with a genomic rearrangement in hMSH2 or hMLH1.

##### Conclusions:

Based on our results it is concluded that using MLPA to identify genomic rearrangements in the above mentioned genes could be considered an economic first step in screening for HNPCC as an initial assessment of gross genomic integrity in leukocyte-derived genomic DNA. This would be especially valuable when individuals undergoing testing are identified by clinical history to be at high risk, as this approach would bypass the logistical difficulties of tumor analysis (assessment of microsatellite instability and immunohistochemistry).

#### P 192

##### THE ROLE OF P53,C-ERB-B2 AND BCL2 IN COLORECTAL TUMORS

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##### Introduction

Colorectal carcinoma is the most common gastrointestinal malignancy and one of the three most common causes of cancer death in western industrialized societies. An important role for P53 gene mutation and c-erb-B2 and bcl2 genes overexpression in the development of colorectal carcinoma had been described. Difficulties in the diagnosis of superficial endoscopic biopsies from papillary rectal neoplasms do exist and this study admits a suggested tool to help the pathologists in making their decision on these lesions.

##### Objective

The aim of the study is to evaluate the use of immunohistochemical stains of p53, c-erb-B2, and bcl2 in the diagnosis of papillary colorectal tumours in endoscopic biopsies and to determine their roles in the pathogenesis of colorectal carcinoma.

##### Materials and methods

Seventy endoscopic biopsies of colorectal tumours were selected. Fifty biopsies were colorectal adenocarcinomas, 10 were villotubular adenomas, and 10 were villous adenomas. Another 10 biopsies of normal and chronic inflammatory bowel disease were also selected. 4 – micrometer sections were prepared and stained with streptavidin –biotin immunohistochemical method using monoclonal antibodies against P53, c-erb-B2, and bcl2 (DAKO). One section of each biopsy was stained with H&E stain. Suitable positive and negative controls were used. Diaminobenzidine (DAB) was used as a chromogen.

The results were compared with surgical specimens of total colectomy and proctocolectomy.

##### Results

Forty two (84%) biopsies of colorectal carcinomas, one tubulovillous adenoma (10%) and two villous adenomas (20%) showed positive staining for P53 while no case of chronic inflammatory bowel disease or normal mucosa showed any staining for P53.

Forty eight (96%) biopsies of colorectal carcinoma, 6 (60%) tubulovillous adenomas, 7 (70%) villous adenomas, 3 (60%) of the chronic inflammatory bowel disease biopsies and 2 (40%) biopsies of normal colonic mucosa showed positive staining for c-erb-B2. Twenty eight (56%) biopsies of colorectal carcinomas, 4 (40%) tubulovillous adenomas, 6 (60%) villous adenomas, 2 (40%) chronic inflammatory bowel disease biopsies showed positive staining for bcl2. While no stain for bcl2 was noticed in normal intestinal mucosa biopsies.

##### Conclusions

P53 immunostaining is an important tool in the differentiation between benign and malignant papillary colorectal neoplasms in endoscopic biopsies especially when combined with c-erb-B2 and bcl2.

**P 193****THYROID TRANSCRIPTION FACTOR-1 IMMUNOREACTIVITY IN COLORECTAL CARCINOMA**

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**Introduction :** Metastatic adenocarcinoma of unknown primary origin is a common but perplexing problem. Among the useful immunohistochemical markers, thyroid transcription factor-1 (TTF1) has revealed to be a sensitive and specific marker for adenocarcinomas of lung and thyroid origin. Two patients with a multinodular liver without known malignancy underwent liver biopsies at Angers Hospital ; the liver specimens showed an adenocarcinoma CK7- CK20+ TTF1+. A colorectal cancer (CRC) was discovered in each patient but no lung tumor. The aim of this study was to investigate TTF1 immunohistochemical expression in CRC.

**Methods :** We studied TTF1 immunoreactivity of 53 consecutive cases of CRC diagnosed between May to December 2004 on coloscopic biopsies (8 cases), surgical resections (34 cases) or both (11 cases).

**Results :** A nuclear TTF1-reactivity was observed in 5 cases (9%) : 2 cases with strong reactivity in more than 50% of cells, 3 cases with weak and focal reactivity in less than 5% of cells. When biopsies and tumor were evaluated for the same patient, the immunophenotype was the same.

**Conclusion :** TTF1 is a useful but not specific marker for lung and thyroid cancer. It must be used with other immunohistochemical markers to guide the clinician toward the primary tumor.

**P 194****TUMOR HYPOXIA AND ACIDITY DRIVE STROMATOGENESIS AT THE INVADING TUMOR EDGE OF COLORECTAL CARCINOMAS**

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Stromatogenesis, i.e. the formation of new stroma at sites of tumour invasion for tumour invasion itself, occurs in complicity with host's peritumorous fibroblasts (Sivridis et al, 2004 Int J Surg Pathol 12: 1-9); it is a phenomenon of utmost importance for tumour growth and progression. In this study, we investigated the activation status of host's fibroblasts at the invading tumour edge, assessed as MIB1 proliferation index and thymidine phosphorylase (TP) expression. Our results were related to vascular density (VD), and certain qualities expected to occur in invading cancer cells, such as MIB1 proliferation activity, expression of TP, expression of endogenous markers of hypoxia (hypoxia inducible factor-1 $\alpha$  or HIF-1 $\alpha$ ) and acidity (lactate dehydrogenase 5 or LDH5). Standard immunohistochemical techniques were applied in a series of 150 colorectal adenocarcinomas. Normal fibroblasts confronting the tumour edge showed a median MIB1 index of 2%, significantly more than the 0.3% growth fraction found in normal submucosal fibroblasts and significantly less than the median MIB1 index of 40% estimated in cancer cells. Normal peritumorous fibroblasts with a proliferation rate above median were virtually loaded with TP and supported by an increased vascular network. Cancer cells confronting these fibroblasts were characterized by a high MIB1 proliferative index, high HIF1 $\alpha$  and LDH5 reactivity, and a clear trend to extramural extension. All these associations were statistically

significant. It is suggested that an activated fibroblastic status at the invading tumor front, sets the stage for stromatogenesis and new blood vessel formation, facilitating deep transmural invasion in colorectal adenocarcinomas. This complicity of peritumorous fibroblasts in the overall aggressiveness of the colorectal tumours, occurring within the framework of cancer-stromal cell interactions, is apparently favoured from the altered micro-environmental conditions of hypoxia and acidity.

**P 195****HEDGEHOG PATHWAY IN COLONIC LESIONS**

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Colorectal cancer is one of the major forms of cancer in industrialized countries. About 10% of the colorectal cancer cases have a hereditary basis, including defined syndromes like familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC). The remaining 90% of colorectal cancers occur sporadic by spontaneous mutation of single genes.

Hedgehog signaling is a main regulation cascade in embryonic differentiation of the whole gastrointestinal tract and mutations of some components have been associated with birth defects like gut malrotation, imperforate anus or intestinal transformation of the stomach. In addition to their function in the embryo, the Hedgehog pathway members are present in postnatal and adult tissues including stomach, pancreas, small intestine and colon. Therefore unusual expression of Hedgehog components has been associated with benign and malignant lesions of stomach and pancreas, leading to our current investigations.

In the present study we examined the presence of the Hedgehog pathway in normal human colon using immunohistochemical methods. The antibodies used are directed against the majority of the components involved in the Hedgehog signaling cascade, including the ligands Sonic, Indian and Desert Hedgehog, the transcription factors Gli1, Gli2 and Gli3 as well as the receptor Patched. Additionally, 14 cases of benign and 14 cases of malignant lesions of the human colon have been investigated to prove alteration of the expression pattern. Malignant lesions of the colon mostly comprise adenocarcinoma and benign lesions comprise tubular, villous and tubulo-villous adenoma and hyperplastic polyps. Up- or down-regulation of the Hedgehog pathway has been examined.

In the normal colon tissue we detected expression of the ligands Sonic (Shh) and Desert Hedgehog (Dhh) within the lining epithelium, as well as the receptor Patched and the transcription factors Gli1 and Gli2 along the whole colonic crypts. Within all benign lesions a positive staining of Shh, Dhh, Gli1, Gli2 and Ptch was detected, but expression was not consistently distributed within the lesions than rather restricted to single cell aggregates. Some malignant lesions also displayed the same unique staining pattern, though Hedgehog activity was not detected in all malignancies examined. We conclude that the Hedgehog signaling cascade may be involved in the normal physiological renewing process of the colonic lining.

**P 196****APPLICATION OF SPECIFIC ANTI-SENSE OLIGONUCLEOTIDS AGAINST HUMAN TELOMERASE RNA (HTR) ON CCL-247 COLON CANCER CELL LINE**

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**Background & Objectives:** Inhibition of telomerase activity is one of the most promising targets for cancer therapy, because telomerase activity is present in most malignant cells but undetectable in most normal somatic cells. Unlike an ordinary enzyme, telomerase has RNA and a protein component that are both necessary for telomerase activity. We targeted the RNA component, the telomerase RNA (HTR), which contains the template for the telomere sequence. It is shown that the majority of gastric cancers express high levels of HTR that is essential for cellular survival. In this study, we evaluated the ability of anti sense oligonucleotides against HTR (anti-HTR) to inhibit telomerase activity in human colon cancer cells.

**Methods & Materials:** Thio-phosphoramidated anti-sense oligonucleotides against HTR were transfected into the CCL-247 colorectal cancer cell line using FUGENE6-mediated DNA transfection technology. Different concentration of anti-HTR was applied and cell growth and cell cycle parameters were analyzed. Telomerase activity was measured by TRAP assay and the apoptotic features were studied by PI and Hoechst 33258 staining. Control oligonucleotides and normal fibroblast were used as control.

**Results:** Anti-sense oligonucleotides against HTR were successfully transfected to the cells and reduced the cell viability to 28%. This treatment also inhibited the telomerase activity of the cells by 70%, in a dose-dependent manner. The apoptotic cells rate was also increased to 33%.

**Conclusions:** Our results demonstrated that application of anti-HTR is a powerful approach that can inhibit telomerase activity in colon cell line and therefore probably have the potential to be considered for anti-cancer therapy.

#### P 197

##### **LABEL-FREE ANALYSIS OF MICROSATELLITE INSTABILITY IN COLORECTAL CARCINOMA BY ON-CHIP ELECTROPHORESIS.**

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Microsatellite instability (MSI) is caused by a failure of the DNA mismatch repair system and occurs frequently in various types of cancer. Since sporadic MSI are associated with approximately 10 to 15% of colorectal, gastric or endometrial carcinoma and impact clinical prognosis, MSI analysis is an important tool in clinical research and molecular diagnostics.

**Aim:** Given that conventional techniques used for MSI detection – e.g. polyacrylamide gel electrophoresis (PAGE) or capillary electrophoresis - turned out to be laborious or expensive, this study aimed to develop a simple and efficient procedure of MSI detection.

**Methods:** Forty cases with no (26 cases) or with MSI (14 cases) were selected out of a panel of 150 patients with colon carcinoma. Amplicons derived from areas of non-tumor (N) and tumor (T) tissues were analyzed by microfluidic based on-chip electrophoresis on the Agilent 2100 bioanalyzer.

**Results:** The results presenting a pattern of five microsatellite loci were compared with the findings obtained by fluorochrome-associated PAGE technology. In all cases, label-free microfluidic separation of the PCR amplicons resulted in highly resolved, distinct patterns of each of the five microsatellite loci. Detection of MSI could be demonstrated by microsatellite loci-associated, well defined deviations in the electropherogram profiles of tumor and non-tumor material and confirmed the classification of the MSI cases performed by conventional technology (95% concordance rate).

**Conclusion:** The presented data demonstrate that microfluidic chip technology is a simple, reliable and robust technology for

MSI detection, which allows label-free analyses of microsatellite amplicons within 30 minutes.

#### P 198

##### **ANTIBIOTIC-ASSOCIATED HEMORRHAGIC COLITIS CAN BE INDUCED BY KLEBSIELLA OXYTOCA IN AN IN VIVO RAT MODEL**

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##### **Introduction**

Antibiotic-associated hemorrhagic colitis (AAHC) is a serious side effect of antibiotic treatment occurring mainly with broad-spectrum penicillins. Its cause is unknown, however, *Klebsiella oxytoca* can be found in the stool of affected patients, whereas *Clostridium difficile* is absent. Concomitant intake of non-steroidal anti-inflammatory drugs (NSAIDs) by patients is common.

##### **Methods**

A strain of *K. oxytoca* resistant to amoxicillin-clavulanate (AC) was isolated from the stool of a patient with AAHC. Sprague Dawley rats received either *K. oxytoca*, AC and indomethacin [A], AC and indomethacin [B], *K. oxytoca* and AC [C], *K. oxytoca* [D], indomethacin [E] or AC [F]. Four days after exposure to *K. oxytoca* the animals were sacrificed and the colon was analyzed for presence of inflammation, epithelial damage, mucosal hemorrhage, submucosal edema and erosions applying an arbitrary histological score (0-3 points).

##### **Results**

Histological changes were most prominent in the right-sided colon while changes in the distal colon were less severe. Inflammation was seen only in group [A] and [C] animals with median scores of 1.4 and 1.0, respectively. Marked epithelial damage was seen in group [A] and [C] animals with median scores of 1.7 and 1.3, respectively, whereas the animals in the other four groups showed only focal epithelial alteration (median scores 0.2-0.3). Mucosal hemorrhage was again noted in group [A] and [C] animals only with median scores of 1.0 and 0.6, respectively. Submucosal edema was prominent in group [A] and group [E] animals with median scores of 2.2 and 2.3, respectively. However, mild changes were also seen in groups [B], [C] and [E] with median scores of 0.5-1. Finally, severe erosive changes were detected in group [E] animals (median score 2.5), and group [A] animals (median score 1.4), while in groups [B] and [C] mild changes (median scores 0.1-0.2) were found.

##### **Conclusions**

*K. oxytoca* isolated from a patient with AAHC induces a predominantly right-sided hemorrhagic colitis in an in vivo rat model. The administration of NSAIDs enhances the severity of disease.

#### P 199

##### **INVESTIGATION OF CMV INFECTION AND ANGIOGENESIS IN PATIENTS WITH REFRACTORY CHRONIC ACTIVE ULCERATIVE COLITIS**

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**OBJECTIVE:** A number of cases with chronic ulcerative colitis (UC) are resistant to therapy. Cytomegalovirus (CMV) infection has been reported to be a cause of it. Besides, angiogenesis has also been reported to have a potent role in the course of the disease. This pilot study investigates the

impact of both CMV infection and angiogenesis in patients with refractory UC.

**PATIENTS AND METHODS:** Twenty patients with UC were studied (ten with chronic active and ten with chronic quiescent form). Six of the cases were resistant to steroid, 5ASA and/or azathioprine therapy. All cases were investigated for the presence of CMV both morphologically and immunohistochemically. Angiogenesis was evaluated quantitatively by measuring the number of newly formed vessels expressing CD34 and VEGF in sections of the bioptic material. Measurements were performed through an ocular millimetric grid at 400X magnification, by two different observers and were expressed as microvessels per square millimeter. Ten intestinal biopsies with normal findings were used as a control group for the above investigation.

**RESULTS:** There were no viral inclusions in H&E stained sections nor was the presence of CMV detected immunohistochemically in any of our cases. Microvessel density (MVD) was increased in all the cases with chronic active disease compared to both the quiescent form and the control groups ( $p < 0.05$ ). Furthermore all patients with refractory UC showed higher MVD within the lamina propria of the inflamed mucosa compared to the responding to therapy cases.

**CONCLUSIONS:** The above results do not indicate that CMV infection is responsible for resistance to therapy in our cases of refractory UC. We however suggest that a deficient response to therapy in chronic active UC may be due to increased angiogenesis which, together with other factors, seems to play an important role in the mechanism of tissue inflammation, ulceration and regeneration in UC

#### P 200

##### **DIFFUSE ENDOCRINE SYSTEM AND LYMPHOCYTE SUBSETS IN COLONIC MUCOSA IN ULCERATIVE COLITIS**

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The diagnostics of ulcerative colitis (UC) is based on a complex of methods with obligatory colonoscopy and colon biopsy. The diagnostics is difficult, UC remains largely the diagnosis of exclusion. So, thorough evaluation of all the components of the mucosa could make the diagnostics more reliable. Cellular lining of the mucosa carries out multiple functions including antigen transfer to MALT of the gut and certain endocrine activity. Little attention has been paid to possible role of APUD elements of the mucosa in progression of the disease and their interaction with lymphocytes of the lamina propria.

**THE OBJECTIVE** of the present investigation was to study the lymphocyte subsets and endocrine cells in the colonic mucosa with special reference to EC-cells.

**MATERIAL, METHODS AND RESULTS.** 141 colon biopsies (3 from each of 47 patients with UC - from macroscopically inflamed and non-inflamed bowel) were studied by means of conventional morphology, morphometry and immunohistochemistry. Patients were placed in 3 groups, varying from remission to exacerbation, according to the degree of activity of the inflammation, judged by lymphocyte and leukocyte count. Endocrine cells were revealed by argyrophilic reaction as well as with antibodies against chromogranin A, serotonin (Dako Cytomation, Denmark). Differential lymphocyte subset count was performed in slices immunostained for CD20, CD8 (Dako Cytomation, Denmark), CD3, CD4 (Novocastra Lab, UK).

The total population of endocrine cells was increased in inflamed bowel, especially in exacerbation, with EC-cells comprising the majority of the population (up to 70%). T/B ratio showed considerable decrease, CD4/CD8 appeared to be slightly decreased. Statistical analysis showed negative strong

correlation between total lymphocyte count and total population of endocrine cells in remission, in exacerbation the correlation was strong and positive. CD8+ lymphocyte count was high in both inflamed and non-inflamed bowel in exacerbation of UC.

**IN CONCLUSION** we speculate that endocrine cells of the bowel may be involved in the pathogenesis of UC.

#### P 201

##### **RADIATION COLITIS: A DETAILED HISTOPATHOLOGIC STUDY AND CORRELATION WITH ENDOSCOPIC FINDINGS**

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**Introduction:** Ionizing radiation is known to cause radiation colitis (RC). The histopathological features of irradiation-induced early and late changes in bowel mucosa have not been analyzed in detail

**Aim:** The aim of the study was to characterize the histopathologic features of radiation-induced colitis and to correlate them with the endoscopic findings.

**Patients-Methods:** Twenty-three patients (12 females, 11 males; median age 63 years), suffering from rectal, cervical or prostate cancer were enrolled in the study and were followed up for a median of 13.5 months (range 6-32 months). All patients were treated by a linear accelerator (6 MV, box technique), with a median daily radiation dose of 1.9 Gy and a mean number of fractions, 28. They also underwent three endoscopies (sigmoidoscopies): before initiation of radiotherapy, immediately after the end of radiotherapy and at least six months after the end of radiotherapy. During sigmoidoscopy at least three colonic mucosa biopsies were taken. A new endoscopic grading system based on the RTOG/EORTC late radiation morbidity scale for large intestine and on endoscopic grading systems for inflammatory bowel disease was used for endoscopic grading of bowel mucosa. Clinical diagnosis of RC was based on patient symptoms, on sigmoidoscopy findings and on laboratory tests.

**Results:** Based on the histological features noted, the cases were allocated to one of four groups: no changes, acute injury, early regenerative changes and late regenerative changes. In total 12/23 patients (52.2%) were diagnosed with radiation colitis (RC). Acute radiation colitis was diagnosed in 4/23 patients (17.4%). None of these cases exhibited "eosinophil crypt abscesses." The correlation of histologically defined phases of RC and clinical/endoscopic findings was poor.

**Conclusion:** Radiation colitis is very frequent in patients irradiated in the pelvic area. The discordance between clinical/endoscopic and histopathologic findings could reflect the absence of relation between the presence and extend of histologically-defined radiation colitis and radiation-induced morbidity and mortality. Furthermore, it strengthens the need for a more global concept of radiation-induced bowel injury, incorporating bowel symptoms, performance status, radiation characteristics (dose-duration-timing), a new endoscopy grading system, a detailed histological analysis, laboratory tests and hospitalization.

#### P 202

##### **FULMINANT CYSTIC FORM OF PNEUMATOSIS COLI ASSOCIATED WITH KAPOSI'S DISEASE IN A MAN WITH A KIDNEY GRAFT.**

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We report a first case of fulminant cystic form of pneumatosis coli associated with Kaposi's disease. A 57 year-old-man with Kaposi's disease (skin and lymph nodes), who had a kidney transplantation five years before because of nephroangiosclerosis, suffered from a pyelonephretic syndrome. TDM revealed a wide parietal pneumatosis coli. A right ileocolostomy was performed. Grossly, pneumatosis coli appeared as round, polypoid lesions covered by normal mucosa. It was associated with several black spotty maculas. Microscopic analysis demonstrated multiple empty spaces in the submucosa lined by histiocytes. The maculas were Kaposi's lesions anti-HHV8 +.

Pneumatosis is primary in only 15% of cases. The other cases are secondary to various predisposing conditions. The fulminant form of pneumatosis coli rarely occurs in adults, in whom it is associated with drugs, chemotherapy or with ischemic and pseudomembranous enterocolitis. Pneumatosis coli is also described in acquired immunodeficiency syndrome (AIDS). Such cases have been reported to follow a benign course, even when associated with infectious colitis. Benign form of pneumatosis coli resolves spontaneously in up to 50% . Rare cases of pneumatosis coli require immediate surgery.

#### P 203

##### THE EFFECT OF LAMIVUDINE TREATMENT ON THE PROGRESS OF HBE NEG-ANTI HBE POS CHRONIC HEPATITIS B (CHB). A THREE YEARS STUDY.

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Introduction: Lamivudine is an effective drug for the treatment of CHB. Its long term effect on the activity and stage of CHB is still under investigation as it may induce viral mutations responsible for drug resistance and recurrence of inflammation.

Purpose of the study: A multicenter clinicopathological study of three years duration was undertaken in order to assess the long term effect of Lamivudine in patients with Hbe neg- anti Hbe pos CHB.

Materials: 137 patients entered the study (115• %, 22• Š) age 46.7+/- 11.7 years . A liver biopsy was performed at zero time (124/137), 12 months (109/124) and 36 months (39/124). HBV DNA serum levels were annually investigated.

Methods: Histological grade and stage were diagnosed using Ishak• fs classification system. Hbs and Hbc antigens were immunohistochemically detected, their topography was assessed and a semiquantitative method was used for evaluating the extent of their expression in hepatocytes. Statistical analysis was performed with the Wilcoxon Signed Rank Test and Mc Nema• fs chi Square statistic for alterations of grade, stage, Hbc, Hbs and serum HBV-DNA at zero time, 12 and 36 months (ms).

Results: Low HBV-DNA levels were sustained in 77% of patients at 36 ms (p=0.003) and they were unrelated to grade, stage or Hbs expression but with the extent of Hbc expression. There was statistically significant reduction of grade at 12 ms (p=0.0001) which was not altered at 36 ms. The stage of the disease remained steady throughout the study period with the

exception of 3 cases with cirrhosis at 12 ms which returned to stage 3-4. There was marginal statistical significance of Hbc and grade (p=0.067) at zero time, which became significant at 12 and 36 ms (p=0.035, p=0.006 respectively). The quantity of Hbs expression was not altered throughout the study but an increase in submembranous localization was observed at 12 ms (p=0.035) with subsequent stabilization.

Conclusion: Lamivudine is a very effective drug for the treatment of CHB patients. It reduces the necroinflammatory response (grade), stabilizes the fibrotic process (stage) and results in sustained low HBV-DNA serum levels.

#### P 204

##### SIGNIFICANCE OF CORRELATION BETWEEN SEROLOGICAL MARKERS OF HEPATITIS B AND PATHOLOGY AND ULTRASTRUCTURAL FINDINGS

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Needle biopsy of liver as reliable method in evaluation of disease activity with serological markers of Hepatitis B infection enable more detailed realization of histopatologic diagnosis. This study includes light microscopy (LM) and electron microscopy (EM) analyses of needle biopsy of 101 patients with Hepatitis B. Results of this analysis were compared with the level of serologic markers and antigens of HBV infection, which were collected contemporary with the biopsy. Analyzing of biopsy specimens demonstrated further types of lesions in liver tissue: minimal lesion (ML) in 10 biopsies and acute hepatitis (AH) in 17; chronic persistent hepatitis (CHP) in 51 biopsies; chronic active hepatitis (CAH) in 20 biopsies and cirrhosis (CH) in 3 biopsies. By recognition of pathohistological finding and correlation with serological markers of HBV infection we found that histology findings with advanced (high grade) necrosis in AH and CAH most frequently demonstrated the presence of HBeAg in distinction to the other antigens and antibodies of HBV infection, which were present with significantly lower quantity. In addition, the EM analysis in these morphologic categories most distinct demonstrated the ultrastructural lesions, particularly affection of the nucleoli of hepatocytes in the presence of HBeAg. This serological marker represents the viral infectivity factor and manifests the viral replication and hepatocyte destruction. This study confirms a direct proportion between hepatocyte necrosis, presence of HBeAg and changes in hepatocyte nucleoli, and therefore it contributes the recognition of pathogenesis of virus B hepatitis.

#### P 205

##### CORRELATION BETWEEN TGF-BETA1, VEGF, HGF, EGF, TGF-ALPHA AND FGF SERUM LEVELS AND NECROINFLAMMATORY ACTIVITY AND FIBROSIS IN CHRONIC HEPATITIS C.

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Introduction: Liver fibrosis can be a result of chronic inflammatory processes of different etiology. The meaning of fibrosis is the accumulation of extracellular matrix components in liver parenchyma. This process is the result of disturbed balance between extracellular matrix protein

synthesis and degradation. It is supposed that growth factors taking part in classic 'wound healing' reaction may play the meaningful role in the similar process in the liver and their serum levels may reflect the intensity of liver lesions.

**Aims:** The aim of the study was evaluation of the mutual correlation between TGF-beta1, VEGF, HGF, EGF, bFGF, TGF-alpha levels and inflammatory activity grade and fibrosis stage, as an attempt to assess the role of growth factors in chronic hepatitis and fibrosis formation.

**Methods:** Study group included 100 chronic hepatitis C (CHC) patients (52 men and 48 women, mean age 42) with fibrosis (Scheuer score: inflammatory activity 1-4 points, fibrosis 1-4 points). Control group consisted of 30 HCVAb positive subjects (16 men and 14 women, mean age 41) with normal ALT level, none or minimal necroinflammation (Scheuer: 0 points) and without fibrosis (Scheuer: 0 points). Serum levels of TGF-beta1, VEGF, HGF, EGF, bFGF, TGF-alpha were measured by immunoenzymatic method. Correlation between studied growth factors levels and inflammatory activity grade and fibrosis stage was evaluated.

**Results:** Levels of TGF-beta1, HGF and TGF-alpha were significantly higher in study group than in control group (TGF-beta1 35,89 vs 32,37 ng/ml,  $p=0,023$ ; HGF 1506,34 vs 1017,11 pg/ml,  $p=0,034$ ; TGF-alpha 1,85 vs 0,26 pg/ml,  $p=0,0001$ ). The significant negative correlation between VEGF, bFGF and TGF-alpha serum levels and the grade of inflammatory activity was observed (VEGF  $R=-0,40$ ,  $p<0,01$ ; bFGF  $R=-0,40$ ,  $p<0,05$ ; TGF-alpha  $R=-0,34$ ;  $p<0,05$ ). Only VEGF level correlated negatively with the stage of fibrosis ( $R=-0,30$ ,  $p<0,05$ ).

**Conclusions:**

- 1) HGF and TGF-alpha may play the role in chronic inflammation and liver fibrosis formation as well as TGF-beta1, which is well known fibrogenetic factor.
- 2) Increasing inflammatory activity is connected with decrease of VEGF, bFGF and TGF-alpha levels. All these factors have angiogenetic potential. It may suggest that liver tissue inflammation is connected with impaired angiogenesis. Levels of VEGF were also lower in patients with advanced fibrosis and cirrhosis.

#### P 206

##### IMPACT OF HEPATITIS C VIRUS PROTEINS ON HEPATIC PROLIFERATION

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The development of the liver carcinoma during chronic Hepatitis C is barely known. Mice transgenic for the full Hepatitis C Virus (HCV), which we have bred, develop hepatocarcinoma without immune disease. That suggest that HCV proteins are sufficient to induce the liver carcinogenesis. The aim of this work was to study the role of hepatocytic proliferation in carcinogenesis during infection with HCV. The liver is the only organ that can regenerate by multiplication of hepatocytes after hepatectomy at least 70%. **Materials:** 16 C57Bl6 male mice transgenic for the full HCV open reading frame (FL-N/35 lineage): HCV+, and 15 control mice: HCV-, were used. 27 animals underwent hepatectomy of 75% (12 HCV+, 15 HCV-) and were compare to 4 mice which underwent a white laparotomy. The anaesthesia used was an association of Ketamine-Rompin (1mg/kg). Animals were sacrificed at 4 points of kinetic (1, 4, 18 and 48 hours after hepatectomy). The evaluation of hepatocytic proliferation was analysed by 3 methods: a-quantification of the weight of liver after death of mouse compared without weight considered left in place after hepatectomy. b-

Immunocytochemistry detection of BrdU after nuclear incorporation. c-transcriptional analysis by Dot blot technical of 9 genes whose expression during hepatic regeneration is known (early expression: c-fos, c-myc, MKP1; delayed expression: p53, p21, mdm2, gadd45; late expression: cyclin A and E). **Results:** liver regeneration were incomplete 48 hours after partial hepatectomy in the mice HCV+ and HCV-. The liver weight quantification and nuclear marking with BrdU reveals a delay of regeneration of HCV+ hepatocytes. Cyclin A, 48 hours after hepatectomy, is the only gene to have been activated in HCV+ hepatocyte compared to control mice and HCV- mice. The difference transcriptional expression of cyclin A between HCV+ and HCV- animals is compatible with an entry of HCV+ hepatocyte delayed in phase S. **Discussion:** The delay of liver regeneration in mice HCV- observed in our study is different from the data in the literature. That can be explained by the hepatotoxicity of anaesthesia. Indeed we observed an early transitory toxic microvacuolar steatosis in all mice. No excessive proliferation of hepatocyte were observed in HCV+ mice. Besides our results suggest delay of liver regeneration in HCV+ animals. These results could be the direct consequence of HCV proteins expression in liver.

#### P 207

##### THE POTENTIAL ROLE OF STELLATE CELL ACTIVATION IN LIVER BIOPSIES WITH HEPATITIS C COMPARED WITH HEPATITIS C/DIABETES MELLITUS

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**Background:** Hepatitis C virus (HCV) infection is a major cause of chronic liver disease. Steatosis is a common finding both in HCV and in diabetes mellitus (DM). Hepatic stellate cell activation, manifested by the expression of smooth muscle actin ( $\alpha$ SMA), is a major factor responsible for fibrosis and its progression in chronic liver disease. In this study we assessed and compared the degree of stellate cell activation in liver biopsies of patients with HCV alone and in patients with HCV/DM.

**Patients and Methods:** A total of 50 liver biopsies were evaluated: HCV (n=35), and HCV/DM (n=15). All biopsies were immunostained with the antibody for smooth muscle actin ( $\alpha$ SMA). A grading system on a scale of 0-3 was applied reflecting the percentage of stellate cell activation which occurred in each zone of the hepatic lobule. Statistical analysis (t-test and Wilcoxon rank-sum test) was implemented to determine if stellate cell activation differed at a given stage of fibrosis, grade of inflammation and steatosis in comparing the aforementioned groups.

**Results:** The mean stage of fibrosis for the HCV/DM and HCV group were 2.8 and 1.6 respectively ( $p<0.0001$ ). The mean grade of inflammation for the HCV/DM group and HCV group were 1.9 and 1.5 respectively ( $p=0.03$ ). The mean grade of steatosis for the HCV/DM and HCV group were 1.1 and 0.5 respectively ( $p=0.01$ ). The average grade of stellate cell activation for all zones for the HCV/DM group and HCV group was 1.3 and 1.2 respectively ( $p=.39$ ). However, in both diagnostic groups combined, a significant stellate cell activation was observed in periportal areas (zone 1) and portal tracts for higher stages ( $>2$ ) vs lower stages ( $<2$ ) of fibrosis ( $p<0.0001$ ). In addition, a statistically significant increase in stellate cell activation was observed in zone 1 for the HCV/DM group (mean=1.5) as compared to the HCV group (mean=1.2) regardless of stage of fibrosis ( $p=0.02$ ).

**Conclusions:** The results of this study imply that the activated stellate cells are predominantly present in periportal areas (zone 1), which correlates with advanced stages ( $>2$ ) of fibrosis. Periportal stellate cell activation is significantly

increased in the HCV/DM group as compared to the HCV group.

#### P 208

##### **THE ROLE OF SIGNALING PROTEINS STAT, JAK, SOCS AND PIAS IN RESPONSE TO COMBINED INTERFERON THERAPY FOR CHRONIC HEPATITIS C.**

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The currently used standard treatment for chronic hepatitis C using a dual combination of IFN alpha/RBV is only successful in 50% cases. With the exception of some clinical and biochemical factors, degree of inflammation (grading) and degree of fibrosis (staging), there are no other known markers which may serve as valid predictors of response to therapy. Interference of HCV with signaling pathways modulated by JAK-STAT, ERK1/2, NF-kappa B and MAP proteins is one mechanism which may influence the interaction between HCV and interferon. These proteins regulate different cell processes such as activation of cytokines, activation of apoptosis, regulation of cell proliferation etc. Therefore, it is possible that impaired signaling or inhibition/dysregulation of some of these proteins by HCV infection may cause resistance to IFN alpha treatment.

The aim of study was immunohistochemical assessment and analysis of expression of STAT 2, 3 proteins, their inhibitors SOCS 2, 3 and PIAS 3 and proteins JAK 1 and ERK1/2 in liver biopsies of 23 patients with chronic hepatitis C treated by dual combination IFN alpha/RBV and subsequent correlation of the results of immunohistochemical analysis (histoscore) with histological picture and clinical response to treatment.

The results show increased expression of STAT 3, STAT 2 and ERK 1 proteins and decreased expression of SOCS 3 and SOCS 2 in hepatocytes of patients with more marked inflammation and fibrosis. In patients with sustained virological response there was increased expression of SOCS 3 and JAK 1 and decreased expression of SOCS 2. Relapse was associated with increased expression of SOCS 3 and PIAS 3. However, owing to the small sample size, the results only approximated statistical significance, but we suggest that proteins of STAT family and their inhibitors SOCS and PIAS probably play an important regulatory role during response to treatment for chronic hepatitis C

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#### P 209

##### **SPECTRUM AND INCIDENCE OF SYSTEMIC MANIFESTATIONS IN CHRONIC HEPATITIS C OF LOW ACTIVITY.**

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The aim of the present study is to evaluate extrahepatic manifestations of chronic HCV-infection with low grade and stage. In 58 patients with chronic hepatitis C (CHC) liver biopsy tissue specimens were histologically investigated. The spectrum of changes from mild piecemeal necrosis in few portal areas, mild or moderate lobular necrosis to mild or moderate portal inflammation and fibrous portal expansion was defined. Grading was done according to Knodell scoring system, and staging - according to Scheuer system. The histology activity index fell between 3 and 8. The stage was 1 in all cases. Systemic manifestations were observed in 23 patients. In 12 of them they were associated with cryoglobulinemia. Systemic manifestations included

cryoglobulinemic vasculitis with skin and joint involvement, cryoglobulinemic glomerulonephritis, arthralgia without cryoglobulinemia. Independent risk factors for development of systemic disorders in CHC of low activity comprised gender, age over 45, the disease duration and presence of cryoglobulinemia. CHC with low activity is characterized by high occurrence of systemic manifestations primarily associated with cryoglobulinemia.

#### P 210

##### **ULTRASTRUCTURAL EVIDENCES OF THE LYMPHOCYTE APOPTOSIS IN CASE OF HEPATITIS C VIRUS INFECTION**

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Recent studies do not provide a definite answer on whether the effect of hepatitis C virus infection (HCV), with respect to other etiologic factors of liver damage, is more an up- or a down-regulation of apoptotic process. An electron microscopy still remains a significant contributor to the study of events in apoptosis. Cell membrane blebbing and nuclear chromatin changes are hallmarks of the apoptotic cell death. This study aimed an ultrastructural characterization of the intralobular lymphocyte involvement in apoptotic process along the course of an acute HCV.

The liver tissue biopsies obtained from patients (11) with an acute HCV were processed for conventional transmission electron microscopy and further analysis of apoptotic events taking place within the liver lobule. The intrahepatic lymphocytes distributed within the sinusoids lumen, sinusoidal wall, perisinusoidal space, and along the intercellular space between the hepatocytes were selectively estimated in this study.

A prominent condensation of lymphocyte nuclear chromatin, compaction of cytoplasmic organelles, blebbing, and fragmentation into a great number of rather small apoptotic bodies were constantly present within the lumen of sinusoids. Macrophages detected ultrastructurally and using an appropriate (CD 68) immunohistochemical marker were detected in the close vicinity to the apoptotic bodies. Paired lymphocytes were rather often in the perisinusoidal space. Lymphocytes apoptotic changes were much more less common in this area comparing with the sinusoids lumen. Lymphocytes invading the intercellular spaces between the hepatocytes were single and paired, in some cases invading lymphocytes extended deep enough toward the bile canaliculi. Many of them caused a prominent depression on the hepatocyte surface, and expansion of the intercellular space. Some of these lymphocytes showed a prominent nuclear chromatin condensation, compaction of cytoplasm, and blebbing. Detection of the hepatocyte apoptosis was rare, and was restricted to the cell cytoplasmic shrinkage.

We can conclude that electron microscopy used in this study provides the evidences on the cellular behavior during apoptosis, and reveals the peculiarities in the lymphocyte distribution, type of invasion and cell death events along the acute HCV.

#### P 211

##### **IMPACT OF APOPTOSIS ON THE SEVERITY OF CHRONIC HEPATITIS C**

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Realizing that chronic hepatitis c (CHC) is one of the important health problems in Egypt, this work is designed to study the impact of apoptosis on the parameters concerned with the severity of the disease. The study involved 57 patients diagnosed as pure CHC, according to full clinical history, examination and laboratory investigations to exclude any other associated pathology. All patients were subjected to pelvi-abdominal sonography, upper endoscopy, rectal snips, liver needle biopsies, serological tests for viral markers and liver functions. Immunohistochemical (IHC) studies using both the monoclonals; anti-fas and anti p53 mutant gene were performed on liver sections. Five control cases were included. Electron microscopic (EM) study for a sample of 8 cases was performed.

Apoptosis was focally demonstrated in liver sections by Hx and E stain and via IHC technique by detecting the localization and distribution of Fas antigen (for apoptosis) within the liver tissue. Pathologically patients were classified according to the grades of inflammatory activity into 3 classes; patients with mild inflammatory activity (Gr 1); 38.6%, patients with moderate inflammatory activity (Gr 2); 29.8% and those with severe inflammatory activity (Gr 3); 31.6%. Clinicopathologically; significant elevation of ALT and AST levels were detected with Grs 2 and 3 inflammatory activity compared to both control and Gr 1, inflammatory groups. Fas positivity within hepatic tissue sections was found significantly correlated with the increasing grades of inflammatory activity, stages of fibrosis, serum ALT, AST, ALP, Albumin and HCV-RNA viremia levels in all the studied patients. EM study identified early and advanced ultrastructural hepatocytic apoptotic changes. P-53 was over expressed in 29.8% of the studied liver cases and showed significant direct correlation with the increase in inflammatory activity.

It was concluded that apoptosis through Fas antigen is a main mechanism of liver cell injury in Egyptian pattern CHC and is in parallel correlation with its biological markers and grades of inflammatory activity. Hepatocytes in variable stages of apoptosis could be localized in periportal areas by fas antigen, light microscope and EM. Mutant p53 is over expressed in the precocious stages of CHC-related liver damage before carcinogenesis. We suggest the term: "apoptotic activity" instead of 'necro-inflammatory' activity, in describing CHC interface areas.

**P 212**  
**HEPATIC EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN CHRONIC HEPATITIS C AND SCHISTOSOMAL LIVER DISEASE**

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Immunohistochemical hepatic tissue expression of endothelial nitric oxide synthase (eNOS) that has important function in inflammation and vasoregulation, and vascular endothelial growth factor (VEGF) which plays a role in new vascularization in liver tissue of patients with chronic hepatitis C (n=25), schistosomal liver disease (n=6), and mixed schistosomal and chronic hepatitis C infection (n=7) were semiquantitated and correlated to hepatic histological features of inflammation and fibrosis. Control liver tissue (n=5) were collected during cholecystectomy. In control liver eNOS and VEGF were expressed in endothelial cells lining sinusoids and portal vessels and in hepatocytes. Hepatic tissue with chronic hepatitis C or Schistosomal liver disease over expressed both eNOS and VEGF, this over-expression was insignificant versus control group and between each other. Hepatic tissue with concomitant chronic hepatitis C and

Schistosomiasis had significant endothelial over-expression of eNOS versus livers with chronic hepatitis C (p >0.05) and control liver (p >0.01), and of VEGF versus livers with chronic hepatitis C (p >0.05). Endothelial expression of eNOS and hepatocytic expression of VEGF significantly raised in higher degree of inflammatory activity and in higher fibrotic stage (p >0.05) in hepatic tissue of chronic hepatitis C (with or without Schistosomal co-infection).

In conclusion: endothelial NOS and VEGF over expressed in chronic hepatitis C and in Schistosomal liver disease without definite correlation between their expression suggesting different regulatory mechanisms. Endothelial NOS is expressed mainly by endothelial cells and to a lesser extent by hepatocytes which are the main cells expressing VEGF. Coincidence of Schistosomiasis with chronic hepatitis C accentuates over expression of both eNOS and VEGF proteins indicating that Schistosomiasis is a risk factor for haemodynamic disturbance in chronic viral hepatitis.

**P 213**  
**POSITIVE CORRELATION BETWEEN IGF-IR EXPRESSION AND EXTENSION OF PARENCHYMAL LESIONS IN CHRONIC HEPATITIS C**

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The Insulin-Like Growth Factor (IGF) system is an attractive target of study, since it may be modified by liver diseases and, reciprocally, it might play a relevant role in the evolution of some hepatic diseases. This system is comprised of two ligands, IGF-I and IGF-II, their specific receptors, IGF-IR and IGF-IIR, and the IGF-binding proteins. IGFs are peptides involved in proliferation, differentiation and inhibition of apoptosis of several cell types. IGF-IR was shown capable to mediate both IGF-I and IGF-II signalling, whereas IGF-IIR is only able to decrease the bioavailability of IGF-II. Although increased IGF-IR gene expression has been reported in hepatocellular carcinoma and in human hepatoma cell lines, studies assessing IGF-IR in chronic hepatitis C (CHC) and cirrhosis are scarce.

The objective of this study was to evaluate the hepatic expression of IGF-IR in patients with CHC. Thirty five patients with CHC were selected according to clinical, serological, and biochemical criteria. IGF-IR expression was determined by semi-quantitative RT-PCR and by immunohistochemical analysis of liver fragments obtained before treatment. In ten patients, liver biopsy was performed within one month after the end of combined treatment with  $f\tilde{N}$ -2a or  $f\tilde{N}$ -2b interferon and ribavirin for 48 weeks.

An increase of IGF-IR mRNA content was observed in hepatic tissue from all patients in comparison to normal liver. The immunohistochemical findings suggested these raises in mRNA content are related to increased IGF-IR expression in hepatocytes. There was a positive correlation between IGF-IR mRNA expression and the extension of parenchymal lesions. A statistically significant decrease in IGF-IR mRNA content was observed in patients who achieved sustained virological response after therapy with interferon- $f\tilde{N}$  and ribavirin, suggesting an improvement in hepatic damage. Previous studies demonstrated that normal hepatocytes in vitro do not proliferate in response to IGF-I probably due to downregulation of IGF-IR promoted by locally produced IGF-I. The finding of increased IGF-IR expression in hepatocytes in CHC raises the question whether IGF-I, together with other growth factors such as HGF and TGF $f\tilde{N}$ , directly participate in liver regeneration in this condition. It remains to be elucidated if IGF-IR upregulation results from direct activation of IGF-IR gene by HCV or if it is a consequence of chronic aggression to hepatic parenchyma.

**P 214****POST-TRANSPLANT RECURRENT HEPATITIS C - IMMUNOHISTOCHEMICAL DETECTION OF HCV CORE ANTIGEN AND POSSIBLE PATHOGENIC IMPLICATIONS**

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**Background:** The mechanisms by which severe cholestatic hepatitis develops after liver transplantation are not fully understood. Reports on immunohistochemical distribution of HCV antigens are still scarce, but recently, HCV immunostaining was suggested for early diagnosis of cholestatic forms of recurrent hepatitis C in liver grafts. **Design:** After purification, Rb246 pab anti-core (aa1-68) yielded specific, granular cytoplasmic staining in hepatocytes. Signal amplification through Peroxidase-anti-Peroxidase avoided endogenous biotin. Rb246 was applied to liver samples of explants of 14 transplant recipients, 7 with the most severe form of posttransplantation recurrence (group 1) and 7 with mild recurrence (group 2). We also detected immuno-reactivity at 2 time-points posttransplantation (6 mos and 2 years) in both groups. HCV-core Ag was semi-quantified from 0 to 3+ in each time-point. HCV-RNA was also measured on the different time-points by branched DNA. **Results and Conclusions:** In the early post-transplant time-point, 1 patient had a mild staining (1+) and 2 patients had a moderate staining (2+) in group 1, compared to 6 patients with no staining (0) and 1 patient with mild staining (1+) in group 2. Late post-transplant liver samples were not available in all patients, but only 2 had a mild staining in group 1, compared with no patients in group 2. Strikingly, on the pre-transplant samples, HCV immunostaining was strongly positive in group 1 and mildly positive in group 2. HCV-RNA was significantly higher in group 1, on both time-points post-transplantation. HCV-core Ag was not direct associated to HCV-RNA on the different time-points. These preliminary results suggest that strong HCV immunostaining in the explant is predictive of more severe disease recurrence.

**P 215****GRANULOMAS IN LIVER BIOPSIES OF CHRONIC HEPATITIS C VIRUS.**

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**Introduction :** Granulomas are well known findings in the liver biopsies of hepatitis C Virus (HCV)-infected patients. Frequency of those lesions is variously appreciated, from less than 1 to more 10%. The prevalence of granulomas and their relation to the severity of the liver disease will be discussed. **Methods :** Between 01/01/2000 and 31/12/2003, 1654 liver biopsies were done in HCV-infected patients and read in a single pathology university laboratory. All results were retrospectively analysed in search for granulomas. **Results :** Sixty four biopsies (4%) showed granulomas. Among those, 34 were steatosis-associated lipogranulomas, and 30 corresponded to true epithelioid granulomas. Only the 30 epithelioid granulomas were included in further analysis. They were classified in 3 groups. Group 1 consisted of 13 biopsies, showing granulomas surrounding exogenous

inclusions (9 from patients undergoing dialysis and 4 drug-induced granulomas). Group 2 consisted of 7 biopsies with known aetiology: 2 sarcoidosis, 2 tuberculosis, 2 primary biliary cirrhosis (PBC), and 1 atypical mycobacterial infection in an HIV-positive patient. Group 3 consisted of 10 biopsies (0.6% of 1654) with no evident aetiology apart from HCV-infection. Two out of these were portal tracts' epithelioid granulomas surrounding biliary ducts, with associated cirrhosis. These patients had negative anti-mitochondrial antibodies (AMA). The last 8 presented with small and lobular epithelioid granulomas, with moderate fibrosis (less or equal to F2 for METAVIR score) excepted in one patient who had cirrhosis.

**Conclusion:** In our study, HCV-related granulomas without any other known aetiology were rare (0.6%) and of mild severity. Nevertheless, we found 2 isolated cases with particular pathological features consisting of cirrhosis with portal epithelioid granulomas surrounding biliary ducts in patients with negative AMA. These histological lesions evoke "PBC-like" disease.

**P 216****STEATOSIS IN CHRONIC HEPATITIS C. CORRELATION WITH SPECIFIC HCV GENOTYPE AND METABOLIC CO-FACTORS**

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**Introduction:** Liver steatosis plays a central role in the pathogenesis and disease progression of chronic hepatitis C. Evidence has shown that HCV genotype 3 infection is associated with higher rates of steatosis and this is due to a cytopathic viral effect. The aim of the present study was to correlate the occurrence and severity of liver steatosis with the presence or absence of co-factors in chronic HCV genotype 1 and 3 infection.

**Material and Methods:** We have retrospectively assessed steatosis in biopsy specimens from 239 patients with chronic hepatitis C. Biopsies were divided in a) Group A: 125 biopsies from patients with no metabolic co-factors and Group B: 114 biopsies from patients with metabolic co-factors (alcohol intake, type II diabetes, obesity, lipidemia). Our results were further analyzed according to HCV genotype.

**Results:** Group A: Steatosis was found to be present in a significantly higher rate in patients with HCV-3 infection (70.7%) compared to 56.2% of patients with HCV-1. In both HCV-1 and 3 infection steatosis was mainly of low grade (HCV-3: grade 1, 44.8%, grade 2, 12.2%, grade 3, 8.6% HCV-1: 37.5%, 12.5%, 6.3% respectively). Group B: The results were similar to those of Group A (57.1% for HCV-1, 70% for HCV-3). However, higher grades of steatosis were noticed, mainly in HCV-3 infection (HCV 3: grade 1, 25%, grade 2, 21.7%, grade 3, 23.3%. HCV-1: 31.4%, 29% and 5.7% respectively).

**Conclusions:** Our results show that a) HCV-3 infection is more frequently associated with the occurrence of hepatocyte steatosis b) Steatosis is mainly related to a viral effect and c) Metabolic factors aggravate steatosis produced by the virus itself.

**P 217****LIVER CELL APOPTOSIS AND PROLIFERATION IN HCV INFECTED RENAL TRANSPLANT PATIENTS**

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**Background-Aims:** The role and prognostic significance of hepatocellular apoptosis and regeneration in liver disease of HCV infected renal transplant patients (RTx) remain unclear. In the present study hepatocellular apoptosis and proliferation activity (PA) were assessed in liver biopsies (LBs) of RT patients with HCV infection and were correlated with hepatitis-necroinflammatory activity (grade and stage), disease outcome, viremia and genotype.

**Methods:** Ninety liver biopsies including 31 follow up biopsies were examined histologically. DNA fragmentation was detected by TUNEL assay and PA by immunohistochemical ki67 expression. Apoptotic index (AI) and PA were defined by the number of positive hepatocytes per 10 High Power Fields (1000 cells) and were classified in three categories ranging from 0-5 cells and from 0-1,35 cells respectively. HCV RNA levels and genotype were also determined.

**Results:** Histological evaluation revealed no significant changes in 8 (13,6%), acute hepatitis in 2 (3,4%) and chronic hepatitis in 49 (83%) cases. A low apoptotic index (AI) (0-2,5) was observed in 31 (52, 5%) and a high (>5) in 12 (20,3%) cases. There was no PA in 22 (37,9%) cases whereas it was >1,35 in 17 (29,3%). By univariate analysis the AI showed a significant correlation with viral load, resulting in 1 log<sub>10</sub> higher HCV RNA levels in those patients with high number of apoptotic cells ( $p=0,003$ ). This finding was verified by multivariate analysis (OR=6,67, 95% CI 1,4-30,9,  $p=0,015$ ). Both variables did not show any significant correlation with hepatitis grade and stage, disease progression and genotype, whereas a significant positive correlation was observed between AI and PA ( $p=0,03$ ).

**Conclusions:** Apoptotic cell death seems to be pathogenetically associated with high viral load probably through a direct cytopathic pathway. The low PA points towards a suppressed liver cell proliferation, which may be linked to viral effects and/ or the immunosuppressive therapy.

#### P 218

##### **A STANDARDIZED AND COMPUTERIZED PATHOLOGICAL REPORT FOR CHRONIC HEPATITIS C : A SIX YEARS EXPERIENCE.**

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Since 1999, we have been using a standardized and computerized pathological report (SCPR) for liver biopsies with chronic hepatitis C in our department of Pathology, with annual revision of its content. Here we present the initial SCPR, its evolution, and the evaluation made by clinicians and pathologists.

The SCRP was developed by one of us in May 1999 on Diamic ® laboratory software (Infologic, France), based on a Word interface. It consists in a basic text with missing items to be filled using a panel of predetermined and preregistered answers, with either simple or multiple choice; after that, modifications/addition of data can be made. At first, the basic text and the answers were strictly based on the grid and the algorithm for histological evaluation published by the METAVIR group.

For 6 years, 1718 reports were made by 3 pathologists. They used the SCPR immediately and systematically. This adhesion was favored by the previous use of a paper form for METAVIR grid. Further changes included the introduction of new answers, the development of multiple choice items and of an arborescence of answers, all resulting in more accuracy and flexibility in the description of histological parameters, and in the conclusion, possibility to notify the degree of diagnostic fiability, and to add a secondary diagnosis with clinical

consequences, such as steatohepatitis, auto-immune hepatitis, iron overload, or liver cell dysplasia. As a mean, the SCPR is now one full page, 400 words, and 31 lines. During the last revision based on 200 consecutive SCPR, we found 149 (74.5%) reports to be strictly conform to the bible text; for the remaining 25.5%, the small variations observed had to do with the evaluation of fibrosis: difficulties in establishing the stage of fibrosis ( $n=22$ ) or quantification of peri-centrolobular/-cellular fibrosis, which lead us to the last modifications. Clinicians find this SCPR very well adapted for the diagnosis, prognosis and follow up of patients with hepatitis C, either isolated or associated with other hepatopathies. Pathologists appreciate the quickness of the making the report (120 seconds as a mean), and its didactic and pedagogic values. Furthermore, all necessary data are present and ready for statistical evaluation without reexamination of the slides.

In conclusion, the SCPR for hepatitis C reporting appears as a simple, quick, systematic, reproducible and flexible tool adapted to the clinicians and pathologists needs.

#### P 219

##### **CORRELATION BETWEEN INTRAHEPATIC EXPRESSION OF HLA-DP / DR, INTERCELLULAR ADHESION MOLECULES AND T HELPER LYMPHOCYTES IN CHRONIC VIRAL B AND C HEPATITIS**

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The specific cellular immune response in the liver against HBV and HCV chronic infections requires the cooperation between professional and occasional antigen presenting cells (APC) e.g.: Kupffer cells, macrophages, hepatocytes, endothelial cells and lymphocytic subpopulations.

The aim of the study was the assessment of HLA-DP / DR expression and intercellular adhesion molecules on hepatocytes, correlated with activated level of CD4 helper T cells in chronic viral hepatitis.

Indirect triserial immunohistochemical ABC method was performed on liver biopsies taken from 20 patients with single and double infections, using HLA-DP, HLA-DR, CD54, CD44, CD4 antibodies.

Using regressive biostatistic analysis, a positive direct correlation between HLA-DP/DR and CD44 was observed ( $r = 0.46$ ) with statistical significance ( $p = 0.04$ ). In double infections ( $n = 5$  cases), HLA-DP/DR expression had a positive correlation with CD4 ( $r = 0.56$ ), but without statistical signification ( $p = 0.3$ ). In HCV infections, a positive correlation statistically highly significant, between CD44 and CD54 was found ( $r = 0.78$ ,  $p = 0.002$ ). Between CD44 expression and CD4+ infiltrating cells, a negative correlation statistically significant was established ( $r = - 0.6$ ,  $p = 0.008$ ). Activated level of CD4+ lymphocytes assessed by CD44 had a negative relationship ( $r = -0.44$ ), statistically significant ( $p = 0.04$ ).

During hepatic chronic viral infections, decreasing of T helper cells number in inflammatory infiltrate is associated with a significant activation degree of the same lymphocytes and with an increased expression of accessory molecules on hepatocytes, suggesting a by-pass of professional APC which can maintain a necro-inflammatory microclimate.

#### P 220

##### **HEPATOCYTE APOPTOSIS IN CHRONIC HEPATITIS C: A PROMINENT FEATURE OF DISEASE SEVERITY IN EGYPTIAN PATIENTS**

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**Background and Aims:** Chronic hepatitis C virus (HCV) is a major health problem in Egypt, where genotype 4 is the most prevalent. There is increasing evidence, from recent studies, suggesting that liver damage in chronic HCV genotype 1 and 3 is mediated by the induction of apoptosis. The aim of this study were to assess hepatocytic apoptosis in chronic HCV patients, correlate it with disease severity, and to identify possible mechanisms of apoptosis induction.

**Methods:** The study included 57 selected patients diagnosed on clinico-pathological and virological basis as chronic HCV , in addition to five control cases. Liver specimens were studied according to the grade of inflammation, stage of fibrosis and extent of steatosis. Immunohistochemical (IHC) studies using both the monoclonals; anti-Fas and anti-p53 mutant gene were performed. Electron microscopic (EM) study for 8 cases were done.

**Results:** Apoptosis was focally demonstrated in routinely stained liver sections and intensely by detecting Fas antigen expression in 84.2% of patients compared to no expression in controls. A positive correlation was observed between Fas expression and grades of inflammation , stages of fibrosis, serum alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP) , and viral load. p53 was over expressed in 29.8% of patients, showing significant direct correlation relative to the grades of activity, stages of fibrosis, serum albumin and bilirubin levels, ALP and viral load. Significant direct correlation were detected between hepatic steatosis and both Fas and p53 positivity. EM study identified the ultrastructural features of apoptotic changes within hepatocytes.

**Conclusions:** Hepatocyte apoptosis through Fas antigen expression is significantly increased in Egyptian patients with chronic HCV and correlates with disease severity. We suggest the term "apoptotic activity" instead of "necroinflammatory activity" in describing chronic HCV interface areas. Mutant p53 is over expressed in the precocious stages of HCV related liver damage before carcinogenesis indicating close follow-up.

#### P 221

##### **HISTOPATHOLOGICAL STUDY OF CHRONIC HEPATITIS C: EVALUATION OF PERISINUSOIDAL FIBROSIS AND COMPARISON OF SCORING SYSTEMS**

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**Background/Aims:** Needle liver biopsy has been shown to be the "gold standard" in assessing the histopathological degree of liver damage which is essential for the routine care of patients with chronic hepatitis C virus (HCV) infection. Several scoring systems have been proposed to standardize the morphological features seen in HCV. Although it is a characteristic feature for steatohepatitis, it is well known that perisinusoidal fibrosis may also be seen in viral hepatitis. In this study, our aims were to determine 1) the interobserver reliability of the different scoring systems, 2) the correlation between the scoring systems, 4) the correlation of necroinflammatory activity in different scoring systems with the serum transaminase levels, 3) the incidence of pericellular fibrosis in HCV and its relation with different histopathological features. **Methods:** Liver biopsies stained

with H&E and trichrome from 96 chronic HCV cases were re-examined and scored by two independent pathologists using Knodell, METAVIR, Scheuer, and Ishak systems. Pericellular fibrosis was scored semiquantitatively according to Brunt et al. (0= no fibrosis, 1= <33%, 2= 33-66%, 3= >66%). The results were analysed using Kappa statistics and chi-square test. **Results:** While all the systems used produced moderate agreement when necroinflammatory score (NIS) was assessed, the ISHAK system produced slightly higher (k=0.64) values. NIS showed poor correlation with serum transaminases in all systems (k< 0.30) except Ishak score which was moderate (k=0.47). Concordance between the different scoring systems is good for NIS, and excellent for fibrosis score. Perisinusoidal fibrosis was present in 54.2 % of the cases and no correlation could be identified with any of the histopathological features including degree of steatosis and pericentral necrosis. **Conclusions:** While all systems produced reasonable agreement, this was greater in the Ishak system, especially concerning NIS. Perisinusoidal fibrosis was not a rare morphological change which had no relation with any of the histopathological features examined and its role in the prognosis of HCV patients needs to be determined with prospective studies.

#### P 222

##### **CORRELATION BETWEEN IFN-MEDIATED SIGNAL PROTEINS EXPRESSION LEVELS IN LIVER TISSUE AND THE PREVALENCE OF HCV INFECTION.**

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Hepatitis C virus (HCV) affects to 2% of occidental population. Interferon (IFN) is the main antiviral agent. At present, patients with chronic hepatitis C are treated with recombinant IFNa in combination with ribavirin. However, only 30% of patients are responders to this therapy. The molecular mechanisms of HCV and IFNa interactions are not fully solved. IFNa subtypes constitute a family of 13 polypeptides coded by different genes that interact with the same receptor while eliciting distinct antiviral and antiproliferative actions. Recently, in vitro data demonstrated that HCV is able to counteract the IFN signal at very early stages affecting Jak/STAT pathways. Thus, it suggests that HCV bypasses endogenous IFN-mediated signal probably making useless the current therapies with IFN in the majority of patients. In the present study we focus our interest on the expression of proteins involved in IFNa mediated signalling pathways in tissue liver samples from HCV patients and control donors. We sought to determine the correlation between the presence of HCV and the physiological expression levels of STAT proteins and their protein activation modulators (SOCS) that could explain the failure of IFN treatment in the majority of HCV patients. To this aim, we have developed tissue arrays technology and analysis by microscopy. We found robust and higher expression levels of STAT1 and STAT2 in HCV patients when compared with healthy donors. Moreover, expression of these proteins are restricted to nuclear localization in hepatocytes, thus indicating that at least the early-IFN response is functional in these patients. SOCS protein expression were also investigated and results are discussed.

#### P 223

##### **HEPATIC STEATOSIS IN EGYPT . A CLINICAL AND PATHOLOGICAL PROFILE**

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Bright liver is not an uncommon abdominal ultrasonographic finding in the daily medical practice. Objectives: This study aims at evaluation of the clinical, laboratory and pathological aspects of cases presenting with Simple Steatosis (SS) and Non Alcoholic Steato-Hepatitis (NASH) in Egypt.

Methods: Seventy five subjects with bright liver by abdominal ultrasonographic examination were enrolled for this study. According to their virological markers they were selected so as: 60 subjects were sero- negative for chronic hepatitis B (HBV) and chronic hepatitis C (HCV), whereas 15 subjects were anti- HCV sero- positive. All patients were subjected to full clinical and laboratory investigations with special emphasis on risk factors of hepatic steatosis, and to needle liver biopsy.

Results: Hepatic Steatosis is a disease of both sexes, female: male ratio is 1.9:1. It occurs mostly in the fourth and fifth decades of life. Risk factors are: obesity, diabetes mellitus, hypertriglyceridemia. Main complaints are easy fatigue, right hypochondrial pain. On histopathological assessment: NASH was present in 60% of the studied patients among whom mixed micro and macro vesicular hepatocytic steatosis was noted with moderate and severe affection. Eighty-seven percent of patients with SS had a micro steatotic pattern with minimal to mild degree of affection. Chronic hepatitis C patients exhibited micro vesicular pattern in 46.7% and mixed pattern in 53.3% of biopsies. ALT and triglycerides were significantly higher in NASH than SS with positive correlation between their levels and histopathological scoring of hepatic steatosis. AST/ALT ratio was <1 in 90% of cases. Conclusion: NASH is an emerging disease entity in Egypt which might be a cause of progressive chronic liver disease.

**P 224**  
**MORPHOMETRICAL STUDY OF HEPATIC FIBROSIS AND NECROINFLAMMATORY GRADE BY REPEAT LIVER BIOPSY IN PATIENTS WITH CHRONIC HEPATITIS C (HCV), THE ROLE OF VIRUS GENOTYPE AND TREATMENT.**

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Introduction. Prognosis of chronic HCV infection depends on fibrosis progression in the liver. Previous data concerning rate of fibrosis progression and results of HCV therapy show possible significance of virus genotype and necroinflammatory grade. Semi quantitative assessments of fibrosis and necroinflammation are currently regarded as a routine procedure, but it is obvious that these methods are subjective. Alternative method is comparative morphometrical analysis of repeat liver biopsies.

Aim. The aims of this study were to assess rate of fibrosis progression by investigation of primary and repeat liver biopsy HCV-infected patients and to identify factors, connected with prognosis.

Methods. Paired liver biopsies from 60 patients (age 26-45 years) with chronic HCV were studied. RT-PCR was used for detection of HCV-RNA and its genotype. Patients were non-treated before primary biopsy, 20 patients were treated by \_ interferon/riboverin in standard dose between biopsies. The same pathologist scored paired biopsies from the same patient, applied V.J.Desmet (1994) and R.G.Knodell (1981) semi

quantitative assessments. Software SigmaScan Pro 5.0 was used for morphometrical analysis. Sections were stained by Masson Trichrome method. Fibrosis index (FI) was calculated as collagen area divided all section area.

Results. Our results showed that 66,6% of patients had 1b genotype of HCV. Genotypes 2a and 3a were found rarely (12,5% and 37,5%). 12,5% of patients were with mixed genotype 1b-3a. Investigation of primary liver biopsy showed predominantly mild and moderate necroinflammation and fibrosis of liver in case of 1a or 1b HCV genotype, without significant difference (Knodell 5, Desmet 1-2, IF 0,01-0,03). Significantly marked necroinflammation (Knodell 9 and more) were in the biopsies from patients, that were infected 3a or mixed 1a-3b HCV genotype. Liver fibrosis stage (IF, Desmet) did not correlate to inflammation degree. Morphometrical investigation of paired biopsies showed that the majority of treated patients showed no progression of fibrosis (invariable IF, or moderate reducing from 0,03 to 0,01). Our data indicated fibrosis progression in repeat biopsy if patients were older than 40 years or had marked fibrosis (Desmet 3, IF 0,1) in primary liver biopsy.

Conclusion. Our results suggest good correlation between histological activity and HCV genotype. Success of therapy depends on fibrosis range and age of patients at the time of primary biopsy.

**P 225**  
**LIVER HISTOLOGY IN PATIENTS WITH SPORADIC ACUTE HEPATITIS E : A STUDY OF 11 PATIENTS FROM SOUTH-WEST FRANCE.**

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Hepatitis E virus (HEV) is responsible for acute hepatitis predominantly in developing countries. Sporadic cases have been described in industrialized countries but information on hepatic histology in these cases is scarce. We describe a series of 11 patients with sporadic acute hepatitis E and a liver biopsy. Methods: Liver biopsy was performed because of clinical suspicion of underlying chronic liver disease or uncertain etiology of the acute episode of hepatitis despite thorough diagnostic evaluation. HEV IgG antibodies were detected using an immuno-enzyme assay. HEV RNA was detected in the serum and feces with a real time PCR. All serums were negative for hepatitis B virus antibodies and DNA, hepatitis C virus RNA and antibodies, immunoglobulin-M class to hepatitis A virus, CMV and EBV. Anti-nuclear, anti smooth-muscle and anti LKM antibodies were also negative. Toxic and drug induced hepatitis were ruled out on anamnesis. Results: Eleven patients, 3 females and 8 males diagnosed with acute hepatitis E from march 1997 to march 2004 had a liver biopsy. Mean +/- SD age was 61 +/- 13. Overall 45% of the patients had an exaggerated alcohol consumption. Liver biopsy was transjugular in 5 cases. Five patients had confirmed histological micronodular cirrhosis. Four had exaggerated alcohol consumption on anamnesis (> 40 g/d). Two of these had lesions of acute alcoholic hepatitis including Mallory bodies, neutrophil infiltrates and steatohepatitis. The other 2 had mild steatohepatitis without Mallory bodies. All the other patients (1 with cirrhosis, 1 with mild fibrosis and 5 without fibrosis) had important spotty necrosis of hepatocytes with polymorph inflammation in the lobule. Anisocaryosis was present in most patients. Ballonisation was observed in only one case. No viral inclusions or epithelioid granuloma were observed. In portal tracts, inflammation was marked with numerous neutrophils in almost all cases and moderate piecemeal

necrosis in only 2 cases. Bile duct damage was mild and ductular proliferation various. Cholestasis was observed in 4 cases. There was no phlebitis. Conclusion. Cirrhosis and alcohol consumption was frequent in this population of patients hospitalized for acute hepatitis E raising the question of their interaction. Characteristic pathology signs of acute hepatitis E were hepatocyte intralobular necrosis, polymorph inflammation and neutrophils in portal tract.

#### P 226

##### THE SIGNIFICANCE OF SMAD4 EXPRESSION IN THE PROGNOSTIC EVALUATION OF THE CHRONIC HEPATITIS

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**Introduction and Objective:** We aimed to compare SMAD4 expression and fibrosis in the pre and post-treatment hepatic biopsies

in cases with chronic hepatitis B, chronic hepatitis C and chronic hepatitis D.

**Materials and Methods:** We examined the hepatic biopsies of thirty one chronic hepatitis C and 40 chronic hepatitis B cases, 4 chronic D hepatitis which were diagnosed at our department. The cases were reexamined, staged and graded according to modified Ishak and Metavir scoring systems. Immunohistochemistry was performed using antibody against SMAD4 and nuclear SMAD4 staining in perisinusoidal cells having oval nuclei and sprouting vacuolar cytoplasm was accepted as positive.

**Results:** When SMAD4 cut-off value 0,263 was chosen, 89 percent of the patients having SMAD4 expression below this level showed regression in fibrosis. Patients having SMAD4 expression more than 0.263 cut-off value showed fibrosis progression. This progression was calculated as 77,7 percent when the cases evaluated according to Metavir system and 81 percent when they were evaluated according to the modified Ishak. In cases with SMAD4 cut-off value below 0,184 the stage decreased in 96 percent of the patients. Median SMAD4 expression was found significantly higher in cases with chronic hepatitis C.

**Conclusions:** Our results showed a significant correlation between SMAD4 expression and fibrosis. Therefore, we conclude that it is necessary to observe the SMAD4 expression in pretreatment hepatic biopsies while defining the prognosis of the cases with chronic viral hepatitis.

#### P 227

##### HISTOPATHOLOGIC PARTICULARITIES OF HEPATITIS D VIRUS CHRONIC HEPATITIS

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Hepatitis D virus (HDV) chronic hepatitis (which is frequent in Romania) shows no specific histopathologic lesions comparing with other viral etiologies.

**MATERIAL AND METHODS:** We studied 58 cases of HBV-HDV chronic hepatitis and 355 cases of HBV chronic hepatitis diagnosed in our department in 14 months; we analyzed various histopathologic parameters (portal and lobular inflammation, interface hepatitis, spotty necrosis, confluent necrosis, apoptosis, fibrosis, ground glass cells, large cell dysplasia, ductal lesions) using Ishak semiquantitative

score to assess necro-inflammatory activity and fibrosis; the results were compared with a group of 355 cases of HBV chronic hepatitis; a statistical analysis was performed – CHITEST, FISCHER and STUDENT tests were used with a level of statistical significance (P) of 0.05.

**RESULTS:** In HBV-HDV chronic hepatitis there were significant more abundant lobular inflammation (P=0.004), interface hepatitis (P=0,002), spotty necrosis (P=0,005), steatosis (P=0.025), Kupffer cells hyperplasia (P=0.007), necro-inflammatory activity (P=2,25428x10-07) and fibrosis (P=9,83716x10-07); the portal inflammatory infiltrate and ballooning degeneration were more prominent in HBV-HDV chronic hepatitis but P values were outside of the confidence range (P=0.052 and P=0.057); there was more hepatocytic hemosiderin deposition, less polymorphic inflammatory infiltrate, more prominent ductal lesions and large cell dysplasia. No significant differences were recorded in matter of sex, age, number of ground glass cells, distribution of portal inflammatory infiltrate or confluent necrosis.

**CONCLUSIONS:** Based on our data (higher necro-inflammatory activity with an elevation of all histopathologic parameters of necrosis and inflammation except confluent necrosis, higher hepatocytic degeneration (ballooning degeneration and steatosis), higher fibrosis) a more aggressive histopathologic appearance is recorded in HBV-HDV chronic hepatitis.

#### P 228

##### IMMUNOHISTOCHEMICAL CHARACTERIZATION OF HEPATIC DUCTULAR REACTION IN WILSON'S DISEASE

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The so called ductular reaction is a mysterious histological response of the human liver. It occurs in a wide variety of hepatic diseases, but its origin and importance is still debated. In the present study the immunophenotype of the ductular cells was investigated in three livers from patients of Wilson's disease.

The immunohistochemistry was performed on paraffin embedded liver tissue by ABC technique using diaminobenzidine as chromogen.

The ductular cells were cytokeratin(CK)7+/19+/14- and were surrounded by smooth muscle actin positive stellate cells. Occasionally, large polygonal, hepatocyte like cells were present in the ductules, which lost CK7 staining but gained nuclear hepatocyte nuclear factor 4 (HNF 4) positivity. Ki 67 staining was confined exclusively to the ductular structures, but copper/orcein positive granules were present only in hepatocytes. Alfa-fetoprotein, deleted in malignant brain tumor-1 (DMBT-1) reactions were negative, CD 34 antibody decorated only the endothelial cells.

These results suggest that the ductular reaction in Wilson's disease represents a stem cell derived regenerative response and shows certain similarity to the oval cell proliferation in the rat liver.

#### P 229

##### HISTOPATHOLOGICAL SCORING OF THE NECRO-INFLAMMATORY ACTIVITY AND FIBROSIS IN AUTOIMMUNE HEPATITIS: A COMPARISON OF TWO SCORING SYSTEMS

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#### Background and Aims:

Histopathological scoring of grade of necro-inflammatory process and stage of fibrosis is widely used for chronic viral hepatitis and the same scoring systems are also applied to the biopsy samples of patients with autoimmune hepatitis. We aimed to compare the results of scorings by Modified Histological Activity Index (HAI) and the METAVIR systems and their validity for determination of severity of disease in Iranian patients with autoimmune hepatitis.

#### Materials and Methods:

Blind histological examination of 20 liver biopsy slide sets from patients with autoimmune hepatitis was performed by two independent pathologists. Grades and stages of the liver lesions were determined according to the HAI and METAVIR systems. The correlations of the scores of the two systems were determined by weighted kappa analysis. Correlation of necroinflammatory activity and fibrosis with serum levels of aminotransferases was analyzed by the Spearman's rho test.

#### Results:

There was moderately well to good correlation between grades and stages obtained by the two systems (weighted kappa 0.58 and 0.56 respectively). However, the scores of none of the two systems showed a significant correlation with serum levels of aminotransferases.

#### Conclusion:

Concordance between HAI and METAVIR scoring systems is good for the histological grading and staging of autoimmune hepatitis. However, neither of the two systems proved to be valid for determination of the activity of the disease when compared to the levels of serum aminotransferases. We conclude that HAI and METAVIR systems give comparable results in autoimmune hepatitis but a more valid system has to be devised for histological scoring of this group of liver diseases.

Key words: Autoimmune hepatitis, Scoring, Metavir, Modified HAI

#### P 230

##### **EFFECT OF SPLENECTOMY ON LIVER FIBROGENICITY IN PORTAL HYPERTENSION PATIENTS**

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This study was designed to assess the role of the spleen on the presence and localization of liver fibronectin, to measure the amount of hepatic collagen and serum level of fibronectin and procollagen III in cases of portal hypertension.

The study was performed on 40 patients divided into two groups: Portal hypertension group (20 patients) included one pure schistosomal hepatic fibrosis, three mixed schistosomal and virus C cirrhosis, ten virus C hepatic cirrhosis, two virus B hepatic cirrhosis and four mixed B&C infections. Control group (20 patients) presented with different splenic insults without hepatic affection. In the first group, through clinical examinations and sonographic findings revealed hepatosplenomegaly and grade III & IV varices with history of injection sclerotherapy in 65% of cases.

Preoperatively, marked hepatic fibronectin deposition in portal tracts and around blood vessels raised the stage of fibrosis. This was accompanied by decrease in plasma level of fibronectin and increase of serum level of procollagen III in relation to the control group.

Six months post-operatively, tissue fibronectin, collagen content and serum procollagen III were insignificantly decreased. However, plasma level of fibronectin was increased significantly.

These results denoted that fibronectin is one of the predominant early fibrogenic components in chronic liver diseases, stimulating further collagen deposition. It is concluded that the role of the spleen in the development of hepatic fibrosis seems ancillary. Multifactorial influences, including etiology, timing of splenectomy, hepatic vascular adjustment, and factors associated with the biology of extracellular matrix of the liver, probably play a more significant role. Further studies were recommended to assess the role of splenectomy on liver fibrogenesis at different stages of fibrosis. Intervention with splenectomy could improve or reverse fibrogenic activity at early stages.

Key words: Splenectomy, fibrogenicity, fibronectin, procollagen III, liver fibrosis, portal hypertension.

#### P 231

##### **PRIMARY SCLEROSING CHOLANGITIS SIMULTANEOUSLY WITH ANCA-ASSOCIATED VASCULITIS**

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Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by inflammation and fibrosis of the intra- and/or extrahepatic bile ducts. Anti-neutrophil cytoplasmic antibodies (ANCA) are a heterogeneous group of autoantibodies directed against cellular components of the polymorphonuclear leucocyte. ANCA directed to proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) are closely associated with systemic necrotizing vasculitis. Atypical anti-neutrophil specific antibodies (atypical p-ANCA) are detected in the majority of patients with PSC.

Methods: Retrospective analysis of patients with diagnosis of PSC between the years 2002-2004.

Results: A total of 38 patients in whom ERCP features of PSC and liver biopsies were evaluated - 25 (65,8%) men, 13 (34,2%) women. Of the 38 patients, 26 (68,4%) suffered from inflammatory bowel disease. 3 patients (2 men, 1 woman) had skin palpable purpuric rash, and underwent skin biopsy with morphological diagnosis of vasculitis. Simultaneously positive ANCA antibodies directed to proteinase 3 were detected in the sera of all of them. All of these patients had been suffering from migrating polyarthralgia. None of them had any renal abnormalities.

Conclusion: The underlying pathophysiology of PSC is unknown but genetic and immunological mechanisms may play a role. Patients with PSC can have positive tests not only to atypical pANCA but also to PR3-ANCA. Some of the extrahepatic complications of PSC can be associated with systemic vasculitis, and these patients need more extensive monitoring of the disease and especially adequate therapy to prevent a more serious course of the disease.

#### P 232

##### **CELL REACTION OF PERISINUSOIDAL FIBROSIS DURING NONALCOHOLIC STEATOHEPATITIS.**

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Nonalcoholic steatohepatitis (NASH) is characterized by perisinusoidal fibrosis, which develops on early stages of the disease. The cellular sources of fibrillar collagens and other matrix proteins are endothelial cells (EC), hepatic cells and mainly hepatic stellate cells (HSC) which proliferate after liver injury and undergo activation to a myofibroblast - like phenotype. Capillarization is a process in which the liver sinusoid became consecutive capillaries with evident basement around and it is followed by expression of CD34 by EC.

**Aims:** To determine the connection between the development of perisinusoidal fibrosis, activation of HSC and capillarization of sinusoids during NASH. **Methods:** 36 liver biopsies from the patients with NASH were studied. We analyzed correlation between grade of disease activity and stage of the disease and expression of  $\alpha$ -SMA (activation of HSC) and CD34 (capillarization). **Results:** Perisinusoidal fibrosis is revealed in all stages of NASH and followed by activation of HSC. Expression of CD34 arises on the basis of perisinusoidal fibrosis, activation of HSC and correlates with the grade of activity. **Conclusion:** Perisinusoidal fibrosis is the result of HSC activation, leads to capillarization and to change of EC phenotype and expression of CD34.

#### P 233

##### **BETA-AMINOISOBUTYRIC ACID ALLEVIATES STEATOHEPATITIS IN A MURINE MODEL OF DIABETES AND OBESITY.**

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Non-alcoholic fatty liver diseases (NAFLD) are characterized either by isolated steatosis or by steatosis associated with necroinflammation, thus constituting non-alcoholic steatohepatitis (NASH). NASH can evolve toward more severe liver diseases such as fibrosis, cirrhosis and hepatic carcinoma. NASH is mainly observed in metabolic diseases such as type 2 diabetes and obesity.

**Background:** we previously reported that administration in lean (Swiss) mice of 100 mg/kg/d of beta-aminoisobutyric acid (BAIBA, a natural catabolite of thymine) for 6 weeks was associated with increased hepatic fatty acid oxidation (FAO) and decreased body fat mass (BFM). In genetically obese and diabetic ob/ob mice this dose of BAIBA stimulated FAO at a lesser extent and did not decrease BFM.

**Aims:** the first goal of the study was to determine whether higher doses of BAIBA and/or a longer exposure could decrease BFM in ob/ob mice. In addition, we asked whether BAIBA could improve liver function and lesions and oxidative stress in this model of obesity. Lastly, we wished to know more regarding the mechanisms whereby BAIBA is able to increase hepatic FAO.

**Materials and Methods:** 100 or 500 mg/kg/d of BAIBA were administered to ob/ob mice for 4 months. BFM was assessed by DEXA before and at the end of the experiment. ALT and LDH were determined in plasma and liver histology was performed with standard stains (hematoxylin-eosin, Masson trichrom, picosirius). In addition, apoptosis was assessed with a TUNEL assay. mRNA expression of 2 mitochondrial FAO enzymes (L-CPT-1, MCAD) was assessed with a RiboProtection Assay. Finally, hepatic glutathion and lipid peroxidation were measured.

**Results:** we showed that administration in ob/ob mice of high dose of BAIBA for 4 months did not decrease BFM. Although hepatic steatosis was not decreased in ob/ob mice, periportal and perisinusoidal fibrosis and necroinflammation and apoptosis were alleviated by BAIBA. This histologic improvement was associated with a trend toward lower ALT and LDH. Hepatic expression of L-CPT-1 and MCAD was increased and oxidative stress was lessened.

**Conclusion:** although the natural compound BAIBA does not decrease fatness in ob/ob mice, it improves liver function in this model. However, despite increased expression of FAO enzymes, steatosis in ob/ob mice was not decreased suggesting that a compensatory de novo lipogenesis may occur in liver. BAIBA is being tested in other models of dysmetabolic diseases.

#### P 234

##### **HEPATIC DYSFUNCTION IN ANOREXIA NERVOSA: CLINICAL, HISTOLOGICAL, ULTRASTRUCTURAL AND PROGNOSTIC ANALYSES**

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Liver tests abnormalities have been described in anorexia nervosa. However, the mechanisms of liver dysfunction in this context are not well established and histological reports are rare.

**Aims:** To analyze the clinical, pathological and prognostic features of hepatic dysfunction observed in patients with severe anorexia nervosa.

**Patients & methods:** 9 patients with anorexia nervosa (7 females, 2 males, mean age: 27.3) were investigated in Hôpital Beaujon because of acute liver failure. The clinical and biological parameters were recorded. Liver biopsy was performed in every patient with electron microscopy study in two cases.

**Results:** At admission, the mean BMI was  $11.3 \pm 1$  kg/m<sup>2</sup>, (range 9.6 – 13.2). Transaminase ranged from 18.1-105.5N; prothrombin time was below 50% in all patients. Five patients were hospitalized because of hypoglycaemic coma and cardiac explorations revealed cardiac dysfunction in 4 out of the 5 patients (left ventricular ejection fraction below 50%). Hepatic gradient was normal in all patients who underwent a transjugular biopsy (8/9).

Liver biopsies performed days 2 to 9 after admission showed swelling and clear hepatocytes in all cases, corresponding to spongiocytic steatosis as proven by Sudan red staining in only 1 case. Periodic acid-Schiff staining showed decrease glycogen amount in all cases and abundant ceroid pigments in hepatocytes of the centrilobular zone, associated with hepatocytic atrophy and inconspicuous sinusoidal fibrosis in 4 of the 9 cases. Centrilobular necrosis without inflammation or congestion was observed in one case. Ultrastructurally, hepatocytes displayed a low density of glycogen particles and few organelles. Endoplasmic reticulum was slightly altered. Phagolysosomes with dense osmiophilic bodies were prominent. Clinico-pathological confrontations showed a higher proportion of centrilobular lesions in patients with cardiac dysfunction and/or severe hypoglycemia. The patient with microvesicular steatosis presented with recurrent vomiting before admission. After glucose therapy, complete normalization of prothrombin time was achieved in all patients. Only one patient with cardiac dysfunction needed dobutamine therapy.

**Conclusion:** Severe hepatic abnormalities may occur in anorexia nervosa with favorable evolution after glucose infusion. Microscopically, fatty deposit was infrequent but hepatocytic lesions were common but mild, possibly related to hypoglycaemic coma and/or cardiac dysfunction.

#### P 235

##### **HISTOLOGICAL STUDY OF POLYCYSTIC LIVER DISEASE REVEALS SIGNIFICANT VASCULAR CHANGES IN NON-CYSTIC LIVER PARENCHYMA.**

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**Background :** Polycystic liver diseases are made of heterogeneous entities associated with specific genetic mutations. Histological analysis focused on liver cysts' characteristics and failed to describe morphological aspects of non-cystic parenchyma. The aim of this study was to assess

histological changes of the non-cystic parenchyma, which could explain the pejorative clinical evolution of some cases. Patients and Methods: Forty-six liver resections obtained from patients with polycystic liver disease were retrospectively examined. The sex ratio was 41 women / 5 men, with a mean age of 50 years + 9 (range: 33-66). The liver resection consisted in a left hepatectomy (n=19), a right hepatectomy (n=12), or a total hepatectomy performed in the context of liver transplantation (n=15). Twenty-two patients had also kidney polycystic disease involvement. Histological analysis evaluated the following features in a semi-quantitative assessment: portal fibrosis (0: absent, 1: mild enlargement of portal tracts, 2: few fibrotic septa, 3: numerous fibrotic septa, 4: cirrhosis), centro-lobular fibrosis (0: absent, 1: discrete perisinusoidal fibrosis around the centrolobular vein, 2: perisinusoidal fibrosis involving < 1/3 of the lobule, 3: perisinusoidal fibrosis involving > 1/3 of the lobule), sinusoidal dilatation and congestion (0: absent, 1: few foci, 2: extensive areas), regenerative nodular hyperplasia (incomplete or complete). The presence of venous thrombosis, venular portal dilatation or other parenchyma anomalies were also noted.

Results: In addition to the presence of numerous cysts disseminated throughout the liver, 40 cases (87%) displayed significant morphological changes in the non-cystic parenchyma. All patients had bilobar disease and 32 of them have complicated cysts (28 hemorrhagic cysts, 9 infected cysts). Significant portal fibrosis (> score 2) and centrolobular fibrosis (score 2) were noted in 16 (34.7%) and 25 (54.3%) cases, respectively. In 18 cases (39%), both sinusoidal dilatation and congestion were extensive. Venous thrombosis was present in 12 cases and venular portal dilatation in 41 cases. Architectural disorders, including regenerative nodular hyperplasia-like and regenerative nodular hyperplasia, were observed in 16 cases (34.7%). Peliosis and steatosis were reported in 12 and 7 case, respectively.

Conclusion: This study shows that non-cystic parenchyma of polycystic liver disease display significant morphological disorders, especially vascular changes, including regenerative nodular hyperplasia, and also fibrosis in at least 1/3 of cases. Such modifications, potentially induced by the secretion of cytokines and growth factors by the liver cyst epithelial cells, may be involved in the progression of the disease.

**P 236**  
**EVALUATION OF THE ANTI-SCHISTOSOMAL ACTIVITY OF THYMOQUINONE IN MURINE SCHISTOSOMIASIS MANSONI: INFLUENCE ON NITRIC OXIDE, ICAM-1 AND COLLAGEN**  
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We have proved previously that *Nigella Sativa* oil reduced *Schistosoma mansoni* (*S. mansoni*) induced hepatic fibrosis, the total worm burden, egg load and granuloma formation. Therefore, we aimed here to study the possible antischistosomal effect of one of its active constituent's thymoquinone (TQ). The drug was given to *S. mansoni* infected mice in two dose levels (5 and 10 mg/kg, p.o.) for two weeks, and its effect was compared to that of the standard antischistosomal drug, praziquantel (PZQ). Two weeks later, the following parameters were estimated, viz., worm load and distribution, oogram changes, egg load in liver and intestine, granuloma diameter, collagen deposition and intercellular adhesion molecule-1 (ICAM-1) expression. Moreover, serum levels of L-alanine aminotransferase (ALT) and nitric oxide (NO), as well as NO hepatic level were estimated. Mice

treated with TQ showed a significant reduction in total number of worm burden, both in liver and portomesentric blood vessels. The oogram pattern recorded a decrease in immature and mature ova, and an elevation in dead ova. Tissue egg load was reduced in both liver and intestine. In infected mice treated with TQ, the elevated serum ALT activity leveled off. However, neither serum NO level was altered, nor in the infected group nor in those treated with TQ or PZQ. In liver homogenate, infection decreased NO level, which was not corrected with PZQ, but in-groups treated with TQ, a further reduction was reported. TQ caused shrinkage in granuloma diameter, and a diminution in collagen deposition. On the other hand, PZQ failed to reduce collagen deposition. Both TQ and PZQ decreased ICAM-1 expression in Kupffer and inflammatory cells. However, neither TQ nor PZQ affected it in endothelial cells. These results point to a possible antischistosomal role of TQ, which may be correlated with modulation in the immune system.

Key words: schistosomiasis mansoni; nitric oxide; granuloma; intercellular adhesion molecule-1 (ICAM-1), collagen; thymoquinone; praziquantel.

**P 237**  
**HEPATIC STELLATE CELLS IN HUMAN SCHISTOSOMIASIS: A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY WITH LIVER CIRRHOSIS.**

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Hepatic schistosomiasis (SCH) frequently results in liver fibrosis, but in the most of cases without functional loss of the liver. Chronic hepatitis C virus hepatitis (HCV) often courses with cirrhosis and liver failure. The hepatic stellate cells (HSC) are involved in both disease, but it is not clear if the proportion of quiescent or activated HSC and their rates of proliferating can be associated with the clinical outcomes. The present study intends to verify if exist difference in quiescent and activated hepatic stellate cells and their proliferating capacity, between SCH and HCV. The liver biopsies were reviewed and the diagnoses were confirmed. It was performed a immunohistochemistry study, with double staining for proliferating cell nuclear antigen and glial fibrillary acid protein (for detection and quantification of proliferating quiescent HSC), and double staining for proliferating cell nuclear antigen and smooth muscle  $\alpha$ -actin (for detection and quantification of proliferating activated HSC). In SCH patients, there was observed a lower number of activated HSC than in HCV patients ( $p < 0.05$ ), without any difference considering the quiescent HSC ( $p > 0.05$ ). The rates of proliferating, both to quiescent and activated HSC were similar in SCH and HCV patients ( $p > 0.05$ ). Thus, the activation state of HSC is more important than proliferating rates. The HSC are important to SCH fibrosis, but, the activated HSC profile, the more proliferating, is predominant in cirrhosis. Probably, the evident inflammatory stimuli present in HCV can activate a higher number of HSC than in SCH, that present a mechanical mechanism of fibrogenesis and courses with lower fibrosis and less important clinical repercussion.

**P 238**  
**PULMONARY EPITHELOID HAEMANGIOENDOTHELIOMA (IVBAT)**

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In our Institute four cases were diagnosed by the authors, they also review the clinicopathological features, differential diagnostic problems and the literature. The epitheloid haemangioendothelioma (EHE) can occur everywhere in the human body, most often in liver, soft tissue, bone and lung. In the literature mentioned occurrences in central nervous system, mamma, head-neck region and lymph nodes. The opinions are divided but the mostly accepted is that EHE is a low grade sarcoma. The tumors that are normally progressing from soft tissue and bone are more aggressive than the ones of other origin.

Pulmonary origin is relatively rare. Patients are usually without complaint and the condition is discovered accidentally. Occurrence in women is four times more frequent. The average age is under forty.

In pulmonary EHE on X-ray and CT scan shows most often subpleural solitary or multiplex nodules. Nodules can reach 2 cm in diameter but most are under 1 cm. Radiomorphology shows metastatic tumor progression. Nevertheless repeatedly scans can not show any progression. The differential diagnostic approach needs to consider multiplex benign tumors, vasculitis, bronchiolitis and granulomatous diseases. Survival of patients are variable from a few months to 15 years and depends on the site of tumor. Pulmonary origin with pleural and lymph node spreading indicates poorer outcome. Therapy is variable, can only be observation or chemotherapy too.

#### P 239

##### **LUNG OSTEOMA – A NEW BENIGN LUNG LESION**

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**Introduction:** The most common benign neoplasm's of the lung are hamartomas. A primary osteoma in the lung has never been reported. Within its differentials hamartoma with osseous foci, osseous metaplasia, secondary ossification, and nodular amyloidosis have to be discussed.

**Material and Methods:** A 38 year-old man presented with a solitary well limited tumor in the right middle lobe. He was treated with chemotherapy and interferon because of multiple myelomas and got a bone marrow transplantation 2 years and a few months before this lesion appeared. The patient underwent surgical resection. Usual routine pathologic work-up followed.

**Results:** The tumor presented with a well defined fibrous capsule and consisted of mature bone. Within the tumor a fatty tissue was seen much as it would be seen in mature bone. Focally most often at the edges small bone spicules could be discerned, most probably the area of new bone formation and appositional growth. There was no amyloid deposition, no immature epithelial tubules as in hamartomas, and no normal lung structure within the tumor, such as in osseous metaplasia.

**Discussion:** We have named this lesion osteoma, because it looks like any other extraskeletal osteoma. For comparison with similar lesions to be excluded, nodular amyloidosis, hamartomas, and various forms of osseous metaplasias will be exemplarily shown. To the best of our knowledge, this is the first case of osteoma arising in the lung.

#### P 240

##### **LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE LUNG: REPORT OF TWO CASES**

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**Introduction** The term lymphoepithelioma-like carcinoma (LELC) has been proposed for histologically unique neoplasms characterized by an undifferentiated malignant epithelial neoplasm with a markedly prominent lymphoid infiltrate. LELC of the lung is a rare lung tumour and is classified as a variant of large cell carcinoma. The aim of the present study is to describe the histopathological features of 2 LELCs and to analyze the association with EBV of LELC.

**Materials and methods** I report two patients (females) with LELC, who underwent surgery. Tumor specimens were routinely fixed and processed. Deparaffinized sections were stained by HE and examined immunohistochemically with cytokeratin, EMA, CEA, LMP-1 and ZEBRA protein expression. For the detection of EBV, non-radioactive DNA-RNA in situ hybridization for EBV-encoded small RNAs (EBER-1) was carried out.

**Results** Tumor cells of the 2 lobectomy specimens showed a syncytial growth pattern of undifferentiated cells. Cancer cells consisted of oval nuclei with a clear to vesicular chromatin pattern and a few prominent nucleoli. The neoplastic epithelial cells were accompanied by an intense lymphoid infiltrate and admixed intimately with small lymphocytes. Some areas of squamous differentiation in the form of well-defined cell borders and focal keratinization were observed within the tumors. The tumor cells of both cases stained positive for cytokeratin and EMA but not for CEA. EBER1 expression was intense and was present in most tumor cells of both cases. No expression of EBV-related proteins (LMP-1 and ZEBRA) was detected in tumor cells.

**Conclusion** I believe that careful histopathological examination of the large cell carcinomas of the lung may help to establish the exact diagnosis of LELC. Although the typical LELC of the lung consists of undifferentiated carcinoma, EBV-infected LELC in Asians is not restricted to undifferentiated carcinoma and includes more differentiated carcinomas

#### P 241

##### **UNDIFFERENTIATED 'LYMPHOEPITHELIOMA-LIKE' CARCINOMA OF THE LUNG A DISTINCT ENTITY WITH BETTER PROGNOSIS**

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##### **BACKGROUND**

lymphoepithelioma – like carcinoma (LELC) is a large cell carcinoma histologically similar to nasopharyngeal lymphoepithelial carcinoma with nests of large malignant cells in a lymphoid rich stroma. LELC of the lung is very rare in western countries, but not in southeast Asia where it is frequently associated with Epstein-Barr virus. Algeria is endemic for Epstein-Barr virus infection and nasopharyngeal carcinoma and Burkitt lymphoma are relatively frequent in our population.

##### **OBJECTIVE**

to attempt to identify cases of LELC from a large series of lung cancer and to assess their association with Epstein-Barr virus and their particularities.

##### **METHODS**

A total of 70 cases of poorly differentiated lung carcinoma reviewed. EBV research was performed by in situ hybridation technique (IHS) using EBER arrays associated with immunohistochemical (IHC) technique for expression of LMP-1 protein.

##### **RESULTS**

10 cases of LELC identified (08 squamous carcinomas and 02 undifferentiated carcinomas with lymphoid stroma). The patients were aged 45 to 79 years (median, 52 years), ratio M/F= 5. All the men were smokers.

We found a high expression of LMP-1 by IHC and EBER-1 by HIS in the two cases of undifferentiated carcinomas with lymphoid stroma. The two patients are females, 60 years old and non smokers. LELC of the lung occurring in Asians is an EBV-associated neoplasm ; it also associated with young age, a history of not smoking, high bcl-2 immunoreactivity, and better survival rate (Fen Fen Chen,1998)

#### IN SUMMARY

These are the first cases of LELC associated to EBV reported in Algerians. The role of EBV in the oncogenesis is poorly understood. LELC is histologically and biologically distinct entity with a better prognosis.

#### P 242

##### **MEDISTINAL LOW-GRADE FIBROMYXOID SARCOMA (A CASE REPORT)**

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#### Abstract:

We report the second case of Low-grade fibromyxoid sarcoma of the mediastinum, a very rare tumor, in a 55-year-old woman presented with dyspnea. Chest X-ray and computed tomography (CT) on admission showed a 30cm mass in the anterior mediastinal space, compressing the lungs and adhered to chest wall. A diagnosis of benign peripheral nerve sheath tumor was established by a CT-guided percutaneous needle biopsy. Systemic examination revealed no metastasis to the contralateral pleural cavity or other distant organs, and the mediastinal mass was resected. Tumor cells were positive for vimentin and negative for actins, EMA, CD34, keratins, and S-100 protein .Pathological and immunohistochemical analyses confirmed a diagnosis Low-grade fibromyxoid sarcoma. The patient is alive without recurrence 10 months after his operation

Key word ; Low-grade fibromyxoid sarcoma- mediastinum

#### P 243

##### **LEIOMYOMA OF THE BRONCHUS: A CASE REPORT AND REVIEW OF LITERATURE**

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Leiomyoma of the lower respiratory tract is a rare benign neoplasm and only limited numbers of cases have been described in the English literature. We recently encountered a case arising in the intrapulmonary bronchus of a Japanese young adult and present clinical and histological summary with brief literature review. A 33-year-old woman complained of old pneumonia and persistent cough for three years and was referred to our hospital in late 2004. Chest computed tomography showed a well circumscribed and enhanced mass lesion as well as a dilation of the distal bronchus and pneumonia. The mass was measured 15 mm in the largest dimension and was located in the eighth segment of the left bronchus. Bronchoscopy demonstrated a smooth whitish mass, which obstructed left basal bronchus. The left lower lobectomy was performed and gross specimen included a polypoid lesion 21• ~11• ~10 mm in dimension, which was covered by the intact bronchial mucosa. Histological examination revealed a spindle cell tumor with bland cellular morphology and readily recognized fascicular arrangement.

Nuclei showed typical bland-end shape and rare mitosis. Neither destructive growth nor infiltration into surrounding tissue was observed. Immunohistochemically, the tumor cells were positive for myogenic markers such as desmin, alpha-smooth muscle actin, HHHF35, calponin and h-caldesmon. Post-operative course is not eventful for six months after operation. Leiomyomas of the lower respiratory tract are uncommon and account for less than 2% of all benign tumors of the lung. They were subdivided into three anatomical varieties: parenchymal, bronchial, and tracheal leiomyomas. As far as we know, less than 75 cases have been reported with female predominance of 1.5:1, and are found rarely in the aged or in children. Bronchial obstruction with atelectasis, pneumonia and hyperlucency are common symptoms as seen in the present case. Outcome is generally excellent.

#### P 244

##### **INFLAMMATORY MYOFIBROBLASTIC TUMORS – LUNG NEOPLASMS WITH UNPREDICTABLE BEHAVIOUR**

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Background: Inflammatory myofibroblastic tumor (IMT) is a mesenchymal lung neoplasm according to a recent WHO classification of lung tumors. Methods: We analysed biological behavior of 20 IMTs diagnosed in last ten years on operated tissue obtained as segmental resection, lobectomy or pneumonectomy. Results: 17 IMTs were solitary nodules without necrosis and 3 IMTs were multinodular with one central, large nodule surrounded by a few smaller ones. Some of "satellite" nodules involved mediastinal pleura, penetrating in mediastinal fat tissue. Morphologically, tumor contained spindle cells arrayed in fascicles with or without mild nuclear pleomorphism and seldom mitoses. Spindle cells showed myofibroblastic differentiation, histochemically by van Gieson, Masson – trichrome and Periodic – Acid – Schiff (PAS) characteristic stains and immunohistochemically by desmin and vimentin positivity. After surgery 17 solitary IMTs didn't recide. Three multinodular, aggressive IMTs were treated as malignant mesenchymal tumors with adequate oncologic therapy. Conclusion: IMTs are mesenchymal neoplasms with benign or locally invasive, malignant growth pattern. The criteria for IMTs malignancy, despite cellular pleomorphism and mitoses, are their multinodular, aggressive growth pattern accompanied with presence of necrosis. Postsurgery oncologic treatment was required in such cases. It means that IMTs are truly neoplasms not pseudotumors.

#### P 245

##### **GRANULAR CELL TUMORS OF THE LUNGS-THREE CASES**

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Background: Granular cell tumors (GCT) are rare tumors most often located in oral cavity, skin or subcutaneous tissue. The occurrence of this tumor in the tracheobronchial tree is extremely rare.

Methods: We describe three cases of GCT found in our institution during fifteen years

period (1990-2004). At all three cases biopsies of tumors were obtained, microscopically examined and pathohistologically diagnosed.

Results: In the first patient, male, 45-years old, the diagnosis was confirmed, after recurrent bronchopneumonias by

bronchological examination, with endoscopic pendular tumor in the left upper lobe.

In the second patient, female, 63-years old, after hemoptysis, the diagnosis was established by bronchological examination, with tumor in the proximal wall of main left bronchus.

In the third patient, male, 84-years old, the diagnosis was confirmed, after fine needle aspiration biopsy.

The histopathologic study of the specimens, supported by histochemical and immunohistochemical techniques, determined the lesions to be a GCT. In all three cases on pathohistological examination large cells with acidophilic cytoplasm were found. In the first case a mild nuclear pleomorphism was present. Tumor cells showed histochemically extremely PAS sensitivity and immunohistochemically S-100 positivity.

All patients were treated locally, with complete remission in two patients.

One year after resection, the first patient developed malignant GCT, with metastatic diseases in the left parietal bone and in the left suprarenal gland and was treated with chemotherapy.

Conclusion: Pathohistological examination of each lung tumor is requested because of adequate oncologic treatment, especially in cases of rarity as GCT. Endobronchial granular cell tumors are in the majority of cases a benign tumors with a good prognosis. Local treatment gives the best results.

#### P 246

### CONGENITAL CYSTIC LUNG DISEASE AND LUNG CANCER

Titl:

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BODY: A retrospective study was carried out on 642 cases. 81 cases / 13% / were malignant lung tumors arising within congenital malformation areas. There were 72 males / 89% / and 9 females / 11% / with ages ranging from 23 to 65 years. In 18 cases / 22% / congenital lung malformations were revealed 1-15 years earlier lung cancer development. Morphologic analysis revealed two groups: 58 cases were different grade squamous cell carcinoma inside hypoplasia, congenital cystic adenomatoid malformations of the lungs; 23 cases were malignant carcinoid or adenocarcinoma or bronchioloalveolar multifocal cancer inside hypoplasia with congenital cystic disease of the lung, introlobar sequestration or congenital bronchioloectatic emphysema.

Signatura of Presenting Autor:

Dear colleagues!

A prompt reply would be appreciated.

God bless you!

#### P 247

### PULMONARY CYSTIC DISORDER RELATED TO LIGHT CHAIN DEPOSITION DISEASE

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Light chain deposition disease (LCDD) is a rare entity that very uncommonly affects the lung. We report 3 cases of LCDD presenting as a bilateral cystic lung disorder with severe outcome leading to lung transplantation. In all cases, a presumptive diagnosis of lymphangioliomyomatosis or Langerhans' cell histiocytosis was proposed based on clinical and radiological data. The diagnosis of LCDD was finally established in 2 cases, on an open lung biopsy specimen, and in 1 case, on the explanted lungs.

In these cases, the disease was characterized by progressive dyspnea associated with numerous joining cysts, diffusely distributed in both lungs. Nodules were also observed in 1 case. Lung function tests showed an obstructive pattern. The histological examination demonstrated an amorphous eosinophilic Congo red negative material located in the alveolar walls, small airways and vessels. It was most often surrounded by multinucleated giant cells. Emphysematous changes were present at the edge or at distance from these deposits. Many small airways were also dilated. Immunofluorescence disclosed staining for  $\epsilon$  but not for  $\delta$  light chains within abnormal deposits and basement membranes. Electron microscopy revealed coarsely granular electron-dense deposits in the same localizations. No other extrapulmonary deposition was ever found except for 1 case in minor salivary glands. No immunoproliferative disorder could be evidenced.

In summary, this report demonstrated that LCDD may 1) primarily affect the lungs, 2) present as a cystic disease resembling lymphangioliomyomatosis and Langerhans' cell histiocytosis on radiological grounds, and 3) lead to severe respiratory failure.

#### P 248

### THYROID TRANSCRIPTION FACTOR-1 EXPRESSION IN LUNG CANCER

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Introduction: It is known that tissue-specific transcription factors control cell determination and differentiation. TTF-1 is a tissue specific transcription factor expressed in the epithelial cells of thyroid gland and the lung.

Aim: In this study we detected the expression of TTF-1 in normal human lung and in lung adenocarcinomas primary or metastatic, by immunohistochemistry, in order to find its diagnostic value, if TTF-1 can be used to distinguish between lung and nonlung origin of a tumor.

Methods: Using AvidinBiotin-Complex / Alkaline Phosphatase immunohistochemical technique method and TTF-1 MAb clone 8G7G3 (DAKO) for the detection of TTF-1 in a retrospective study from our laboratory material (177 adenocarcinomas, 64 diagnosed in biopsies tissue, and 113 in surgical specimens, 20 NE neoplasms and 40 small cell lung carcinomas / SCLCs).

Results: (1) TTF-1 was detected in normal human lung with nuclear immunoreaction in bronchial and alveolar epithelial cells (2) Nuclear immunoreaction of TTF-1 was detected in the majority of lung adenocarcinomas (157/177) (3) TTF-1 was not expressed in neoplasms having a NE cell origin (4) Expression of TTF-1 was identified in 35 cases of SCLCs.

Conclusions: TTF-1 immunoreaction was absent in 20 cases of metastatic lung tumor with clinical or histological suspicion (from colon, breast, renal cell adenocarcinoma) and this finding supports the potential diagnostic value of this marker in lung and nonlung origin of a tumor.

#### P 249

**CONGENITAL CYSTIC ADENOMATOID MALFORMATION OF THE LUNG AND BRONCHIOALVEOLAR CARCINOMA IN A YOUNG WOMAN**

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**Introduction:** Congenital cystic adenomatoid malformation (CCAM) is a hamartomatous mass of lung tissue that has varying degree of cystic changes. CCAM is a relatively uncommon pulmonary lesion that presents mainly in neonates, rarely this lesion manifests in older children and adults. There are on records only a few patients with CCAM or history of CCAM who has developed bronchioalveolar carcinoma.

**Case report:** A 29-year-old nonsmoking woman was admitted to Department of pulmonary diseases because of persisting fever, cough and the left chest pain suspicious of chronic pneumonia in the last several months. On the chest X-ray paracardial consolidation of the left lung was found. Physical examination was unremarkable. Chest CT-scan revealed a picture suspicious of intralobar pulmonary sequestration. However, the chest and abdominal aortography did not confirm the diagnosis. Lung functions were within normal limits and bronchoscopy did not reveal any abnormality of bronchial tree, or other pathology.

**Results:** Resection of the left lower lobe was performed. Grossly and histologically there was found a congenital cystic adenomatoid malformation in the excised tissue containing areas with numerous mucinous cells, which in some foci had transitions to mucinous bronchioalveolar carcinoma. Molecular genetic investigation revealed a deletion mutation located on the exon 19 of EGFR gene. Patient recovered without major complaints after surgery. In the follow-up period, the chest X-ray and CT-scan did not show progression of tumor.

**Conclusion:** CCAM with transition to bronchioalveolar carcinoma is an extraordinary rare disorder. The presence of mucinous epithelium in CCAM may led to the suspicion, that this epithelium is premalignant. Mutation of EGFR gene is currently recognized as a marker of early phase of cancerogenesis in this lesion. Because this type of tumor becomes growth factor-dependent, it typically shows a good response to biological therapy and the prognosis of such patients, who undergo radical surgery, is usually favourable.

**P 250  
COMBINED AND SYNCHRONOUS PULMONARY TUMOURS-CASES REPORT**

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We report two cases of rare lung tumours. The first case is a 51-years-old-male which on CT of the chest had a single centro-hilar tumour. The bronchoscopy revealed a polypoid intrabronchial mass infiltrating the lung parenchima. On bronchoscopic biopsy the histologically diagnosis was typical carcinoid. Also, on frozen sections, the right tracheobronchial lymph node has shown micrometastases of typical carcinoid. The surgery specimen, represented by the middle lobe of the right lung, had a tumour of 5 cm in the main diameter. On routine stain (H&E), we diagnosed two different histopathological patterns: typical carcinoid (IHC positive diffuse for chromogranin and synaptophysin) and squamous cell carcinoma (IHC negative for neuroendocrine markers). The second case is a 59-years-old-male which presented in the superior lobe of the left lung two tumours: the first one was a

polypoid intrabronchial mass of 2.5 cm which at light microscope had a basaloid scumous cell carcinoma appearance. The second one was a peripheral mass measuring 3 cm in diameter which microscopically proved to be an adenocarcinoma. The two cases of combined and synchronous lung tumours are rare occurrence. The clinical significance of these types of tumours are uncertain.

**P 251  
PRIMARY LYMPHOID NEOPLASMS OF THE LUNG**

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**Introduction:** Primary lymphoma of the lung is a rare clinical entity (0.5-1% of the primary malignancies of the lung). Less than 100 cases of primary Hodgkin disease of the lung have been reported in the literature, worldwide. We present five cases of primary lymphoid neoplasms of the lung.

**Patients and Methods:** Over a 6 years period, 5 patients (3 men, 2 women, mean age: 70.6 years) were admitted with symptoms of bronchial occlusion and/or signs of parenchymal disease. Minimal invasive diagnostic techniques were performed to all patients. Radiologically, 4 patients demonstrated signs of diffuse nodular lesions, while 2 of them had concomitant pleural effusion. Lower lobe atelectasis was demonstrated in one patient. Bronchoscopy was performed in one patient, Medical Thoracoscopy (MT) in one patient and Video-assisted Thoracic Surgery (VATS) in 3 patients. In all cases histological examination of the excised specimens was followed.

**Results:** Primary non-Hodgkin lung lymphoma was diagnosed in 3 patients (one patient with solitary endobronchial lesion). Primary Hodgkin disease of the lung was diagnosed in 2 patients. All patients received appropriate treatment. One patient with non-Hodgkin lung lymphoma died 4 months after the diagnosis, due to treatment complications, while the other 2 patients with non-Hodgkin lung lymphoma died 12 and 13 months respectively, from non disease-related causes. Two patients with primary Hodgkin disease of the lung are disease-free 9 and 10 months respectively from the time of diagnosis.

**Conclusions:**

The tremendous progress in the fields of histopathology and immunochemistry along with the advent of minimally invasive diagnostic techniques (MT, VATS) have facilitated the diagnostic approach to the primary lymphoid lung neoplasms. With the use of these minimally invasive techniques by which large sized biopsy specimens can be obtained, unnecessary diagnostic thoracotomies can be avoided.

**P 252  
SCLEROSING HEMANGIOMA OF THE LUNG:  
REPORT OF 5 CASES WITH  
IMMUNOHISTOCHEMICAL AND  
CLINICOPATHOLOGICAL FINDINGS**

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Sclerosing hemangioma of the lung (SHL) is a rare tumor which is thought to be benign. Although the differentiation of tumor cells has been controversial, it is believed to be

originated from fetal pulmonary epithelial cell. Also, with various histological patterns, it leads to diagnostic difficulties having no specific immunohistochemical marker.

The aim of this study is to demonstrate the immunohistochemical profile of SHL in addition to the analysis of clinicopathological features.

The clinicopathological features of 5 SHLs were reviewed and analyzed. Immunohistochemical analysis could be performed in 3 cases for antibodies TTF-1, Surfactant protein-B, NSE, Chromogranin A, Synaptophysin, CD31, CD34, estrogen and progesteron receptors, Cytokeratin 7, EMA, p53 and Ki67.

All patients were female with an average age of 54.4 years (36-65). The tumor size ranged from 2 to 5 cm with a mean of 3.7 cm. The combination of observed histological patterns was different in each case. Four cases revealed papillary pattern, 1 case revealed sclerotic pattern, 4 cases revealed solid pattern and 3 cases revealed hemorrhagic pattern. No mitotic activity was detected in all cases. Ki-67 proliferation index ranged from 1.4 to 2.2% with an average of 1.8%. Both the surface and round stromal cells were diffusely positive for EMA and TTF-1 in two cases, whereas NSE was demonstrated focally in all. Only in one tumor TTF-1 and Surfactant protein-B were detected to be weakly focal positive, but EMA was diffusely strong positive. None of the tumors exhibited positivity for endothelial markers. Cytokeratin7 and Surfactant protein-B were both positive in only surface cells. Progesteron receptor was detected both in surface and round stromal cells but estrogen receptor was negative.

SHL is most probably a benign tumor of primitive pulmonary epithelium with various histological patterns. There is no specific immunohistochemical marker for SHL but EMA, TTF-1 and Surfactant proteins are helpful in differential diagnosis. This entity must always be kept in mind in the diagnosis of solitary pulmonary nodules because of its similarity to the histology and immunophenotype of the primary and metastatic pulmonary tumors. Positivity for progesterone receptor antibody may be associated with the female predominancy of SHL. However, this suggestion is needed to be studied on large series of SHL.

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##### **TTF-1 IMMUNOHISTOCHEMISTRY AS AN INTRA-OPERATIVE DIAGNOSTIC TOOL AT FROZEN SECTION FOR DISTINCTION BETWEEN PRIMARY AND SECONDARY LUNG TUMOURS**

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Although undertaking frozen sections is a well established, diagnostically accurate procedure in intra-operative lung cancer diagnosis, differentiation of primary from metastatic carcinomas remains problematic. This study assesses the value and practicality of undertaking immunohistochemistry (IHC) for thyroid-transcription factor-1 (TTF-1) at the time of frozen section when faced with this clinical situation.

Thirteen patients presented with a mass in the lung and a history of previous carcinoma (colon n= 8, renal n= 1, breast n=3, thyroid n=1). Specimens were taken during operation for confirmation of malignancy and at this time additional sections were taken for intra-operative localisation of TTF-1. The antigen was visualised using the Envision® System with abridged methodology. Slides were assessed as positive, equivocal or negative. Conventional IHC was then repeated both on the paraffin block from the frozen section and a separate tumour block from the resection specimen for comparison.

Of 13 cases, seven cases were positive for TTF-1 at frozen section, one of which was metastatic thyroid carcinoma. The

remaining six were either positive (n=5) or equivocal (n=1) on the subsequent paraffin section and positive on resection specimen (n=6) and felt morphologically to be primary pulmonary adenocarcinomas. Six cases were negative on frozen section. However, one case of colonic carcinoma showed focal staining for TTF-1 on sections from subsequent paraffin block and resection. IHC confirmed metastatic disease in the resection specimens (colonic carcinoma (n=5), cytokeratins (CK)20 +ve, CK7 -ve TTF-1 -ve; renal cell carcinoma (n=1), TTF-1 -ve with clear cell morphology). The average time above that of the basic frozen section was 24 minutes per frozen section. The extra cost per test was £32 per slide.

Frozen section IHC using TTF-1 shows specificity and sensitivity similar to those seen for fixed tissues, and appears feasible within the time frame of a thoracotomy. However, its practice should be properly planned within an operative procedure as liberal usage will likely have significant staff and cost implications.

#### P 254

##### **COX-2 EXPRESSION IN NEUROENDOCRINE TUMOURS OF THE LUNG**

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##### **Background:**

Recent studies have highlighted the role of COX-2 in human cancers and the protective effect of COX inhibitors. Cox-2 can be found in a large proportion of lung adenocarcinoma and squamous cell carcinoma. However, little is known about the frequency of COX-2 expression in neuroendocrine tumours of the lung.

##### **Materials and Methods:**

Paraffin-embedded material from 64 neuroendocrine tumours were retrieved from the archives. Immunohistochemistry showed COX-2 staining in all twelve carcinoids (100%), in two of four atypical carcinoids (50%) and in 13 of 48 small cell carcinomas (27%). While a dense and intensive cytoplasmic COX-2 staining (++ - +++) could be seen in carcinoids, the findings in the two positive cases of atypical carcinoids were weak respectively moderate. In SCLC, seven cases showed a weak (+) expression of COX-2, four cases presented a moderate staining (++) . Normal bronchial epithelium showed an apical staining, pneumocytes II were positive in regions with chronic inflammation.

##### **Conclusion:**

This study is one of the first to investigate systematically the expression of COX-2 in neuroendocrine tumours of the lung. It demonstrates that the different entities of these tumours show a very distinct immunohistochemical pattern. The clinical impact of these findings has to be further investigated.

#### P 255

##### **EXPRESSION AND PROGNOSTIC IMPLICATIONS OF APOPTOSIS-RELATED PROTEINS IN LOCALLY ADVANCED NON-SMALL CELL LUNG CANCERS.**

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**Introduction:** In advanced lung cancer at present little parameters are available to predict outcome.

**Purpose:** To evaluate prognostic significance of apoptosis related proteins in locally advanced non-small cell lung cancer (NSCLC).

**Patients and Methods:** Bronchoscopically procured tumor biopsies of NSCLC patients were stained

immunohistochemically and rated for expression of eight different cellular proteins. Staining  $\geq 10\%$  of the tumor cells was classified as negative,  $> 10\%$  was considered as positive. Patients were treated with 60 Gy radiotherapy with or without carboplatin as radiosensitizer.

Results: Apoptotic proteins in tumors that showed positive staining were the highest for Bax (99%), Fas (92%), FasL (87%), Rb (87%), p21(WAF1) (73%), and p53 (70%), and the lowest for c-myc (58%) and Bcl-2 (58%). In the Cox regression analysis stage (stage 3B versus stage 3A, relative risk (RR) = 0.63, 95% CI, 0.43-0.92,  $p < 0.02$ ), the presence of mediastinal lymph nodes (N2 versus N3, RR=1.69, 95% CI, 1.02-2.8,  $p = 0.04$ ) and Bcl-2 positivity (RR=0.61, 95% CI, 0.37-0.98,  $p = 0.04$ ) were predictive for overall survival. Only Bcl-2 staining percentage (RR10 (RR associated with an increase in stained cells of 10%) = 0.93, 95% CI, 0.89-0.99), p53 (RR10=0.94, 95% CI, 0.89-0.99,  $p = 0.027$ ) and FasL (RR10=0.92, 95% CI, 0.86-0.99) were predictive for a longer time to progression. No specific constellation of apoptotic proteins was associated with tumor response except that the loss of Rb expression was associated with the absence of any tumor response.

Conclusion: Bcl-2 expression in tumor tissue of patients with locally advanced NSCLC predicts a better overall survival, while Bcl-2, p53, and FasL expression predict for a longer time to progression.

#### P 256

##### EXPRESSION OF SOMATOSTATIN RECEPTORS 2-5 IN NEUROENDOCRINE TUMOURS OF THE LUNG

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##### Background:

Somatostatin receptors 1-5 can be found in a large proportion of human tumours. Due to the fact, that receptor-positive tissue can be detected by scintigraphy, several investigations have taken place to demonstrate somatostatin-receptors also at the immunohistochemical level. Recent studies have concentrated mainly on the detection of the Somatostatin receptor type 2 (SSTR2) in small cell carcinoma. We were interested to investigate systematically the presence of SSTR 2-5 by immunohistochemistry in various entities of neuroendocrine tumours of the lung.

##### Materials and Methods:

Paraffin-embedded material from 45 neuroendocrine tumours were retrieved from the archives. Of twelve carcinoids, 8 expressed SSTR2, three SSTR3. None were positive for SSTR 4 or 5. All four atypical carcinoids were negative for SSTR2, in 3 cases a cytoplasmic staining for SSTR3 was found. One case was weakly positive for SSTR4 and two cases for SSTR5. In 10 of 28 small cell carcinoma SSTR2 was found, 13 cases were positive for SSTR3, 3 cases for SSTR4 and one case for SSTR5. The case of large cell neuroendocrine carcinoma was positive for SSTR3 and SSTR4, SSTR2 and SSTR5, however, were negative.

##### Conclusion:

This preliminary study may help to correlate the findings by receptor autoradiography with immunohistochemical results in various types of lung neuroendocrine tumours. This may provide further information concerning diagnosis as well as therapeutic approach in these tumours.

#### P 257

##### PULMONARY METASTASIS SECONDARY TO "BURNED OUT" TESTICULAR TUMOR. A CASE REPORT.

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Metastatic germ-cell tumor of the primary testicular lesion has rarely reported. The majority of cases described in the literature have occurred in patients with retroperitoneal metastasis. The authors present a case of pulmonary metastasis with complete regression of the primary testicular tumor.

A 34-year-old Caucasian man presented with a 3-month history of chronic cough. The chest X-ray and the thoracic computed tomography scan showed a 10x14 cm mass in the lower lobe of the left lung without lymph node involvement. The flexible bronchoscopic examination revealed an obstructive mass in the upper segment of the left lower lobe. Histological examination proved the mass to be a malignant germ-cell tumor containing embryonal carcinoma elements. Serum  $\beta$ -human chorionic gonadotropin and  $\beta$ -fetoprotein were elevated. Ultrasonography visualized a heterogeneous testicular mass. Histological examination of the testicular tumor showed stromal fibrohyalinoses with no evidence of a tumor. The patient was successfully treated with orchiectomy, chemotherapy and lung surgery. Four years later, he remains disease free.

Confronted with a young man presented an isolated pulmonary tumor and apparently normal testes, the physician should suspect a metastatic malignancy, especially metastatic germinal germ-cell tumor with regression of the primary testicular tumor. Tumor marker assays and ultrasonography should be performed and in-depth histological examination of the testis is essential when lesions are identified.

#### P 258

##### THYROID TRANSCRIPTION FACTOR-1 (TTF-1) IN THE DIFFERENTIAL DIAGNOSIS OF THE PRIMARY AND METASTATIC LUNG CANCER. OUR 4-YEAR FOLLOW-UP STUDY.

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Introduction: Lung adenocarcinoma is very frequent in adult population. The tumor may be primary, but metastasis from malignancies of other organs often appears as a solitary nodule. From the treatment's point of view it is very important to know if the tumor is of primary or metastatic origin. TTF-1 is a sensitive marker for pulmonary and thyroid adenocarcinomas.

Material and methods: In order to determine the usefulness of TTF-1 in distinction between primary and metastatic lung adenocarcinoma we have examined the expression of TTF-1 in 100 solitary pulmonary nodules. They included 50 stage I. peripheral primary bronchial adenocarcinomas and 50 metastatic pulmonary adenocarcinomas of different origin such as breast, colon, rectum, kidney, stomach and thyroid gland. TTF-1 was performed on formalin-fixed and paraffin-embedded tissues. All primary adenocarcinomas were diagnosed in 2000. In November, 2004 follow-up study was performed. We investigated the correlation between the strong and the weak nuclear immunostaining and the survival of patients.

Results: We found immunopositivity in 46/50 cases, among them 30 cases showed strong nuclear immunostaining. In the remaining four cases the immunopositivity was localized to the cytoplasm. All metastatic adenocarcinomas were negative, apart from the 2 thyroid carcinoma metastases that showed strong immunopositivity. Follow-up data were available in 49/50 patient. There was no tumor recurrence in 27 cases (12 strong immunopositivity), in 6 cases the disease progressed (5

strong immunopositivity), and 14 patients died (9 weak immunopositivity). Kaplan-Meier survival analysis and chi square test showed strong correlation between strong immunopositivity and free survival time ( $p=0.03$ )

Conclusion: Our results confirm, that the TTF-1 immunohistochemistry is a very sensitive and highly specific method in the differential diagnosis of primary and metastatic lung adenocarcinomas and should be used in the everyday routine practice. Moreover, TTF-1 immunopositivity proved to be a good prognostic factor in stage I lung adenocarcinomas.

#### P 259

##### TISSUE MICROARRAY IMMUNOPHENOTYPING OF PRIMARY NSCLC AND BRAIN METASTASES

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Introduction. A proportion of non small-cell lung cancers (NSCLC) form metastases almost exclusively into the brain irrespectively of their primary sizes. Purpose. To identify markers which can predict metastatization of NSCLC into the brain. Methods. Twenty one squamous cell carcinomas and 5 adenocarcinomas of the lung with brain metastases using immunohistochemistry with a selected panel of antibodies were tested. Twenty squamous cell carcinomas and 15 adenocarcinomas with no brain involvement were used as a control. Tissue cores of 2 mm in diameter were incorporated into 24-sample microarray blocks resulting in 117 samples in 5 paraffin blocks using duplicate samples in most cases. Tested antibodies were specific for molecules of cell adhesion (e.g. BP180/Collagen XVII, CD44, E-Cadherin, b-Catenin), cell growth (e.g. Akt1, EGFR, nm23) cell cycle regulation (e.g. Cyclin D1, -D3; Ki67, p16, p21waf1/Cip1, p27kip1, DNA repair (Topoisomerase IIa) and apoptosis (e.g. Bax, Bcl-2, CAS, Caspase-3, -8,-9, FAS, p53). The results were assessed by 2 independent observers a 4-scale scoring system (0 negative, 1 weak, 2 moderate, 3 strong), where cut-off levels were based on literature data considering the patho/biological role of the given marker. The consensus based final scores were converted using the TMA-Deconvoluter software. Non-supervised hierarchical clustering was applied for all data and for selected data groups, which then were visualized using the TreeView program. Results. Phenotypic marker expression of primary NSCLC and their brain metastases showed very similar profile without major loss or gain of any marker. None of the exclusive groupings of markers, i.e. analyzing cell cycle, apoptosis or cell adhesion associated markers grouped separately, resulted in obvious clustering of our cases. However, when the expression profile was set up combining immunoreactions for Cyclin D1, Cyclin D3, Ki67, p16, Collagen XVII/BP180 molecules, the majority of brain tumors clustered with a high positive correlation (0.86) with these markers. Conclusion. Based on tissue microarray analyses, the metastatic capacity of NSCLC cases into the brain seems to be associated with cell cycle activation and the upregulation and delocalization of the hemidesmosomal Collagen XVII/BP180 antigen. Analyses of further restricted combinations involving the expression of a few more marker molecules are still under way.

#### P 260

##### STROMAL TUMOR OF THE ILEUM WITH PULMONARY METASTASIS.

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Gastrointestinal stromal tumors (GISTs) constitute the largest category of primary non-epithelial neoplasms of gastrointestinal tract. Extra-abdominal metastases of GISTs occur in less 10 percent of patients.

A 34-year-old Caucasian man was admitted for abdominal pain, anorexia, nausea vomiting occur, weight loss of 10 kg. No abdominal mass was found. The endoscopic findings were not specific. Abdominal computed tomography scan showed multiple intramural masses of jejunum and ileum. Thoracic CT scan showed a right hilar mass with small mediastinal lymph nodes. The intestinal masses were resected by laparotomy and hilar mass by mediastinoscopy. Histological and immunohistochemical studies on the surgical resection specimens revealed a GIST with pulmonary metastases. The patient died after 2 years.

GISTs are rare and represent the 0.2% of all malignancy of this site. They occur predominantly in older individuals over 40 years of age with equal sex incidence. They are most common in stomach (60-70%), followed by small intestine (20-30%), colon and rectum (5 %), oesophagus ( $f=5$  %), and occasionally in omentum and mesentery. Recent work has indicated that the interstitial cells of Cajal, which exhibit both myoid and neural features, could be candidates for tumor histogenesis.

20-30 % of GISTs show malignant behavior, including peritoneal dissemination and hematogenous metastases. Metastases are often present at the time the primary tumor is discovered. The most common metastatic sites are the peritoneal surfaces and liver, following by retroperitoneal soft tissues. Lymph nodes, bone and lung metastases are rare. The survival rates at 5 years of malignant tumors vary of 19 to 54 %.

#### P 261

##### UNUSUAL METASTATIC PATTERN OF A RADIATION INDUCED ANGIOSARCOMA OF THE SKIN – A CASE REPORT

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We report on a 67 year old female patient in whom hysterectomy and oophorectomy as well as adjuvant telecobalt radiation were performed 27 years ago. 25 years later multiple tumours occurred in the abdominal wall. Histological examination of these tumours showed an epitheloid neoplasm with vascular proliferation and a fine framework of reticular fibres. Immunohistochemistry (pancytokeratin, cytokeratin 8/18, CA125 and CD45 negative, vimentin and CD31 positive, CD34 focally positive) revealed angiogenic differentiation and an angiosarcoma was diagnosed. It was treated surgically.

Another two years later the patient was admitted to hospital with severe dyspnoea. Chest x-ray and CT-scans showed abundant bilateral predominantly interstitial infiltrates in both lungs. Bronchoscopy revealed a diffuse thickening of the bronchial mucosa. Bronchoalveolar lavage, biopsies of the bronchial mucosa and transbronchial lung biopsies were performed. A malignant small round blue cell tumour was identified in all specimens. Immunohistochemistry (cytokeratin and CD45 negative, CD31 intensely positive) confirmed metastases of the angiosarcoma.

Post-mortem examination revealed relapse of the angiosarcoma in the abdominal wall as well as metastases in the lungs, which showed a predominantly interstitial and

peribronchial growth pattern. Further metastases were found in the lymph nodes, in the myocardium, in the abdominal fatty tissue and additionally in a synchronous renal cell carcinoma. Radiation induced angiosarcoma is very uncommon. Furthermore the metastatic pattern with the clinical presentation as an interstitial lung disease on the one hand and with a metastasis in another malignant tumour, which occurred independently, on the other hand are extreme rarities.

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##### **ASSOCIATION BETWEEN MALIGNANT MESOTHELIOMA OF THE PLEURA AND OTHER MALIGNANCIES**

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The association between malignant mesothelioma and other primary tumors has repeatedly been described. In such reports, however, a denominator is frequently not available. In the present study, a series of 169 malignant pleural mesotheliomas, examined at necropsy at the Hospital of Monfalcone, Italy, in the period 1980-2002, were reviewed. The group included 139 men and 30 women. All mesotheliomas were classified as asbestos-related. The co-existence of mesothelioma with other malignancies had been detected in 33 cases (19.5%). Four different tumors were seen in two cases. A 77-year-old man showed, besides mesothelioma, a lung carcinoma, a small carcinoma of the kidney, and a small carcinoma of the prostate. A 85-year-old woman showed three carcinomas of the large bowel. Triple malignancies were observed in 6 cases; in particular, non-Hodgkin lymphoma or leukaemia were associated with mesothelioma in three cases, and prostate adenocarcinoma in two. Double tumors were observed in 25 cases, with prostate adenocarcinoma (5 cases), bladder carcinoma (4 cases), and hepatocellular carcinoma (3 cases), being the most frequent additional malignancies. Of the four non-Hodgkin lymphomas observed in the whole series, two were extra-nodal. In some of the present cases the co-existence of different tumors was a cause of major difficulties in the clinical diagnosis. Only small percentages of people exposed to asbestos develop mesothelioma. The analysis of the cancer varieties associated with mesothelioma may furnish information about the background, in which mesothelioma develops, and/or about the co-factors playing a role in the genesis of asbestos-related mesothelioma. The co-existence of mesothelioma with non-Hodgkin lymphoma or with hepatocellular carcinoma may suggest a role of immune impairment in the genesis of mesothelioma.

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##### **CELL PROLIFERATION RATE AND TELOMERASE ACTIVITY IN THE DIFFERENTIAL DIAGNOSIS BETWEEN BENIGN AND MALIGNANT MESOTHELIAL PROLIFERATIONS**

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The differential diagnosis of malignant mesothelioma (MM) from benign mesothelial proliferations (BMP) based on histopathologic criteria is sometimes not satisfying and causes diagnostic problems for the histopathologists. We investigated whether the immunohistochemically determined cell proliferation rate and telomerase activity, using Ki-67 and hTERT immunohistochemistry, respectively, are useful in the differential diagnosis of MM from BML. Sixty six cases of MM (33 epithelioid, 30 biphasic and 3 sarcomatoid) and 22

cases of BML (15 reactive mesothelial proliferations and 7 fibrous pleuritis/pericarditis) were included in this study. The mean value of Ki-67 proliferation index (pi) in MMs was significantly higher than that of BMLs. Biphasic MMs have higher Ki-67pi than epithelioid and sarcomatoid types. Ki-67 immunohistochemistry has the sensitivity of 86%, specificity of 74% and positive predictive value of 94% in detecting MM. hTERT immunohistochemistry detected MM with sensitivity and specificity of 68%. Furthermore, the sensitivity and specificity of hTERT for detecting MM among the cases with low Ki-67pi were 58% and 78%, respectively. As a result, being cheap and simple methods, Ki-67 and hTERT immunohistochemistries can be used in differentiating malignant and benign mesothelial lesions in routine formalin-fixed, paraffin-embedded material.

#### P 264

##### **DIAGNOSTIC VALUE OF PSEUDOINCLUSION IN THYROID PAPILLARY CARCINOMA AND HASHIMOTO'S LYMPHOCYtic THYROIDITIS**

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Objectives and background: Papillary carcinoma is the most common malignant tumor of the thyroid.

Intranuclear inclusions (pseudoinclusions) that represent an invagination of the cytoplasm into the nucleus are frequently seen in papillary carcinoma, and in fact this is one of characteristic features.

Chronic lymphocytic thyroiditis (Hashimoto's); is one of the thyroid diseases that could be present with thyroid cold nodule and FNA is recommended for evaluation.

Materials & Methods: This is an experimental study. In this study 33 cases of patients with cold nodules or diffuse enlargement of one or both lobes of thyroid were candidates for FNA. In clinical studies, these 33 cases were diagnosed as suggested for malignancy or Hashimoto's thyroiditis. All prepared smears were stained with papanicolao and geimsa, and studied.

Findings: In all cases showed nuclear pseudoinclusion in papanicolao and geimsa stain.

Cytologic findings in 16 smears included variable degrees of lymphocytic infiltration with some hurthle cells in scanty colloid background and diagnosed chronic lymphocytic thyroiditis (Hashimoto's thyroiditis). 17 of them were diagnosed as suggested for papillary carcinoma, due to seen pseudoinclusion, but in histologic study of them showed Hashimoto's thyroiditis and the result of TPO test in these 17 cases was positive.

As a result, pseudoinclusion showed clearly in smears stained with papanicolao.

Conclusion: Most of cytomorphologic findings in FNA of papillary carcinoma are not specific, for example, papillae could be seen in papillary hyperplasia, nuclear features, so ground-glass chromatin, longitudinal folds or nuclear groove, although are important for papillary carcinoma, but are neither constant nor specific. Psammoma bodies are observed in a small number of cases which are not specific to papillary carcinoma of the thyroid, because they also have been described in other lesions such as toxic diffuse goiter and Hashimoto's thyroiditis. In contrast to previous reports, nuclear pseudoinclusion are not characteristic for papillary carcinoma of thyroid and it could be seen in Hashimoto's thyroiditis.

#### P 265

##### **ACCURACY OF FINE NEEDLE ASPIRATION CYTOLOGY IN THYROID NODULES.**

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**Introduction:** FNAC has become accepted as a cost-effective procedure in the assessment of thyroid nodules and is an important first-line diagnostic test for the evaluation of goiter and the single most effective test for the preoperative diagnosis of a solitary thyroid nodule. To evaluate the accuracy of FNAC in the diagnosis of thyroid nodules did this study.

**Material & Methods:** This study was carried out at Shahid Beheshti Hospital in Babol Medical University during the period of 1998 and 2003. There were 745 FNACs of which 98 had definite histology after thyroidectomy. FNAC was correlated with histology and sensitivity, specificity, accuracy with NPV and PPV were calculated.

**Results:** The overall sensitivity of FNAC detecting thyroid neoplasia was 86.84%, specificity 73.3% and accuracy 78.57%. NPV and PPV were 89.79%, 69.38%, respectively. There was no false positive malignant FNAC. 32 malignant cases were detected, that of 22 males, 13 (59%) had malignancy compared with 19 of 76 females (25%).

**Conclusion:** FNAC is a useful test in assessment of solitary thyroid nodules especially in males who have high index of suspicion of thyroid cancer. Malignant or suspicious for malignancy cytology are absolute indicators for thyroidectomy.

**Keywords:** Solitary thyroid nodule, FNAC, Thyroid neoplasia, Accuracy.

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#### P 266

**HISTIOCYTOID-LIKE CELLS IN FINE-NEEDLE ASPIRATION BIOPSY (FNAB) OF THE THYROID**  
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**INTRODUCTION:** Histiocyte-like cells, characterised by vacuolated cytoplasm without classic nuclear changes of papillary carcinoma (PC), isolated or in clusters, have been described recently as highly specific for PC in thyroid FNAB specimens.

**AIMS:** Immunohistochemical study and cytohistologic correlation in cases with histiocyte-like cells at FNAB without classic features of PC.

**MATERIAL AND METHOD:** 327 FNA of thyroid were performed in our Hospital last year. Cases with histiocyte-like cells observed isolated in both smears and cell blocks were selected; Cytokeratin 19 (CK-19) (Novocastra, b170), Galectin 3 (Gal-3) (Novocastra, 9C4) and CD-68 (DAKO, PG-M1) were performed on the cell block; histologic correlation with surgical specimen was made.

**RESULTS:** Of 327 FNAB, only in 3 cases histiocyte-like cells were identified in both smears and cell block. These cells were immunoreactive for CK-19 and Gal-3, and were negatives for CD-68. All resection specimens correspond to nodular hyperplasia (NH), showing at microscopic level cystic follicles lined by follicular cells with histiocytic appearance like those identified in cytologic samples.

**CONCLUSIONS:** Histiocyte-like cells are a rare finding. Although these cells present the same immunohistochemical

profile as PC cells, they are not pathognomonic, raising a potential pitfall for the interpretation of thyroid FNAB. These cells could be altered follicular cells from the cyst lining, in NH, with repair-like features or scamous metaplasia based on their immunoreactivity.

#### P 267

**OUR EXPERIENCE IN THE EFFECT OF FINE-NEEDLE ASPIRATION ON THE PAPILLARY THYROID CARCINOMA SCAN**

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Papillary carcinoma represents approximately 75-85% of all thyroid malignancies. We report comparative analyses: fine-needle aspiration (FNA) cytology before operation and definitive histopathology undergo surgical excision in evaluation of it.

During the last 2 years in 600 examinee thyroid aspirates 19 cytological diagnosis of papillary carcinoma was made. These diagnostic guidelines were used: so-called "tumor cellularity" of smears, papillary arrangement of cells, tumor cells are larger than normal follicular cells with slight anisocytosis, ground-glass nuclei, presence of intranuclear inclusions and scanty or absent colloid. Tumor was more frequent in females in 74%. The middle ages (51,2) was older than expected. Classic cytological characteristics had 89% aspirates. One papillary carcinoma contained psammoma body. In 26,3% degenerative changes (hemorrhage and necrosis) were presented. In 2 smears papillary carcinoma were associated with Hashimoto's thyroiditis and oxyphilic lesion.

Definitive histopathology undergo surgical excision was confirmed papillary carcinoma (15, or 79%), Hashimoto's thyroiditis with degenerative changes (2), nodular goiter with degenerative changes (1) and oxyphilic lesion (1).

We conclude that FNA cytology has a high sensitivity and specificity for the diagnosis of papillary carcinomas. Significant number of thyroid papillary cancers could be detected by fine-needle aspiration before operation.

#### P 268

**FINE NEEDLE ASPIRATION CYTOLOGY IN THE BREAST SCREENING PROGRAM IN VERONA. FIVE YEARS OF EXPERIENCE.**

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**Introduction:** Fine needle aspiration cytology (FNAC) is a quick, low expensive diagnostic technique widely used in the management of breast lesions both in screening programs and clinical senology. Efficacy of cytology is variable in different experiences. A European standard system of reporting the classification of cell samples (C1-C5) is now used to perform quality assurance statistics in cytology units. This system allows to compare results between units with different experience. Purpose of the study: To evaluate the cytology quality standard in Breast Screening Program (BSP) in first five years of activity according to the European guidelines. Material and methods: Compared with the "classic" screening, the Verona program has a different organization, similar to that of a Breast Clinic, and operates in a building exclusively devoted to the BSP. First level mammograms and all second level exams (US, FNAC, core biopsy, mamotome) are performed in the same structure by a staff composed of two radiologists, two cytopathologists, four technicians and two nurses. All women are submitted to a double projection mammography with real time analysis of mammograms by Radiologists. Results are immediately communicated. Negative patients are dismissed after

reassurances; suspicious patients are immediately submitted to second level examination. FNAC is evaluated in real time for adequacy and a diagnostic report is immediately given to the Radiologist. Results: 1374 FNAC have been performed; the cytology diagnosis was C1: 10.8% (149 cases); C2:51.7% (710cases); C3:6%(83 cases); C4: 9.2% (124cases); C5:22.3% (308cases). Absolute sensitivity was 73%, Complete sensitivity:95.4%, Specificity (full):75.2%, PPV:99%, False positive rate:0.4%, False negative rate:2.1%, Inadequate rate:10.8%, Inadequate rate from cancers:2.4%, Suspicious rate:15%. Conclusions: In the Verona BSP all thresholds for FNAC quality performance are within the preferable value suggested by European quality guidelines for screening programs. From our results it seems that FNAC can be highly effective particularly within a screening program with real time microscopic analysis coupled with multidisciplinary consult between radiologist and pathologist.(Supported by Fondazione Cassa Risparmio VR-VI-BL-AN.Bando 2004.Progetto Cr.Mammella)

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##### CLINICAL AND CYTOLOGIC FEATURES OF PAPILLARY NEOPLASMS OF BREAST

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**INTRODUCTION:** The cytologic pattern of papillary neoplasm of the breast is described as a cellular aspirate with complex papillary fronds and a variable number of single, high, columnar epithelial cells. Differentiating a benign papillary lesion from an atypical or malignant one is difficult using fine needle aspiration cytology owing to their overlapping cytologic features.

**OBJECTIVE:** To compare the cytologic features of benign and malignant papillary breast lesions.

**STUDY DESIGN:** We reviewed the clinical and cytologic features in 29 cases of intraductal papilloma and 26 cases of atypical papilloma or papillary carcinoma that had been diagnosed by histologic examination. The diameter of the mass was examined as a clinical feature. The cytologic features evaluated were as follows: bloody background, row of tall columnar cells, naked bipolar nuclei, hemosiderin-laden macrophages, myoepithelial cells, single scattered atypical cells, cellularity, nuclear atypia, nuclear grade, apocrine metaplasia, eosinophilic cytoplasmic granules, papillary clusters, small papillae, cell balls, and large sheets.

**RESULTS:** Of the features evaluated, the diameter of the mass, naked bipolar nuclei, and cell ball differed significantly between benign and atypical or malignant papillary neoplasm. The average diameter of a benign papillary neoplasm was 1.8 cm, and that of an atypical or malignant papillary neoplasm was 2.2 cm ( $p=0.042$ ). Naked bipolar nuclei were found in 27 cases of benign papillary neoplasm (93.1%) versus 19 cases of atypical or malignant papillary neoplasm (73.1%) ( $p=0.050$ ). Cell balls were found in 14 (48.3%) and 21 (80.8%) cases, respectively ( $p=0.012$ ). All six cases in which cell balls were present and naked bipolar nuclei were absent proved to be atypical or malignant papillary neoplasm. Of 17 cases where cell balls were absent and naked bipolar nuclei present, 13 (76.5%) were benign papillary neoplasm.

**CONCLUSION:** Most of the cytologic features overlap in benign and atypical or malignant papillary neoplasm.

Although they were not pathognomic, naked bipolar nuclei and cell balls were cytologic features that differed significantly between benign and atypical or malignant papillary neoplasm. When papillary neoplasm of the breast is suspected in a cytologic smear, the combination of clinical

examination, mammography, and cytologic features should be considered to make the correct diagnosis.

#### P 270

##### THE ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) IN CLINICAL DECISION MAKING FOR PATIENTS WITH ADVANCED BREAST CANCER

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**Background:** Fine needle aspiration cytology (FNAC) is a well established procedure for the diagnosis of primary tumor, recurrence, and metastasis. Biomarkers of breast cancer cells (hormone receptor status and Her-2 oncogene expression) play a key role in the prognosis and treatment response. These biomarkers can become modified during progression of disease (PD).

**Purpose:** To demonstrate the utility of FNAC to confirm the clinical diagnosis and to re-evaluate the biomarker status in recurrent disease.

**Materials and Methods:** From January 2003 to April 2005, fine needle aspiration was performed on 98 cutaneous nodules or lymph nodes from 86 women. Smears were alcohol fixed (95%) and/or air-dried and stained with hematoxylin and eosin (H&E), Papanicolaou stain, or May-Gruenwald Giemsa. In 42 cases, the remaining specimen was rinsed in a 50% alcohol solution prior to preparation in a paraffin-embedded cell block. Slides from the cell block were submitted for H&E, and when necessary, immunohistochemistry (IHC) and fluorescent-in situ hybridization-dual colour (FISH) were performed. Cytomorphology, IHC, and FISH results were compared with those of the primary tumour, when possible.

**Results:** The majority of FNAC were positive (76 cases) for recurrent disease. 14 cases were negative and 8 were unsatisfactory for evaluation. 56 of the 76 positive cases were re-assessed for biomarker status. There was an 83.9% concordance in biomarker status (hormone receptor status and Her-2 expression) in the recurrent tumour when compared to the primary tumour. The discordant rate was 16.1% giving rise to the hypothesis that in a few cases one tumour cell clone has dominated and metastasized.

**Conclusions:** FNAC is a well-tolerated procedure that is easy to perform and less invasive. A well managed cytologic specimen is sufficient for establishing a diagnosis and biological characterization. IHC and FISH can be easily performed on FNAC and facilitate the management of breast cancer patients with PD.

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##### CYTOLOGICAL FEATURES OF PROSTATIC INTRAEPITHELIAL NEOPLASIA

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**INTRODUCTION.** Fine needle aspiration cytology (FNAC) is an acknowledged method for diagnosing prostate cancer. Although core biopsies have become more common, FNAC is still useful in elderly men and in patients with advanced disease. False positive results are uncommon but concerns have been raised that prostatic intraepithelial neoplasia (PIN) could be misinterpreted as carcinoma. **PURPOSE.** The aim of this study was to describe cytological features of PIN.

**METHODS.** Macroscopically normal areas of peripheral zone in 177 radical prostatectomy specimens were sampled by scraping cells from cut surfaces. The obtained material was

smear, air-dried and Giemsa-stained. Histological slides from these areas were reviewed and 17 samples with high-grade PIN and no invasive cancer were selected. An equal number of smears from invasive cancer were randomly chosen for comparison. The cytological specimens were reviewed without knowledge of the histological diagnosis.

**RESULTS.** Cancer smears showed high cellularity and dissociation, while PIN smears only contained a few clusters of atypical cells. Pronounced nuclear atypia, prominent or multiple nucleoli and mucin were more often seen in cancer than in PIN. Crystalloids, cytoplasmic granules and size of atypical clusters did not distinguish between PIN and cancer.

**CONCLUSION.** PIN should not be diagnosed by FNAC alone. However, a highly cellular smear with pronounced nuclear atypia seems to preclude PIN.

#### P 272

##### **URINE CYTOLOGY IN THE PREVENTION OF NEPHROPATHY BY POLYOMAVIRUS IN PATIENTS WITH RENAL TRANSPLANTATION.**

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**INTRODUCTION:** The polyomavirus (PV) is a member of the papovavirus family; two strains are associated with disease in humans: BK virus and JC virus. It has a small size (45 nm. diameter), it is not enveloped, and it contains a double-stranded DNA genome. Primary infection occurs in childhood, having an asymptomatic course, and residing in a latent state that can be activated without clinical consequences. 0.3% of the immunocompetent individuals have asymptomatic virurias, 3% of the pregnant women, and 30-40% of the recipients of a renal allograft. In this last group the reactivation can lead to nephropathy and allograft rejection.

**PURPOSE OF THE STUDY:** We aim to demonstrate the usefulness of urine cytology in the detection of reactivation of PV infection in patients with renal allograft.

**MATERIALS AND METHODS:** The 26 transplanted patients in the study were randomly assigned into two groups: group A with 17 patients with monthly control cytologies and group B with 9 patients without urine cytological studies. A cytology was considered to be positive when PV-infected intranuclear inclusion cells (decoy cells) were found. When positive, an additional cytology was made 15 days later, and, if positive too, the immunosuppression treatment of the patient was reduced. Besides, if renal dysfunction was found a biopsy was performed.

**RESULTS:** 11% of the patients of group B developed PV nephropathy, while none of the patients of group A developed it, in spite of having 29% of positive urine cytologies.

**CONCLUSIONS:** The repeated detection of decoy cells in urine cytologies of patients with renal transplantation allows the modulation of the immunosuppression treatment in order to control the viremia and to prevent the nephropathy that could lead to the loss of the renal allograft. As a consequence, in our hospital the patients with a renal allograft follow periodic urine cytology controls.

#### P 273

##### **FNAB OF LYMPH NODES – IS IT WORTH USING THIS DIAGNOSTIC METHOD?**

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**Introduction:** Although lymph node examination by fine needle aspiration biopsy (FNAB) is a quick and cost-effective method, which is well tolerated by the patient, it is not widely used by hematologists. In our opinion, it is partly due to misapprehension and bad local experience.

Our purpose was to give a non-aligned statistical proof of the efficacy of this diagnostic method, based on our data.

**Method:** Between 1st of January 1995 and 31st of December 2004, 2796 ultrasound (US) guided lymph node FNABs were performed in the neighbouring US laboratories, that were examined by our cytological team. ( 734 aspirations were performed by radiologists, the rest by cytopathologist). The smears were wet fixed with ethanol, stained with H&E.

The results are the following: lymphadenopathy – 1334, metastatic disease-880, unsatisfactory for diagnosis - 307, (264 of them were performed by the clinician, the rest by the cytopathologist), hematological malignancy: 152 cases, „other” 119 (the latter are cases, in which the smears are satisfactory, but the result cannot be pushed into either of the above-mentioned categories. 76 of the hematological malignancies were non Hodgkin lymphomas (NHL), and found 18 HL. 17 of the 18 HL diagnosed by FNAB, were histologically confirmed. In further 58 cases, the cytological examination raised the possibility of a hematological malignancy, which was confirmed in 41 patients. (No more data in 9 patients). In 8 cases, the histological examination proved no malignancy ( fals positive by cytology). In this series of 2796 lymph node FNAB, one single false negative case was found.

**Conclusion:** our data prove, that FNAB of enlarged lymph nodes is a very efficient method in the diagnosis of different hematological and non- hematological diseases in experienced hands . The examination can be repeated in case of the suspicion of false negativity. We suggest the use of this easy, cost effective and quick method, by which unneeded operations might be avoided, and based on the quick diagnosis many patients can benefit. We would like to share some of our „favourite cases” with the audience.

#### P 274

##### **EFFECT OF VITRIFICATION ON FERTILIZING CAPACITY OF SPERMATOZOA IN FERTILE AND INFERTILE MEN**

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Cryopreservation is widely used in Assisted Reproductive Techniques to maintain male fertility. Evaluation of the acrosome and its disruption and motility are important tasks within the diagnostic tests of male infertility to predict the fertilizing capacity of sperm. The aim of the present study was to evaluate the effect of vitrification on acrosome reaction and motility of sperm in fertile and infertile men. Semen samples were collected from ten fertile and twenty infertile men after a minimum of 3 days of sexual abstinence. After seminal liquefaction, a routine semen analysis was performed using Semen Analyzer Quality to determine sperm concentration and motility according to World Health Organization specifications. This was immediately followed by the evaluation of acrosome quality by triple staining technique. The remainder of each sample was immediately cryopreserved by vitrification method and thawed after two weeks. Analyses of concentration, motility and acrosome quality were performed after thawing and the results were statistically analysed by paired t-test.

After vitrification, the percentage of live spermatozoa with intact acrosome was significantly decreased from  $33.37 \pm 4.07$  to  $9.87 \pm 2.97$  ( $p < 0.002$ ) in fertile and from  $26 \pm 3.64$  to  $11.16 \pm 1.82$  ( $p < 0.001$ ) in infertile groups. The percentage of sperm progressive motility (a + b) were significantly

decreased from 60 and 35 to 40 and 20 in fertile and infertile groups after vitrification, respectively. It is concluded that vitrification impairs fertilizing capacity of sperm in fertile and infertile men.

#### P 275

### SECONDARY TUMORS TO THE PANCREAS. STUDY OF 17 CASES WITH FINE NEEDLE ASPIRATION CYTOLOGY

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**Introduction:** Secondary tumors to the pancreas are unusual and in most cases part of an advanced metastatic disease. They account for 3-16% of all pancreatic malignancies. They present as solitary or multiple lesions, sometimes with clinical and radiological features that can be confused with a primary pancreatic adenocarcinoma. Fine needle aspiration cytology (FNAC) is a quick, low-expensive technique that allows a rapid identification of metastatic carcinoma and the patient's management in pre-surgical diagnostic phase.

**Purpose:** To report the clinical and cytological findings of secondary tumors to the pancreas observed in the Department of Pathology of Verona University.

**Methods:** Between January 1990 and December 2004, we studied 17 secondary tumors to the pancreas diagnosed by FNAC.

**Summary:** The mean age of patients was 62 years. 10 patients were males (59%) and 7 females (41%). In 16 cases (94%) FNAC was performed with ultra-sonography guide on outpatients and in 1 case FNAC was performed during abdominal laparotomy for suspected pancreatic adenocarcinoma. The metastatic lesion involved the head of the pancreas in 41% of cases (7/17) and the body-tail in 35% (6/17). In 24% of cases (4/17) the anatomic site of pancreatic metastasis was not available. Radiological imaging showed in 94% of cases (16/17) a suspicious solitary mass and in a single case multiple lesions; the diameter of suspicious nodules ranged from 2 to 8 cm. The histotype of metastasis was renal cell carcinoma, classic type: 35%(6/17), large bowel carcinoma: 18%(3/17), gastric carcinoma 6%(1/17), breast carcinoma: 4%(1/17), prostate carcinoma: 6%(1/17), carcinoma of the lung: 6%(1/17). In 24% of cases the primitive site of neoplasia could not be assessed. The clinical history was available in all cases of renal cell carcinoma with a mean interval of 13 years between the primitive renal carcinoma and the pancreatic metastasis.

**Conclusion:** Most secondary tumors to the pancreas occur as a solitary lesion, both on the head and tail of pancreas, simulating a primary pancreatic carcinoma. The most frequent metastatic tumor to the pancreas is renal cell carcinoma, often many years after the primary renal tumor. FNAC allows a distinction between primary pancreatic cancer and secondary tumors and represents an important diagnostic tool in the follow up of oncologic patients.

#### P 276

### VIRAL MARKERS IN CYTOLOGIC AND HISTOPATHOLOGIC PREPARATIONS OF CERVICAL LESIONS

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Consistent epidemiologic evidence had demonstrated that human papillomavirus (HPV) infection plays a central role in

the development of cervical cancer precursors and invasive cervical cancers. Over 20 types of HPV are associated with cervical intraepithelial lesions and cervical carcinomas. The purpose of our study was to analyze viral markers expression in cervical lesions. 105 Pap smears and 84 biopsies were selected as they exhibited viral cytopathic effects. We performed immunohistochemical staining for HPV on both types of preparations, in cases with SIL diagnosis. Cytologic aspects were: koilocytosis associated with diskeratocytes, and parabasal cells ( $\pm$  atypia), low grade squamous intraepithelial lesion (L-SIL) and high grade squamous intraepithelial lesion (HG-SIL). Histology revealed chronic cervicitis, condyloma acuminatum, L-SIL and H-SIL. HPV was detected in 61.1 % of smears, exhibiting a double localisation: nuclear and cytoplasmic and in 65.71 % of biopsies, with a nuclear localisation and an enhanced expression within superficial layers of condylomas. Concluding, HPV may be evidenced in cytologic and histologic preparations from cervical lesions, the negative preparations being attributed to (co)existence of other infectious agents or to the limited sensitivity of the immunohistochemical method.

#### P 277

### COMPARATIVE STUDY OF CONVENTIONAL AND LIQUID-BASED CYTOLOGY IN A POPULATION WITH OPPORTUNISTIC CYTOLOGICAL SCREENING

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**Background:** In the last decade the conventional Pap smear (CPS) has frequently been replaced by the liquid-based cervical cytology (LBC) in the routine clinical practice due to improved technical quality and increased efficacy in detecting squamous intraepithelial lesions (SIL) and cervical cancer by LBC.

**Aims:** The purpose of this study is to report the preliminary results of the performances of LBC versus those of CPS, in a country with an opportunistic cytological screening and a high incidence of cervical carcinoma.

**Methods:** This comparative retrospective study is an initial part of a wider project, anticipated to assess the validity of the new method and its impact on clinical practice. It includes two groups of women referred to a tertiary centre for gynaecologic care, in different six-month periods. In each group, the Pap smear was taken by one of the methods. Data from unselected, consecutive series of 3,261 LBC tests and of 4,102 CPSs were analysed. Cytological diagnoses were classified using the Bethesda system. The differences in specimen adequacy and the rates of atypical squamous cells (ASC) category between the two groups were compared using  $\chi^2$  test. The histological correlation with histopathological findings could be established in 901 patients with the LBC and in 1,408 with the CPSs. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of the findings were evaluated as well.

**Results:** The improvement in the specimen adequacy for LBC was documented by the more than fivefold reduction of the proportion of unsatisfactory specimens (0.4% for LBC vs. 2.1% for CPS,  $P < 0.001$ ). The rate of satisfactory but limited by  $\bar{c}$  was also reduced by 40% for LBC ( $P < 0.001$ ). However, the rate of ASC category was similar in both methods (0.9% for LBC vs. 1.2% for CPS). The performances of LBC for detection of cervical SIL or cancer lesions versus those of CPS, such as sensitivity (94% vs. 79%), PPV (85% vs. 69%) and NPV (90% vs. 66%) were significantly different ( $P < 0.001$ ). There was no statistically significant difference in

specificity between the methods (65% vs. 62%,  $P < 0.15$ ). Ultimately, the diagnostic accuracy was higher in LBC than in CPS (84% vs. 73%,  $P < 0.001$ ).

Conclusions: Our preliminary results suggest that the implementation of LBC significantly improved the technical quality of the smears and resulted in statistically significant improvement of the diagnostic value of the Pap test.

#### P 278

##### **IMMUNOPHENOTYPING OF CSF SPECIMENS BY SLIDE BASED LASER SCANNING CYTOMETRY**

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###### Background:

In cerebrospinal fluid (CSF) cytology analysis of cell surface markers is frequently required for diagnosis or subtyping of lymphoproliferative disorders, but insufficient cells may be present for flow cytometry. Laser scanning cytometry provides a simplified and inexpensive means of immunophenotyping paucicellular samples. We have assessed our experience with this method in CSF samples requiring immunophenotyping over an 18-month period.

Materials and Methods: Cyto centrifugation and rapid Giemsa stain were used for immediate assessment of 521 consecutive CSF samples. Slide based immunophenotyping by Clatch's method was attempted in 60 samples that showed some increase in lymphoid cells. Twenty-five cases had a history of previous hematolymphoid malignancy, and in 12 HIV positive patients neurologic decompensation led to suspicion of CNS lymphoma. 37 samples contained 10,000 cells or less, with the remainder ranging up to 150,000 cells total. In 12 samples with over 60,000 cells a full immunophenotyping panel of 20 antibodies was performed. In the samples with fewer cells at least kappa and lambda light chains and CD19 were initially assessed. By repetitive re-staining of the unfixed cells on the slide more surface antigens could be documented. After fixation and permeabilization intracellular markers could be assessed. All analyses were performed in the cytology laboratory using an LSC (Laser Scanning Cytometer - Compucyte®)

Results: 43 cases showed a reactive immunophenotype and a diagnosis of negative or chronic inflammation was rendered. The 17 remaining cases were diagnosed as suspicious for lymphoma (7) or malignant lymphoma (10). In 3/7 suspicious cases the LSC result was supportive of, but not conclusive for, lymphoma. In the remainder it was not contributory. In the 10 definite lymphoma cases the LSC immunophenotype confirmed B-cell clonality or an abnormal T-cell phenotype consistent with the clinical and morphologic diagnosis.

Conclusions: Slide based cytometry using Clatch's method is useful in the evaluation of CSF samples containing a hematolymphoid population suspicious for lymphoma, allowing specific reactive or malignant diagnoses in a number of cases containing inadequate cells for flow cytometry.

#### P 279

##### **UTILITY OF CYTOLOGIC IMPRINTS IN INTRAOPERATIVE DIAGNOSIS**

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Intraoperative diagnosis has been traditionally based on frozen histologic sections because provide architectural information, cytologic smears allow us to evaluate morphology on well preserved cells, without the well-known artifacts associated with freezing. Both methods thus complement each other. However, utilizing only the cytologic technique is possible if performed by experienced hands in special situations.

Material and Methods

We study 416 consecutive specimens submitted for intraoperative biopsies over a two-year period, Imprint cytologies were done on all specimens, and frozen sections were performed on 384 of them. The diagnoses obtained from both methods were recorded and compared with the final diagnosis yielded by the definitive histological sections.

###### Results

9 of 416 cases (2.2%) were misdiagnosed by IC, and there were diagnostic errors in 8 / 384 (2.1%) cases by frozen sections. Diagnostic accuracy was 97.8% for imprint cytology and 97.9% for frozen sections. Six cases of 416 (1.4%) were misdiagnosed by both imprint cytology and the frozen section. The accuracy rate of both methods used jointly was 98.5%.

###### Conclusions

Intraoperative imprint cytology is a quick, easy-to-perform, and low-cost technique that provides morphological details on intact cells without the artifacts resulting from freezing. It is therefore a highly valuable adjunct to the frozen section.

The benefits obtained from the use of imprint cytology are unquestionable, particularly under the following situations and considerations: 1) assessment of small specimens; 2) necrotic lesions; 3) to prevent cryostat contamination in infectious processes; 4) to facilitate identification of microorganisms; 5) no special equipment is required; 6) to preserve material for special studies (ultrastructure, immunohistochemistry, etc.); 7) for the assessment of some specimens for which it is not possible to perform frozen sections, such as bone specimens; 8) it shows cytogenetic characteristics (e.g. parathyroid tissue, cytological type of lymphoma); and 9) experience is gained in cytology.

#### P 280

##### **THE IMPORTANCE OF P-16'S EXPRESSION CORRELATED WITH KI-67 IN ESOPHAGEAL INTRAEPITHELIAL HPV-INDUCED SQUAMOUSE LESION**

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**BACKGROUND** The esophageal malpighian epithelium may be the siege of HPV infections, thus determining intraepitheal squamous lesions, similar to those found at the level of the uterine cervix. P-16 is a cyclin-dependent kinases inhibitor and a key regulator of the G1-S, and check point in the cell cycle. The over-expression of P-16 marker has been observed in CIN and invasive carcinomas, associated with high-risk hPV types.. Ki-67 is a nuclear antigen expressed in G1-S-G2-M phases, also being an aggressivity indicator.

**DESIGN** A number of 31 esophageal biopsies and 8 esophagectomy specimen material was formalin-fixed, paraffin embedded , HE stained and analysed immunohistochemically using specific antibodies against Ki-67 and P-16 by ABC immunoperoxidase procedure. Out of those 31 biopsies, 7 were showing HPV-induced low grade intraepithelial squamous lesion ,and 18 were showing high grade intraepithelial squamous lesions (CIN II-6, CIN III-9, CIS-3) and 6 cases of invasive carcinoma.

**RESULTS** :Our study demonstrates that HSIL-s have uniform and diffuse nuclear staining (Ki-67+) 100% (24 cases) and (P-16+) 80% (19 cases) through all cell layers of the lesion. None of our 7 LSIL cases showed (+) Ki-67 in the superficial layer. In the lower third of the epithelium our LSIL cases showed positive staining for both P-16 and Ki-67 but the upper third of the epithelium showed no such staining for either of them.

**CONCLUSION** Although the classical morphological features are the main diagnostic mean in the case of HPV

infection, the P16 and Ki-67 immunohistochemical markers and their staining patterns are useful in distinguishing between two biologically different groups of esophageal neoplastic lesions.

#### P 281

##### **INTESTINAL METAPLASIA AT THE GASTRO-ESOPHAGEAL JUNCTION: PREVALENCE AND ASSOCIATED CONDITIONS**

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**AIM:** Junctional intestinal metaplasia (JIM) is defined as intestinal metaplastic change found at the esophago-gastric junction and its pathogenesis is controversial. The aim of our study was to determine the prevalence of JIM and the frequency of coexisting esophageal and gastric lesions. **METHODS:** we studied a continuous series of 485 unselected patients with various digestive symptoms who underwent upper GI endoscopy including multiple esophageal, junctional, and gastric biopsies (body and antrum). All samples were stained with Haematoxylin-eosin, PAS-Alcian blue and Giemsa for *H. pylori* detection. All the slides were simultaneously revised by two expert GI pathologists who assessed the presence of microscopic esophagitis, Barrett's metaplasia, intestinal metaplasia at the junction and in the stomach and gastritis in accordance with the up-dated Sydney system.

**RESULTS:** JIM was found in 91/485 cases (18.8%); 11 had complete-type metaplasia, 77 incomplete-type and 3 both types. The male/female ratio was 1,5/1 for cases with JIM and 1/1 in 394 controls without JIM and the median age was 59.5 in the former and 53.3 in the latter group ( $p=0.0003$ ). The prevalence of associated lesions in cases with and without JIM respectively was as follows: microscopic esophagitis 50.5% vs 58.6%, Barrett's esophagus 31.9% vs 0%, *H. pylori* gastritis 22.0% vs 23.9%, gastric intestinal metaplasia 27.5% vs 17.3% ( $p=0.037$ ), no gastro-esophageal lesions 4.4% vs 24.1%. Since numerous patients showed more than one associated lesion the sum of % exceeds 100%.

**CONCLUSIONS:** both esophageal and gastric lesions may be found in association with JIM with a trend of esophageal lesions to be more frequent in cases with JIM than in controls. The very low prevalence of normal esophageal and gastric findings in cases with JIM as compared with controls suggests that JIM is not an isolated change but esophageal, gastric or combined factors contribute to its pathogenesis.

#### P 282

##### **ESSENTIAL MARKERS FOR THE EVALUATION OF METAPLASIA, DYSPLASIA AND CANCER IN BARRETT'S ESOPHAGUS**

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**Introduction:** The last 20 years are characterized by increased incidence of distal esophageal adenocarcinoma, being considered as a complication of gastroesophageal reflux disease and Barrett's esophagus. The major risk factors for adenocarcinoma are intestinal metaplasia and high grade dysplasia.

The purpose of this study was to select essential markers of dysplasia and metaplasia that could be included in standard panel for diagnostics of precancerous conditions of distal esophagus.

**Methods:** In 2003 – 2004 we performed an immunohistochemical (IHC) analysis of distal esophagus biopsy specimens (histologically showing features of metaplasia and/or dysplasia) in 42 patients (38 male and 4 female). Our common panel included antibodies to the following proteins: markers of intestinal or cardiac differentiation (CK 17 and CK 120, villin), markers that could be used for evaluation of dysplasia grade (CEA, p53) and marker of proliferation (Ki67). In difficult cases (where cancer is being supposed) we defined additional markers: basement membrane marker - collagen IV, marker of histiocytes - CD 68, vimentin and a wider set of cytokeratins. **Results:** The high grade dysplasia and adenocarcinomas were revealed due to the strong uniform cytoplasm reaction for CEA with nuclear reaction for p53 and a higher proliferation index. Using the above mentioned panel of markers we have diagnosed the following cases: 16 (38,2%) - Barrett's esophagus with cardiac metaplasia, 8 (19,2%) - Barrett's esophagus with intestinal metaplasia, 7 (16,6%) adenocarcinoma, 7 (16,6%) squamous-cell carcinoma, 2 (4,7%)—poorly differentiated carcinoma and 2 (4,7%) - chronic esophagitis.

We have concluded that the usage of the wider panel of biomarkers could significantly help to diagnose the distal esophagus precancerous conditions and to determine effective treatment.

#### P 283

##### **EVALUATION OF BARRETT'S MUCOSA IN TERMS OF CK7, CK20, KI67, P53, AND COX2 EXPRESSIONS USING CHROMOENDOSCOPIC OESOPHAGEAL BIOPSIES**

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#### P 284

##### **GLYCOPROTEIN EXPRESSION PATTERNS IN THE BIOPSIES OF GASTROESOPHAGEAL JUNCTION : A PRELIMINARY STUDY**

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To determine incomplete intestinal metaplasia (IM) in endoscopic biopsies obtained from the gastroesophageal junction or proximal, may represent IM of the cardia (CIM) due to *H. pylori* (HP) and gastroesophageal reflux disease (GERD) or Barrett's esophagus (BE), which have different clinical implications. In this study, we aimed to determine the glycoprotein expression patterns in BE, CIM (HP and GERD related), multilayered epithelium (ME) and columnar blues (CB) in order to relate their expression to BE development. Archival biopsies and endoscopy reports of 138 patients biopsied from gastroesophageal junction (or proximal) were re-evaluated. CIM was demonstrated in 51% of 64 patients diagnosed as carditis due to GERD. Fifty-eight patients had HP carditis with complete and incomplete intestinal metaplasia in 16% and 27% of the cases, respectively. BE was determined in 16 patients, 3 biopsies revealing dysplasia. ME was detected in 3 BE and 12 GERD associated carditis. CB was identified in 4 HP related carditis, 16 GERD related carditis and 12 BE. Sequential sections of representative formalin-fixed paraffin-embedded tissue sections in 16 BE, 12 GERD and 8 HP associated CIM were stained by immunohistochemistry for Human Gastric Mucin (HGM), MUC1, MUC2, MUC5AC. Submucosal glands of esophageal mucosa expressed MUC5B, whereas squamous epithelium

MUC1. MUC1 was rarely expressed in BE and CIM compared to ME and CB ( $p < 0.05$ ). All BE cases showed gastric mucin (MUC5AC, HGM), but only 53.4% MUC2 expression. Gastric mucins (MUC5AC, HGM) were detected in all CIM cases, but expression intensity was low in columnar cells and usually HGM staining was limited to goblet cells in HP associated CIM cases. MUC2 expression differed between GERD and HP associated CIM being 45% and 22%, respectively. Gastric mucins (MUC5AC, HGM) with low intensity of staining was limited to basal cytoplasm in CB, but to the superficial columnar cells in ME. Our preliminary results suggests that ME may be a precursor in the development of BE. In regard to mucin expression the specificity of any pattern is insufficient for distinction in individual cases with BE and CIM in GERD are virtually indistinguishable, limiting their use in this differential and raising the question of whether they are biologically related.

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### IMMUNOHISTOCHEMICAL MARKERS IN THE DIAGNOSIS OF BARRET'S ESOPHAGUS AND ESOPHAGEAL ADENOCARCINOMA

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#### IMMUNOHISTOCHEMICAL MARKERS IN THE DIAGNOSIS OF BARRET'S ESOPHAGUS AND ESOPHAGEAL ADENOCARCINOMA

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Introduction: Barrett's esophagus /BO/ is associated with increasing incidence of the adenocarcinoma of the esophagus. Histopathological staging of BO is associated with high inter- and intraobserver variation .There have been many attempts to find adjunctive methods for improving the diagnose.

Purpose: As the progression of Barrett's metaplasia to adenocarcinoma is related to impaired control of cell cycle the question arised whether the application of tumor markers involved in increased proliferation and inhibition of apoptosis could be useful for an exact diagnosis. ..

Material and methods: Tissue samples from 25 patients were examined: eight with BO and seventeen with adenocarcinoma, associated with BO. The tissue pieces for the immunohistochemistry analysis were obtained by endoscopic biopsy or after surgical resections and included in paraffine blocks. Several tumor markers involved in cell proliferation and apoptotic pathways were studied: Ki-67, PCNA, RB, p16, p53, Bcl-2 and Bax .Routine immunohistochemical procedures were applied with antibodies produced from Dako, Denmark.

Results:Expression of the markers for proliferation Ki-67 and PCNA was increased in adenocarcinoma, especially in undifferentiated forms.Mutated tumor marker-p53 was strongly expressed in adenocarcinoma and Barrett's epithelium with high grade of dysplasia.Expression of the proapoptotic marker Bax was increased in adenocarcinoma.The antiapoptotic marker Bcl-2 showed no expression in most of the samples; in some samples the staining was weak in tumor cells and strong positive in immune cells of the lamina propria. Expression of the markers associated with cell cycle-p16 and Rb was increased in adenocarcinoma and high grade Barrett's epithelium . Conclusion: The results suggest that the investigation of tissue tumor markers may contribute to improve the diagnosis , assessment of the prognosis and risk of progression .

#### P 286

### PROGNOSTIC AND POTENTIALLY PREDICTIVE FACTORS IN EARLY GASTRIC CANCER

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BACKGROUND: The incidence of gastric cancer is decreasing worldwide, but the prognosis is still generally poor. It is the second cause of death in men and fourth one in women. Detection of the early gastric carcinoma is the crucial factor to improve the results

of treatment. 5 -year survival rate in T1 stage is 80-95%. Lymph node metastasis are observed in 10.4% of early gastric cancer. The depth of invasion and lymph node metastasis are the most significant prognostic factors in the early gastric cancer. AIM: The E-cadherin-catenin complex is important for cell-cell adhesion of epithelial cells. We analyze the E-cadherin expression with clinicopathological features of the early gastric cancer. METHODS: 39 patients were diagnosed and total gastrectomy with lymphadenectomy was done. Histological report included: location and size of the tumor, surgical margins, histologic type and grade, pTNM stage. Immunohistochemical staining of E-cadherin was performed. Normal membrane E-cadherin staining was observed in control gastric mucosa. RESULTS: Abnormal expression of E -cadherin was demonstrated in 37 cases (94.9%). High (3+) membranous expression of E-cadherin was seen in 2 well differentiated adenocarcinomas (5.1%). Less or absent immunoreactions of E-cadherin was observed in 14 well differentiated adenocarcinomas and all (23) poorly differentiated adenocarcinomas or diffuse type of Lauren classification. Moreover, abnormal expression of E-cadherin occurred more frequently in macroscopic ulcerated type (0-IIc, 0-I II) and was associated with lymph node metastasis. CONCLUSION: We suggest that E-cadherin play an important role in the macroscopic growth as well as microscopic differentiation of the early gastric carcinoma.

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### HISTOLOGIC CHANGES OF THE GASTRIC MUCOSA ASSOCIATED WITH GASTRIC CARCINOMAS AND PRIMARY LYMPHOMAS

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In order to formulate proper surveillance and therapy protocols in epithelial changes of the gastric mucosa, it is important to determine the frequency and type of preneoplastic lesions in gastric carcinogenesis. For comparative purposes, we evaluated gastric mucosa surrounding 93 gastric adenocarcinomas (GCa), 19 primary gastric lymphomas (PGL) and gastric mucosa of 30 autopsy cases who died of reasons other than gastric malignancies, according to the updated Sydney System and Padova International Classification. In addition, in order to determine the role of G-cell and enterochromaffin-like cell hyperplasia in gastric carcinogenesis, we studied gastrin and chromogranin A (ChrA) immunohistochemistry in the surrounding mucosa of each case. In our study; atrophy, intestinal metaplasia (IM) type I and III, foveolar hyperproliferation (FHP) were all observed in high frequencies in gastric carcinoma cases ( $p < 0.001$ ). Hyperproliferative intestinal metaplasia (HIM), low (LGD) and high grade dysplasia (HGD) were only detected in carcinoma cases. When diffuse ( $n=43$ ) and intestinal ( $n=50$ ) type of GCa cases were compared, atrophy, IM type III, HPIM, LGD, and HGD were statistically more common in intestinal GCa's ( $p < 0.05$ ). High H.pylori density and FHP were recorded mainly in diffuse GCa's. In our study, gastrin and ChrA overexpression were observed in GCa and PGL cases but not in autopsy cases. Gastrin expression was related to intestinal metaplasia ( $p < 0.05$ ) but not to the H. pylori

density and atrophy. ChrA expression was related to intestinal metaplasia ( $p < 0.01$ ) and atrophy ( $p < 0.05$ ) but not to the H. pylori density. G cell hyperplasia correlated well with the linear and diffuse neuroendocrine cell hyperplasia ( $p < 0.05$ ). Our study revealed that FHP and HPIM which comprises inconspicuous for dysplasia group in Padova International Classification were associated with carcinoma cases in high frequencies. We think that these lesions could be preneoplastic lesions rather than being a part of a regenerative process. Our results show that although intestinal GCa's develop as a consequence of a sequence of events, some mechanisms acting in the active phase of gastritis could be an important factor in the carcinogenesis of the diffuse type. Our data related to gastrin and ChrA immunohistochemistry gives the idea that both G cell and enterochromaffin cell hyperplasia may be involved in gastric carcinogenesis.

#### P 288

### INTESTINAL AND DIFFUSE TYPES OF GASTRIC ADENOCARCINOMA: CLINICOPATHOLOGIC FEATURES

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Gastric carcinoma is classified into intestinal type (IC) and diffuse type

adenocarcinoma (DC) by Lauren histological classification. There are reports that these two types have different prognostic implications according to different clinicopathological factors. We examined 40 DC and 49 IC in radical gastrectomy specimens to determine the importance of factors affecting prognosis within these two groups of carcinomas. Patients' age, sex and tumor localisation were determined from clinicopathologic files. We retrospectively evaluated all the H&E stained

slides of the cases and recorded histological grade, WHO histological types, invasion pattern (submucosal, pushing or infiltrative), depth of invasion, venous, lymphatic and perineural invasion, intratumoral and peritumoral lymphocytic infiltration, desmoplasia, metastatic foci in lymph nodes and other organs. In our study, we observed that both types were more common in males and IC was more frequent in elderly. In the literature; lymph node metastases, perinodal, perineural, serosal invasion, surgical margin positivity are features accepted as determinants of poor prognosis and these are all observed in high frequencies in our DC cases. No statistical difference was found between the two groups in respect to WHO histological types but DC mostly had signet ring cell component. In our study, we also found that the two types had different invasion patterns: DC, infiltrative or submucosal; IC, infiltrative or expansile. There were no difference in intratumoral lymphocytic infiltration (including lymphoid aggregate and follicle formation) between two histological types. Although no statistical difference could be found in regard to desmoplasia formation between the two groups, the extent of desmoplasia was higher in IC. In summary, factors defined as poor prognostic factors in the literature were more common in DC and we conclude that these altogether bring out the poor outcome in DC.

#### P 289

### HISTOLOGICAL CHANGES IN HYPERPLASTIC POLYPS AND SURROUNDING GASTRIC MUCOSA

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Introduction: Hyperplastic polyps were considered to represent the regenerative changes in a background mucosa

with chronic atrophic gastritis and rarely harbor true precancerous lesions themselves.

Aim: The aim of this study was to determine on which morphological background gastric hyperplastic polyps develop and to assess the frequency of precancerous changes in the hyperplastic polyps and background mucosa.

Material and Method: We studied 73 patients with gastric hyperplastic polyps and characterized endoscopic and histologic features of the polyps (topographic location, multiplicity and the presence of dysplasia and adenocarcinoma) and the background gastric mucosa (intestinal metaplasia, dysplasia, carcinoma, and classification of gastritis). The histopathologic features seen within the hyperplastic polyp and the background mucosa were assessed according to the revised (2004) Vienna classification.

Results: Hyperplastic polyps were most common in the antrum (42.5%) followed by the corpus (27.4%), both antrum and corpus (15.1%) and the site of anastomosis (15.1%). There were multiple polyps in 37% of the patients which were mostly situated in antrum (40.7%), followed by both antrum and corpus (33.3%). Intestinal metaplasia of the polyp was present in 19% and low-grade dysplasia in 9.5% of patients. Only one patient (1.3%) had intramucosal adenocarcinoma within the polyp which also showed adenocarcinoma in the surrounding mucosa. Evaluation of the surrounding gastric mucosa showed intestinal metaplasia in 36% of patients, at least low-grade dysplasia in 12%, and synchronous carcinoma in 9%. Of the seven cases with hyperplastic polyps harboring low grade dysplasia, four also demonstrated low grade dysplasia in the surrounding gastric mucosa. All hyperplastic polyps with dysplasia were located in the antrum and all but one were multiple. Ninety percent of the hyperplastic polyps were associated with various forms of gastritis, most commonly superficial gastritis (44.2%), followed by chronic active H. pylori gastritis (23.1%), chronic atrophic gastritis (21.2%) and chemical gastritis (11.5%).

Conclusion: These results show a strong association between mucosal inflammation and development of hyperplastic polyps. As the frequency of dysplasia and adenocarcinoma in the background mucosa was higher than that of hyperplastic polyps, a meticulous endoscopic examination and multiple biopsies of the surrounding mucosa would reveal early precancerous changes.

#### P 290

### EFFECT OF TRANSFORMING GROWTH FACTOR-BETA (TGF-B) ON PROLIFERATION OF GASTRIC EPITHELIAL CELLS IN CULTURE

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BACKGROUND: Helicobacter pylori has a well-established role in the development of gastric cancer. In vitro studies reveal increased proliferation of the gastric mucosa in the presence of H. pylori infection. It has been also shown that production of some cytokines, such as interleukin-1 beta (IL-1b) is increased in H. pylori infection. In addition, IL-1b increases proliferation of gastric epithelial cell in culture study. In this study, The effect of transforming growth factor beta (TGF-b) on gastric epithelial cell proliferation has been examined.

METHODS: AGS cells were cultured with TGF-b. DNA synthesis was assayed by bromo-deoxyuridine (BrdU) test and total viable cell numbers by MTT assay.

RESULTS: TGF-b decreased DNA synthesis and cell numbers. This effect was both time- and dose-dependent ( $p < 0.05$ ). Both tests, BrdU test & MTT assay revealed this suppressive effect, but it was more evident in BrdU test. Simultaneous exposure to TGF-b & IL-1b, obliterated the results of each one, alone.

CONCLUSIONS: TGF-b suppresses proliferation of malignant gastric epithelial cells. It appears that modulation of tyrosine kinase activity is essential to anti-proliferative effect

of TGF- $\beta$ . This effect is reversed by IL-1 $\beta$ , and it may be due to opposed effects of these cytokines.

#### P 291

### PROGNOSTIC VALUE OF BAX, BCL-2 PROTEINS AND FAS/FASL SYSTEM IN PATIENT WITH GASTRIC AND COLON CANCER.

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**Background.** The inhibition of apoptosis is one of possible mechanism of tumor formation. The family Bcl-2 proteins and Fas/FasL system play the important role in apoptosis regulation. The family Bcl-2 protein includes the proteins-inhibition of apoptosis (such as Bcl-2), and activators of apoptosis (such as Bax). Fas protein is known to induce cell death by apoptosis in susceptible cells. Change of a level gene expression these protein lead to disturbed of apoptosis regulation. The prognostic importance of Bax, Bcl-2 proteins and Fas/FasL system in this tumor is yet to be evaluated.

**Aims.** To examine the prognostic significance of Bax, Bcl-2 and Fas protein expression in patient with gastric and colon adenocarcinoma.

**Methods.** The content of proteins Bcl-2, Bax and Fas was definite in fragments of the frozen tissues by immunohistochemical research serial paraffin section both tumor and not transformed tissues along patients (n=15) with the streptavidin-biotin method.

**Results.** It was establish the modification in level Bcl-2 and Bax protein in gastric and colon cancer. The level of Bcl-2 protein in tumor tissue increase in 1,5 time in comparison with not transformed tissue. The Bax level is increased or remains like not transformed tissue. Is shown, there is the high level of Bcl-2 and Bax in majority of cells in gastric and colon cancer. Fas expression were noted in all cancer specimens and all their noncancerous counterparts. The level of Fas-positive cells was significantly lower in patient without metastasis (by 89%,  $p=0,04$ ) and then in non cancerous tissue (by 54%,  $p=0,04$ )

**Conclusion.** The increase of synthesis Bcl-2, apparently, is one of mechanisms of apoptosis inhibitors in tumor cells. The increase of Bax level testifies to activation apoptosis-inducing mechanisms in tumor cells. However the determining importance has not a level of protein in a cell, and its ability to carry out the function. By us is earlier shown, that the gene Bax frequently changes at gastric and colon cancer. Expression of Fas in gastric and colon epithelium is progressively reduced during the malignant transformation and is frequently associated with metastasis in gastric and colon adenocarcinoma.

#### P 292

### CLAUDIN 1, 3, 4 AND 5 IN GASTRIC CARCINOMA, LOSS OF CLAUDIN EXPRESSION ASSOCIATES WITH THE DIFFUSE SUBTYPE

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**Introduction:** Claudins are tight junctional proteins present in epithelial and endothelial cells.

**Purpose of the study:** Purpose was to study the expression of claudins 1, 3, 4 and 5 in gastric carcinomas and their significance for tumor behaviour

**Methods:** 118 cases of gastric carcinoma including additional samples from 50 metastatic lesions were collected.

Immunohistochemistry was performed for claudins 1, 3, 4 and 5 Ki67 and E-cadherin. Additionally, cases were studied for apoptosis by the TUNEL method.

**Results:** Expression of all these claudins could be seen in gastric carcinoma, most prominently for claudin 4 and least expression was found for claudin 5. All claudins showed significantly more expression in gastric carcinomas of intestinal type. Their expression was significantly associated with each other. Expression of claudin 4 and 5 was associated with E-cadherin. Strong expression of claudin 5 was associated with higher cell proliferation and apoptosis. Claudin 3 expression had an association with a better prognosis of the patients, especially in the intestinal type.

**Conclusions:** The results show that expression of claudins 1, 3, 4 and 5 is lower in diffuse type gastric carcinomas. Possibly they play a role in determining the diffuse phenotype and lowered cohesion of cells in diffuse type of gastric carcinoma in a similar manner as E-cadherin. The loss of their expression does not clearly associate with poorer prognosis of the patients, except for claudin 3 where strong expression was associated with a better outcome of the patients, a feature especially related to intestinal type tumors.

#### P 293

### CYTOKERATIN EXPRESSION PATTERNS OF GASTRIC CARCINOMAS ACCORDING TO LAUREN AND GOSEKI CLASSIFICATION

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**INTRODUCTION:** Goseki system is a new gastric carcinoma classification proposal that classifying gastric carcinomas as according to their gland formation and mucin production patterns. In Goseki system, grade I tumors are in intestinal type; grade II tumors correspond to mucinous tumors; grade III tumors include mucin poor, diffuse infiltrating morphology and grade IV tumors contain signet ring cell carcinomas. The signet ring cell carcinoma of the stomach is believed to be originated from superficial foveolar epithelium of the gastric mucosa while mucinous carcinoma is a specific entity originating from goblet cell metaplasia. It seems likely that the main advantage of Goseki classification is to separate signet ring cell carcinomas, mucin poor diffuse infiltrating carcinomas and mucinous carcinomas of the gastric mucosa in to different groups. Cytokeratin 7 (CK7) is observed only in chronically inflamed gastric mucosa and co-exists with incomplete intestinal metaplasia. CK20 can be observed in the superficial foveolar epithelium and mature goblet cells.

**PURPOSE:** The aim of this study was to examine the cytokeratin expression profiles in gastric carcinomas that are classified according to both Goseki and Lauren systems.

**MATERIAL METHOD:** CK7, CK8, CK19 and CK20 were applied to the paraffin sections of 66 gastric carcinoma cases. The cytokeratin expression patterns of cases were grouped as CK20(+)/CK7(+), CK20(+)/CK7(-), CK20(-)/CK7(+), CK20(-)/CK7(-). The results were compared statistically with chi-square test.

**RESULTS:** CK20 immunoreactivity was observed in 18% of the grade I carcinomas, 24% and 31% of grade III and IV case respectively. CK20(+)/CK7(-) pattern was observed in 20% of the grade IV and 66.7% of the grade II carcinomas and was not observed in the grade I or grade III tumors ( $p<0,0001$ ).

**CONCLUSION:** In conclusion, both Goseki grade III and IV carcinomas originate from superficial gastric mucosa but grade III carcinomas are poorly differentiated. Goseki grade II carcinomas have a specific immunophenotype than intestinal type gastric tumors. Goseki classification seems to be superior in identifying poorly and well differentiated forms of diffuse infiltrating carcinomas and mucinous carcinomas.

**P 294****UNEXPECTED TTF-1 EXPRESSION IN GASTRIC CARCINOMA**

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**Background:** Thyroid transcription factor 1 (TTF-1) is a nuclear transcription factor selectively expressed in thyroid, lung and diencephalon. Immunoreactivity against TTF-1 in an adenocarcinoma is thought to be highly specific for pulmonary or thyroid origin.

**Design:** The morphologic characteristics of a case of gastric adenocarcinoma exhibiting strong staining for TTF-1 are reported and relevant literature data is reviewed.

**Results:** A biopsy specimen from a polypoid lesion of the cardia in a 68-year-old caucasian patient showed neoplastic glands with immunoreactivity against TTF-1 (MAb 8G7G3/1). Exploration of lung and thyroid was unremarkable. After surgical resection, the gastrectomy specimen revealed a mixed diffuse-type and intestinal type adenocarcinoma of the oesocardial junction, with strong nuclear TTF-1 positivity limited to the intestinal-type component. Moreover, TTF-1 staining was also focally present in the surrounding gastric mucosa, within intramucosal glandular cysts partly lined by metaplastic ciliated epithelium. Distant mucosal changes in stomachs resected for carcinoma and especially ciliated metaplasia are common findings in Japanese patients, but are rarely observed in European subjects. Expression of TTF-1 in ciliated metaplasia has never been reported before. Among the numerous studies concerning TTF-1 expression in carcinomas other than those derived from lung and thyroid, positivity in gastric carcinoma has only been shown in two cases, without details of histological data.

**Conclusions:** We describe a case of gastric carcinoma showing strong nuclear TTF-1 positivity within the tumor cells themselves and in intramucosal glandular cysts partly lined by ciliated epithelium. This finding suggests that the rare gastric cancers with TTF-1 expression may possibly derive from ciliated metaplastic cells. Moreover, detection of TTF-1 in a gastric carcinoma calls into question the absolute specificity of this marker for thyroid or pulmonary cancer.

**P 295****RCAS1 EXPRESSION IN GASTRIC CANCER: CORRELATION WITH CLINICOPATHOLOGICAL VARIABLES AND PATIENTS' OUTCOME**

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**Background-Aims:** The tumor associated antigen "receptor-binding cancer antigen expressed on SiSo cells" (RCAS1) inhibits the growth of receptor expressing cells and induces apoptosis. In this study, RCAS1 expression was examined in tumor samples obtained from gastric cancer patients and was correlated with histopathological parameters and patients' survival.

**Materials and Methods:** RCAS1 protein expression was examined immunohistochemically in paraffin embedded tissue sections from 38 gastric cancer cases. RCAS1 protein positivity, overexpression (positivity in more than 75% of tumor cells) and intensity (mild, moderate, intense) of staining were correlated with patients' age, gender and TNM stage, tumor histological type, grade, proliferative capacity (Ki-67 labeling index), and the patients' final outcome.

**Results:** RCAS1 protein positivity was noted in all of the examined cases, while in 15 out of 38 (39%) RCAS1 protein

was overexpressed. RCAS1 overexpression and intensity of staining were statistically significantly correlated with tumor histological type, being mainly expressed in tumors of intestinal type ( $p=0.027$  and  $p=0.002$ , respectively). The other clinicopathological parameters examined and patients' survival were not correlated with RCAS1 protein overexpression and intensity of staining.

**Conclusions:** RCAS1 protein is widely expressed in gastric cancer cases and its overexpression is correlated with intestinal type gastric tumors, suggesting the participation of RCAS1 in gastric carcinogenesis.

**P 296****RELATION OF NEUROENDOCRINE DIFFERENTIATION TO TGF-ALPHA AND EGFR EXPRESSION IN GASTRIC ADEOCARCINOMAS: THEIR PROGNOSTIC IMPLICATIONS**

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Coexpression of transforming growth factor-alpha (TGF-a) and epidermal growth factor receptor (EGFR) in carcinomas is believed to confer growth advantage to tumor cells. It is well known that TGF-a is one of the products of neuroendocrine cells, but there is no evidence concerning the possible effect of neuroendocrine differentiation (NED) in gastric adenocarcinomas (GCa) on the expression of TGF-a and EGFR. In order to assess this and their role on the survival of carcinoma patients, we analyzed Chromogranin A (ChrA), TGF-a and EGFR immunohistochemistry (IHC) in partial/total gastrectomy specimens of 101 GCa (diffuse type = 41, intestinal type = 60) cases. ChrA IHC was performed on the tissue sections prepared from formalin fixed paraffin embedded tissue blocks containing GCa areas. ChrA IHC was evaluated semiquantitatively (0-4). For each case IHC was performed for TGF-a and EGFR in sequential sections of a representative block selected based on NED. Evaluation of the IHC staining for TGF-a and EGFR was done according both to the intensity (0-2) and quantification of the positively stained areas (0-3). When a total score (sum of the intensity and quantification measurements) was 4 or greater, the tumor was considered positive for either TGF-a and EGFR.

According to the results of TGF-a and EGFR staining the carcinomas were scored as 0 if neither TGF-a and EGFR was stained, as 1 if either TGF-a or EGFR was stained, and as 2 if both TGF-a and EGFR were stained. Follow-up data was available in 54 patients. Twenty seven patients was alive, while 27 died of disease with a follow-up of 7-101 and 1-131 months, respectively. NED in GCa's was related to TGF-a expression ( $p<0.01$ ) but not to EGFR expression and TGF-a / EGFR coexpression. The percentage of cases expressing ChrA was higher in diffuse type GCa's, not with statistical significance ( $p>0.05$ ). When the cases were grouped according to Lauren's classification, NED correlated with TGF-a but not with EGFR in both intestinal and diffuse type GCa's. Correlation of NED with TGF-a / EGFR coexpression reached statistical significance only in diffuse type GCa's. Although histologic type, NED, and TGF-a had no effect on survival, EGFR ( $p<0.05$ ) and TGF-a / EGFR coexpression ( $<0.001$ ) were related to survival. The results of our study suggest that the autocrine mechanism between TGF

**P 297****EXPRESSION OF HUMAN GASTRIC MUCUS AND PCNA IN INTESTINAL METAPLASIA ASSOCIATED WITH GASTRIC CARCINOMA: AN IMMUNOHISTOCHEMICAL STUDY AND A PRELIMINARY REPORT**

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**Background:** Gastric adenocarcinoma is an important cause of cancer related death and historically is known to be frequently associated with intestinal metaplasia (IM). Because IM is an important intermediary in the gastric carcinogenic sequence, understanding how an IM progresses to cancer is important. HGM is a well known gastric phenotypic mucus marker that is expressed in normal gastric epithelial cells and various carcinomas. To clarify the heterogeneous nature and significance of IM, we have examined the relationship between proliferating cell nuclear antigen (PCNA) and human gastric mucus protein (HGM) expression in areas of IM adjacent to gastric cancer and chronic gastritis.

**Methods:** In this study, we reevaluated biopsy samples obtained from 110 cases of chronic gastritis associated with IM (CG+IM) and 30 cases of gastric carcinoma associated with IM (GC+IM). We examined PCNA and HGM expression in the proliferating zone, in the areas of IM and tumor separately via immunohistochemically and correlated with each group of CG+IM and GC+IM. Immunohistochemical study could not be evaluated in all of the cases due to technical problems.

**Results:** The immunohistochemical staining of HGM was positive in normal gastric mucosa adjacent to CG+IM and GC+IM groups. In CG+IM group, HGM expression in the areas of IM was shown lower than GC+IM groups, this was statistically significant according to Mann-Whitney-U test ( $p: 0,005$ ). Although statistically insignificant, PCNA labeling index of the IM areas in GC+IM was higher than in CG+IM ( $p: 0,07$ ).

**Conclusion:** The findings suggest that cases of IM with overexpression of HGM and high PCNA labeling index are more commonly observed in GC+IM group. We concluded that, overexpression of HGM and evaluating the PCNA labeling index may be useful for early detection of IM with high risk of gastric carcinoma. But it is necessary to study these parameters within large series to establish the precise decision.

#### **P 298**

##### **EXPRESSION OF MUCINS IN GASTRIC CARCINOMA AND THEIR CLINICOPATHOLOGICAL SIGNIFICANCE.**

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**AIM:** To investigate the expression of three types of mucin (MUC1, MUC2, MUC5AC) and in human gastric carcinomas and their clinical significance

**METHODS:** Tumours were classified according to WHO criteria. The mucin phenotype was determined immunohistochemically using markers for MUC1, MUC2, MUC5AC in 95 cases of human gastric cancer.

**RESULT:** There were significant correlations between MUC1 and MUC2 expression and types of cancer. MUC1 and MUC2 expression was shown to have a tendency to lower expression in well and moderately differentiated tubular adenocarcinoma and higher expression in advanced stage cancers (poorly differentiated adenocarcinoma, signet-ring cell carcinoma and mucinous carcinoma) ( $P > 0.05$ ); MUC5AC expression was shown to have a tendency to higher expression in well and moderately differentiated tubular adenocarcinoma and lower expression in advanced stage cancers ( $P > 0.05$ );

**CONCLUSIONS:** MUC1 ve MUC2 is significantly influenced by histological grade. These findings suggest that MUC1 ve MUC2 expression may be an indicator for prognosis of gastric cancer patients and correlated with early

tumorigenesis and initiation of invasive growth in gastric cancer.

#### **P 299**

##### **HELICOBACTER PYLORI GASTRITIS-UPDATED SYDNEY CLASSIFICATION APPLIED IN OUR MATERIAL**

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**Hp** inhabits the stomach more than 50% of humans and is the most frequent cause of chronic gastritis worldwide.

The purpose of this research was to present the importance of combining topographical, morphological and etiological information of diagnostic evaluation on grading gastritis in our material according to Updated Sydney Classification.

There have been investigated 154 cases of gastric mucosa (endoscopic biopsies) which were fixed in buffered neutral formalin and embedded in paraffin. Tissue sections (5mm thick) were cut and stained with H&E, May Grunwald Giemsa and Silver stain.

The biopsy cases were analyzed in attempt to assess the major histopathological features of gastritis. The histopathological variables (Hp density, neutrophil infiltration, mononuclear infiltration, atrophy, intestinal metaplasia and dysplasia) were graded on a scale of 3 (mild, moderate and severe). Minor histopathological features were not graded, but simply assessed in case of their presence or absence.

36 (23.37%) cases were positive for Hp (22.2%, 72.2%, 5.5%). The atrophy was positive in 23 (14.93%) cases with the scale (47.8%; 47.8%; 4.34%). Dysplasia was positive in 13 (8.44%) cases with the scale (84.6%; 7.6%; 7.6%). Intestinal metaplasia was positive 25 (16.2%) with the scale (76%; 20%, 4%).

After eradication of Hp, neutrophils disappeared for 6-8 weeks, but chronic inflammation persisted longer as well as other major Hp features.

There were 6 (3.8%) cases of MZL, which were treated properly.

The presence of H. pylori increases the chances of developing adenocarcinoma and MALT lymphoma; therefore, it is important to look for possible early eradication of H. pylori to prevent the eventual development of gastric cancer. These findings should influence the treatment of gastric cancers.

#### **P 300**

##### **COMPARING 200 GASTRIC TOUCH CYTOLOGY STAINED BY GIEMSA AND GRAM METHODS WITH HISTOLOGY STAINED BY H&E FOR HELICOBACTER PYLORI INFECTION**

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**Introduction and purpose:**

Many observations suggesting that Helicobacter pylori (H. Pylori) to be closely associated with acute gastritis (Type B) and gastric ulcer, and some malignancy of stomach. Multiple tests are available for determining H. pylori infection. Culture is the standard method, but as the histologic examination the result is delayed. Serologic method and phase contrast microscopy is also used for detection of H. pylori.

Among the methods based on endoscopic biopsies only the rapid urease test and cytology may give result available before the patient leaving endoscopy room. The purpose of this study is to compare histologic examination with touch cytology stained by Giemsa and gram methods in 200 cases.

**Materials and Methods:**

For each patients 2-5 biopsies were obtained put into 10% formalin for histologic examination. Additional single gastric biopsy was transferred from the biopsy forceps to a glass slide then delicately rolled onto the surface of the slide. This technique is called touch cytology. Then the slides were air dried. For each patient one slide stained by Giemsa and another slide stained by Gram method.

**Results:**

In histologic examination 93 out of 200 cases were positive for *H.pylori*. In Giemsa method 135 out of 200 cases, and in Gram method 92 cases out of 200 were positive for *H.pylori*. Additional result consist of 4 cases of *Candida albicans* with yeast-like organism and pseudohyphae in touch cytology stained by Giemsa or Gram method but not detected in histologic examination.

**Conclusion:**

We believed that touch cytology method is useful, simple and rapid in diagnosis of mucosal infection of stomach with *H.pylori* organism, specially when the level of infection is mild. Touch cytology stained by Giemsa method yield more positive result and easier and faster to screen the smear, but in touch cytology stained by Gram method no advantage obtained over histologic examination. The combination of the histologic examination with touch cytology examination (Giemsa) are the test of choice for detection of *H.pylori* and evaluation of inflammation and degree of injury/

**P 301****EFFECT OF TRIPLE THERAPY IN HELICOBACTER PYLORI ASSOCIATED GASTRITIS.**

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*Helicobacter pylori* (*HP*) infection is a cause of gastritis in most of the patients with duodenal ulcer. Clearance of the organism leads to improvement or even total resolution of gastritis.

**Aim:** To evaluate the histological outcome of gastritis in the patients with duodenal ulcer after HP eradication.

**Methods:** A total 87 patients with duodenal ulcer with positive HP, were given triple therapy for one week. The gastric antral biopsies were made before and 6 months after the end of treatment. Histological investigation was performed according to the Sydney system on a four-point scale.

**Results:** The scores of gastritis histological feature before and after HP eradication are as following: 1) 2.1 and 0.9 for inflammatory cells infiltration, 2) 0.7 and 0.4 for atrophic changes, 3) 0.2 and 0.2 for intestinal metaplasia, 4) 0.4 and 0.3 for lymphoid follicles. Gastric mucosa was normalized in 58.6% (51 / 87) out of the patients.

**Conclusions:** In this study, HP eradication improved gastritis in the patients with duodenal ulcer. Gastric mucosa normalizes in 58.6% of the patients, 6 months after triple therapy.

**P 302****REGRESSION OF GASTRIC MALT LYMPHOMA AFTER HELICOBACTER PYLORI ERADICATION.**

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**Background :** *Helicobacter pylori* (*H p*) is considered the leading cause of gastric mucosa associated tissue lymphoid (MALT) lymphoma (GL) and the majority (60 – 90%) of low grade gastric MALT lymphoma are reported to regress after eradication of *H p* (Fischbach, 2004).

**Objective:** to evaluate the histologic response of low grade gastric MALT lymphoma after eradication of *H p*.

**Methods and results:** 186 cases of gastric MALT lymphoma were analyzed at the pathological department during eight years (1996 – 2003). They represent 30, 8% of all gastric cancer diagnosed during the same period. Sex ratio was 0, 97 and median age was 42 years (17 – 80 y). Abdominal pain was the most common presenting symptom (81%); a suspect antral ulceration was the endoscopic finding in 80%.

124 patients (66%) had low grade gastric MALT lymphoma. *Hp* infection was found in all cases. 61 of them (53%) were treated with triple therapy (amoxicillin, proton pump inhibitor and metronidazol) for seven days. Subsequently, they were followed up by sequential endoscopy and biopsy. Eradication of *Hp* infection was achieved in all patients; 35 of them were free of lymphoma (complete remission, 28 months median follow up); 03 patients were in partial remission (median follow up, 10 months). 02 patients relapse after 21 and 27 months; they had surgical cure and adjuvant chemotherapy.

Eradication therapy permitted the regression of more than 50% of early low grade gastric MALT lymphoma. Our results are similar to those reported in the literature

**Conclusion:** Actually, GL therapy isn't well codified. However eradication of *Helicobacter pylori* in low grade gastric MALT lymphoma is widely accepted as a first line therapy. Although complete *Hp* eradication is necessary and a long time follow up needed to confirm the regression.

**P 303****MOLECULAR DETECTION AND GENOTYPING OF HELICOBACTER PYLORI STRAINS FROM GASTRIC BIOPSY SPECIMENS USING PCR-RFLP**

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*Helicobacter pylori* is a gram-negative microaerophilic organism that colonizes human gastric mucosa. It has been proved that *H. pylori* is an etiologic agent of chronic gastritis and peptic ulcer diseases and is also a risk factor for gastric cancer. The World Health Organization has classified *H. pylori* as a class I carcinogen. Epidemiological studies have shown that *H. pylori* infection occurs world wide at a high prevalence rate.

Recurrence of infection after apparent eradication has also been reported and is associated with recurrence of ulcers. However, it is unclear whether the recurrence of ulcers following *H. pylori* eradication therapy is due to recrudescence of the previous infection or to exogenous re-infection by another strain. An accurate method for the detection and differentiation of *H. pylori* strains in patients both before and after therapy is therefore of great importance for diagnosis, monitoring of treatment, and reduction of the long-term consequences of continued but undetected disease.

Attempts to differentiate *H. pylori* strains have been made using a variety of conventional typing schemes, like haemagglutination, biotyping, cytotoxin activity, plasmid profiles and immunoblotting. However, each of these methods due to some inherent difficulties did not gain widespread use for precise differentiation of *H. pylori* strains.

Gastric biopsy specimens were obtained from 79 patients, 36 women and 43 men. Extracted DNA amplified using primers specific for *UreC* gene. The amplified products were purified using PCR products purification kit and then digested with 10 U of *CfoI*, *MboI* and *AluI* restriction enzymes separately for at least 3h at 37°C.

Five patterns for *MboI*, five patterns for *AluI* and 4 restriction patterns for *CfoI* were observed. The patterns were classified based on the number of bands detectable by gel electrophoresis.

From our study it is concluded that, the PCR-based RFLP technique is a useful approach for genotyping of specific *H. pylori* strains. Using this method we can determine

reinfection, infection with new strains, recrudescence or drug resistance in *H. pylori* infected patients.

#### P 304

### THE POSSIBILITIES OF IMMUNOHISTOCHEMICAL STAINING METHOD FOR DETECTION OF HELICOBACTER PYLORI (HP) INFECTION IN CASES WITH CHRONIC GASTRITIS, ULCERS AND GASTRIC CANCER AND GASTRIC LYMPHOMA BRACHKOVA Yordanka, MARKOVA Elena

Background: The HP infection affects more than 50% of the human population and plays an important role in the pathogenesis of chronic gastritis, chronic peptic ulcer of stomach and duodenum, gastric cancer and gastric lymphoma. Thus the eradication of HP has definite therapeutic and preventive effect.

Aims: 1. To improve the diagnosis of HP infection putting into practice the immunohistochemical / IHCh/ staining of bacteria on endoscopic biopsies. 2. Determine the rate of HP infection in patients with chronic gastritis, chronic peptic ulcers, gastric cancer and gastric lymphoma.

Methods: Two hundred forty endoscopic biopsies prepared with routine histological and histochemical methods (HE, V.G., PAS) are studied retrospectively and analyzed. Two hundred twenty three (92%) are stained with Giemsa for HP and 99 (42%) are tested immunohistochemically with Polyclonal rabbit anti HP antibody (DACO)

Results: Analyzing the material we found that the identification of HP with Giemsa staining was respectively 55% and 58% in cases with chronic gastritis and ulcers and 66% and 69% in the surrounding mucosa in patients with gastric cancer and gastric lymphoma. Immunohistochemically HP was detected in 83% and 93% in chronic gastritis and ulcers and 70% and 77% in gastric cancer and lymphoma. The efficiency of the proposed method in number detection is mostly evident in the group of patients with chronic gastritis, where all the negative and suspicious for HP cases were examined immunohistochemically. The percentage of cases with identified HP bacteria in this group increased from 55% to 93%. For the group of ulcers the rise is from 58% to 83%, while for gastric cancer and lymphoma cases it was inconsiderable. The reason for this is the lack of appropriate material from the surrounding mucosa in the tissue samples for IHCh staining. Conclusions: The examination with IHCh staining is a reliable method for identification of HP infection in gastric mucosa. By the means of this method we reduce the number of suspicious cases. The increased exposure of HP is due to the detection of the attached to the cell membranes bacteria and the coccoid forms too.

Key Words: HPylory, immunohistochemical detection, gastroscopic biopsies.

#### P 305

### HELICOBACTER PYLORI INFECTION IN CHILDREN: CORRELATION BETWEEN CAGA, ICEA AND VACA GENOTYPES AND HISTOPATHOLOGICAL CHANGES IN GASTRIC MUCOSA

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#### Background

Despite strong association of distinct *Helicobacter pylori* (HP) genotypes with degree of histological changes in gastric mucosa of adults, such an association is still controversial in paediatric population.

Aims of the study

To analyse correlations between *cagA*, *iceA* and *vacA* HP genotypes and the degree of histological changes in the gastric mucosa in paediatric population.

#### Material and methods

The entry criteria for the study was a positive rapid urease test, and subsequent detection of *ureA* and/or *ureC* genes by polymerase chain reaction (PCR) in the same tissue specimen. Histological changes in gastric mucosa were graded according to the updated Sydney system. The *cagA* gene, signal (s) and middle (m) regions of *vacA* gene and *iceA* gene were determined by PCR in the total DNA, isolated from tissue samples used for the rapid urease test.

#### Results

Altogether 186 samples of gastric mucosa (158 from antrum and 28 from corpus) from 140 children, aged from 4 to 18 years (mean age 13 years) were analysed histologically. The degree of activity and chronicity in antrum and corpus correlated with the density of HP colonisation ( $p < 0.05$ ). A statistically significant decrease in the degree of activity and chronicity after HP eradication was observed only in antral mucosa ( $p < 0.05$ ). The HP eradication rate was 69% for antrum and 75% for corpus. *cagA* was detected in 63% (75 of 119) of samples and correlated with the degree of activity and chronicity in antrum ( $p < 0.05$ ). No correlation was found between *iceA* and histological changes. Regardless of the subtype, a borderline statistical association was present between *vacA* s and antral intestinal metaplasia ( $p = 0.05$ ), and between *vacA* m and corpus atrophy ( $p = 0.03$ ). Co-infection with various HP strains, found in 8% of antral mucosa samples, did not correlate with histological changes.

#### Conclusions

Our results confirm the need for treatment of symptomatic children with HP infection in order to diminish and/or abolish the damaging effect of acute and chronic inflammation on the gastric mucosa, a hallmark of HP infection. Both have been previously shown in adults to correlate with the subsequent development of atrophy and intestinal metaplasia, possible precursor lesions in gastric carcinogenesis. Finally, it appears that at least the *cagA* gene influences the degree of activity and chronicity in antral mucosa of children, and that the mechanism of mucosal damage by HP is different in corpus and antrum.

#### P 306

### GASTROINTESTINAL LYMPHOMAS AND HELICOBACTER PYLORI INFECTION – CLINICOPATHOLOGIC STUDY

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Background: Chronic *Helicobacter Pylori* /HP/ infection is strongly associated with the development of primary gastric B-cell lymphoma of MALT type. The choice for gastric surgery or stomach concerning treatments, should be based on an accurate histopathological diagnosis, grading and clinical staging. The effect of curing HP infection on the course of a high-grade gastric MALT lymphoma is uncertain.

Aims: 1. To study the primary gastrointestinal lymphomas /GIL/ for the last 5 years, obtained gastroscopically, after surgical treatment or diagnosed on autopsy.

2. To follow up and analyze the effect of HP eradication therapy /HPET/ and chemotherapy on gastroscopic biopsies.

Patients and methods: For the period 2001-2005, 36 patients between 38 and 84 years with primary GIL were studied /32 of the stomach, 3 on small bowel and 1 on large bowel/. Twenty six cases were diagnosed on gastric biopsies, 5 after surgical biopsy and 5 after autopsy. All lymphomas were immunophenotype identified, graded and clinical staged by Ann Arbor and Musshoff system.

Results: From 36 GIL 35 were classified as B-cell NHL and 1 as high grade T-cell NHL, 28 were high-grade B cell

lymphoma /HGBCL/ or diffuse large B cell lymphoma /DLBCL/ and 7 low-grade B cell lymphoma /LGBCL/. A follow up examination, about 30-36 months, was performed on 7 cases/3 with HGBCL and 4 with LGBCL/. Complete histological remission was found in 2 patients with successful HPET and chemotherapy /CHOP, MABTHERA/ and unsuccessful HPET with incomplete histological remission in 5 cases. Twelve patients /aged 50-57/ with DLBCL were operated in stage IIE -10 gastric and 2 intestinal. Half of them were with large ulcerated lesions. All GIL diagnosed after autopsy were in advanced stage /1 high-grade T-cell and 4 DLBCL/.

**Conclusions:** Our results revealed that primary GIL were mostly high grade B-cell/DLBCL and in 80% were associated with HP infection. The effect of HPET was not successful in most cases. We found that they were rarely suspected by clinicians and were diagnosed mostly in IIE clinical stage. This settled down the question about revision of HPET and the responsiveness of lymphomas to the therapy.

**Key words:** Gastrointestinal lymphoma, Low grade MALT, DLBCL, HP eradication, Chemotherapy, Surgical treatment.

### P 307

#### **GASTROINTESTINAL STROMAL TUMOUR: ROUND CELL ANAPLASTIC TYPE LACKING C-KIT/CD117 IMMUNOEXPRESSION**

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**INTRODUCTION AND AIMS:** Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract with the stomach as the most common site. CD117/KIT immunopositivity has been accepted as a specific marker for GISTs preferentially used for confirmation of the diagnosis. However, GISTs constitute a group of phenotypically heterogeneous mesenchymal neoplasms with uncertain biological behaviour that often coexpress CD34 and/or myoid, neural and other markers.

**MATERIAL AND METHODS:** We reexamined clinically, histologically and immunohistochemically 121 cases of paraffin-embedded GISTs noted in the Tumor Registry of Clinical Centre of Serbia from the total of 412 patients operated for abdominal mesenchymal mostly visceral tumours between 1992 and 2004. In addition, evaluation of malignancy of GISTs was based on clinico-pathological criteria proposed both by Fletcher in 2002 and Bucher in 2004.

**RESULTS:** Most of gastric GISTs were composed of spindle cells (73%), epithelioid cells (20%) or both and/or rare cell types (7%), and nearly 30% showed obvious clinical malignant behavior. We present three cases of primary GISTs with similar small round cell morphology, two gastric (in 47 year old male and 56 year old female patients) and one esophageal (63 year-old male patient). In one case, hepatic metastases were resected during primary surgery, in two other cases they developed after 8 and 11 months. None of them were treated with imatinib (Glivec) and only one patient had conservative chemotherapy. Follow-up showed short survival interval (9 to 16 months). All cases expressed only strong vimentin immunopositivity and were preexcluded for other small round cell differentiation. Immunohistochemical examination showed weak cytoplasmic CD117 expression in one case, evident CD34 expression in two of cases (one of them exclusively CD34 positive) and confirmed non-myogenic and non-neurogenic differentiation. In addition, all cases

showed Ki-67 index higher than 10% and PCNA higher than 30%.

**CONCLUSION:** Round cell anaplastic type of GIST might represent small distinct clinically aggressive subgroup of these tumours with incomplete immunophenotypic expression or dedifferentiated type of GISTs different from immunohistochemically similar group of so-called fibroblastic, mostly spindle cell type of GISTs (GIFT).

### P 308

#### **GASTROINTESTINAL STROMAL TUMORS OF THE DUODENUM.**

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#### **Abstract**

**Introduction:** The majority of gastrointestinal tumors (GIST) express c-kit, a growth factor receptor with tyrosine kinase activity. Mutations in the c-kit proto-oncogene may lead to constitutive ligand-independent activation of c-kit and subsequent neoplastic transformation, but alterations in chromosomes 14 and 22 may also play a role in the molecular pathogenesis. The purpose of the study is to distinguish GISTs from the other mesenchymal tumors of the GI tract and specially duodenal tumors, because of differences in natural history, as well as the efficacy of treatments targeting the GIST tyrosine kinase.

On immunohistochemical staining, tumor duodenal cells were positive for vimentin, CD34, CD117(c-Kit), Ki-67 and negative for muscle-specific actin, desmin, S100, neurofilament and keratin. GISTs tumors have a spectrum of clinical behavior at all sites of their occurrence. Tumors that show low mitotic frequency (5 or fewer mitoses per 50 HPF) usually have a benign behaviour. However, there is a definite percentage of mitotically inactive tumors that subsequently metastasize, emphasizing the fact that low mitotic count does not rule out a malignant behaviour. GISTs with mitotic counts over five per 50 HPF are customarily designated as malignant, and tumors over 50 mitoses per 50 HPF are designated as high-grade malignant.

The small intestinal tumors and duodenal tumors were more aggressive than other localizations, in our cases the tumors shows high risk and malignant potential in comparison with gastric tumors. Tumor size, location, mitotic and proliferation indices, and mutations in the juxtamembrane domain of c-kit (exon 11) appear to correlate with risk of malignant behaviour.

The molecular targeting of the critical pathogenetic mechanism underlying GIST has given patients new hope, and has provided physicians with a highly effective and well tolerated therapeutic option for a disease for which no systemic therapy existed previously.

Moreover GISTs serve as the model solid tumor for a molecular biology-based diagnosis and treatment of cancer.

### P 309

#### **CLINICOPATHOLOGIC FEATURES AND IMMUNOHISTOCHEMICAL STAINING FOR C-KIT PROTEIN (CD117) IN METASTATIC AND PRIMARY MALIGNANT MELANOMA OF THE GASTROINTESTINAL TRACT**

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**Introduction:** Malignant melanoma (MM) is the most common metastatic tumor of the gastrointestinal tract and can present with fairly common constitutional symptoms. The small and large intestines are the most common sites for metastases from cutaneous MM. However, primary MMs in these sites are exceedingly rare. Genuine primary MM of the gastrointestinal tract is a rare, highly malignant anorectal neoplasm of poor prognosis.

**Aim:** This investigation was performed to determine the clinicopathologic features and immunohistochemical staining for c-kit protein in metastatic and primary gastrointestinal malignant melanomas.

**Material and Method:** 26 gastrointestinal MM cases were retrieved from the archives of pathology departments of four different medical schools and were evaluated for histologic features. Formalin-fixed, paraffin-embedded tissue sections were immunostained with c-kit protein by the avidin-biotin-peroxidase procedure.

**Results:** These cases included 17 anorectal, 6 small intestinal, 2 duodenal, and 1 gastric MM. Most of the anorectal MMs (56.2%) were presented with gastrointestinal hemorrhage, whereas small intestinal (SI) MMs with intussusception (44.4%) and acute abdomen. Comparison of gender and tumor size (median, 5.5cm in anorectal MM, and 5cm in SI) showed no significant difference. Patient age at presentation (median, 62 years in anorectal, and 49 years in SI) was not statistically different ( $p=0.08$ ). Resection materials revealed that anorectal cases were solitary (12/12), whereas small intestinal cases were multiple (4/4),  $p=0.0005$ . Histologically, tumor cell (epitheloid and spindle) type ( $p=0.667$ ), and diffuse and intense cytoplasmic expression of c-kit did not differ significantly in between the anorectal, small intestinal and duodenal MM ( $p=0.627$ ).

**Conclusion:** Metastatic MM in the gastrointestinal tract should be suspected in patients with history of melanoma of the skin and acute gastrointestinal symptoms. Other than clinical presentation, location and multiplicity, the clinicopathologic parameters did not differ between primary and metastatic MM. This study also emphasizes on diffuse positive immunorexpression of c-kit protein in gastrointestinal MMs which diminishes the discriminatory value of c-kit protein for differential diagnosis between the cases of gastrointestinal stromal tumor and poorly pigmented malignant melanoma.

**P 310**  
**FIRST RESULTS OF THE NATIONAL PROGRAM OF STANDARDIZED GISTS DIAGNOSIS IN SLOVAKIA: RETROSPECTIVE VERSUS PROSPECTIVE ANALYSIS**

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**Introduction:** The definition of gastrointestinal stromal tumors (GIST) has evolved over time and its true incidence is still unknown, probably underestimated. GIST showing an increasing incidence after molecular discovery (KIT gene) and clinical-pathological diagnostic tools (CD117) represent targets for new treatment by anti-CD117 therapy.

**Purpose of the study:** Comparison of incidence, morphology and clinical data of GIST of two series: The 1st series representing a retrospective study of 10 years material reported at ESP Congress in Ljubljana (2003) versus (vs) 2nd series of GIST diagnosed during the last 2 years under cooperation with other Slovak departments involved in national program.

**Methods used:** For both the series, identical methodology and interpretation of the findings based on the criterias of Fletcher et al. (2002) were used. In addition to histological stainings,

the immunohistochemical analysis included primary antibodies against vimentin, desmin, actins, S-100 protein and CD117 (all DakoCytomation) and CD 34 (Immunotech). Because of lacking molecular biology examinations, only CD117-positive cases were included to the study.

**Summary of results:** The 1st series includes 32 and the 2nd 33 cases. The tumors of both the series showed predominant localisation in the stomach (18 vs 13 cases) and small intestine (11 vs 15 cases), while other localisations were rare (colon 1 vs 2 cases, extramural GIST 2 vs 2 cases). One of the 2nd series cases represented a liver metastasis of the case diagnosed previously as „gastric leiomyoma“ 19 years ago, without a possibility for the review. However, both the series showed different distribution of the risk categories: predominance of the lower risk categories in the 1st series (2 cases of very low, 16 of low, 8 of intermediate and 6 of high risk category) vs predominance of high risk categories in the 2nd series (4 cases of very low, 8 of low, 7 of intermediate and 14 of high risk category).

**Conclusion reached:** In contrast to literary data and results of our 1st series, the intestinal localisation was predominating in the 2nd series. Although both files are relatively small, the localisation might be related to the increased number of the higher risk categories cases. The study shows first benefits of the program, resulting in a higher „sensitivity“ of the pathologist to recognize the GIST. Therefore the program will continue and should involve molecular diagnostic techniques of GIST diagnosis.

**P 311**  
**GASTROINTESTINAL STROMAL TUMOR AND OTHER SPINDLE CELL TUMORS; PATHOLOGICAL AND CLINICAL ANALYSIS OF 30 CASES.**

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**BACKGROUND:** Gastrointestinal stromal tumors (GISTs) are mesenchymal neoplasms of the gastrointestinal tract. These lesions have been a source of controversy in regards to classification, histogenesis, differentiation and biologic potential. Recently with the advent of new therapeutic interventions, proper characterization and conceptualization of these tumors has acquired a new meaning, and emphasis is being placed on the role that various diagnostic techniques play in the evaluation of these neoplasms.

**PURPOSE OF THE STUDY:** We conducted a study to identify true GISTs with known pathological and immunohistochemical criteria from other tumors of the gastrointestinal tract.

**METHODS:** We selected from clinical records of patients with diagnosis of schwannoma, leiomyoma, leiomyosarcoma and tumor of uncertain biological behavior of the gastrointestinal tract, 79 cases. All the cases studied were surgically treated. To confirm the usefulness of an immunohistochemical panel of antibodies and histological analysis we have been selected 30 cases of GISTs. The antibodies used were: c-kit/CD117, CD34, desmin, smooth muscle actin (SMA), S-100 protein.

**RESULTS:** Thirty cases of GISTs were identified, based on histological and immunohistochemical analysis. All the confirmed GISTs were positive at immunohistochemistry for c-kit expression and most of them for CD34, desmin and S-100. SMA have only limited value. The two predominantly location of the tumors were the stomach (75%) and small intestine (6%). Various pathological and clinical parameters have been evaluated (size, mitotic rate, invasion into mucosa,

presence or absence of coagulative necrosis, status of surgical margins, location, chronic or acute bleeding).

**CONCLUSIONS:** Tru GISTs can be recognized from other tumors by histological analysis. Immunohistochemistry to identify c-kit protein expression is useful to certify the diagnosis. For predicting behavior in GISTs, the authors proposed some criteria.

#### P 312

##### **IDENTIFICATION OF GASTROINTESTINAL STROMAL TUMORS IN PRIMARY BENIGN AND MALIGNANT MESENCHYMAL NEOPLASMS OF THE GASTROINTESTINAL TRACT AND INCIDENCE IN DOUBS COUNTY. CLINICOPATHOLOGICAL RETROSPECTIVE STUDY OF 230 CASES FROM 1990 TO 2000.**

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Gastrointestinal stromal tumors (GISTs) are KIT-positive spindle cell or epithelioid mesenchymal tumors (MTs) occurring anywhere in the gastrointestinal (GI) tract or abdomen. The majority of GI-MTs are therefore now considered as GISTs. However, the frequency of GISTs in GI-MTs is not well known and their incidence too. The purpose of this study is to identify GISTs in the Franche-Comté region (eastern France) in a ten year-period (1990-2000) in all primary benign or malignant MTs of the GI-tract and abdomen.

This study includes MTs diagnosed in all Franche-Comté region pathology laboratories as leiomyomas, leiomyosarcomas, leiomyoblastomas, schwannomas, schwannosarcomas, neurofibromas, fibromas, fibromatosis, spindle cells sarcomas, and GISTs. The Franche-Comté (population : 1,200,000) is a region composed of 4 counties in particular the Doubs county (population : 499,062). Over the 10 year-period, 230 cases were retrieved. For each case, a standard morphologic study and an immunohistochemical study using anti-CKIT, CD34 antibodies was carried out. The proliferative index was performed with the Ki67 (MIB-1) antibody. The diagnosis was drawn up according to GIST Symposium 2002 criteria.

Out of the 230 cases, 29 were initially identified as GISTs (13 %). After this second study, 102 / 230 are GISTs (44 %), and 28 / 29 are true GISTs. In stomach 67 / 81 are GISTs (83 %), in small bowel 24 / 40 (60 %), in omentum 4 / 5 (80 %), in mesentery 3 / 9 (33 %), in colon 3 / 53 (6 %), in retroperitoneum 1 / 12 (8 %). In esophagus (21 tumors), in liver (5), in anus (2), in appendix (1) and in gallbladder (1), no GIST was identified. GISTs were developed in stomach in 66 % of cases, in small bowel in 24 %, in omentum and mesentery in 7 %, in colon in 3 % and in retroperitoneum in 1 %. Over 102 GISTs, 47 patients live in Doubs county at time of diagnosis. The mean "floor" limit of the raw incidence rate was estimated at 1 case / year / 100,000 in Doubs county. The proliferative index over 10 % of neoplastic cells stained was correlated with poor prognostic.

The occurrence of GISTs in our study is close to that reported in literature. Stomach and small bowel locations are the most frequent. Furthermore, our study shows that 74 benign or malignant GI-MTs were reclassified as GISTs and that GISTs are the most common mesenchymal neoplasm in the gastrointestinal tract.

Supported by grants from the French Research Ministry (PHRC 2003), NOVARTIS, and Ligue Contre le Cancer

#### P 313

##### **NATIONAL PROGRAM OF A STANDARDIZED BIOPSY DIAGNOSIS OF GASTROINTESTINAL STROMAL AND NEUROENDOCRINE TUMORS OF THE GI-TRACT IN SLOVAKIA**

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**Introduction:** The gastrointestinal stromal and neuroendocrine tumors (GISTs, resp. NETs) of the gastrointestinal tract show unique biological behavior. Their appropriate management requires a standardized approach and methods used for the biopsy diagnosis.

**Purpose of the study:** Because of their relatively low incidence, the experiences of the pathologists with the biopsy diagnosis and differential diagnosis of GIST and NET are limited. Therefore the aim was to develop a national system offering a standardized biopsy examination and interpretation of all the clinical and laboratory results for the patients with GIST and NET.

**Methods used:** The authors introduced in 2004 a retrospective analysis of previously diagnosed GISTs and NETs and attended the Italian course INTUIT („Identification and New Treatment of Uncommon Intestinal Tumors“). Due to a cooperation of Slovak pathologists and involved clinical specialists, a national network system of a GISTs and NETs biopsy diagnosis based on the accepted regional centralisation and using standardized protocol for all the obtained data was introduced in Jan. 2005. The system includes a second look biopsy examination of the cases at accepted specialized centres in relation to clinical data evaluation. In addition to the unified panel of morphologic and immunohistochemical methods of the GISTs and NETs biopsy diagnosis, all hormonal and necessary prognostic markers may be examined in situ at the coordination centrum of the project.

**Summary of the results:** The early preliminary results summarizing first months of the project document its first benefits, e.g. increasing number of the biopsically identified GIST cases. An integral part of the project is represented by the educational activities. In association with the traditional IAP meetings of the Slovak pathologist, first two minisymposias discussing the topics of GIST and NET biopsy diagnosis were organized and the project was introduced at the national conference of the gastroenterologists. Next educational activities will involve also other specialists, incl. the participations of the molecular biologists.

**Conclusions reached:** The described program starts to offer: a./ for clinical practice standardized results representing a good basis for the management and therapeutical considerations in the cases of GISTs and NETs, incl. their targeted therapy, b./ good outcomes for further optimization of their diagnosis including also molecular biological techniques.

#### P 314

##### **VARIABILITY ASSESSMENT OF IMMUNOPHENOTYPICAL PATTERN OF DIFFERENTIATION IN GASTROINTESTINAL STROMAL TUMORS**

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Gastrointestinal stromal tumors (GIST) are mesenchymal neoplasms of gut with origin in interstitial cells of Cajal which express the tyrosin-kinase receptor of c-kit (CD117). According to phenotypical features, GIST's can be classified in tumors with smooth muscle differentiation, neural, mixed or non neuro-muscular differentiation.

The aim of the study was to investigate and to test the efficiency of a panel of antibodies in immunophenotypical pattern variability analysis of differentiation in GIST.

The indirect triserial ABC method was performed on formalin fixed paraffin embedded tissues taken from 13 patients with GIST, using 5 antibodies : CD117, CD 34, vimentin, actin, S-100.

GIST's were immunoreactive to CD117 in 80% cases, to CD34 in 91% cases. Using regressive biostatistics analysis, it was observed a direct positive correlation between CD117 and CD34 ( $r = 0.7$ ) and highly significant ( $p = 0.002$ ). Between CD117 and actin, a positive polynomial relation was found ( $r = 0.4$ ), without statistical signification ( $p = 0.6$ ). Between CD34 and actin, a slightly positive linear correlation ( $r = 0.3$ ) was observed, as it was between CD34 and S-100 ( $r = 0.23$ ), but none with statistical signification ( $p = 0.2$ ). Tumors with a diffuse stain to CD117 and CD34 shown a focal expression to S-100, actin and vimentin.

The study confirmed the coexpression between the main diagnostic markers of GIST's - CD117 and CD34 ; CD34 high percentage in correlation with actin and S-100 expression, may provide to this heterogen antigenic association, a significant diagnostic value with future therapeutical involvements.

### P 315

#### INFLAMMATORY CELL-RICH GASTROINTESTINAL STROMAL TUMOR IN THE STOMACH: A CASE REPORT

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We report the case of a 62-year-old woman with two primary neoplasms: adenocarcinoma of the colon with hepatic metastase and stromal tumor of the stomach, that had unusual histologic findings. Abdominal-pelvic ultrasonography and computed tomography revealed that there was wall widening in the descending colon and posterior wall of the antrum.

Preoperative endoscopic examination revealed a hemispheric submucosal tumor in the anterior wall of the antrum.

Histologic diagnosis of colon mass was adenocarcinoma and tumor metastased to liver. The tumor of the stomach, which was 3 cm in diameter, was resected by antrectomy. Histologic examination revealed that it was composed of spindle shaped cells with elongated nuclei and no mitoses. Inflammatory cells were intense and patchy throughout the tumor and a peripheral cuff of lymphoid aggregates was observed in the tumor. Tumor cells expressed vimentin, S-100 protein and CD117 strongly and diffusely and neuron-specific enolase weakly and focally. Tumor was uniformly negative for smooth muscle actin, desmin, cytokeratin, CD34, synaptophysin, and chromogranin. A positive reaction for MIB-1 was only a few. This appears to be the first report of the stromal tumor which has a cuff of lymphoid cells in peripheral and dense plasma cells in GISTs.

### P 316

#### C-KIT MUTATIONS ANALYSIS OF A SERIES OF GISTS FROM PORTUGUESE PATIENTS

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Gastrointestinal Stromal Tumors (GISTs) are rare primary mesenchymal tumors of the gastrointestinal system. GISTs diagnosis is partially dependent on the immunohistochemistry c-Kit over expression, a type III tyrosine kinase receptor. The majority of GISTs have a constitutively active form of

oncogene c-kit, which is the reflex of activation mutations in the extracellular domain (exon 9), juxtamembrane domain (exon 11), and intracellular kinase domains (exons 13 and 17). Approximately 80% of c-kit mutations occur in exon 11, and are clinically important due to their association with high sensitivity and response to Imatinib, a specific kinase receptor inhibitor, when compared to mutations in the other c-kit exons. The reported frequency of c-kit mutations ranges from 20 to 70% of GISTs cases. In Portugal, the incidence of c-kit mutations in GISTs tumors is, to the best of our knowledge, unknown.

The aim of this work was to assess the frequency of oncogene c-kit mutations, in a Portuguese series of GISTs and other mesenchymal tumors, to determine the frequency of patients that would benefit from Imatinib therapy.

DNA was extracted from formalin-fixed and paraffin-embedded tissues of 22 GISTs and 7 mesenchymal non-GISTs tumors (3 Leiomyosarcomas, 1 Malignant Triton, 2 Leiomyomas, 1 Sarcoma). Mutation analysis of c-kit exons 9, 11, 13 and 17 was done by PCR-SSCP. Samples with a SSCP pattern different from the normal blood pattern were further sequenced to confirm the genetic alteration.

Of the twenty-two GISTs cases, eight (36.4 %) contained a mutation in exon 11, one (4.5 %) in exon 9, and no mutation was identified in exons 13 and 17. Mutations in exon 11 included four deletions, two duplications and two base substitutions. The mutation in exon 9 was duplication. All deletions and duplications were in-frame. In total, c-kit mutations were present in 9/22 (41%) of GISTs. No mutation was found in any of the mesenchymal non-GIST cases studied.

We characterized for the first time the occurrence of c-Kit mutations in Portuguese GISTs series. The incidence of c-kit mutation is 41%, in agreement with described range. As previously reported, the great majority (8/9, 89%) of mutations are located in exon 11, and lead to constitutive activation of c-kit kinase activity. No mutations were identified in non-GISTs stromal tumors.

### P 317

#### A NATIONAL STUDY OF GISTS IN GREECE

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KALEKOU, C. KAFIRI,

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PAPADOPOULOS

Gastrointestinal study group of HELLENIC SOCIETY OF PATHOLOGY

GISTs are rare neoplasms of the GI tract with unpredictable prognosis, differentiating towards Cajal cells; most GIST express CD117 and carry genetic changes in chromosome 11 which encodes for the c-kit receptor, a tyrosine-kinase transmembrane molecule get involved in cell signaling. An inhibitor of this molecule has been discovered recently, giving rise to new therapies.

The aim of this study is to investigate the incidence of GIST in Greece and analyze the morphological and immunohistochemical characteristics, so as to create a GIST database in Greece.

The mesenchymal neoplasms of GI tract and mesentery diagnosed in 23 laboratories in Greece during a 5-year period (1998-2002) were collected. They were studied by ten couples of Pathologists for morphological subtypes, mitotic activity (50hpf), necrosis, cystic degeneration and atypia in H&E and by immunostaining with CD117 for the staining pattern and the percentage of positive cells (DAKO 1/100,

polyclonal, without pretreatment, using the Ventana automated NeXes system). Follow up the patients for 24 –96 months for overall survivor, partial or complete remission and relapsing in comparison to the morphological and immunohistochemical characteristics of GISTs.

209 out of 311 cases of mesenchymal neoplasms were positive for CD117 (67.2%). 60.3% of the patients were 61-80 years old, 51% were men and 41% women (M/F: 1.44). 57.8% of GISTs affected stomach, 28.6% small bowel, 6.6% large bowel and 3% mesentery. 48.2% of neoplasms were measured < 2cm, 36% >2<5cm, 37.6% >5<10 and 21.60% >10cm. Less than <2 mitoses per 50hpf were counted in 48.2% of the cases, 2-5 in 20.3%, >5<10 in 13.7% and >10 in 17.8%. The most common neoplasm was the spindle cell type (52.50%) with moderate atypia (54%). In 51.2% of GISTs 81%-100% of neoplastic cells express KIT protein. A diffuse staining pattern was found in 80.2% of the cases. Since now we follow up 80 patients.

Epidemiology and Histology of GISTs in Greece share the same characters with GISTs worldwide apart from a lower expression of KIT protein, a finding needing further investigation. Follow up of patients is in process and the results should be discussed.

### P 318

#### MULTIPLE GASTROINTESTINAL STROMAL TUMORS IN SMALL BOWEL

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**Objectives:** In most cases, gastrointestinal stromal tumors (GIST) are sporadic in nature, however, they may occur as multiple lesions in familial forms associated with KIT or PDGFRA germline mutations, but may also develop in the setting of type 1 neurofibromatosis. In these situations, GISTs arise in a background of interstitial cell of Cajal hyperplasia and may be associated with systemic manifestations including skin hyperpigmentation and mast cell disorders. We report four patients with multiple GIST in small bowel and one of them was a child who was diagnosed as a very rare multiple GISTs and gastrointestinal autonomic nerve tumor (GANT).

**Methods and Results:** We identified four patients with multiple GISTs in small bowel. One of the adult patients has also type 1 neurofibromatosis. Rests of the patients have no type 1 neurofibromatosis and familial disorders. Immunostains for CD117, CD34, desmin, actins, S-100 protein, and keratins were performed on all of the tumors. Patient's ages/genders were 66/M, 65/F, 48/F, 10/M. All of the patients had multiple GISTs in small bowel, large bowel mesothelial surface (operation findings); at least 10 nodules and average 3.0 cm (0.2mm-12cm). First patient also had pancreatic adenocarcinoma, third patient with type 1 neurofibromatosis also had periampullary somatostatinoma. Our child patient had suffered from diarrhea and had familial tuberculosis history. He had multiple (the largest was 12cm diameter) serosal nodules. Tumor cells appear like ganglion cells and immunostains for CD117 was focal positive, NSE, Synaptophysin were diffuse positive, CD34, desmin, actin, myoglobin were negative.

Our cases had no hepatic metastases. However they had multiple residual tumor nodules, only child patient has been treated with Imatinib Mesylate for two months.

**Conclusions :** Multiple GISTs are unusual tumors that have been rarely described in children. GISTs require resection and close observation for hepatic metastases.

### P 319

#### REDUCED PROLIFERATIVE ACTIVITY DOES NOT EXPLAIN LOSS OF AVIDITY ON PET SCAN, IN A CASE OF METASTATIC GIST

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#### INTRODUCTION

Positron Emission Tomography (PET) is commonly used to monitor disease progression in patients with metastatic Gastrointestinal Stromal Tumour (GIST). We report a case of Imatinib-refractory disease with very low avidity on PET scan.

A malignant GIST was resected from the stomach of a 60 year old woman in 2000. She represented with metastases in the liver and lesser sac in 2001, and was treated with Imatinib. In 2003 further metastases were identified, although tracer uptake on PET was so low that the scan was initially reported as negative. One possible explanation for this could be that the chemotherapy had resulted in a metabolically inactive tumour, which would not take up tracer. A likely result of this would be lower proliferative activity; therefore the pathology was reviewed in an attempt to ascertain whether this could provide an adequate explanation.

#### METHODS

All slides were reviewed from both the original resection specimen and the subsequently resected metastatic tumour. Mitotic counts were performed on two separate tissue blocks from each specimen, along with immunoperoxidase staining for Ki-67 (MIB-1, Dako). For the mitotic counts, 50 high power fields (hpf) per block were examined, and only definite mitotic figures were counted. Ki-67 staining was assessed by counting the number of positively stained cells per 100 tumour cells, conducting several counts over each slide.

#### RESULTS

In the original specimen, there were 55 and 66 mitotic figures (mf) in 50hpf; up to 4mf/hpf and 40mf/10hpf. In the metastatic tumour the counts were 58 and 51mf/50hpf; up to 4mf/hpf and 29mf/10hpf.

Ki-67 staining showed zonal variation in both instances, with staining per centages ranging from 2-58% in the primary tumour and 2-54% in the metastasis. In both cases, the overall average staining rate was estimated at around 40%.

#### CONCLUSION

There does not appear to be any significant difference in proliferative activity between these tumours, whether assessed by traditional mitotic counts or by staining for Ki-67. Thus differences in proliferative activity do not explain the lack of tracer uptake in this case. It is postulated that Imatinib therapy may have been sufficient to inhibit tracer uptake and/or phosphorylation, but insufficient to inhibit tumour cell proliferation. Regardless of the cause, this loss of avidity has important implications for therapeutic monitoring of patients with recurrent GIST.

### P 320

#### THE HISTOPATHOLOGIC PATTERN OF GASTRO-INTESTINAL STROMAL TUMORS (GIST) AFTER TREATMENT BY IMATINIB.

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**INTRODUCTION** – Mesenchymal neoplasms derived from stroma of gastrointestinal tract (GIST) are restive for classic chemotherapy. These tumors become from Cajal cells and show immunopositivity for tyrosine kinase receptor – CD 117. This reaction is a base to recognize them and allows to treat by Imatinib (Glivec). It is no a predictive factor.

**MATERIALS AND METHODS** – Thirty patients with GISTs were treated by Imatinib in Cancer Center in Warsaw

between 2001 and 2004 year because of dissemination of neoplasms. Eight cases become from stomach, twelve from small intestine, ten tumors were of unknown origin. The response of treatment was monitoring by computer tomography. The cases with radiologically regression were resected. The resected specimen were fixed in 10% formalin and further tissue sections were stained with routine hematoxylin and eosin. Immunohistochemical analysis for CD 117 were also done.

**AIM** – To show histopathologic curative effects in GISTs treated by Glivec **RESULTS** – Complete response appeared in 3 cases, partial response in 20 ones, in others (8) there were no any changes. Grossly: Tumors of diameter from 2 to 6 cm with gelatinous consistency were present with many haemorrhages and necrotic slough. Microscopically – the cells of neoplasm show a little degree of damage (about 30% of neoplasm volume). The remainder or tumor cells were confirmed by positive reaction CD 117. The results of therapy were: necrosis, haemorrhage, foci of connective tissue, hyaline deposits.

**CONCLUSION** – Partial or complete regression of GISTs was observed in patients cured by Imatinib. This medicine makes possible to resect non – operative tumors within healthy limits.

### P 321

#### **IMMUNOHISTOCHEMICAL EVALUATION OF P16, MCM-2, AND KI-67 EXPRESSION AS MARKERS OF TUMOR PROGRESSION IN GISTS BY TISSUE MICROARRAY AND CORRELATION WITH THE RISK CLASSIFICATION OF THE NIH CONFERENCE CONSENSUS**

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**Introduction:** Risk stratification for gastrointestinal stromal tumors (GISTs) is crucial but sometimes remains challenging if based on histology alone. Inactivation of p16 gene is common in human cancers, which results in aberrant loss of its encoding protein and consequently promotes G1/S progression of cell cycle. Minichromosome maintenance protein-2 (MCM-2) is associated with cell proliferation and represents one member of the MCM hexamers that unwind DNA during replication. Few series have addressed the role of dysregulated p16 in disease progression of GISTs with a sufficiently large sample size. Moreover, the prognostic value of MCM-2 has never been examined in GISTs. **Purpose:** We attempted to elucidate the association between expression of p16, MCM-2, and Ki-67 and disease progression in GISTs by tissue microarrays (TMAs). **Methods:** Six TMAs were prepared from 343 GISTs confirmed by CD117 and/or PKC- $\zeta$  with each case punched in quadruplicate. All cases were stratified by NIH consensus criteria and immunostained with p16 (Novacastra), Ki-67 (DAKO), and MCM-2 (Novacastra). Only cases with  $\geq 2$  preserved cores were scored for statistic analysis. **Results:** Of cases with scoring data, aberrant loss of p16 (<15%) was observed in 63% of GISTs: 47/88 (53%) of very low/low-risk (VL/L-R) cases and 134/201 (67%) of intermediate/high-risk (I/H-R) GISTs ( $p=0.032$ ). Overexpression of Ki-67 ( $\geq 5\%$ ) and MCM-2 ( $\geq 10\%$ ) was found in 34% and 48% of GISTs, respectively: 15/81 (18%) of VL/L-R cases and 79/192 (41%) of I/H-R GISTs for Ki-67 ( $p<0.001$ ); 29/82 (35%) of VL/L-R cases and 106/197 (54%) of I/H-R GISTs for MCM-2 ( $p=0.005$ ). Both Ki-67 and MCM-2 were highly related to mitoses ( $p<0.0001$  for each) and tumor size ( $p<0.0001$  for Ki-67,  $p=0.017$  for MCM-2),

but aberrant loss of p16 was not so. Besides, MCM-2 labeling was not only highly correlated with ( $p<0.0001$ ) but also significantly higher ( $p<0.0001$ ) than that of Ki-67. However, expression of p16 was inversely associated with MCM-2 labeling alone ( $p=0.011$ ). **Conclusions:** I/H-R risk GISTs preferentially express higher MCM-2, higher Ki-67, and loss of p16, indicating their roles in tumor progression. However, aberrant loss of p16 protein still occurs in a considerable proportion of early GISTs, which may contribute to tumorigenesis by promoting G1/S transition with a consequent increase in cell proliferation. MCM-2 may help identify non-high risk GISTs that will behave aggressively because of the higher sensitivity than Ki-67.

### P 322

#### **GIST AS UNEXPECTED CAUSE OF DYSPHAGIA IN MULTIPLE SCLEROSIS PATIENT**

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**Introduction:** Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Majority of cases arise in stomach and small intestines, while occurrence in other sites is rare. We report a case of GIST causing dysphagia in patient with multiple sclerosis.

**Patient and methods:** A 50 year old female patient with multiple sclerosis suffered from dysphagia of unknown cause. Preoperative endoscopic and CT procedures discovered large tumor mass in the lower posterior mediastinum. Left posterolateral thoracotomy revealed tumor basis emerging from the esophageal wall. Excised tumor was pathohistologically analyzed using standard HE and immunohistochemical stains against vimentin, S-100, SMA, CD34 and CD117.

**Results:** Tumor mass measured 9.5 cm in diameter. Cut surface was fleshy with areas of cystic degeneration and necrosis. Histologically tumor consisted of short fascicles of spindle cells mixed with areas of epithelioid cells, both positive for CD34, CD117 and vimentin, while S-100 and SMA were negative. There were 8 mitosis per 50 high power fields.

**Discussion:** Less than 2% of GISTs occur in the esophagus, mostly in the lower parts, causing dysphagia. High majority of patients are middle-aged males without other symptoms which makes GIST difficult to diagnose. Rareness of such cases explains why there is only one study found in literature that reports 17 patients with GIST of esophageal localization. That survey enabled Miettinen and associates to undoubtedly confirm that size of tumor and high mitotic index are major prognostic factors. According to these prognostic elements, GIST revealed in our patient was characterized with high risk of aggressive behavior. The specific targeted medication therapy was recommended. Postoperative period was uneventful and patient was discharged on the ninth postoperative day.

The occurrence of GIST in patient with multiple sclerosis is considered to be coincidental; tumor symptoms were masked by known neurological disease and proper diagnostic evaluation was delayed. Overall survival will depend not only on response to given therapy but also to concomitant neurological disease.

**Conclusion:** Although rare, GIST ought to be considered in differential diagnosis of tumor masses in posterior mediastinum. Pathologist should be aware of this possibility because adequate gross and microscopic examination followed by immunohistochemical analysis is essential.

**P 323****SMALL INTESTINAL STROMAL TUMORS: A CLINICOPATHOLOGIC STUDY OF 7 CASES**

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**Introduction:** Stromal tumors of the digestive tract, or gastrointestinal stromal tumors (GIST), originate from Cajal cell. Small intestinal stromal tumors (SIST) are infrequent tumors. Their worse prognosis compared to other GIST is clearly established. The aim of this study was to report the clinicopathologic features and immunohistochemical characteristics of SIST through a series of 7 cases.

**Material and methods:** We identified seven cases of c-kit positive SIST from 40 cases of GIST reviewed in our center over a 13-year period (from January 1990 to December 2002). **Results:** Three patients were men, four were women and the average patient age was 56 years. Five tumors were located in the ileum, two in the duodenum and one in the jejunum. The presenting clinical symptoms were gastrointestinal bleeding, either acute or occult, in 4 cases and abdominal pain in 2 cases. In the remaining patient, SIST was an incidental finding during abdominal ultrasonography for unrelated condition. Tumour size was ranged from 2 to 10 cm (median: 5.5 cm). Histologically, the majority of tumours in this group (6 cases) were composed of spindle cells with relatively scant amounts of cytoplasm. In all 6 of these tumours, the cells were arranged in a predominant fascicular growth pattern. In one tumour (case 3), the majority of cells had an epithelioid morphology and a focal organoid growth pattern was seen. Nuclear pleomorphism was absent in two cases, moderate in 3 and severe in 2 tumors. Mitotic counts ranged from 0 to 10 mitotic figures per 50 HPF. Treatment options were segmental intestine resection in all cases. None of the patient had lymph node involvement or distant metastasis at time of surgery. Four of five patients on whom there was follow-up information were free of disease. One patient died at 8 days from postoperative complications.

**Conclusion:** The authors discuss the clinicopathologic features including immunohistochemical staining profile of the SIST and define morphologic criteria which can help the pathologist to distinguish between low and high malignant potential SIST. The SIST express CD117, and mutations of the c-kit gene are central to their pathogenesis and likely the key to effective therapeutic intervention (Glivec®).

**P 324****IMMUNOHISTOCHEMICAL EXPRESSION OF CYCLOOXYGENASE-2 AND CYTOKERATIN 8/18 IN GASTROINTESTINAL STROMAL TUMORS**

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**Introduction:** Cyclooxygenase (COX) is the key enzyme in the prostaglandins synthesis, catalyzing the conversion of arachidonic acid to PGH<sub>2</sub> and exists in two isoforms (COX-1 and COX-2). The inducible form COX-2 plays an important role in tumorigenesis and has been shown that it is expressed in colorectal cancer, as well as in lung, breast and prostate cancer. Little is known about the expression of COX-2 in nonepithelial tumors, such as the gastrointestinal stromal tumors (GISTs). As regards expression of cytokeratin in GISTs, it seems to be very rare.

**Purpose:** To examine the expression of COX-2 and cytokeratin 8/18 in GISTs.

**Materials and Methods:** Twenty-six patients (15 females and 11 males, median age: 63.8 years, range: 36-79), with GISTs were evaluated. Paraffin-embedded tumor tissue sections were stained with hematoxylin-eosin. COX-2 and cytokeratin 8/18 expression were determined immunohistochemically.

**Results:** COX-2 expression was noted in the cytoplasm of tumor cells in 19 of 26 tumors (73,1%), with variable intensity of staining. The expression was stronger in epithelial than in spindle cell tumors and in epithelial areas of mixed tumors. There was no statistically significant difference of the COX-2 expression between benign and malignant tumors, but there was a greater extent of expression in tumors located within the stomach. Cytokeratin 8/18 expression was focally found in tumor cells in 2 gastric tumors (7,7%), one benign and one malignant.

**Discussion:** COX-2 is upregulated in many epithelial tumors, particularly in those of the gastrointestinal tract. The enzyme is implicated in the process of carcinogenesis and its overexpression has correlated with tumor size, infiltrative growth, local recurrence, haematogenous metastasis and reduced survival. However, up to now little is known about the expression of COX-2 in non epithelial tumors. COX-2 expression has been described in the sarcomatoid component of some uterine tumors, in pleural tumors and in sarcomas of infancy. It is also known that expression of simple epithelial keratin in GISTs is much rarer than that observed in typical leiomyosarcoma and occurs especially in malignant epithelioid tumors.

**Conclusions:** COX-2 overexpression was noted in the majority of cases of GISTs, suggesting the possible role of the enzyme in the tumor growth and the use of COX-2 inhibitors in treatment. On the contrary, expression of cytokeratin 8/18 in GISTs was very rare.

**P 325****GASTRITIS IN CHILDREN AND ADULTS : A COMPARATIVE HISTOPATHOLOGIC STUDY.**

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**Introduction :** Helicobacter pylori (HP) is highly associated with gastritis, peptic ulcer, gastric carcinoma and lymphoma. Since HP infection usually occurs in childhood and remains for life unless treated, we compared gastric biopsies in children and adults.

**Material and methods :** The study was performed on random biopsies from gastric mucosa of 100 patients (50 adults and 50 children). Sex and age were recorded for each patient. Following parameters were evaluated on H&E biopsies according to Sydney system: HP infection (0/1/2/3+), lymphocyte infiltrate (0/1/2/3+), activity (0/1/2/3+), atrophy (0/1/2/3+), and intestinal metaplasia (present/absent). Comparison of histologic variables between two groups (children and adults) was performed using the Fisher exact test or khi-2 analysis, as appropriate.

**Results :** The male to female ratio was 1.17 in both groups. HP was present in 90% of children biopsies and 82% of adult biopsies (p=0.249). A significant trend toward increased lymphocyte infiltrate in children was detected (p=0.019), but there was no difference in the prevalence of follicular pattern (p=0.838). There was no statistical difference in activity (p=0.58). The mucosa in children (18%) showed significantly less atrophy than in adults (36%) (p=0.043). Intestinal metaplasia was positively correlated with age (22% in adults/2% in children, p=0.02).

**Conclusion :** In children, gastritis shows increased lymphocyte infiltrate and less damaged epithelium. In adults,

the immune response seems to be decreased and the capability of epithelium regeneration is altered since atrophy and metaplasia are increased.

#### P 326

##### **IMMUNE RESPONSES INDUCED AFTER MUCOSAL ADMINISTRATION OF THE NON TOXIC CHOLERA B SUBUNIT IN HUMAN VOLUNTEERS: USEFULNESS OF ELISPOT METHOD**

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Oral administration of antigens, including allergens and autoantigens, maybe an efficient way to prevent diseases associated with untoward immune responses to self- and non-self antigens. However, this approach has met with limitations because it usually requires repeated administrations of large doses of antigen. Tolerance induction, based on oral administration of small amounts of antigens conjugated to the B subunit of cholera toxin (CTB), may find broad applications for preventing untoward immune responses. The aim of this study was, by using a commercial CTB (SBL vaccine), 1) to evaluate the CTB-specific systemic and mucosal immune responses after oral, nasal, or sublingual human immunization; 2) to evaluate the predictive markers of immunogenicity. We showed that homing receptor expression profile depends on the site of immunization: 100% of CTB-specific IgA SCs express alpha4 beta7 after oral, nasal and sublingual immunization; 100% of CTB-specific IgA SCs express CD62L after nasal and sublingual immunization, whereas 40% of CTB-specific IgASCs express CD62L after oral immunization. The detection of CTB-specific antibody-secreting cells in blood was performed in cell suspensions par ELISPOT method. According to the route of immunization and systemic or mucosal CTB-specific response, ELISA (IgA2) or search for antibody secreting-cells in blood (IgA and IgG ELISPOT) were performed. Moreover, a selection of cells expressing chemokine receptor or integrin such as CCR9, CCR6, CCR10 and integrin alpha4 beta7 was performed by magnetic cell sorting and detection of antibody secreting-cells in positive and negative fraction was done by ELISPOT.

#### P 327

##### **GASTRIC MUCOSAL ENDOCRINE CELLS IN THE EXPERIMENTAL BREAST CARCINOGENESIS. AN IMMUNOCYTOCHEMICAL STUDY**

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It is well known the role of many regulatory peptides in clinical course of human cancers. However, these are no reported data about endocrine cells of the stomach in the experimental breast carcinogenesis. Because of that, the aim of this report is to study G-, EC-, ECL- and D-gastric mucosal cells, during chemically induced breast carcinogenesis. We used the female albino Wistar rats, 42 days old and 120±10gr. of weight. One milligram of 9,10 dimethyl-1,2 benzantracene (DMBA) was implanted in the 5-th mammary gland. The experimental group included 15 rats, as well as control group. The animals were killed after 60 days after the implantation.

Specimens from stomach were taken only from the rats with developed mammary gland cancer. Five micrometer thin

sections were routinely stained with HE and immunocytochemical SAB-method with antibodies against gastrin, serotonin, histamin and somatostatin. Obtained results were statistically analysed by Students T test.

We have found a characteristic hyperplasia of endocrine cells, with hyperplastic G, EC and ECL-cells, compared to these cells of control animals. Simultaneously in this study revealed the development of multifocal dysplastic lesions in the antral mucosa.

The authors suggests direct stimulatory effects of DMBA on endocrine cells of the gastric mucosa. It can be concluded that DMBA cause preneoplastic effects on the gastric mucosa in rats.

#### P 328

##### **AUTOMATED PROLIFERATION/APOPTOSIS DETERMINATION IN GASTROINTESTINAL FRESH FROZEN SECTIONS USING TRIPLE FLUORESCENT LABELLING AND SCANNING FLUORESCENT MICROSCOPY CORRELATES TO IMMUNHISTOCHEMICAL STAINING AND VISUAL COUNTING**

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Background: Proliferation/ apoptosis balance is an important information in gastrointestinal ulcerative and malignant diseases. Until now immunohistochemical staining and visual counting was the routine procedure. Recently we reported a new scanning fluorescent technique for automated motorised microscopes (SFM).

Aims: development of triple fluorescent labelling method for proliferating/apoptotic/ resting cells and application of SFM for the automated analysis and counting on gastric biopsy specimen.

Materials and methods: Routine gastroscopy antral biopsy specimen (30) were fresh frozen and 5 um sections were prepared. Proliferation was detected using a PCNA antibody and an antimouse-rhodamine labelling system. Apoptosis was labelled using the TUNEL reaction with FITC bound nucleotids. DAPI nuclear counter staining was applied.

The labelled sections were scanned and digitised in the three fluorescent channel. SFM was modified to detect epithelial surface, glands in the biopsy specimen. Automated nuclei detection, PCNA and Tunnel detection was performed, ratio was calculated. In parallel standard biopsy specimen were labelled with PCNA and AEC labelling. TUNEL reaction was performed also performed. Visual counting was performed upto 1000 epithelial cells.

Results: The mean PCNA labelling in healthy samples were 45,3±12,4%, TUNEL positivity was found in 23,2± 7,8%. Significant correlation in apoptosis/proliferation ratio between the two methods could be observed ( p<0,05). The SFM procedure proved to be more time efficient both in the labelling, both in the detection procedures.

Conclusions: Triple fluorescent labelling and automated fluorescent microscopy is an applicable tool for the proliferation, apoptosis determination in fresh frozen samples.

#### P 329

##### **EXPERIENCES IN AUTOMATIC CLASSIFICATION OF COLON AND GASTRIC DIGITAL SLIDES**

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##### **INTRODUCTION**

Thanks to the fast evolution of digital microscopy new ways have been opened for image analysis on whole histological

tissue slides. Moreover recently new pattern recognition techniques were introduced for cell detection on the complicated histological images working on single field of views.

#### AIMS

The aims of present study were to adopt new development of image processing algorithm for whole biopsy slides to detect higher structures as gland, epithelium surface and finally to automatically classify gastric and colon samples using digital slide format.

#### METHODS

Altogether 69 colon (24 normal, 11 aspecific colitis, 25 colitis ulcerosa and 9 crohn disease) and 79 gastric (14 normal, 17 atrophy, 6 gastritis with intestinal metaplasia, 12 gastritis and 30 adenocarcinoma) well oriented mucosal biopsy specimens were selected. To digitalize the selected H/E stained slides Zeiss Mirax slide scanner system was used. Automatic histological evaluation modules were developed in C++. Altogether 45 parameters described the area, cell density and cellular characteristics of the basic tissue components as the surface epithelium, glands, connective tissue and the inflammatory cell compartment. Area and contained cell number ratios of different tissue compartments were calculated which we named to tissue cytometric features.

#### RESULTS

We could find that that newly developed tissue cytometric features efficiently can be used to classify both gastric and colon digital slides by their disease state. The most important parameter was the ratio of total cell number and cell number in interstitial region (Colon: healthy  $1.57 \pm 0.17$ ; aspecific colitis  $1.34 \pm 0.16$ ; colitis ulcerosa  $1.18 \pm 0.09$ ; Crohn disease  $1.28 \pm 0.11$ ,  $p < 0.01$  - Gastritis healthy  $1.43 \pm 0.12$ ; gastritis  $1.23 \pm 0.13$ ; carcinoma  $1.1 \pm 0.05$   $p < 0.01$ ).

#### CONCLUSION

This preliminary study proved again that the development and evaluation of quantitative tissue metric features can be used in the automated classification of histological colon biopsy specimen.

#### P 330

##### WEGENER'S GRANULOMATOSIS MASQUERADING AS MASTOIDITIS: REPORT OF AN UNUSUAL CASE

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**Introduction:** Wegener's granulomatosis is a rare, immunologically mediated inflammatory disease (WHO, 1991). The diagnosis is based on the clinical picture, the presence of cANCA by immunofluorescent microscopy and by biopsy of the affected tissue. Our purpose is to present a case in which the unusual clinical picture and the atypical histologic findings posed a diagnostic problem.

**Material and methods:** A 27 year old woman was referred to our hospital (NMC) with symptoms of severe mastoiditis. During a period of 7 months she had 7 operations in 4 different hospitals for progressive chronic inflammation of the right mastoid process. Microscopically, all surgical specimens showed nonspecific granulation tissue with chronic inflammation, fibrosis, and once, suppurative granulomatous inflammation. This process also involved adjacent bone, causing focal osteonecrosis. Laboratory tests, including bacteriologic cultures failed to reveal a definite etiology of the progressive, destructive process which later necessitated also resection of a part of the first cervical vertebra. The patient's

condition deteriorated and she became critically ill. Consultation was requested and the entire histologic material was reviewed (K.B). Special stains (Gram, PAS) for microorganisms, fungi and parasites, as well as immunohistochemical reactions to exclude differential diagnostic considerations were all negative.

**Results:** The main features of the reviewed histologic material were

- Ulcerative granulomatous inflammation with focal fibrosis and destruction of underlying tissues
- Capillaritis and venulitis without arteritis
- Confluent, partly well circumscribed histiocytic granulomas with central microabscesses
- Irregular („geographic”) necrosis (in one small area only)

These histologic characteristics and the exclusion of differential diagnostic considerations (infectious granuloma, sarcoidosis, histiocytosis, eosinophilic granuloma) strongly suggested a localized variant (”forme fruste”) of Wegener's granulomatosis and was confirmed by the cANCA test, which initially was negative. The patient was treated with steroids and cyclophosphamide and has been in remission for almost two years. Follow-up studies failed to disclose any upper respiratory, pulmonary or renal involvement.

**Conclusion:** In young or middle age patients ”mastoiditis” resistant to conventional medical or surgical therapy may be due to Wegener's granulomatosis.

#### P 331

##### A CLINICOPATHOLOGICAL STUDY OF KIMURA'S DISEASE IN THE HEAD AND NECK REGION. A REPORT OF THREE CASES.

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**Background:** Kimura's disease (KD) is an uncommon inflammatory disorder of unknown aetiology, primarily seen in young Asian males. Its characteristics are painless subcutaneous masses in the head and neck region, blood and tissue eosinophilia, and markedly elevated serum IgE.

**Material and methods:** Over a ten-year period, 3 cases of KD of the head and neck region were diagnosed in our department and retrospectively reviewed.

**Results:** The three male patients (25, 26, and 29 years old at diagnosis) were natives from Madagascar, Cambodia and Mauritius. All had hypereosinophilia and elevated serum IgE with normal renal function. The first patient presented with a parotid localization, while the second had a temporal dermohypodermic nodule with ipsilateral involvement of parotid gland. The third patient showed a preauricular mass. Histologically, the soft tissue and the parotid gland were infiltrated by lymphoid follicles with folliculolysis and eosinophils forming eosinophilic abscesses. There were numerous thin walled vessels with neither epithelioid nor vacuolated endothelial cells. Adjacent lymph nodes also showed hyperplastic follicles with paracortical eosinophilia and folliculolysis. The immunohistochemical studies revealed CD20+ follicles with prominent staining of dendritic reticular cell CD23+, surrounded by numerous interfollicular CD3+ and CD4+ or CD8+ T-cells. CD1a, HHV-8, and LMP1 were negative. In situ hybridization confirmed the lack of EBV infection. FVIII-related antigen and CD34 decorated the thin-walled blood vessels.

Recurrence was observed in the first patient both at original and contralateral sites.

**Conclusion:** KD is a rare chronic inflammatory disease with genetic predisposition that typically presents as a deep pre-auricular nodule and/or parotid gland involvement. The

immunohistological study confirms its reactive nature. Excision is considered as the treatment of choice, but recurrences can occur even in a prolonged period.

### P 332

#### **MORPHOFUNCTIONAL PECULIARITIES OF THE GUM TISSUES AT PATIENTS WITH CHRONIC PERIODONTITIS AND PRE-IMPLANTATION TREATMENT**

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Periodontal diseases have high incidence rate worldwide. Quite often treatment plans involving tooth replacement, are used for sites diagnosed with chronic periodontitis (CP). Thus, there is a problem of finding pathogenically based methods of structural changes correction in a gum. Present study was undertaken to help further evaluate gum tissues remodelling with and without pre-implantation treatment of CP.

**Methods.** 30 volunteers were enrolled while receiving dental care. 10 gums biopsies were taken from patients without CP symptoms (group of comparison), 10- from patients with CP undergoing surgical operation without preoperational therapy (group 1) and 10- from CP patients with pre-implantation treatment by glutarginum and vector-therapy (group 2). Histological slides were stained with H&E, Van Gison, and then stereological evaluation by point count method was performed. The volume fractions of inflammatory infiltrates, vascularity, areas of dystrophy and necrosis of connecting tissue and others were determined. Collagen I and ? type's expression were studied immunohistochemically with the use of specific antibodies.

The results have shown that the increase of volume fractions of inflammatory infiltrates, vessels, foci of periodontal connective tissue dystrophy and necrosis are determined in group I. Immunohistochemical analyses showed that both expression and distribution of the I type collagen was diminished here too. Unlike this, the ? type collagen was registered here more often with increased intensity of expression.

The data in group 2 demonstrate a reduction of hyperemia, vessels number, inflammatory infiltrates and areas of connecting tissue dystrophy. The collagen type's distribution has changed to the mode seen in group of comparison: type I collagen predomination over the ? type collagen.

**Conclusion.** These findings suggest that chronic periodontitis stimulates the forming of immature connective tissue and persistent inflammation in a gum. On this background there is braking of repair processes, which can unfavorably affect implant osteointegration. The use of glutarginum, possessing antioxidant and membranes protective properties, in conjunction with the mechanical debridement by vector-therapy, allows normalizing gum tissue's metabolism, to recover the normal parameters of collagen stromal components. These seem to be effective in maintaining structure and function of the gums and periodontal tissues at pre-implantation period.

### P 333

#### **PRESENCE AND DISTRIBUTION OF MERKEL CELLS IN ORAL MUCOSA.**

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**Introduction:** Merkel cells (MC) are endocrine elements located in the basal layer of epidermis and oral mucosa. MC are difficult to be found by routine light microscopy; but can

be visualized by electron microscopy and immunohistochemistry. Cytokeratin 20 (ck20) is a sensitive marker for MC. A few data are available on the distribution of MC in human oral mucosa. The aim of the present paper is to better define the presence and distribution of MC in normal and pathological oral mucosa.

**Materials and Methods:** 89 surgical consecutive biopsies of oral mucosa were evaluated. Patients age ranged between 3 and 84 years (mean 48). Sections were stained with haematoxylin/eosin and immunohistochemistry applying a monoclonal antibody anti ck20 (Dako, clone KS 20.8, diluted 1:40). Semiquantitative evaluation of ck20-positive cells, consistent with MC, was performed on 300 basal keratinocytes, in the area with higher number of MC. Location (basal or supra-basal) of MC and shape (oval or dendritic) were recorded.

**Results:** MC Distribution according to the site: the highest number of MC was observed in the gingiva (range 1 to 80 MC, mean 14 MC), palate (range 1 to 40 MC, mean 11 MC) and buccal mucosa (range 2 to 19 MC, mean 11), while MC were rare in the tongue (1 to 8 MC, mean 4 MC), and cheek (no more than 1 MC for biopsy), and were not observed in tonsil mucosa. Distribution according to the histological diagnosis. MC were present in 7/14 biopsies from normal oral mucosa (range 1 to 27 mean 8); 7/14 in acute inflammation with ulceration (range 3 to 18, mean 8); 9/11 chronic inflammation with hyperkeratosis (range 1 to 80, mean 18); 4/6 lichen (range 1 to 14, mean 5); 7/12 epithelium overlying chorion fibrosis (range 1 to 33, mean 13); 12/15 epithelium overlying salivary gland tumours and ameloblastoma (range 1 to 40, mean 13); 3/17 dysplasia and in situ carcinoma (range 1 to 8, mean 5). MC were not localized in the ulcerated or dysplastic epithelium, but when present they were observed in the epithelium adjacent to the lesion. In all cases MC were oval or dendritic in the same proportion., they were mainly located in the basal layer, in two cases only reaching the superficial layers of the epithelium.

In conclusion the present data confirm that MC are mainly localized in gingival, palate and buccal mucosa. In addition their number increases in chronically damaged mucosa, thus suggesting a possible function in reparative processes.

### P 334

#### **HPV GENOTYPING BY DNA CHIP STUDY IN AMELOBLASTOMA**

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**Background:** Ameloblastoma is a relatively common tumor, however, the etiologic factor or biologic activity are not clearly defined. This study is designed to determine the relation of ameloblastoma with a possible etiologic factor, human papilloma virus (HPV), and to evaluate the clinicopathologic characteristics of HPV-positive ameloblastoma.

**Methods:** The materials were 31 formalin-fixed, paraffin-embedded tissue samples from 22 ameloblastoma patients. HPV DNA chip study was done, and clinicopathologic parameters were compared according to the recurrence and HPV status.

**Results:**

1. In sixteen cases without recurrence, HPV DNA was not detected. However, three of 15 tissue samples from six recurred cases were HPV 16-positive. One of them showed positive for HPV 16 and HPV 40.
2. Nine of 16 cases without recurrence were under 20 years old, in contrast, there was no case under 20 years old in 6 recurred cases.
3. There were no significant differences of sex ratios and tumor locations according to the recurrence.

4. Radiologically three of 16 cases without recurrence was multicystic mass, but four of 6 recurred cases were multicystic. Three HPV DNA-positive cases were also multicystic.

5. Ten of 16 cases without recurrence and five of 6 recurred cases were follicular type histologically, and one recurred cases showed conspicuous cytologic atypia.

6. Two of 3 HPV-positive cases showed relatively rich stromal component, and remaining one case exhibited conspicuous inflammatory reaction.

Conclusion: In the half of recurred ameloblastoma cases, HPV 16 was detected, in contrast, no HPV-positivity in 16 cases without recurrence. The results suggest that HPV could be regarded as a possible etiologic factor of ameloblastoma with a tendency of recurrence. In addition, the risk of recurrence might be increased in cases showing multicystic mass, follicular type with cytologic atypia or rich stromal components and HPV-positivity.

### P 335

#### AMELOBLASTOMA WITH MALIGNANT TRANSFORMATION: A CASE REPORT - 20 YEARS EVOLUTION

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INTRODUCTION: Ameloblastomas comprise nearly 1% of the oral tumors. They have slow growth, but they are locally aggressive. Malignant transformation and metastasis occurrence are described in the literature. In 1971 Malignant ameloblastoma was included in WHO's classification of odontogenic tumors.

OBJECTIVE: Report a case of ameloblastic carcinoma in jaw.

CASE REPORT: We present a case of a 30 year-old female patient, with primary ameloblastoma in left jaw, without evidence of metastatic disease to the diagnosis. This patient was submitted to partial resection of the left jaw. The evolution demonstrated local progression, with eight recurrences in jaw, buccinator muscle, infratemporal fossa and cervical area, in 20 years of observation, showing evident malignant transformation in the last three recurrences.

DISCUSSION: The term malignant ameloblastoma and ameloblastic carcinoma have been raising discussions in the literature. Peter et al revising WHO's odontogenic tumors classification (1992) suggests a subdivision of malignant potential ameloblastic tumors, proposing the terms malignant ameloblastoma and ameloblastic carcinoma. The literature demonstrates that the expression of P53 and a high proliferation index is related to a higher tendency of tumor's recurrence and aggressiveness. Morphologically we observed a neoplasia composed of strings or islands of basaloid epithelial cells, with fence appearing outlying cells, intermixed with a reticular collagenized stroma. Pleomorphic cells with atypical and hyperchromatic appearing nucleus, beyond numerous mitosis, were observed in the last three recurrences, as well as the high p53 and Ki67 expression. Indeed, it is a highly aggressive tumor with evident malignant transformation, testified by cytological alterations, initial negative expression for p53 protein, which is over expressed in the last three recurrences, and the cellular proliferation index progression (Ki67).

CONCLUSION: The term ameloblastic carcinoma can be applied to our case. It demonstrated malignant cytological alterations, besides the classical ameloblastoma morphology. The morphological and immunohistochemical features of this case can represent a tumoral progression model of the ameloblastic tumors.

### P 336

#### EWING'S SARCOMA OF THE JAW BONES. A REPORT OF THREE CASES.

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Background: Ewing's sarcoma is an uncommon neoplasm usually arising in long bones or pelvis. The involvement of jaw bones is very rare.

Patients and methods: Three patients with Ewing's sarcoma of jaw bones treated between 1996 and 2005 were clinicopathologically reviewed. The diagnosis was based on the histological, immunohistochemical and genetic molecular data.

Results: Two women and one man were 22, 24, and 25 years-old at diagnosis. Two tumours localized in the mandible, while the other was in the maxilla. The dominant symptoms were pain, as well as a rapidly destructive and expansile mass. Histologically, the tumour consisted of sheets of small, round and undifferentiated cells that were PAS-positive. Abundant blood vessels, haemorrhagic foci, and necrotic areas were seen. Immunohistochemical study revealed that the tumour cells were reactive for CD99 (MIC2) and vimentine, but EMA, pancytokeratin, S-100 protein, chromogranin A, and CD45 negative. The Ki-67 proliferation rate was 10, and 80% in two cases. In one case CD117 (c-Kit) was slightly expressed as well as p53 in 30% of cells. MDM2 was negative in each case. EWS rearrangement was evidenced by FISH in one case and RT-PCR in another case. Patients were treated by surgery followed by chemotherapy. One patient had three local recurrences without metastasis during a 5 years follow up.

Conclusion: Ewing's sarcoma is a rare and aggressive tumour of jaw bones that occurs in young adults. Immunohistochemical and genetic molecular analyses are essential for diagnosis. The recurrence is quite common and the prognosis is poor.

### P 337

#### ATYPICAL LIPOMATOUS TUMORS OF THE TONGUE

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Aims: To report 5 cases of rare low grade lesion, atypical lipomatous tumor (ALT) of the tongue.

Patients: The patients were 3 males and 2 females, aged 11-78 years (average 51.2 years, median 65 years). The tumors were in all cases present at the lateral side of the tongue. In one case, there were multiple tumor nodules, localized at both sides of the tongue. Follow up was available in four cases (8-96 months). After surgical excision, none tumor recurred or metastasized.

Results: The size of the tumors ranged from 7 mm to 20 mm (average 11.2 mm, median 10 mm). All cases shared similar microscopic features. They showed infiltrative growth into surrounding structures (skeletal muscle, fibrous tissue). They were of lobular arrangement, composed of adipocytes with slight variation of size and shape. Isolated sparse lipoblasts (both univacuolar and multivacuolar) were present. In two cases, there was prominent interstitial sclerosis and the features thus coped more with the diagnosis of sclerosing ALT, whereas three cases had the features of lipoma-like

o cases, isolated spindle-shaped primitive looking found, presumably of precursor nature. Mitotic very low, only several typical mitoses were found examined. Immunohistochemically, the tumor showed positivity of vimentin (4/4), S100 protein (4/4), and CDK4 (3/4). Proliferative antigen Ki-67 was present in isolated cells only (less than 0.5 %). Two cases were examined by FISH. Amplification of mdm-2 gene was present in both cases, whereas amplification of CDK-4 was present in one case only.

Atypical lipomatous tumors of the tongue are rarer, they have characteristic morphologic features and immunophenotype, enable their diagnosis. The herein presented results are in agreement with conclusions of the previously reported series. They behave in an indolent fashion; they can recur if not fully excised, however they virtually never metastasize.

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## HAEMANGIOPERICYTOMA OF THE OCCIPITAL CONDYLE: A CASE REPORT

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Haemangiopericytoma ( HPC ) a rare neoplasm arising in somatic soft tissue, or in a central nervous system. HPC of central nervous system is highly cellular and well circumscribed tumour almost always attached to the dura. The histology of meningeal haemangiopericytomas can be variable but local recurrences are almost inevitable in the long term while postoperative irradiation appears to delay recurrence.

A case report published in 1979 still contained " haemangiopericytic tumour of meningeoma, however this concept has now been abandoned.

In the skull, HPC occurs more often in occipital region, near the confluens sinuum.

A 69 years old female patient refers to the Clinic for Maxillofacial Surgery, Faculty of Stomatology Belgrade, with a swelling in occipital region. From personal history it could be concluded that pathological formation of tumour had long duration ( " since childhood" ).

The tumour feature was slightly lobulated, firm, cut surface fleshy and yellow white to red brown, with zones of necrosis and hemorrhage.

Histology : Highly cellular lesions with numerous channels ( " stag-horn type" ) surrounded by and lined by thin nests of spindle-shaped cells. The nuclei were sometimes elongated with moderate chromatin density. Cellular atypia was found as well as prominent mitotic figures and dense reticulin network surrounded individual tumor cells.

A definitive diagnosis was made : Haemangiopericytoma WHO grade II.

## INTRAMUSCULAR MYXOMA OF THE TEMPORALIS MUSCLE: A CASE REPORT

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Intramuscular myxoma is a benign tumor of soft tissue. It is characterized by a proliferation of myxoid stroma with scattered spindle-shaped cells. The diagnosis is based on histological findings and immunohistochemical studies. The treatment is surgical excision.

Myxomas are commonly found in the heart, jaw and soft tissues of the body; however these lesions are

unusual in the soft tissue of the head and neck. The lesion is composed of undifferentiated stellate cells set in a myxoid stroma.

Case report : In June 2000, a 63-year-old male was evaluated for a slowly growing, asymptomatic mass in his left temple region, which had been present for more than 4 years.

Clinical examination showed a 5x4x2 cm, firm, smooth, painless, oval mass in the left temporal region. The overlying skin surfaces were yellowish and gelatinous.

Microscopic examination showed a relatively well circumscribed lesion. The spindle and stellate cells were embedded in an abundant myxoid stroma with a reticulin fiber network. No cellular pleomorphism or mitotic activity was evident. A diagnosis of intramuscular myxoma was made. 3 years after surgery, there was no evidence of recurrence.

Discussion: In the head and neck, intramuscular myxoma is extremely rare, because it commonly arises from large skeletal muscles.

It is difficult to diagnose this lesion before biopsy, because of the lack of typical clinical manifestations or features in radiographic studies. It usually appears as a painless and slow-growing mass.

The basic histology of intramuscular myxoma is indistinguishable from myxoma that occurs elsewhere in the body; however, on close inspection, the delicate fibrous capsule is incomplete, and most of all lesions infiltrate adjacent muscle.

Differential diagnosis of intramuscular myxoma must include malignant tumors such as myxoid liposarcoma, malignant hyaline cartilage and myxoid chondrosarcoma.

Treatment for intramuscular myxoma is surgical excision. There is no report of metastasis or malignant changes, but occasionally recurrences resulting from incomplete excision.

## P 340

### APOPTOSIS DISORDERS IN SJÖGREN'S SYNDROME

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It has been suggested that defects in the modulation of programmed cell death (apoptosis) might lead to autoimmune disease, such as sjögren's syndrome (SS).

OBJECTIVES: The aim of our study is to determine the role of apoptosis in Sjögren's syndrome. MATERIAL AND METHOD: Salivary glands biopsies from 21 patients suffering of primary and secondary SS (Chisholm 3 and 4) were analysed by apoptosis regulators: Bcl-2, Bax, P53 and Fas (CD95) using immunohistochemistry method. Control group had 15 normal subjects. RESULTS: Bcl-2 was strongly expressed in lymphocytic lesions of all cases. Its associated with a bax and a p53 reactivity respectively in 76% (16/21 cases) and 85% (18 cases/21) of acinar and ductal epithelial structures. A fas antibody was detected in 5 cases only in epithelial component and none in lymphocytic area.

CONCLUSION: Present data suggest a lymphoid defective apoptosis, by Bcl2 activation, inducing lymphoid infiltrate. SS salivary epithelial cell death requires the cooperation of both Fas and Bax. However, P53 duct expression may be a defensive mechanism that provides ductal cells with time to repair DNA damage.

## P 341

### STUDY OF B-CELL MONOCLONALITY, HELICOBACTER PYLORI AND BCL-10 EXPRESSION IN SJÖGREN'S SYNDROME.

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**INTRODUCTION:** Sjögren's Syndrome (SS) is an autoimmune disorder characterized by lymphocytic infiltration and MALT acquisition at the exocrine glands, as well as an increased risk of developing malignant lymphoma, especially extranodal marginal zone lymphoma of MALT type.

*Helicobacter pylori* (Hp)-gastritis predisposes to the development of acquired MALT and eventually MALT lymphoma, associated in 5% of cases with the mutant form of Bcl-10, while the bacterium is a common habitant of oral microbial plaque and saliva, only rarely investigated in salivary lymphoid tissues in SS.

**PURPOSE:** To evaluate the presence of acquired MALT and B-cell monoclonality at the labial salivary glands of patients with SS and their possible association with *Helicobacter pylori* and Bcl-10 expression.

**MATERIALS AND METHODS:** Formalin-fixed paraffin embedded tissue from 40 cases with SS and 20 control cases with non-specific sialadenitis was stained by immunohistochemistry for CD3, CD20, kappa, lambda, *Helicobacter pylori* and Bcl-10 protein.

**RESULTS:** Acquired MALT was observed in 26/40 (65%) cases with SS, while 9/26 (34,6%) showed areas of light chain restriction. 7/26 cases (27%) were positive for Hp, 3 of which were also monoclonal (43%). Cytoplasmic expression of Bcl-10 was observed in lymphoid follicles in all 26 cases, while no nuclear expression was detected. None of the controls showed either MALT and B-cell monoclonality or Hp. and Bcl-10 expression. Acquired MALT, *Helicobacter pylori*, and Bcl-10 expression statistically significant correlated with the emergence of B-cell monoclonality ( $p$  value < 0,05).

**CONCLUSIONS:** *Helicobacter pylori* may be linked to MALT acquisition and B-cell clonality in SS, at least in some cases. Adherence to saliva may facilitate lipopolysaccharide (LPS) activation of Toll-like receptors on ductal cells and Bcl-10-mediated NF- $\kappa$ B antigenic signalling of B-cell receptor (BCR) may result in persistent B-cell proliferation in genetically susceptible individuals, thus predisposing to the emergence of pathologic clones. Alternatively, Hp. presence in the salivary glands may induce MALT accumulation as a result of acquired gastric MALT dissemination, as observed in MALT lymphomas, through  $\alpha$ 7-associated recruitment of gastric memory B-cells. Bcl-10 may be of importance in the interruption of the neoplastic cascade, while further studies are needed to clarify Hp's precise role in SS's pathobiology.

#### P 342

##### **ROLE OF EPSTEIN BARR VIRUS IN PATHOGENESIS OF SJÖGREN'S SYNDROME**

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Sjogren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration of salivary and lachrymal glands which results in xerostomia and keratoconjunctivitis sicca. The pathogenesis of this syndrome remains unclear but viral infections have been suggested as a possible aetiologic factor.

**OBJECTIVES:** The aim of our study is to determine the potential role of Epstein Barr Virus (EBV) in pathogenesis of SS. **MATERIAL AND METHOD:** Salivary glands biopsies

from 35 patients suffering of primary and secondary SS were analyzed to determine the presence of EBV by immunohistochemistry method (LMP, ZEBRA, EBNA-2) and in situ hybridization study with EBV-encoded small RNAs termed EBER1 and early lytic PNA probe. Control group had 15 normal subjects. **RESULT:** 14 cases were positive for ZEBRA, 17 were positive for LMP and EBNA-2. All cases were EBV positif after in situ hybridization. None case of control group was EBV (+). **CONCLUSION:** EBV has a strong salivary tropism. Our results suggest that EBV could be implicated in the pathogenesis of SS. Its T-cell responses might contribute to labial salivary gland destruction and could have a role in lymphoid proliferation.

#### P 343

##### **INVASIVE SPHENOID ASPERGILLOSIS: A CASE REPORT WITH AN UNUSUAL PRESENTATION**

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**Backgrounds:** Fungal sphenoid sinusitis is rare, and *aspergillus fumigatus* is the most common pathogen. The invasive form of the disease is usually encountered in immunocompromised hosts with a potentially unfavorable outcome. We report a case of invasive sphenoid aspergillosis with pseudo-tumoral features on imaging, developed in an immunocompetent patient.

**Observation:** A 51-year-old male patient presented in January 2005 with a right otorrhea. He underwent in November 2004 a total thyroidectomy which revealed a multifocal papillary micro-carcinoma of the thyroid. He presented with an eight-year history of a chronic intermittent headache with a negative complete work up. Physical examination was unremarkable except for a right tympanic perforation, and no neurological defect was noted. A computed tomography revealed a 4x2.7cm mass of the left sphenoid sinus associated with bone erosion and suspicious for metastasis. Biopsies of the mass were performed. Histologic examination showed an extensive granulomatous inflammatory process invading the mucosa and bone, with fungal hyphae, identified as *Aspergillus flavus* by culture. Serologies for soluble *aspergillus* antigen and HIV were negative. The diagnosis of chronic invasive aspergillosis of the sphenoid sinus was established, and one-year of voriconazole treatment was started.

**Discussion:** In addition to immunosuppression states, predisposing factors for sinus invasive mycosis include maxillofacial trauma, drug sniffing and radiotherapy, none of which was present in our patient. The disease is usually symptomatic, most frequently with headache, followed by visual changes, cranial nerve palsies, seizures and pain in the trigeminal distribution. Diagnosis relies on endoscopy, imaging and biopsy for histologic examination and culture.

**Conclusion:** Sinus invasive aspergillosis is a rare condition, encountered most frequently in immunodeficient patients. We present an unusual case of a pauci-symptomatic sphenoid invasive aspergillosis in an immunocompetent patient with a pseudotumoral aspect. This entity should be kept in mind in the differential diagnosis of pseudo-tumoral lesions of this area.

#### P 344

##### **THE SIGNIFICANCE OF HISTOLOGICAL ESTIMATION IN FUNGAL INFECTIONS OF THE ORAL AND MAXILLOFACIAL REGION**

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**Introduction:** Mucormycosis (zygomycosis, phycomycosis) is an opportunistic and often fatal fungal infection caused by aerobic saprophytic fungi of the class Zygomycetes (Phycomycetes), order Mucorales, family Mucoraceae and most common genera *Absidia*, *Mucor*, *Rhizopus* and *Rhizomucor*. Different clinical forms are described and most common the rhinocerebral form. Aspergillosis of the maxillary and paranasal sinuses is an endemic disease in countries with hot and dry climate. The causative factor is *Aspergillus* fungus of *Actinomyces* race. Recent studies have shown an increasing incidence of the disease in mild and cool climates. The non invasive (localised and allergic) and invasive (benign and fulminant) forms are accepted. Most cases of the non invasive form are prescribed in otherwise healthy individuals imitated an acute, chronic or allergic sinusitis with a remarkable resistance in a classical therapy of sinusitis.

The purpose of this work is to discuss about the significance of histological estimation in relation to diagnosis, treatment and prognosis in cases with these fungal infections.

**Methods:** The clinical, radiographical and histological pictures in cases with mucormycosis and aspergillosis in oral and maxillofacial area are presented and the particularities in histological sections and stains are referred.

**Conclusion:** Definite diagnosis in the above mentioned fungal infections is emphasized by histological evidences. The systematic laboratory examination of the excretions, material and tissues of the affected oral and maxillofacial areas is the exclusive method for the earliest diagnosis and a successful treatment of the disease, even if negative clinical and radiographical evidences exist.

#### P 345

##### **ROLE OF NERVE GROWTH FACTOR RECEPTOR IN ATROPHIC SALIVARY GLAND IN CASE OF SIALOLITHIASIS**

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Involvement of nerve growth factor cell surface receptor p75 (p75) has been shown to be essential in maintaining the integrity of the salivary glands. Furthermore, in human salivary glands no immunoreactivity for the p75 was observed in the serous or mucous acinar cells. This study was undertaken to analyze a possible role of p75 in human submandibular gland atrophy in case of sialolithiasis using immunohistochemistry.

The study was carried out on 14 human salivary glands obtained along sialadenectomy performed because of the presence of intraglandular sialoliths. The salivary glands were morphologically subdivided into two groups on the basis of the presence (9 cases) and absence (5 cases) of parenchymal atrophy. Formalin fixed, paraffin embedded tissue sections were processed for a traditional streptavidin-biotin-peroxidase technique.

p75 immunoreactivity was restricted to the ductal myoepithelial cells and nerve fibers supplying salivary glands. There was a co-localization of p75 and an alpha-smooth muscle actin reactivity within the myoepithelial cells. A ductal p75 expression was much more stronger displayed in cases with a prominent gland atrophy and severe fibrosis. This

ductal p75 expression correlated with a degree of ductal wall cell proliferation assessed by routine morphological visualization and proliferating cell nuclear antigen immunohistochemistry.

The present study revealed a strong co-incidence between p75 expression and proliferation within a ductal wall in case of prominent salivary gland parenchymal atrophy. These findings suggest that p75 present on the surface of the myoepithelial cells may be intimately involved in the pathophysiology of salivary gland disturbances in case of sialolithiasis contributing to the interplay of multiple factors that balance homeostasis at the appropriate level.

#### P 346

##### **P63 EXPRESSION IN SALIVARY GLAND TUMOURS: ROLE OF DELTANP73L IN NEOPLASTIC TRANSFORMATION.**

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**Introduction:** p63 gene has two different functions: TAp63 favours cell differentiation, whereas DeltaN p63 favours cell proliferation. Two new isoforms have been described, Delta4TAp63 and DeltaNp73L, lacking exon 4. DeltaNp73L is present in squamous cell carcinoma, but absent in normal epithelium.

Purpose of the present study was to illustrate the role of p63 and its truncated variants in salivary gland tumours.

**Materials and methods:** Twenty three consecutive tumours and six normal salivary glands were studied immunohistochemically with anti-p63 monoclonal antibody (NeoMarkers, Fremont, USA, clone 4A4, diluted 1:200) and by RT and Nested PCR to detect p63 isoform expression.

**Results:** Normal salivary glands: p63 antibody stained basal and myoepithelial cells; by RT and nested PCR, the two main isoforms, TAp63 and DeltaNp63 were present, whereas DeltaNp73L was absent.

**Tumours:** p63 antibody was positive in 20 out of 23 cases, specifically in Warthin tumour (WT) (3/3), oncocytoma (OC) (1/1), pleomorphic adenoma (PA) (7/7), polymorphous-low-grade adenocarcinoma (PLGA) (3/3), adenoid-cystic carcinoma (ADCC)(3/4), epithelial-myoepithelial-cell carcinoma (EMC)(1/1), myoepithelial-cell carcinoma (MCC)(1/1). By RT and nested PCR all tumours expressed p63 irrespective of their morphological differentiation. Furthermore the DeltaNp73L isoforms were present in neoplastic tissue but absent in normal salivary gland.

In conclusion, p63 antibody stained basal and myoepithelial cells in normal and neoplastic salivary gland tissues. By RT and nested PCR all salivary gland tumours appeared to express at least one isoform of p63. The DeltaNp63 variant lacking exon 4, named DeltaNp73L, was present only in neoplastic lesions, whereas it was lacking in normal salivary gland tissue, a feature that indicates the crucial role of this molecule in neoplastic transformation.

#### P 347

##### **A CASE OF MUCIN-RICH VARIANT OF SALIVARY DUCT CARCINOMA EX PLEOMORPHIC ADENOMA**

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Salivary duct carcinoma (SDC) is an established aggressive salivary gland neoplasm resembling intraductal and infiltrative ductal carcinoma of the breast. Diagnostic criteria and concept of SDC have been expanded along with subtype and new entity case reports such as sarcomatoid SDC, minor salivary gland origin SDC, invasive micropapillary SDC, and mucin-rich variant SDC. SDC often occurs as the malignant component of carcinoma ex pleomorphic adenoma. @ Lewis et al. reported that SDC was many as the malignant component of carcinoma ex pleomorphic adenoma next to adenocarcinoma, not other specified from retrospective study (Hum Pathol 32: 594, 2001). Mucin-rich variant SDC was first reported by Simpson et al. (Am J Surg Pathol 27: 1070, 2003). They reported four mucin-rich variant SDC cases. However, there is no report of other mucin-rich variant SDC case after Simpson's description. Here we report a case of mucin-rich variant of SDC ex pleomorphic adenoma arising from submandibular gland.

A 73-year-old Japanese male had noticed a painless swelling at the right submandibular area for about 35 years. This lesion had rapidly increased in size for a period of about 10 months. Macroscopically, the resected tumor was a solid mass with multiple mucinous cysts and necrotic areas in the submandibular gland. Microscopically, large areas of tumor nests infiltrated to normal salivary gland with abundant fibrous and/or hyalinizing connective tissue. Malignant tumor has many forms, such as, arranged solid, ductal, cribriform, and mucinous (colloid) carcinoma-like patterns. Some nests exhibit Roman bridge-like appearance, central necrosis, and intraductal patterns. In the mucinous carcinoma-like area, irregularly shaped small nests with fibrous connective tissue floated in pools of mucin. In this tumor it was composed of large atypical round to polygonal eosinophilic cells with round shaped nuclei. Most of these tumor cells were positive for diastase-PAS and mucicarmine, and androgen receptor, gross cystic disease fluid protein-15 and Her2/neu, histochemical and immunohistochemical stainings respectively. Immunohistochemistry of this tumor was the same as general SDC. In a small part of tumor, we recognized small bilayered duct and mixed appearance-like region, such as resembling pleomorphic adenoma in strongly hyalinized stroma. Furthermore @this case has a long term history. Finally, we diagnosed this tumor as a mucin-rich variant of SDC ex pleomorphic adenoma.

**P 348**  
**LYMPHOEPITHELIAL CA OF PAROTID GLAND. REPORT OF TWO CASES**

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Two cases of lymphoepithelial Ca, a rare salivary gland malignant epithelial neoplasm, are presented. This neoplasm is also referred to as "malignant lymphoepithelial lesion" and "undifferentiated Ca with lymphoid stroma".

The patients were males 50 and 61 years old and presented a slowly growing, painless mass located at the left parotid gland, measuring 3 and 2.5 cm, respectively.

In the case of the elder patient a biopsy of a left cervical LN preceded the tumor's surgical excision. The diagnosis on this material was a metastatic high-grade undifferentiated Ca resembling the nasopharyngeal lymphoepithelioma. A simultaneously performed nasopharynx biopsy was negative.

The microscopical examination of the two subtotal parotidectomies revealed malignant epithelial tumors. The neoplasms consisted of irregular shaped islands of large, anaplastic, eosinophilic, epithelioid cells within a mature type lymphocytic-rich stroma. Presence of epimyoepithelial islands, characteristic finding of benign lymphoepithelial lesion, was also evident. The immunohistochemical study

confirmed the epithelial nature of the neoplasms and the concomitant benign lymphoepithelial lesion. EBV infection was revealed by a positive expression to LMP1. Particularities of this tumor, problems of differential diagnosis and terminology, are discussed.

**P 349**  
**EPSTEIN-BARR VIRUS AND WARTHIN TUMOR IN TUNISIA: MOLECULAR AND IMMUNOHISTOCHEMICAL STUDY**

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Purpose: Tunisia belongs to a zone at high risk for several neoplasms associated to Epstein-Barr virus (EBV), particularly for the nasopharyngeal carcinoma. The salivary glands are considered as one of the major reservoirs of replication and latency of this virus. Warthin tumor of the salivary gland is composed of oncocytic epithelium with a prominent follicular lymphoid infiltrate. The histogenesis of this tumor is still debated and its association with EBV is controversial. The purpose of this study was to determine the prevalence of EBV in Warthin tumor in Tunisia.

Methods: Nineteen formalin-fixed, paraffin-embedded blocks of Warthin tumors from the parotid gland were examined for the presence of EBV, using polymerase chain reaction (PCR), EBERS in situ hybridization and immunohistochemistry for the detection of LMP1, EBNA2 and ZEBRA.

Results: Thirteen cases were PCR b-globin positive. The EBV was detected by PCR in 2 of 13 cases (15.4%). In the two EBV positive cases, EBERS in situ hybridization showed a positivity in few lymphocytes; these cells does not express LMP1, EBNA2 or ZEBRA.

Conclusions: The low frequency of EBV in Warthin tumor indicate that the virus is not the cause of this tumor, however the possibility that the virus acts as a cofactor cannot be ruled out.

**P 350**  
**MUCINOUS ADENOCARCINOMA OF THE SUBMANDIBULAR SALIVARY GLAND**

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Mucinous adenocarcinoma is a rare malignant neoplasm characterized by large amounts of extracellular epithelial mucin that contains cords, nests and solitary epithelial cells. It is a tumor analogous to mucinous eccrine carcinoma and mucinous carcinoma of the breast and colon. Few cases of primary salivary gland mucinous adenocarcinoma have been reported and all these cases arose in the major salivary gland. We report a new case in 41-year-old man presented with an enlarging, painless left submandibular mass. On physical examination, a 6-cm firm mass was noted and no adenopathy was detected. Ultrasonography revealed a 4-cm heterogeneous mass. The patient underwent excision of the submandibular gland and lymph node dissection. Histological study concluded at mucinous adenocarcinoma without lymph node metastasis. After two years, the follow-up has been good.

The aim of this study is to review diagnosis difficulty of this rare entity and differential diagnosis.

**P 351**

**IMMUNOPROFILE OF REACTIVE SALIVARY MYOEPIHELIAL CELLS IN AREAS OF IN SITU CARCINOMA EX-PLEOMORPHIC ADENOMA.**

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Myoepithelial cell (MEC) is the component of various secretory glands including salivary and breast glands and it is located between the basement membrane and the luminal cells. The MEC has pinocytotic vesicles, microfilaments and dense bodies resembling smooth muscle cells and they are recognized immunohistochemically by the positivity of myofilaments. Since the beginning, its function as a contractile cell which facilitates the excretion of the products of both salivary and breast glands is well known. With the scientific advances in molecular and biologic studies, new functions were added. The studies in this area are controversial since some have indicated a tumour suppressor function and others a tumour facilitating function for the MEC. In order to increase some knowledge to this subject, we have investigated the immunoprofile of benign MEC in areas of in situ carcinoma ex-pleomorphic adenoma (ISCXAP) comparing with the MEC in areas of pleomorphic adenoma (PA) source of the malignant tumour. Tissue samples from 4 cases of ISCXAP have been selected for immunohistochemistry study using MEC markers (SMA, calponin, CK14, p-63), extracellular matrix protein (laminin) and protease inhibitor (maspin). The results revealed that all MEC markers plus laminin and maspin are stronger expressed in benign MEC of ISCXAP areas than in benign MEC of pleomorphic adenoma. These results have led us to conclude that MEC which surrounds the malignant epithelial component of ISCXAP is phenotypically altered and more differentiated than the benign MEC of pleomorphic adenoma and probably, exerts, at this moment of the oncogenesis process, a tumour suppressive function. Further studies are necessary in order to clarify if the MEC is also implicated in the transition of in situ to invasive carcinoma.

**P 352**

**CD10 EXPRESSION USEFULNESS IN DIFFERENTIAL DIAGNOSIS OF PRIMARY AND METASTATIC CLEAR CELL TUMOURS OF SALIVARY GLANDS**

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**INTRODUCTION:** The expression of CD10, or common acute lymphoblastic leukemia antigen, has been widely studied in normal tissues and neoplasms. CD10 is characteristically expressed in renal clear cell carcinomas, a neoplasm with tendency to metastatize in the head and neck region. Its positivity has been shown to be helpful in the differential diagnosis with other tumours with clear cells in other organs such as the ovary. However, the expression of CD10 in clear cell neoplasms of salivary gland origin has not been previously studied in detail.

**PURPOSE OF THE STUDY:** To study the expression of CD10 in primary clear cell tumours of salivary glands and metastatic renal cell carcinoma, in order to determine the usefulness of this marker for the differential diagnosis.

**MATERIAL AND METHODS:** Thirty-five tumours of salivary glands with a major clear cell component were selected. Thirty-two were primary tumours and three were

metastatic renal clear cell carcinomas. The immunohistochemical study for CD10 (Novocastra Laboratories. Newcastle, UK. Dilution 1:25) was performed in all cases from formalin-fixed and paraffin embedded material.

**RESULTS:** All primary tumours composed of myoepithelial cells showed a strong and diffuse cytoplasmic CD10 positivity: myoepitheliomas (4/4), myoepithelial carcinomas (3/3), and epithelial-myoepithelial carcinomas (3/3). CD10 was also positive in other primary tumours with clear cells: mucoepidermoid carcinomas (3/6) showed a predominant apical and luminal positivity in glandular differentiated areas; some acinic cell carcinomas (2/8) and oncocytomas (1/3) had a cytoplasmic pattern. Three salivary gland clear cell carcinomas and two sebaceous differentiated tumours were negative for CD10. Metastatic renal clear cell carcinomas showed a strong membrane positivity (3/3).

**CONCLUSION:** CD10 is expressed in a variety of primary tumours of salivary glands, with a cytoplasmic or luminal pattern; whereas this marker shows a strong membrane positivity in metastatic renal cell carcinoma. The different immunoreactivity pattern may be useful for differentiating renal cell carcinoma metastasis from other primary salivary gland tumours.

**P 353**

**EXTRA PAROTID PARAPHARYNGEAL SPACE TUMOR WITH BIPHASIC PATTERN.**

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A 54 year old woman presented with a nodular lesion of the parapharyngeal space. This lesion was well-circumscribed hypoechoic on echography, separated from the parotid gland by a narrow band of fat tissue on CT scan. On MRI a possible connection with the parotid gland and a T2 moderate hypersignal were noticed. At surgery the tumor appeared as a firm encapsulated greyish nodule, 3,5 x 5,5 cm, without connection with the parotid gland.

Microscopically, tumor was predominantly composed of spindle cells intermixed with mature adipose tissue and epithelial cells, forming small solid nests with squamoid features, rarely tubes or microcysts. No necrosis was observed. Both epithelial and spindle cells were strongly positive for AE1/AE3. Spindle component was PS100 + and faintly smooth muscle actin +. Proliferation index was less than 3% . Cells were not stained by CD34, desmine, GFAP and CD99 antibodies. Diagnosis of ectopic hamartomatous thymoma was first proposed on account of location at the skull base without connection with parotid gland, predominant spindle shaped pattern and squamoid features, but PS100 positivity was unusual. On serial sections cartilage tissue was found, leading to the diagnosis of pleomorphic adenoma.

Tumors in the parapharyngeal space (PPS) are rare and constitute less than 0.5% of head and neck neoplasm. Of these, pleomorphic adenoma (PA) is the most common (40%) followed by schwannoma and paraganglioma. PA can arise either de novo from accessory salivary glands or from the deep lobe of the parotid gland and extend through the stylomandibular tunnel into the PPS. Typical PA has a biphasic appearance resulting from the intimate admixture of epithelium and myoepithelial cells, and generally contain myxochondroid areas and sometimes adipous tissue. Some pleomorphic adenomas are extremely cellular, the tumor cells being either round or spindle shaped. The myoepithelial component is immunoreactive for keratins, myosin, fibronectin, PS-100 and often for actin. The main differential diagnosis include ectopic hamartomatous thymoma, biphasic

synovial sarcoma, schwannoma, malignant peripheral nerve sheath tumor and myoepithelioma of soft tissue.

Conclusion: Immunohistochemical study is important for the diagnosis of parapharyngeal space tumor with biphasic pattern.

#### P 354

##### PRIMARY AMYLOIDOSIS OF THE LARYNX

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Introduction In 1851 Virchow coined the word amyloid to describe the strachlike reaction of a waxy material when treated with iodine and sulphuric acid. Amyloidosis is defined as a pathologic proteinaceous substance deposited between the cells of tissues and organs leads to various clinico-pathologic conditions. Localized and idiopathic amyloidosis in the head and neck is a rare and benign process.

Methods We present a clinical case of a 63 years female with hoarseness due to a laryngeal mass and also a review of literature regarding localized amyloidosis. A laryngoscopic examination revealed a smooth, non ulcerated submucosal mass involving the ventricle, false cord extended to vocal process. Physical and laboratory evaluation showed no evidence of systemic amyloidosis or multiple myeloma. The lesion was treated by endoscopic laser excision.

Results At light microscope amyloid is a homogeneous acellular eosinophilic and extracellular material with haematoxylin and eosin stains. It stains metachromatically purple red with crystal violet and characteristically manifests a Congo red staining of its fibrils with apple-green birefringence. Amyloid stains dark brown with iodine. Exposure of the Congo red to potassium permanganate and dilute sulphuric acid may be used to partly subclassify amyloid. Immunohistochemical examination revealed a polyclonal staining pattern of light chain of immunoglobulins.

Conclusion Localized amyloidosis of head and neck region is rare. Borow in 1873 first reported amyloidosis of the larynx and at the present about 350 cases were reported in literature. It should be recognized, understood, evaluated and properly treated.

#### P 355

##### ANGIOLEIOMYOMA OF THE NECK MIMICKING THYROID GLAND DUCT CYST

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INTRODUCTION. Angioleiomyoma (AL) is an uncommon benign tumour composed of smooth muscle cell and vascular endothelium. After Shout reported the first 11 cases in 1937, about another thousand have been reported and most authors have accepted that angioleiomyoma originates from the smooth muscle in the wall of vascular channels. In 1973 Morimoto proposed to subdivide AL into capillary (solid), cavernous and venous type. Clinically AL is more frequent in female; they are usually located in lower extremities and present as a solitary, painful cutaneous or subcutaneous mass of solid type. Only about 10% of AL arises in the head and neck area and they usually present as a painless mass of venous or cavernous type. Frequently imaging study or cytological examination is not helpful for preoperative diagnosis. Recurrence is rare.

PURPOSE OF THE STUDY. In this paper we report the case of an angioleiomyoma of the neck mimicking a thyroglossal

duct cyst and to our knowledge this is the first case described in this location.

METHODS. A 67-year-old male presented with a painless mass of the neck. Physical examination revealed a 2 cm subcutaneous solid mass in the upper neck. There was neither associated lymphadenopathy nor abnormality of the skin. Ultrasound examination showed a 20 x 15 x 21 mm solid hyperechogenic mass, with hypoechogenic peripheral rim. The preoperative diagnosis was of a thyroglossal duct cyst. The patient underwent surgery and a 2 cm solid mass was found attached to the geniohyoid muscle but without relation with the hyoid bone.

RESULTS. Histologic examination showed a well demarcated nodule composed of smooth muscle with thick-walled venous vessels with inner layers of smooth muscle and without lamina elastica. The tumour contained also mature fat cells. Immunohistochemistry for smooth muscle actin was positive and confirmed the diagnosis of angioleiomyoma of venous type.

CONCLUSION. AL is a rare benign tumour in head and neck area. This lesion is difficult to diagnose before surgery, especially when it occurs as a painless mass and is in a site where other lesions are more frequent. Surgical excision is necessary to make a correct diagnosis and gives good results with rare recurrence.

#### P 356

##### EWING /PNET FAMILY SARCOMA ARISING FROM BRANCHIAL CYST WALL

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INTRODUCTION: The Ewing sarcoma family tumors (ESFT) include Ewing sarcoma, peripheral primitive neuroectodermal tumor, neuroepithelioma, atypical Ewing sarcoma, and Askin tumor. ESFT research is ongoing to further characterize the biology of the EWS/FL1 fusion protein and its role in transformation, cell growth and chemosensitivity. Unusual location of such tumors may cause diagnostic problems and cytogenetic analysis on tumor tissue samples may solve the problem in diagnostic approach. We present an ESFT arising from a branchial cleft cyst and discussed the diagnostic difficulties.

CASE: A 21 year old female complained dysphagia for 2 years duration. During the last 2 months a mass was palpated on the right side of the neck. Physical examination revealed cystic cervical, nontender mass with 4x3x3cm diameters, located along the lower one third of the anterior border of the sternocleidomastoid muscle between the muscle and overlying skin. Histopathological examination of the excised mass showed cystic wall lined with partially stratified squamous and respiratory epithelium. Lymphoid tissue was seen in the cyst wall in accordance with branchial cleft cyst. Next to this lymphoid area a malignant neoplasm consisting primitive cells arranged in cords and with a vague nesting pattern was observed. Immunohistochemical studies showed CD99 positivity, while cytokeratin, CD45, NSE, S-100, chromagranin, synaptophysin, smooth muscle actin, and desmin were all negative. Cytogenetic analysis performed by using RT-PCR technique on paraffin embedded tumor tissue demonstrated the chimeric fusion transcripts EWS/FLI-1 and confirmed the diagnosis of ESFT.

CONCLUSION: This location was unique when we reviewed the English written literature as far as we could reach. Because the ESFT is a rare tumor in extraskelatal site it is often not considered in clinical differential diagnosis until a biopsy reveals a small blue round cell tumor. Especially unusual presentation like the presented case, a panel of immunohistochemistry may not be helpful in all instances. Cytogenetic analysis may solve the problem if the material belongs to a tumor with a known characteristic transformation. In our case EWS/FL-1 fusion which is present in 85% of ES/PNET cases was helpful in diagnostic approach.

Because of the cystic nature and branchial cyst association malignancy was not in the differential diagnosis clinically, consultation with an oncologist is critical.

#### P 357

### SPINDLE CELL PROLIFERATION IN FOLLICULAR ADENOMA OF THE THYROID. CASE REPORT IN PATIENT TREATED FOR HIGH GRADE LYMPHOMA

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**Introduction:** Spindle cell proliferations of the thyroid are rare and they include anaplastic carcinoma and mesenchymal tumors. There are few reports of spindle cell change in epithelial tumors of the thyroid as such as have been described in follicular adenoma and papillary carcinoma.

**Purpose:** to report the clinical and pathological features of a thyroid adenoma with diffuse spindle cell change in a patient treated for high grade lymphoma.

**Methods:** In 2003, a 31-year-old woman was admitted to "G.B.Rossi" hospital of Verona University and underwent chemotherapy because of high grade lymphoma. In 2004, she received bone marrow transplantation (BMT). During follow-up, a Positron Emission Tomography (PET) was performed and a suspicious single nodule in the thyroid was detected. Following fine needle aspiration cytology (FNAC) was not conclusive and the nodule was surgically removed.

**Summary:** Cytologically, the lesion was composed by cohesive, plump spindle cells with amphophylic cytoplasm, irregular nuclei with granular chromatin. No bizarre nuclei nor necrosis or mitoses were detected. On gross examination, the nodule was 2.5 cm in diameter, with whitish cut surface and a well-defined capsule. Histologically, it was composed, almost completely (90%), of spindle cells growing in diffuse, haphazard pattern, surrounding residual thyroid follicles. There was no vascular neither capsular invasion. Immunohistochemically, spindle cells were strongly positive with TTF-1, CK8-18 and vimentin, positive with keratin, but negative with CD34, S-100, desmin, HMB-45 and calcitonin. Spindle cells showed weak and focal immunoreactivity for thyroglobulin. Immunohistochemical results demonstrated the follicular origin of the lesion and suggested a diagnosis of spindle metaplastic transformation in follicular adenoma.

**Conclusion:** Spindle cell adenoma of thyroid is an extremely rare lesion. In our case FNAC was not conclusive and histological examination was necessary for a correct diagnosis. An association between spindle cell adenoma and the treatment for lymphoma cannot be ruled out.

#### P 358

### EVALUATION OF FIBRONECTIN EXPRESSION IN LARYNGEAL CANCER: AN IMMUNOHISTOCHEMICAL STUDY\*

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Fibronectin is one of the extracellular matrix glycoprotein, that plays an important role in cancer invasion and progression. Our purpose was to evaluate fibronectin expression and its possible prognostic value in laryngeal cancer. Forty-one patients (six female and thirty-five male) surgically treated for laryngeal cancer were included in the study. The expression and distribution of fibronectin was immunohistochemically determined by using monoclonal antibody against fibronectin. Low expression of fibronectin was detected around the individual carcinoma cells, but large

amounts were present in the invasive tumour front and the cancer surrounding stroma was strongest positive for fibronectin. By univariate analysis the level of fibronectin expression on the border of the invasive tumour front was proved to be correlated with tumor stage ( $p=0,03$ ). There was a significant association between the defect of ECM and overall survival ( $p>0,05$ ). These observation indicates that testing the distribution and the expression of ECM seems to be useful to evaluate the histological grading of malignancy of laryngeal cancer and to be helpful to prognosticate the overall survival while measured on the border of tumour proliferative front in laryngeal patients. The results demonstrate that fibronectin amount may be useful in evaluating the invasion potential of the tumour.

#### P 359

### MALT LYMPHOMA IN SJOGREN'S SYNDROME

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**Introduction and aim:** Sjogren's syndrome is a chronic autoimmune and rheumatic disorder with prominent sicca complaints from the mucous membranes because of lack of proper exocrine secretions. A large number of autoantibodies have been reported in Sjogren's syndrome where the antibodies are correlated with the extent and severity of disease. It is world-wide disease and may occur in all ages. However, the peak incidence is in the fourth and fifth decades of life with a female/male ratio of 9:1. Mucosa Associated Lymphoid Tissue (MALT) accounts for about 7,2% of all of non-Hodgkin's lymphomas. Most arise at sites without lymphoid tissue and appear following a period of chronic inflammation. This is the result of autoimmune disease as occurs in the thyroid or salivary glands, or in the stomach following local bacterial infection. In contrast to many reports on MALT lymphoma induced H.pylori chronic infection, on MALT lymphoma in salivary glands induced autoimmune disease there are a few data, that is the reason for our study.

**Material and methods:** Five patients with tumours of the parotid glands and surrounding lymph nodes, were surgically treated and sent to pathologist for histological analysis. Formaldehyde-fixed and paraffin-embedded tumorous tissue were cut and stained with H&E, AB-PAS, Van Gieson's and immunohistochemical LSAB2 methods. For immunohistochemical analysis, antibodies to cytokeratin and to CD20 and CD45RO antigens were used.

**Results:** MALT lymphoma in the salivary glands has a mean age incidence 56 years with a female to male ratio of 3:1. Macroscopical characteristics are not specific. Histological characteristic of MALT lymphoma was the presence of lymphoid follicles and diffuse infiltrate of lymphocytes occupying the marginal zone and both infiltrating and replacing glandular epithelium (pathognomonic lymphoepithelial lesion). The marginal zone, more developed, contained a mixed population of cells, with plasma cells and many mature B-cells, including centrocytoid and monocytoid B-cells. The pathognomonic lymphoepithelial lesions were more numerous than in

gastric MALT lymphoma. Immunohistochemically, lymphoepithelial lesions and B-cell origin of MALT lymphoma were confirmed.

**Conclusion:** MALT lymphoma in Sjogren's syndrome is autoimmune genesis. Characteristics of MALT lymphomas induced by chronic H.pylori infections are similar to characteristics of MALT lymphoma in Sjogren's syndrome.

#### P 360

### MICROPAPILLARY CARCINOMA OF THE BLADDER: REPORT OF TWO CASES

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#### INTRODUCTION

Micropapillary carcinoma (MC) of the bladder, first described in 1994, is a rare and highly aggressive variant of urothelial cell carcinoma (UCC) that accounts for 0.6% to 1% of all urothelial carcinoma. Histologically, the micropapillary component (MPC) is characterized by superficial pattern of slender, filiform processes or small papillary clusters of tumor cells and a deep pattern of tight infiltrating clusters contained within tissue retraction spaces. Herein, we report two additional cases of MC of the bladder including one with a long follow-up.

#### CASE 1:

A 68-year-old man presented with macroscopic hematuria. Ultrasonography and cystoscopy showed a sessile papillary tumor of 4 cm involving the anterior wall. The transurethral excision was complete. Microscopic findings demonstrated a grade G3 TCC with focal superficial and deep MPC (20%). Evidence of vascular invasion without muscular extension was found. A complementary BCG therapy was delivered. Four months later, the tumor relapsed and complete transurethral resection showed a recurrent pTa G1 UCC without MPC. He benefited from a second BCG therapy cure. He is now free of disease since more than six years.

#### CASE 2:

A 70-year-old man, treated for terminal chronic renal failure of unknown cause, exhibited a gross hematuria. Ultrasonography coupled with cystoscopy were consistent with a multifocal papillary tumor. The pathological examination after complete transurethral resection revealed a G3 papillary UCC with extensive MPC (60%) which infiltrate the lamina propria. The resection did not include muscle tissue. No cystectomy nor BCG therapy were scheduled due to his poor general condition. Currently, the follow-up is only one month.

#### DISCUSSION

While MC of the bladder is considered as a highly aggressive tumor with a high grade and stage at presentation, its prognosis is not very well documented due to the low number of cases in the literature. We report two additional cases including one with a favourable clinical course, the follow-up is too short for the second one. The prognosis depends on the extent of the invasion and on the proportion of the MPC. Deep muscle biopsies are recommended to rule out invasion when MPC is encountered. BCG therapy benefit should be better evaluated. A review of a large number of cases is needed to conclude whether or not the prognosis of patient with MC is poorer than that of patients with UCC G3 bladder cancer.

#### P 361

### PROGNOSTIC IMPACT OF INVASIVE PATTERN, SQUAMOUS DIFFERENTIATION AND SARCOMATOID TRANSFORMATION IN TRANSITIONAL CELL CARCINOMA OF THE UPPER URINARY TRACT

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#### Introduction

Initial tumour stage and grade and extent of surgery have been documented as major prognostic factors in patients with upper

tract transitional cell carcinoma (TCC). Our study aimed to evaluate the prognostic impact of other histopathological factors, such as invasive pattern, squamous differentiation and sarcomatoid transformation, in a large series of consecutive patients.

#### Methods

239 consecutive patients underwent surgery for upper urinary tract TCC between 1984 and 2004 at our institution. Patients with non-invasive TCCs (pTa) and multifocal (both pelvic and ureteral) TCCs were excluded. H&E slides of the remaining 188 cases (133 pelvic and 55 ureteral TCCs) were systematically re-evaluated regarding invasive pattern (nodular, trabecular and infiltrative), squamous differentiation and sarcomatoid transformation. The prognostic impact (regarding metastasis-free survival) was analyzed using the Kaplan-Meier method and the log-rank test. For multivariate analysis a Cox proportional hazard regression model including tumour stage and grade was used.

#### Results

The pattern of invasion was nodular in 47%, infiltrative in 31% and trabecular in 22% of cases, respectively. Squamous differentiation was identified in 22%, sarcomatoid transformation and marked tumour cell anaplasia were noted in 8% of cases, respectively. Follow-up data were available from 182/188 (97%) patients. After a mean follow-up of 4.1 years, metastatic disease was noted in 80/182 (44%) patients. Mean time to progression was 22 months. Significant prognostic factors in univariate analysis were pattern of invasion ( $p < 0.001$ ), squamous differentiation ( $p = 0.009$ ) and sarcomatoid transformation ( $p = 0.002$ ). In multivariate analysis, infiltrative pattern of invasion ( $p < 0.001$ , risk ratio [RR]=7.1, 95% confidence interval [CI]=2.4-19.8) and pT category  $> 1$  ( $p < 0.001$ , RR=4.0, 95%CI=1.9-8.3) proved to be independent predictors with respect to metastasis-free survival, whereas for sarcomatoid transformation only a trend was noted ( $p = 0.06$ ).

#### Conclusions

The infiltrative pattern of invasion proved to be a new independent predictor of metastasis-free survival in upper tract TCCs, whereas squamous differentiation and sarcomatoid transformation lacked independent influence on patient outcome.

#### P 362

### PROGNOSTIC IMPACT OF TUMOUR STAGE, GRADE AND ANGIOINVASION IN TRANSITIONAL CELL CARCINOMA OF THE UPPER URINARY TRACT

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#### Introduction

Initial tumour stage and grade and extent of surgery have been documented as major prognostic factors in patients with upper tract transitional cell carcinoma (TCC). However, data are limited due to small sample size and the new two-tiered grading system of the WHO has not yet been systematically tested with respect to prognostic significance. Therefore, our study aimed to evaluate possible conventional prognostic factors, such as tumour stage and grade as well as angioinvasion, in a large series of consecutive patients.

#### Methods

239 consecutive patients underwent surgery for upper urinary tract TCC between 1984 and 2004 at our institution. Patients with non-invasive TCCs (pTa) and multifocal (both pelvic and ureteral) TCCs were excluded. The slides of the remaining 188 cases (133 pelvic and 55 ureteral TCCs) were systematically re-evaluated regarding pT-category, tumour grade according to the recent revision of the WHO classification and angioinvasion (L1, V1). The prognostic

impact (regarding metastasis-free survival) was analyzed using the Kaplan-Meier method and the log-rank test. For multivariate analysis a Cox proportional hazard regression model was used.

#### Results

Follow-up data were available from 182/188 (97%) patients. After a mean follow-up of 4.1 years, metastatic disease was noted in 80/182 (44%) patients. Mean time to progression was 22 months. Significant prognostic factors in univariate analysis were pT-stage > 1 ( $p < 0.001$ ), high tumor grade ( $p < 0.001$ ) and angioinvasion ( $p < 0.0001$ ). No significant prognostic influence was observed for tumour location. In multivariate analysis, pT-stage > 1 ( $p < 0.001$ , risk ratio [RR]=4.7, 95% confidence interval [CI]=2.3-9.5) and angioinvasion ( $p < 0.001$ , RR=4.9, 95%CI=2.7-8.9) proved to be independent predictors with respect to metastasis-free survival, whereas for high tumour grade only a trend was noted ( $p = 0.09$ ).

#### Conclusions

Advanced tumour stage and presence of angioinvasion were independent predictors of metastasis-free survival in upper tract TCCs. No impact was seen for tumour localization (pelvic vs. ureteral), thus supporting the current concept of the TNM system to summarize pelvic and ureteral TCCs in one tumour category.

#### P 363

##### **CORRELATION BETWEEN APOPTOSIS AND HISTOLOGICAL GRADE OF TRANSITIONAL CELL CARCINOMA OF URINARY BLADDER**

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Purpose: TO evaluate the relationship between histological Grade and Apoptotic Index (AI) in TCC of urinary bladder

Methods & Materials: Formalin-fixed and paraffin-embedded tissue blocks from 75 patients with TCC who underwent Transurethral resection (TUR) were studied. One 3 micron sections were provided from each TUR samples. In one section after Hematoxylin and Eosin (H&E) staining, grade was determined according to WHO/ISUP criteria. The apoptotic cells were determined using a terminal deoxynucleotidyl transferase (TdT) mediated dUTP biotin nick end labeling (TUNEL) technique. Apoptotic index was then obtained as the percent of TUNEL positive cells from observations of at least 1000 cells in each section.

Results: Forty-nine patients were men and 26 were women. The mean age was 56.34, b9 years. The mean AI was 2.30, b0.50. The relationship between grade and AI was significant ( $P = 0.000$ ,  $rs = 0.551$ ). A higher grade was associated with a higher AI.

Conclusion: The AI has a direct correlation with bladder TCC grade. For determination of definite role of it in prognosis, other analytical studies are needed.

Key Words: Transitional cell carcinoma, Grade, Apoptotic Index, Tunnel

#### P 364

##### **THE RELATIONSHIP BETWEEN C-ERB B2 ONCOPROTEIN OVER EXPRESSION AND HISTOLOGIC GRADE ON TRANSITIONAL CELL CARCINOMA OF URINARY BLADDER**

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Background and Objective: Transitional cell carcinoma of urinary bladder is the fourth most common cancer in men and eighth in women. Similar to other cancers, this cancer needs to determination of prognosis and type of treatment, after diagnosis. Several parameters such as clinical and

pathological parameters, chromosomal and genetically abnormalities and molecular factors in cells surface have rules in determination of prognosis. In this research, for the first time in Iran, abundance of c-erb B-2 oncoprotein and its relation with tumor grade were evaluated.

Material and Method: A prospective cross-sectional study was performed in paraffin embedded tissue from 75 patients with TCC of urinary bladder. Two 3m sections were provided. In one section, according to WHO/ISUP grading system, grade of tumor was determined, by H&E staining. In the other section, the presence of c-erb B-2 oncoprotein was determined by IHC Method.

Results: Increasing in number of patient with c-erb B-2 protein over expression, was associated to increase in grade of tumor, and a meaningful relation between over expression of c-erb B-2 and grade of tumor was found.

Conclusion: c-erb B-2 oncoprotein over expression had a direct relation with tumor grade. Results of this study are the same as often previous; therefore c-erb- B-2 oncoprotein over expression could be a prognostic factor in TCC of urinary bladder. Other studies for confirmation of this hypothesis are recommended.

#### P 365

##### **IMMUNOHISTOCHEMICAL EXPRESSION OF CK 7, CK20, P53, AND MIB1 IN UROTHELIAL LESIONS**

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Morphological aspect in vesical lesion is sometimes difficult to distinguish from a reactional dystrophy, and it could be difficult to distinguish low and high grade carcinoma, specially in G2 tumour. In these cases immunohistochemistry with p53 and Mib1 may be useful.

Aim of the study : to evaluate the expression CK 20 in the diagnosis of urothelial carcinoma, and to compare to the expression of p53 and Mib1 and CK 7.

Materials and methods : 69 cases of urothelial carcinoma (54 TUR and 15 biopsies), interesting 3 reactional dystrophic lesions, 32 low grade urothelial carcinoma, 34 high grade urothelial carcinoma ; corresponding in 59 superficial tumours (38 pTa, 16 pT1a, 5pT1b) and 8 infiltrative tumours (pT2). For each cases one paraffin block of representative with normal urothelium were tested with CK 7 (Immunotech, 1/50); CK 20 (Immunotech, 1/50); p53 (Dako, 1/80) and Mib1 (Ki67, Dako, 1/100), after antigenic restoration, on an automate stainer Nexes Ventana ®.

Results : Normal superficial cells expressed CK7 in 98,5% of the cases. With CK 20, in normal urothelium, and low grade tumors, only superficial cells were stained. In high grade tumours all the height of the urothelium was stained. CK 20 expression was correlated with grade  $p = 0,01$  (WHO 73: G1 vs G2 vs G3 or WHO 2004: High grade vs Low grade), but not with stage  $p = 0,15$ . Expression of p53 and Mib1 varied from 5 to 90%. Mib1 expression was strongly correlated with grade :  $p = 0,013$  (WHO 2004: High grade vs Low grade or WHO 73: G1 vs G2 vs G3) and with stage  $p = 0,05$ . p53 expression was correlated neither with the grade  $p = 0,58$  nor stage  $p = 0,18$ .

Conclusion : High expression of CK 20 and Mib1 is found in high grade urothelial carcinoma and in CIS. This should be exercised in using CK 20 and Mib1 immunohistochemical staining for the diagnosis of papillary urothelial tumour of intermediate grade (G2, OMS 73), or in case of atypical plane lesion

#### P 366

##### **SIGNIFICANCE OF P21 AND C-MYC EXPRESSION IN BLADDER UROTHELIAL LESIONS**

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Immuno-histochemical tissue expression of the anti-proliferative marker P21 and the oncogenic marker C-myc were estimated in 40 cases with non-neoplastic and neoplastic urinary bladder lesions with or without schistosomal infection to assess the significance of their urothelial expression as a diagnostic tool in patients with higher risk of developing cancer bladder and as a prognostic indicator in malignant lesions. P21 expression was detected randomly in normal urothelial nuclei, while 50% of simple cystitis cases and all cases with premalignant changes were positive for P21 immuno-reactivity expressed into about 16% of urothelial cells. Eighty five percent of malignant cases expressed P21 in 48-55% of urothelial cells without significant variance between different histologic tumor types. Extent of P21 expression inversely correlated with bilharzial association, upgrading of malignancy and tumor invasiveness.

C-myc was detected in 50% of normal urothelium ( as cytoplasmic staining ), in 80% of simple chronic cystitis cases (75% cytoplasmic, 25% cytoplasmic and nuclear expression), and in all cystitis cases with premalignant changes ( as cytoplasmic and nuclear expression). Eighty nine percent of cancer cases were C-myc positive with predominance of nuclear expression to be seen in 16.7% and mixed with cytoplasmic expression in another 58.3% of positive cases. Malignancy upgrading and invasiveness raised C-myc positivity. In conclusion: P21 expression increases in an attempt to check cellular proliferation, but when the matter is out of hand, P21 is down-regulated, while the increase in the oncogen C-myc goes ahead. Loss of P21 and increased C-myc expression in a malignant lesion is a predictor of malignancy progress to higher grade or stage.

**P 367**  
**IMMUNOHISTOCHEMICAL PROGNOSTIC FACTORS OF UROTHELIAL CARCINOMA.**

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AIMS : Immunohistochemical study structural component of extracellular matrix and cellular adhesion molecules in bladder urothelial carcinoma of different grade.

METHODS : Using formalin – fixed and paraffin – embedded tissue, we examined by immunohistochemical method tenascin, collagen IV, laminin, E-cadherin, beta-catenin, CD44 in 39 cases of urothelial carcinoma: G1 – 15, G2 – 12 and G3. Staining intensity of E-cadherin, beta-catenin and CD44 were evaluated using a 0 to 3+ scale.

RESULTS: The strong positive staining (3+) with antibody to E-cadherin was observed in most of cases G1 carcinoma. Low intensive (0/1+) reaction was found in all cases G3 urothelial carcinoma. Immunohistochemical staining with antibody to beta-catenin was the same in localisation and intensity of expression with E-cadherin: G1 carcinoma – 3+ and 2+ in 80%, G2 – 2+ in 40% and 1+ in 60%, G3 – 0/1+ in 100% cases. It is important, that it was low expression E-cadherin and beta-catenin (0/1+) in a few cases high-grade urothelial carcinoma.

CD44 level was high in tumor cells in G1 and low – in extracellular matrix. But in G3 cases we observed strong reaction in extracellular matrix and low reaction – in tumor cells membrane.

Expression collagen IV and laminin was high intensively around tumor complexes in G1 carcinoma, but low – in cases G3. The overexpression of tenascin was found only in bladder carcinoma G1.

CONCLUSION: Our data suggest, that expression E-cadherin, beta-catenin, CD44, tenascin, collagen IV and laminin may play a role of prognostic factors in urothelial carcinoma and they correlate with different level of malignancy. .

**P 368**  
**PROGNOSTIC VALUE OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF : KI67, P53, PCNA AND BCL2 IN THE SUPERFICIAL TUMORS OF THE BLADDER.**

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Introduction: Superficial tumors of the bladder present 2 prognostic problems: a risk of recurrence and a risk of progress in grade and in stage. Predictive factors of recurrence and of tumoral progress were identified. These factors are essentially endoscopic and pathological ones. Recent works underlined the prognostic value of the immunohistochemical study of the markers of cellular proliferation and of apoptosis in these tumors.

Aims: To study a series of superficial tumors of the bladder and to assess the prognostic significance of p53, Ki67, PCNA and bcl2 in these tumors.

Materials and methods: We studied 59 bladder tumors: 43 pTa and 16 pT1. All Patients had cystoscopy 3 months after the resection and tumors that did not recur had a minimum follow up of 5 years.

Results: Age more than 65 years (p = 0,001), multifocality (p = 0,022) and tumoral size  $\geq$  3 cm (p = 0,022) were correlated with the recurrence. The expression of p53 was correlated with the recurrence in the year following the tumoral resection (p = 0,035). That of Ki67 was correlated with the histological grade (p = 0,03) and stage (p = 0,002).

Conclusion : Our results suggest to practise regular endoscopic controls during the first year following the resection of the primitive tumor if it expresses p53. The immunohistochemical expression of Ki67 being correlated with the grade, this marker could help to better classify the tumors of intermediate grade.

**P 369**  
**KI67 EXPRESSION IN RECURRENCE PAPILLARY TRANSITIONAL CELL BLADDER CARCINOMA**

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This work has been sent

but the category miscellaneous is fault

The category of this work is uropathology

We beg your pardon.

**P 370**  
**EXPRESSION OF TETRANECTIN IN INVASIVE AND NON-INVASIVE PAPILLARY BLADDER CANCER**

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**Introduction:** Tetranectin is a plasma protein, which can be detected immunohistochemically in many normal cells. It is able to bind to the kringle-4 region of plasminogen and to participate in proteolytic processes that are considered to play an important role during tumor invasion and metastasis. Tetranectin expression was reported in breast, gastric and colon cancer being associated with a reduced survival in breast cancer.

The aim of the study was to investigate the expression of tetranectin in the normal bladder and bladder cancer.

**Material and methods:** Expression of tetranectin was studied in 20 invasive and non-invasive bladder tumors, including 9 low grade (5 grade 1, 4 grade 2 tumors) and 11 high grade tumors (3 grade 2, 8 grade 3 tumors) and in normal urothelium by means of immunohistochemistry using a monoclonal antibody against human tetranectin.

**Results:** Tetranectin was detected in normal urothelial cells, in the extracellular matrix, in inflammatory cells, in the smooth muscle of the bladder wall and in endothelial cells.

Non-invasive bladder tumors showed a weak tetranectin expression in superficial and intermediate cells.

In invasive tumors high expression of tetranectin was found in the invasive tumor component.

The extracellular matrix stained positive for tetranectin in non-invasive and invasive tumors, exhibiting a more intense staining around invasive tumor.

**Conclusion:** Stromal tetranectin expression is observed in the normal bladder and bladder cancer. The significance of this finding remains to be clarified.

The observed high cytoplasmic expression in invasive tumor cells suggests a potential role for tetranectin during invasion of bladder cancer.

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#### **MINICHROMOSOME MAINTENANCE PROTEIN (MCM) 2 AND 5 EXPRESSION IN MUSCLE INVASIVE UROTHELIAL CANCER : A MULTIVARIATE SURVIVAL STUDY INCLUDING PROLIFERATION MARKERS AND CELL CYCLE REGULATORS**

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Minichromosome maintenance (MCM) proteins are components of the prereplicative complex and thus essential for eukaryotic DNA replication. Their presence only in proliferating cells suggests a role as a novel proliferation marker. There is no previous study investigating MCMs in muscle-invasive bladder carcinomas. We analyzed immunohistochemically the expression of MCM-2 and MCM-5 proteins in 65 patients with muscle-invasive urothelial bladder carcinomas, in relation with clinicopathological parameters, patients' overall and disease-free survival and the expression of the conventional proliferation index Ki-67 and other cell-cycle modulators (p53, pRb, p21WAF1 and p27Kip1).

MCM-2 and MCM-5 levels were significantly higher in high grade ( $p < 0.0001$ ), advanced stage ( $p = 0.001$ ) and non-papillary

tumors ( $p < 0.0001$ ). MCM-2 and MCM-5 protein expression significantly associated with the conventional proliferation index Ki-67 ( $p = 0.0001$  for each protein). MCM-2 or MCM-5 expression positively correlated with p53 labeling index ( $p = 0.014$  and  $p = 0.009$  respectively). Also median p21WAF1 labeling index was higher in MCM-5 high expressors ( $p = 0.028$ ). Finally, both MCM-2 and MCM-5 associated significantly with adverse patients' outcome in both univariate ( $p = 0.0072$  and  $p = 0.0074$  respectively) and multivariate ( $p = 0.0001$ ) analysis.

In conclusion, MCM-2 and MCM-5 proteins appear to be reliable proliferative indices and useful prognostic markers in patients with muscle-invasive urothelial bladder carcinomas.

### P 372

#### **QUALITATIVE AND QUANTITATIVE ANALYSIS OF ANGIOGENIC FACTORS IN TRANSITIONAL CELL BLADDER CARCINOMA: RELATIONSHIP WITH CLINICAL COURSE AT 10 YEARS FOLLOW-UP**

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**Aims:** Many authors have shown that tumour hypoxia exerts its own influence on malignant progression by inducing angiogenic factors and new blood vessels toward and within the tumour. This event usually suggests poor prognosis and/or aggressive tumour behaviour. The objective of this study is to compare molecular analysis of angiogenic factors with microvessel density (MVD) in bladder cancer.

**Materials and methods:** Twenty-nine consecutive patients underwent transurethral or open surgery for bladder tumours. Neoplastic tissue samples, normal-appearing bladder mucosa and blood samples were taken from each patient. All the tissues underwent mRNA extraction and Northern Blot analysis, marked with specific probes for inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase-2 (COX-2) and Vascular Endothelial Growth Factor (VEGF), and evaluated by gel-electrophoresis. MVD, a quantitative analysis for neoangiogenesis, was also evaluated by using CD31 immunohistochemical assay and compared with molecular analysis and patient follow-up. Two follow-ups for recurrence or progression were performed at 74 months and 10 years from surgery, respectively.

**Results:** Pathological evaluation demonstrated the presence of superficial transitional cell carcinoma (sTCC) in 15 patients, while 14 had an invasive bladder tumour (iBT). At both 74 months and 10 years follow-up, all patients with lower MVD had a shorter survival time. No significant results were obtained by comparing disease progression or survival rate with VEGF, iNOS and COX-2 levels. A proportional increase of VEGF expression and MVD associated with poor prognosis was the expected outcome of our study. These results were disregarded at both the first and the second follow-up. A strong correlation between MVD  $> 20$  and survival rate was noted both in sTCC ( $p = 0.024$ ) and iBT ( $p < 0.001$ ) patients.

**Conclusions:** These results confirm that MVD could be considered a good prognostic factor. The angiogenic cytokine overexpression found in control tissue samples of sTCC could have clinical significance in either a macroscopically unidentified diffuse carcinogenetic process or a systemic immune response against tumour cells.

### P 373

#### **MICROSATELLITE INSTABILITY AND SOMATIC DOWN-REGULATION OF MISMATCH REPAIR CHARACTERIZE SOLID MUSCLE-INVASIVE**

## TRANSITIONAL CELL CARCINOMA OF THE BLADDER

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**Background:** Histologic patterns have been demonstrated prognostically useful in transitional cell carcinomas (TCC) of the bladder, but no information is available on the prognostic significance and genetic profile of muscle-invasive TCC by infiltration pattern. The contribution of DNA mismatch repair abnormalities to this profile remains unknown.

**Design:** The predominant infiltration patterns of 72 muscle-invasive TCC was assessed in the deep compartment and classified as "solid" (diffuse infiltration effacing the muscle fibers, 45 cases) or "single-file" (tumor infiltration dissecting the muscle and inducing an intense stromal reaction, 27 cases). Tumors were studied by compartments (superficial and deep to muscularis mucosa), DNA being extracted from both compartments to analyze the microsatellite pattern of TP53, RB1, WT1, and NF1 by polymerase chain reaction-denaturing gradient gel electrophoresis. Mitotic indexes, Ki67 index, in-situ end labelling (ISEL) and DNA ploidy analyses were evaluated in the same areas. Statistical differences were tested using ANOVA and Fisher's exact test. Mismatch repair was assessed by mlh1 and msh2 sequencing and immunostaining in TCC with >2 abnormal microsatellite loci.

**Results:** Single file TCC showed lower cell turnover (Ki67 index 14.94±4.28, ISEL 14.1±10.0), lower incidence of aneuploid DNA content, and shorter mean survival (20 months) than solid TCC (Ki67 index 20.65±4.94, ISEL index 20.2±22.7, and 37-month survival, respectively).

The genetic profile was demonstrated significantly different for RB1 (p=0.0003) and NF1 (p=0.0023) only. Single-file TCC showed a low incidence of genetic deletions/single nucleotide polymorphism(s), not involving RB1 locus, and very occasionally NF locus (2 cases, 13%). A significant decrease of mlh1/msh2 protein expression (absence of any of these proteins) with no gene mutations were identified in TCC with high microsatellite instability and solid growth pattern.

**Conclusions:** 1. Somatic down-regulation of mismatch repair proteins (mlh1/msh2) in solid muscle-invasive TCC results in microsatellite instability characterized by deletion/single nucleotide polymorphism(s) in RB1 and NF1. 2. This microsatellite instability profile correlates with the higher cellular turnover (both proliferation and apoptosis) and longer survival observed in patients with solid TCC (as compared with those of single-file TCC).

### P 374

#### CYTOKERATIN 20 EXPRESSION IN UROTHELIAL CARCINOMA OF THE BLADDER: A HIGH-THROUGHPUT TISSUE MICROARRAY ANALYSIS CONFRONTING THE PRIMARY TUMOR AND ITS METASTASIS.

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#### Introduction

Cytokeratin 20 (CK20) expression has been reported in a high percentage of urothelial carcinomas (UC) of the bladder, with no clear correlation with stage or grade, and it has poorly been studied in metastatic carcinoma.

The aim of our study was to determine whether CK20 was consistently expressed in metastatic UC.

#### Material and Methods

We studied all the cystectomy cases performed in our department from 1995 until 2004 with a diagnosis of UC, and found 45 cases which had developed metastatic disease. A Tissue Microarray (TMA) was built with duplicates of 0,6

mm cores of the 45 primary UC and their metastasis (31 nodal, 4 pulmonary, 4 osseous, 4 peritoneal and 2 to the urogenital area). CK20 immunohistochemistry was performed on 5mm sections (clon Ks20.8, Dako, dilution: 1/400).

#### Results

CK20 was positive in 45,4% of the primary UC and in 42,9% of the metastasis. In 54% of the cases, primary and metastatic disease showed the same pattern of expression (23% both positive, 31% both negative), while in 19% of cases there was a loss of CK20 expression in the metastasis compared to primary tumor. Unexpectedly, 27% of the primary UC were negative for CK20 and their metastasis were positive.

#### Conclusions

CK20 is generally considered a good marker of UC of the bladder, specially in combination with CK7. In our series, less than half of the primary UC express CK20, probably owing to high-grade histology. Furthermore, there is a decrease of CK20 expression in metastatic disease compared to primary UC. We think that CK20 is a good marker of UC of the bladder, specially in combination with CK7, and specially in low-grade and low-stage UC.

### P 375

#### EXPRESSION OF CELL CYCLE PROTEINS IN BLADDER CARCINOMA

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The cell cycle is known to be deregulated in cancer. We therefore analyzed the immunohistochemical expression of cell cycle related proteins p53, Rb, p21, p16, p27 and cyclin D1 in 128 cases of transitional cell bladder carcinomas. The proliferation status was determined by Ki67, cyclin A and cyclin B1 protein expression. The expression of the proteins was correlated with clinicopathological parameters.

High expression of p53, p21 and cyclin D1 proteins (>10% of neoplastic cells) was detected in 64.7%, 16.5% and 32.5% of the cases, respectively. Low expression (<10% of neoplastic cells) of pRb, p16 and p27 proteins was found in 55.65%, 22.10% and 9% of the cases, respectively. Expression of Ki67, cyclin A and cyclin B1 proteins was found in all cases. Significant positive correlations were found between a) p53 and p21, Ki67, cyclin A, cyclin B1, b) pRb and Ki67, cyclin A, c) p21 and cyclin D1, cyclin A, when their expression was compared as continuous variables.

The following combined p53/p21 expression patterns (p53 pathway) were observed: p53+p21+ (13.91%), p53+/p21- (53.04%), p53-/p21+ (3.48%), p53-/p21- (29.56%). The combined Rb/p16/cyclin D1 expression patterns (Rb pathway) were: Rb+/p16+/cyclin D1+ (9.63%), Rb+/p16+/cyclin D1- (25.3%), Rb+/p16-/cyclin D1- (3.6%), Rb+/p16-/cyclin D1+ (7.23%), Rb-/p16+/cyclin D1+ (15.67%), Rb-/p16+/cyclin D1- (26.5%), Rb-/p16-/cyclin D1- (10.84%), Rb-/p16-/cyclin D1+ (1.20%). A significant correlation was found between combined p53/p21 expression patterns and cyclin A (p=0.002) and cyclin D1 (p<0.0001) expression. Moreover, cyclin A expression was correlated with combined Rb/p16/cyclin D1 expression patterns (p=0.040).

High expression of p53, Ki67, cyclin A and cyclin B1 (>10% of neoplastic cells) was negatively correlated with the papillary morphology of the tumor (p=0.026, p<0.0001, p<0.0001 and p=0.001, respectively) and positively with tumor grade (p=0.014, p<0.0001, p=0.002 and p=0.027, respectively) and T-stage (p=0.014, p<0.0001, p=0.004 and p=0.025, respectively).

These results suggest that alterations of cell cycle regulatory proteins are common in bladder carcinomas and may act in cooperative or synergistic ways to promote tumor progression.

#### P 376

##### **HER-2/NEU OVEREXPRESSION AND AMPLIFICATION IN METASTATIC UROTHELIAL CARCINOMA OF THE BLADDER.**

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##### **Introduction**

Studies reporting Her2Neu expression, both immunohistochemical and by fluorescence in situ hybridization (FISH) have shown varying results: some authors have suggested that Her2Neu gene amplification rates are low, in comparison with protein overexpression. The pattern of expression of Her2Neu in metastatic disease has not been almost studied.

The aim of our study was to determine Her2Neu status by immunohistochemistry (IHC) and FISH in metastatic bladder cancer.

##### **Material and Methods**

We studied all the cystectomy cases performed in our department from 1995 until 2004 with a diagnosis of UC, and found 45 cases which had developed metastatic disease. A Tissue Microarray (TMA) was built with duplicates of 0,6 mm cores of the 45 primary UC and their metastasis (31 nodal, 4 pulmonary, 4 osseous, 4 peritoneal and 2 to the urogenital area). FISH was performed using the Pathvysion kit (Vysis, Abbott), and IHC was performed on 5mm sections (CB11, Ventana) and evaluated as 0, 1+, 2+, and 3+.

##### **Results**

Only 6% of the primary UC and 8,6% of the metastasis show amplification by FISH, and correspond to the 3+ results by IHC. 91,1% and 3% of the primary neoplasms and 80% and 11,4% of the metastatic tumors are IHC negative and show 2+ positivity for Her2Neu, but none of them are amplified by FISH.

##### **Conclusions**

Her2Neu is not frequently amplified in bladder carcinoma, not even in metastatic disease. All the cases with a 2+ IHC result are not amplified in our series, and perhaps the performance of FISH is not necessary in those cases.

#### P 377

##### **IMMUNOHISTOCHEMICAL EXPRESSION OF UROPLAKIN-III IN PRIMARY BLADDER ADENOCARCINOMAS AND LESIONS WITH GLANDULAR PHENOTYPE.**

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**Introduction:** Primary bladder adenocarcinomas (PBAs) represent a small fraction of bladder tumors (1-2%). Their differential diagnosis from other metastatic adenocarcinomas can be difficult on histological basis alone, and the combined use of Cytokeratin (CK) 7 & 20 does not always seem to resolve the problem. Relatively new, urothelial specific diagnostic markers, such as Uroplakin-III (UPIII), are commercially available for immunohistochemistry.

**Purpose:** To investigate the possible diagnostic value of UPIII immunohistochemical detection in PBAs, as well as, in a

small number of lesions with true glandular differentiation, arising in the bladder.

**Methods:** Seven cases of PBA, one villous adenoma with mucin producing cells and two cases of enteric type urothelial metaplasia were retrieved from our files. The material was re-evaluated and stained with monoclonal antibodies against CK7, CK20 and UPIII using appropriate positive and negative controls, according to standard procedures. A streptavidin-biotin based detection system was employed, using diaminobenzidine as chromogen. The staining reaction was assigned semiquantitatively; the cut-off point was set at 5%.

**Results:** Regarding the panel of CK7/20 four PBAs were (+/+); one (+/-); two (-/+). The adenoma and the metaplastic lesions were (+/+). UPIII was positive in almost all cases (9/10), including (6/7) PBAs regardless of their histologic subtype. The staining pattern was cytoplasmic with focal membrane accentuation. The staining intensity and the percentage of positive cells were variable.

**Conclusions:** UPIII seemingly represents a valid, constant diagnostic immunohistochemical marker for PBA and can contribute to their differential diagnosis, providing evidence for their urothelial origin, even in cases with frankly enteric phenotype.

#### P 378

##### **LOH AND FISH ANALYSIS FOR THE DETECTION OF BLADDER CANCER FROM VOIDED URINE SPECIMENS: A PRELIMINARY STUDY REPORT**

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**Introduction:** Successful treatment of bladder cancer depends largely on early detection of primary and recurrent disease. As cytology alone is too unreliable to serve as a basis for therapy decision, improved laboratory tests are needed. Today, loss of heterozygosity (LOH) and fluorescence in situ hybridization (FISH) are the most promising assays to detect early tumor-specific genetic alterations in cytological specimens. We chose to set up and validate a LOH method, which involves analysis at 10 microsatellite loci on chromosomes 8p, 9q, 9p, 13q, 16q and 17p. Ambiguous cases were additionally analysed by FISH.

**Methods:** Matched biopsies and urines from patients scheduled for tumor resection were collected prospectively and examined by routine histology and cytology. LOH analysis was performed using fluorescence-labeled primers and capillary electrophoresis. FISH analysis was done according to the manufacturer's recommendations (UroVysion, Vysis).

**Results:** Preliminary analysis were performed on 62 urine samples from patients with histological confirmed diagnosis of bladder cancer (carcinoma in situ pTis: 8; non-invasive papillary pTa: 10 G1, 22 G2, 3 G3; invasive papillary pT1: 2 G2, 9 G3 and pT2: 8 G3). Chromosomal aberrations were detected in 47 urine samples (76%), while 15 were LOH negative (mostly pTa G1-2 tumors). Remarkably, the sensitivity of LOH for low grade tumors (G1-2) was superior to cytology (69% vs. 26%). Most genetic aberrations were associated with losses at 9p and 9q, confirming a pivotal role of these regions for bladder tumorigenesis, while LOH on chromosome 8p, 13q, 16q and 17p rather correlated with advanced tumor grade and stage. To date, FISH analysis was performed on 13 urines. There were 10 histologically positive carcinoma cases (2 pTa G1-2, 2 pTa G3, 3 pT1-2 G3 and 3 pTis), of which 7 exhibited chromosomal instabilities, while 3 were scored as normal. Out of 3 histologically tumor-free cases, 2 were FISH-negative and 1 FISH-positive. Comparing

the two methods, identical LOH and FISH positive as well as negative results were documented in 7 histologically corresponding cases. Clinical follow-up is currently ongoing for the other 6 ambiguous cases.

Conclusions: Preliminary data show that both LOH and FISH analysis improve the sensitivity of voided urine specimens for the detection of bladder cancer.

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#### DETECTION OF PRIMARY UROTHELIAL CARCINOMA IN VOIDED URINE SPECIMEN USING FOUR PROBE FISH ASSAY COMBINED WITH AUTOMATED MICROSCOPIC SYSTEM, A PROSPECTIVE STUDY

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**INTRODUCTION:** The gold standard to diagnose urinary cancer is urinary cytology combined with cystoscopy. The low-grade urothelial cancer and early detection of recurrences are the greatest diagnostic challenges and many attempts are made to make the urinary cytology screening more sensitive and specific especially in these two critical cases. The literature is flooded with different methods including different immunologic and genetic testings like microsatellite instability assays and FISH. It seems that FISH assays will play an important role in the future in the cytology laboratory.

**PURPOSE:** Determination of the effectiveness of an automated microscopic system in the detection of chromosomal changes in uroepithelial cancer.

**METHODS:** We have analyzed 19 patients with primary uroepithelial cancer. Conventional urinary cytology coupled with cystoscopy and biopsy in each case was performed. From the collected voided urine we have analyzed three preparations processed with three methods. After optimizing the cell count in cyto-chamber we used the alcohol fixed specimen for FISH analysis. The preparations were evaluated by Zeis Axioplan2 automated microscope combined with Metasystem/Metacyte-Metafer4 software and/or manually counted (150-300 cells).

**RESULTS:** Histologically evident uroepithelial carcinoma was present in 16 cases (grade I/II/III: 3/8/5 respectively). The cytology evaluation of the voided urine specimen could prove the presence of neoplastic cells in 10 cases (62.5%) and was suspicious in 4 cases (25%). One result was negative and one was unsatisfactory. Automated detection system could identify surprising number of chromosomal abnormality even in histologically negative cases (average 31 cell; 7.81%). The number of cells carrying chromosomal abnormality was significantly lower in low grade vs. high grade tumors (G1:45 cells; 5,18% vs. G2:248,5; 16,77% and G3:146,8; 17,39%). Similar correlation could be observed in in situ vs. invasive (pTa:78 cells, 7,41%; vs. pT1:272,67, 19,67%; pT2:102,5, 16,3%). The control manual counts gave similar results.

**CONCLUSION:** Using Zeis Axioplan2 automated microscope combined with Metasystem/Metacyte-Metafer4 software is a powerful method to detect chromosomal changes in voided urine cytology specimen. The criteria defining positive diagnosis need to be adjusted, because the original Urovysion criteria was established on manual evaluation which required only the presence of 25 morphologically abnormal cells.

#### P 380

#### LIMPHOEPITHELIOMA-LIKE CARCINOMA OF THE BLADDER. CASE REPORT AND REVIEW OF THERAPEUTIC MANAGEMENT

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#### Introduction

Lymphoepithelioma-like carcinoma (LELC) of bladder is an extremely rare tumor. In the bladder, Zuckerberg and col. first describe this tumor as a part of a series of carcinoma simulating lymphoma. The origin of this tumor is unknown although it is suspected that it originates from modified urothelial cells. Initially it was classified as a neoplasm with a poor prognosis, but actually it is recognized its sensitivity to chemotherapy and a more long-term prognosis when compared with other types of carcinomas of bladder of equivalent stage and grade.

#### Case report

We present, a case of LELC diagnosed and treated at our Institution. The case is that of a 70 years old male with two years history of hematuria. A transurethral resection (TUR) of the bladder tumor was carried out after multiple inconclusive imaging studies, cystoscopy and urine cytologies. The histologic diagnosis of LELC with tumor infiltration of the muscle layer, led to 4 cycles of chemotherapy treatment with cisplatin and etoposide based regimens, and no additional surgical interventions. 18 months later, the patient was well and free of disease.

#### Results and conclusion:

Microscopically, the tumor showed a neoplastic cell proliferation composed of sheets and nests of atypical cells with scarce eosinophilic cytoplasm and large vesicular nuclei with prominent nucleoli and numerous mitosis. The tumor invaded into the muscle layer of the bladder, and was accompanied by a prominent inflammatory reaction. The bladder mucosa was focally ulcerated, and we did not identify areas of carcinoma in situ or conventional bladder carcinoma elsewhere. Immunohistochemically, tumor cells showed strongly positive cytoplasmic staining for Keratin (CK) AE1/AE3 and epithelial membrane antigen; and were focally positive for CK 7 and 8. Neoplastic cells were negative for CK 20. Immunostaining for Epstein-Barr virus was negative. All these findings are concordant with those of reported LELC cases in this location. Invasive LELC of bladder, according to the criteria of Amin et al., are classified as pure (all lymphoepithelial elements), predominant (more than half) or focal (less than half). It is well established that the two first types, are tumors that respond well to chemotherapy. It is important to recognize these tumors, and distinguish them for conventional transitional tumors with focal LELC differentiation, in order to administrate appropriate treatment and avoid cystectomy in these patients.

#### P 381

#### PRIMARY SMALL CELL NEUROENDOCRINE CARCINOMA OF THE URINARY BLADDER : AN IMMUNOHISTOCHEMICAL STUDY OF 15 CASES.

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**Introduction :** Primary small cell neuroendocrine carcinoma (SCC) of the urinary bladder is a very rare entity (0.5% of urinary bladder tumors). Its morphology may overlap with poorly differentiated urothelial carcinoma but SCC must be clearly distinguished because of differences in treatment and prognosis. We investigated the immunohistochemical expression of epithelial growth factor receptor (EGFR) and some other markers in a series of 15 SCC.

**Methods :** We studied synaptophysin, CD56, chromogranin A and B, cytokeratin (34betaE12) p16, p53 and EGFR reactivity in 15 consecutive cases diagnosed between 1990 to 2005 on

transurethral resection (9 cases), radical cystectomy (1 case) or both (5 cases). Ten cases were fixed in formalin, 5 in Bouin's fluid.

Results : In 13 cases (87%), the SCC was associated with an urothelial carcinoma which was a minor component in all but one case and was invasive in 8 cases, in situ in 2 cases or both in 3 cases. A reactivity with 2 or more neuroendocrine markers was observed in all cases. Synaptophysin was positive in all cases, CD56 in 13 cases (87%), chromogranin A in 12 cases (80%) and B in 9 cases (60%). 34betaE12 was always negative on SCC but was expressed by the urothelial carcinoma component while p16 was positive on both components in all cases. All tumors fixed in formalin presented a high expression of p53. Nine SCC (60%) expressed EGFR in at least 5% to more than 50% of cells.

Conclusion : In poorly differentiated tumors of urinary bladder, neuroendocrine markers must be used with 34betaE12 to differentiate SCC from poorly differentiated urothelial carcinoma. As in small cell carcinoma of the lung, we found an over-expression of p53 and p16 that might be related to a disruption of the Rb pathway. EGFR is expressed in a majority of SCC of the urinary bladder thus suggesting a possible therapeutic role for EGFR inhibitors.

**P 382**  
**NEUROENDOCRINE DIFFERENTIATION IN**  
**PRIMARY ADENOCARCINOMAS OF THE URINARY**  
**BLADDER**

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Introduction: Adenocarcinoma (AC) of the bladder is a rare entity and has several architectural variants: the "enteric-type" is the most common one and shares morphological and immunophenotypic features with conventional colorectal carcinoma. Although the occurrence of neuroendocrine (NE) differentiation in ACs from various sites, lung, colon and prostate, among the most relevant has been widely reported, in the bladder only rare case reports have been documented. cells, as known in colorectal, prostatic (but not in urothelial) carcinomas, among others.

The purpose of this study was to investigate the occurrence of NE differentiation in bladder AC and non-neoplastic glandular lesions.

Methods: Sixteen primary bladder ACs (6 urachal) and 4 urothelial carcinomas with glandular differentiation (UGCC) were collected. The UGCC had high grade urothelial carcinoma areas admixed with enteric-type glands (5-80% of the tumor). ACs were subclassified into enteric-type (7), mucinous (6) and NOS (3 cases) variants. To classify a case as "urachal adenocarcinoma" we applied the criteria proposed by Johnson and coworkers [1985]. In addition 20 glandular cystitis, 3 urachal cysts, 15 urothelial carcinomas and 3 small cell carcinomas were also collected as control groups. NSE, synaptophysin and chromogranin A immunostainings were performed. Clinico-pathological and outcome data were available for all but one case.

Results: Panendocrine markers gave heterogeneous results: NSE was negative, synaptophysin was focally positive in two cases, and chromogranin-A revealed NE differentiated cells in 12/20 cases (60%) (all enteric-type and mucinous carcinoma variants). All urachal cysts and 70% of glandular cystitis also had chromogranin-A reactive cells. Control urothelium and urothelial carcinomas (but not 3/3 small cell carcinoma) were chromogranin-A negative. No correlation was found between NE differentiation and clinical (including outcome) or pathological parameters. At follow up, 50% of patients had

fatal outcome. In patients died of disease, the mean survival was 40 months (ra

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**PARAGANGLIOMA OF THE URINARY BLADDER:**  
**REPORT OF THREE CASES**

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We present three cases of urinary bladder paraganglioma. One of them is a tumor resection of a patient with typical micturition attacks. The other two are transurethral resection materials consulted to our department. One of these two patients was diagnosed as urothelial carcinoma and the other one was diagnosed as adenocarcinoma and urothelial carcinoma in two different pathology departments. As we learned later, one of these patients had an hypertension attack during the biopsy procedure.

Microscopical examination of all three cases revealed the typical cellular and vascular arrangement of paragangliomas. Focal pleomorphism was remarkable in one of the cases. Submucosal invasion was detected in the transurethral resection materials. In the partial cystectomy material the tumor was located in the muscular layer. The negative immunohistochemical reaction of the tumor cells with pancytokeratin, cytokeratin 7 and cytokeratin 20 helped to rule out urothelial carcinoma. The tumor cells showed diffuse immunoreactivity with chromogranin A, synaptophysin and neuron specific enolase and the sustentacular cells stained positive for S100 protein. Based on these immunohistochemical findings we diagnosed the tumors as paraganglioma. The Ki67 proliferation index of the tumors were 1-2%.

The invasion of lamina propria and the muscular layer of paragangliomas is the misleading point of the transurothelial resection materials. The pathologist must take paraganglioma into consideration in the differential diagnosis of these materials not to miss out the cases.

**P 384**  
**PARAGANGLIOMA OF URINARY BLADDER**  
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Urinary bladder is a rare site of paraganglioma. We describe a paraganglioma of the urinary bladder in a 51-year-old female who presented with painless gross hematuria. Abdominal ultrasonography revealed a protruding well-defined mass, about 3Cm. in diameter over the left lateral wall of the urinary bladder. The tumor was not completely resected by transurethral resection of bladder tumor (TURBT) due to involvement of the bladder wall. Histologic examination of the tumor indicated clusters of polygonal chromaffin cells, arranged in nests (zellballen). Although there was marked pleomorphism and atypia in nuclei, no evidence of recurrence or metastasis has been seen since about 1 year. There is no reliable method for predicting their clinical behavior, so long-term follow-up is required. We also provide a brief review of the literature for comparison.

**P 385**  
**SARCOMATOID TRANSITIONAL CELL**  
**CARCINOMA OF URACHUS: A CASE REPORT**  
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Tumors of urachus duct which is remnant of embryonic

allantoic duct are rare. We report a case of transitional cell carcinoma of urachus in a 54 Y/O man with chief complaint of abdominal wall mass. The mass had adhesion to anterior abdominal wall and also extension to dome of bladder. By operation the mass and small part of wall of bladder were excised.

Cut section of mass showed a cyst inside, with tumoral lesion around. Histologically the tumor was transitional cell carcinoma of variable degrees and also sarcomatoid pattern in some areas. Smooth muscles of urachus wall and also bladder wall were invaded by tumor, but surgical margins and two detected lymph nodes were free.

It seems that this patient is the first reported case of sarcomatoid carcinoma of urachus.

#### **P 386**

#### **SARCOMATOID TRANSITIONAL CARCINOMA OF URACHUS (A CASE REPORT)**

SETARESHENAS Roya, AZIZI Rasoul

Urachus duct which is remnant of embryonic allantoic duct, may show neoplastic lesions rarely. The most common tumor of urachus is adenocarcinoma, but squamous cell carcinoma and transitional cell carcinoma (TCC) has also been reported.

We report a case of sarcomatoid TCC of urachus in a 54 year old male who presented with abdominal wall mass.

Abdominal CT-Scan and sonography detected a mass in lower abdomen between umbilicus and symphysis of pubis with minor attachment to bladder. Cystoscopy was normal.

Operation consisted of excision of mass and small part of bladder wall. Cut section of mass showed a cyst inside with tumoral lesion including necrosis around, surrounded by muscular and fatty tissues.

Microscopically the tumor was TCC with variable degrees and also sarcomatoid pattern in some areas. Smooth muscle of urachus and bladder wall were invaded by tumor but striated muscles, surgical margins and 2 detected lymph nodes were free.

It seems this is the first reported case of sarcomatoid carcinoma of urachus.

#### **P 387**

#### **HISTOPATHOLOGIC ASSESSMENT OF ANGIOGENIC RESPONSE IN DIFFERENT URINARY BLADDER LESIONS.**

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This study was designed to evaluate the angiogenic activity in chronic bladder lesions (chronic cystitis and schistosomal cystitis) in comparison to bladder tumors, as well as its relation to the stage of invasiveness of bladder carcinoma. The pattern and state of vascularization of bladder lesions could provide novel therapies using anti-angiogenic agents.

Methods: The current study included 75 cases of urinary bladder lesions representing 12 cases of chronic non-specific cystitis, 12 cases of schistosomal cystitis, 31 cases of transitional cell carcinoma (TCC), 13 cases of squamous cell carcinoma (Sqcc), 7 cases of urothelial dysplasia; in addition to 5 control cases of apparently normal bladders. All cases were first diagnosed and studied by hematoxylin and eosin. Then all were assessed for the expression of both basic fibroblast growth factor (bFGF); within epithelial cells, endothelial cells, fibroblasts and macrophages, and Factor VIII-related antigen (von Willebrand Factor, vWF); delineating endothelial cells at the wall of blood vessels, using a standard immunohistochemical technique. Positive expression for bFGF was scored as 3+, 2+ & 1+, while microvascular density (MVD) was calculated through counting the microvessels positively stained for Factor VIII-related antigen in five successive hot spots. Results:

Expression of bFGF and MVD were significantly higher in malignant bladder cases, especially in advanced stages, compared to chronic cystitis cases. Schistosomal cystitis cases showed marked angiogenic activity. Conclusion: High angiogenic response in non-invasive early stage of bladder cancer could predict recurrence and metastatic potentiality. Tumor invasive capability is directly correlated to the increase in its angiogenic activity. Marked angiogenesis in schistosomal cystitis could provide a favorable environment for tumorigenesis, thus they are commonly recorded in association.

#### **P 388**

#### **ADENOCARCINOMA OF THE URINARY BLADDER ASSOCIATED WITH SCHISTOSOMIASIS IMMUNOHISTOCHEMICAL PROFILE**

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#### **INTRODUCTION**

Primary nonurachal intestinal adenocarcinomas of the urinary bladder are frequently associated with chronic irritation of bladder mucosa and a background of intestinal metaplasia and/or intestinal type cystitis glandularis.

#### **PURPOSE OF THE STUDY**

The morphology and immunophenotype of 102 cases of bladder adenocarcinoma associated with schistosomiasis were reviewed, and special attention was paid to the presence of associated metaplastic or preneoplastic changes.

#### **MATERIAL & METHODS**

One hundred two partial or radical cystectomy specimens performed at the National Cancer Institute, Cairo, Egypt with the diagnosis of primary adenocarcinoma were studied. Biographic data and clinical information including clinical stage, type of therapy and follow-up were reviewed. The histopathology of tumor, pathologic stage and variation in cell type was recorded in all samples. Special attention was paid to the status of non-involved bladder mucosa for the presence of intestinal metaplasia and/or cystitis glandularis. Five representative paraffin blocks from each case were selected for immunohistochemical studies.

Four micron paraffin sections were stained by a standard labeled streptavidin-biotin method using antibodies against cytokeratin 7 and cytokeratin 20 (M7018 and M7019), p53 (M3566), HER-2/ neu (A0485) and Cathepsin D (A0561) [DakoCytomation, Carpinteria, CA], heat-induced antigen retrieval using citrate buffer in a vegetable steamer. DAB was used as chromogen in the presence of hydrogen peroxide. The presence, pattern and intensity of immunohistochemical reactions were evaluated in both tumor cells and in non-neoplastic epithelium.

#### **RESULTS**

The tumors occurred predominantly in trigone, lateral, or posterolateral walls. None of the tumors involved bladder dome or the anterior wall. The study cohort consisted of 64 male and 38 female patients (male-female ratio, 1.7:1.0) ages 25 to 78 (mean, 50.3 years). Most frequent presenting symptoms were hematuria and irritation. In all patients, partial or radical cystectomy was performed followed by radiation therapy. The clinical stage of disease and the follow up data are as follows; 73 patients (72%) presented with advanced disease (T3 and T4), 56 of whom (78%) were either dead from the disease or alive with cancer during the 36 month follow up period. Five patients with the predominant signet ring cell type adenocarcinoma were all dead of the disease in less than 15 months after the diagnosis.

#### **P 389**

### **PREVALENCE OF P53 AND KI-67 IMMUNOREACTIVITY IN CHRONIC URINARY BLADDER LESIONS**

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Early detection of alterations affecting p53 and/ or ki-67 control pathways will affect the clinical course of the disease and tumor progression.

The aim of this study was to investigate the expression of mutant p53 oncoprotein and the proliferative marker ki-67 in a series of non-neoplastic and neoplastic urinary bladder lesions with or without schistosomal infection using immunohistochemical staining on formalin-fixed, paraffin embedded sections. Its relation to traditional prognostic indicators (grades and stages) of malignant lesions was assessed.

Cystoscopic biopsies from (66) cases, received in the Pathology Department of TBRI were examined including; 22 cases of chronic cystitis (16 were schistosomal in etiology and 10 were associated with premalignant changes) and 44 cases of malignancy (34 cases of transitional cell carcinoma (TCC) and 10 cases of squamous cell carcinoma (SqCC)). Four normal controls were also added. Grades and stages of malignant cases according to World Health Organization Scheme were studied in sections stained with Hx.&E.. Scoring of the immunohistochemical nuclear expression of p53 and ki-67 for all cases was performed. Results were compared with those of control bladder specimens. Nuclear staining for p53 could not be detected in normal urothelium or in cases with cystitis (whether bilharzial or not), but it was expressed in presence of premalignant changes with significant over-expression in bilharzial versus non bilharzial cases. p53 over-expression accentuated in malignant lesions and correlated with upgrading of malignancy. Ki-67 was variably expressed in hyperplastic urothelium of cystitis cases, over-expressed in premalignant and malignant lesions with significant correlation to schistosomal association in malignant cases, to grade of tumor differentiation, and to p53 overexpression. Assessment of both markers can yield prognostic information that is more powerful than what behavior alone can provide.

### **P 390**

#### **E-CADHERIN AND EPITHELIAL MEMBRANE ANTIGEN EXPRESSION IN SCHISTOSOMAL URINARY BLADDER LESIONS.**

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Urothelial immunohistochemical tissue expression of the epithelial cell-to-cell adhesive molecules: E-cadherin and Epithelial Membrane Antigen (EMA) were assessed in 66 cases with chronic urinary bladder lesions (benign, premalignant, and malignant) with or without schistosomal infestation to estimate a possible role of schistosomiasis in disruption of these adhesive antigens with its consequent relationship to pathological prognostic features of bilharzial related bladder carcinoma versus non bilharzial one. Normal urothelium expressed E-cadherin and EMA as uniformly membranous immunostaining of high intensity, localized to epithelial cell membranes. The staining intensity of both E-cadherin and EMA declined in chronic non specific cystitis, with significant limitation in extent of positively stained urothelial cells in chronic schistosomal cystitis. In advance, both antigens were expressed in a heterogenous pattern with loss of staining in basal urothelial layers and its preservation in superficial cells in cystitis cases with dysplastic changes. In malignant cases, the extent and intensity of staining of both antigens significantly diminished with alteration of their membranous immunoreactivity into heterogenous staining

pattern and even cytoplasmic or nuclear accumulation which was more evident in T.C.C than in Sq.C.C cases. Upgrading and invasiveness of malignancy correlated with changing pattern of expression of E.M.A and E-cadherin, and with loss of urothelial positivity extent and intensity of E-cadherin rather than EMA. Schistosomal association in malignant cases correlated with more diminution in staining intensity of both antigens, and with advanced alteration in their pattern of expression which may facilitate tumor invasiveness and explain presentation of schistosomal associated cancer bladder at advanced stages.

In conclusion, it is the altered expression of E-cadherin and EMA that reflect neoplastic urothelial process rather than percentage of stained cells or their staining intensity. Schistosomal cystitis progressively minimize EMA and E-cadherin secretion than do non specific cystitis with advanced alteration in their expression pattern when complicated by malignancy process versus non schistosomal related bladder malignancy. Assessment of E-cadherin and EMA immunoreactivity may be a useful histologic prognostic marker in schistosomal cystitis and schistosomal related cancer bladder.

### **P 391**

#### **COMPARATIVE PATHOLOGIC ANALYSIS OF RENAL NEOPLASMS DEVELOPED IN PATIENTS WITH ERDS AND BACKGROUND RENAL CHANGES**

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KRISHNAN Bhuvanewari, TROUNG D. Luan

#### **INTRODUCTION:**

Patients with end-stage renal disease (ESRD) show increased incidence of acquired cystic kidney disease (ACK) and development of renal tumors. In a recent study demonstrated prominent calcium oxalate (CO) deposition in these carcinomas.

#### **PURPOSE:**

Complete phenotypic and immunologic profiling of benign and malignant neoplasm and determination the extent of CO deposition in the tumors and parenchyma.

#### **METHODS:**

We performed a retrospective analysis in a 13-year period to identify ACK-associated renal neoplasms. We compared the main pathologic features, immunologic profile and proliferative rate of the malignant and benign tumors. The severity of CO deposition was semiquantitated (0-4+) under polarizing microscope. RCC Marker, CD10, EMA and kidney specific cadherin (KSC) stains were performed and the intensity was graded (0-4+). The number of proliferating cell was determined by Ki-67 in 10 high power fields.

#### **RESULT:**

We identified 30 renal cell carcinomas with background changes of ESRD. Two histologic appearances were dominant: clear cell carcinoma (13, 43.33%) and oxalate type (8, 26.67%). We also identified 4 papillary (13.3%), 1 chromophobe (3.33%) and 1 sarcomatoid carcinoma (3.33%). Three tumors were necrotic and were not classified in either group. CO deposition was present in 8 neoplasms. Immunologic profile of the clear cell and the 3 necrotic tumors, which probably represented also clear cell carcinoma judged by the residual morphological features, showed EMA positivity (>3+; 76.92% and 100%, respectively); oxalate and sarcomatoid type had dual expression of RCC marker (>3+; 62.5% and 100%, respectively) and CD10 (>3+; 100% both). Papillary carcinoma expressed strongly EMA (4+; 100%) and had and intermediate CD10 intensity (2+; 100%). Chromophobe type displayed a combined expression of EMA and KSC (4+ each). No increased mitotic activity was detected. In the kidneys we identified 21 adenomas: one necrotic and 20 with either oxalate (8, 38.09%) or papillary phenotype (12, 57%). CO was identified in 4 cases, all associated with oxalate phenotype. Cystic changes were

present in all kidneys and combined with CO crystals in 11 cases.

#### CONCLUSION:

CO deposition in ACK-associated renal lesions is common. Distinct, recently described tumor morphology is evident, which defies the current classification. Proximal tubular cell differentiation in conjunction with ESRD-mediated high serum level of CO may be important in the pathogenesis

#### P 392

#### MORPHOLOGIC, ULTRASTRUCTURAL AND HISTOCHEMICAL STAINING CHARACTERISTICS OF CHROMOPHOBE RENAL CELL CARCINOMAS

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Morphologic, ultrastructural and histochemical staining characteristics of Chromophobe Renal Cell Carcinoma

Introduction: Approximately 5% of renal cell carcinoma in surgical series are chromophobe renal cell carcinoma. It is rare in the first two decades of life. Although obesity, smoking, hypertension and exposure to industrial chemicals have been implicated as risk factors in the genesis. An association with von Hippel-Lindau disease is seen in occasional cases. Associations also exist with tuberous sclerosis and autosomal dominant polycystic kidney disease. The genetic hallmark of chromophobe renal cell carcinoma is the loss of multiple chromosomes.

Case: 70 years old male presented with complaint of hematuria and pain in the flank. Ultrasonography and computerized tomographic scan show a solid mass in the left kidney. The diagnosis was obtained of the hematoxylin and eosin (H&E), histochemical stained slides and ultrastructural findings.

Results: On gross examination a well-circumscribed, solid tan mass is identified. Microscopically, characterized by mosaic pattern composed of large and small cells with reticular cytoplasm, well-defined thick cytoplasmic membranes. The Hale's colloidal iron stain colors the cytoplasm blue. Ultrastructurally, the cytoplasm of chromophobe cells is crowded with many small vesicles.

Conclusion: The eosinophilic variant of chromophobe cell renal cell carcinoma was recognized. This variant shares the ultrastructural and colloidal iron staining features of the typical variant but in H&E stained slides often closely resembles renal oncocytoma. For this reason, it is worthwhile routinely to collect specimens for electron microscopy from renal tumors, especially those which do not have the yellow color characteristic of clear cell renal cell carcinoma.

#### P 393

#### YOLK SAC TUMORS : A REPORT OF 9 CASES

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SELLAMI Tahya

#### Introduction

A single institution review of epidemiological, clinicopathological and immunocytochemical features, and prognosis of yolk sac tumor

#### Materials and Methods

We report a retrospective study performed of 9 cases of yolk sac tumor accessioned at the pathologic anatomy and cytology laboratory of the University Teaching Hospital of Sfax between 1983 and 2003.

#### Results:

Presentation occurred at a median age of 18 months (range 8 months to 24 months). The interval between children's first

symptoms and diagnosis was 2,2 months (range 1 to months). Clinical presentation occurred most often (88, 88%) with testicular lump. Seven patients had stage I and two patients had stage II according to the Baden classification.

The anatomopathological study confirmed the diagnosis. The germinal testicular tumor presented a structure that showing the yolk sac of the human embryo. Two pathognomonic histological signs were most often presents (66, 66): the Schiller Duval body and hyaline globules. The immunohistochemical study showed that this tumor over expressed the  $\alpha$ -fetoprotein (AFP) in 100% of the cases. The radical orchidectomy was indicated in all cases. It was associated with chemotherapy in advanced tumors.

Prognostic factors included age, clinical stage, rate of AFP, vascular or lymph node involvements, local extension, tumor's size, proliferated molecular factors = AND1's index, proliferated cellular nuclear Antigen PCNA, ki 61 and molecular genetically factor = oncogen hst-1

#### Conclusion:

The testicular tumors in pre-pubertal children and especially the yolk sac tumors have an excellent prognosis the overall 5 years survival range 95 to 100%.

Prospective study will be effective to establish a therapeutic consensus between different equipments.

#### P 394

#### TWO UNUSUAL SITES OF SOLITARY FIBROUS TUMOR (SFT)

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#### INTRODUCTION

Solitary fibrous tumor is a rare spindle-cell neoplasm of uncertain histogenesis and with potentially aggressive behaviour. It usually arises on serosal surfaces with infrequent involvement of other sites. Renal and paratesticular SFTs are extremely rare and only 9 intrarenal, intrapelvic and parapelvic and 3 paratesticular cases have been reported in the international literature respectively.

#### AIM

Two cases of SFTs, one large renal and one paratesticular are reported with review of the literature and emphasis on the differential diagnosis from other mesenchymal or epithelial spindle cell neoplasms.

#### MATERIALS - METHODS

Case 1. A 79 years old man was operated for an asymptomatic solid tumor of the left kidney at CT-scan. The tumor was 12 cms in greatest diameter, well circumscribed, round, with smooth surface, firm consistency, whorled appearance and grey-white in colour with hemorrhagic areas without involvement of the pyelocalycic system.

Case 2. A 27 years old male had a palpable, nodular, painless left intrascrotal mass. At operation, a well circumscribed 3 cms binodular tumor between the tunica vaginalis and the dartos was removed. Sections were examined with H+E, PAS, PAS-D and the immunophenotype was determined with the Envision-HRP method for the detection of MCK, Vimentin, Desmin,  $\alpha$ -SMA, CD31, CD34, CD99, CD68, Bcl-2, HMB-45, GFAP, NSE, NF, Ki-67 and C-kit.

#### RESULTS

Both tumors were encapsulated, non-invasive, composed of spindle cells with storiform, diffuse or fascicular growth pattern of varying cellular density and scattered wide collagen bundles. The renal tumor at the centre was vascular with loosely arranged cells and an atypical mitosis. Additional features: myxoid areas with scattered multinucleated cells, a marked chronic perivascular inflammation and focal

hyalinization of the vascular walls. The neoplastic cells of both tumors were Vimentin, CD34 and CD99 positive. Bcl-2 expression was either diffuse in the paratesticular SFT or reduced/absent in the central vascular area of the renal SFT. NSE and CD68 focal positivity was observed in the paratesticular SFT. Ki67 <2% and <2 mitoses per 10 HPF.

#### CONCLUSIONS

SFT has a characteristic growth pattern and immunophenotype (Vimentin, CD34, CD99, Bcl-2<sup>+</sup>) which enables differential diagnosis from other tumors of fibroblastic, myofibroblastic or epithelial origin. Follow-up, even after complete excision is recommended as it may recur or even metastasize regardless of cellular atypia.

#### P 395

##### MYCOPLASMOSIS OF MALE UROGENITAL TRACT IN IRAN

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Mycoplasma are the smallest bacteria capable of growth in artificial media. Their contribution to male urogenital infections and infertility are lesser known. The most common complaints of male patients who refer to urologists include urogenital tract irritations and infertility problems. Although the cause of these complications are very diverse and multifactorial, urogenital infections with Mycoplasma and Ureaplasma are among the known etiological factors. Very little attention has been paid to these infections in the male patients in Iran. A total of 302 clinical samples including semen, urethral secretions, and first urine drops were enriched and cultured for Mycoplasma. We also sought to compare the isolation rate for the bacteria by the filtration (0.45 µm pore size) versus non-filtration (multi-antibiotic use) method of enrichment. The bacteria were cultured from 27 of these samples. Of these, 11 were contaminated with Mycoplasma hominis, 9 with Ureaplasma urealyticum, and the remaining 7 individuals were co-infected with both of these bacteria. Six percent of the sperm secretions, 10% of the first urine drop samples, and 11% of the urethral discharges were culture positive. Co-infections were seen only in the first urine drops. Overall, Mycoplasma and Ureaplasma were cultured in 8.9% of the male samples collected. This points to the relatively high prevalence of mycoplasmosis among sexually active male population in Iran. It also emphasizes a probable detrimental role mycoplasmosis plays in the overall quality of sperm cells.

#### P 396

##### MOLECULAR AND IMMUNOHISTOCHEMICAL STUDY OF CELL CYCLE REGULATORS IN URINARY BLADDER TUMORS

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**INTRODUCTION:** The optimal management of patients with bladder neoplasms requires the assessment of multiple predictive markers in addition to classical clinical and pathologic (grade and stage) factors. **PURPOSE:** Our main goal was to quantify diverse molecular and immunohistochemical (IHC) markers in order to accurately predict the clinical evolution of these patients in terms of tumor recurrence, progression and survival. **METHODS:** Clinical and pathologic data from 84 patients were collected including all tumor grades and stages. DNA from all these cases was analyzed for the presence of loss of heterozygosity (LOH) of the 9p21 locus; homozygous deletions (HD) and

promoter methylation of the p14ARF, p16INK4A and p15INK4B genes; gene amplification of the MDM-2, CCND1 and CDK4; and P53 mutations; by means of diverse PCR-based techniques. Paraffin-embedded material from the cases was also studied at the IHC level by means of p53, Ki-67 (MIB1), p14, p16, p21, p27, pRb, MDM-2, and cyclin D1 antibodies using the avidin-biotin peroxidase procedure. Statistical analyses were performed with a specific software program package (STATISTICA version 6.0, Statsoft Inc, USA). **RESULTS:** In univariate studies, HD of the p14ARF, p15INK4A, and p16INK4B genes showed a statistical correlation with recurrence prediction; whereas CDK4 and CCND1 amplifications together with Ki-67 labeling index (LI), p27, p53, p16 and pRb immunoreexpression were statistically associated with tumor progression. Likewise, LOH of the 9p21 locus along with p14, p53 and cyclin D1 protein expression showed strong statistical correlation with relapse-free survival (RFS). In multivariate studies, tumor stage and Ki-67 LI appeared to be independent prognostic factors in relation to overall survival, whereas HD of the 9p21 locus was related to RFS. **CONCLUSION:** Three factors demonstrated in the present study independent prognostic significance: clinical tumor stage, overexpression of Ki-67 antigen and HD of the 9p21 locus.

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#### P 397

##### HEAVY EOSINOPHILIC INFILTRATE OF THE BREAST

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**Introduction:** Significant eosinophilic infiltrate of the breast is encountered in eosinophilic mastitis, granulomatous mastitis, Churg-Strauss syndrome, Crohn's disease, parasitic infection and rarely in carcinoma.

**Aim:** Our aim is to identify cases of breast reported with heavy or significant eosinophilic infiltrates.

**Materials & Methods:** Seven cases were identified after reviewing all breast cases reported during the period of 1999-2005. Three cases diagnosed as granulomatous mastitis, one eosinophilic mastitis, two ductal carcinoma and one gynecomastia. The ductal carcinoma cases exhibited florid eosinophilic infiltrates with periductal fibrosis and destruction. One case associated with prior history of herbal medication.

**Conclusion:** Significant eosinophilic infiltrate is identified in cases of mastitis and rarely in ductal carcinoma. Although eosinophils can degranulate and release toxic inhibiting factors into tumours, they are uncommonly encountered in breast carcinoma.

#### P 398

##### FEMALE MALIGNANT BREAST TUMORS IN CAMBODIA

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**Background:** Little is known about breast cancer in Cambodian women living in Cambodia.

**Objectives:** To describe the various histological types of invasive breast cancers, their frequency, grade and stage.

**Methods:** We selected all breast biopsies and surgical specimens that were processed during 2003 and 2004 in our newly set up laboratory in Phnom Penh. Tissue samples were embedded after 10% formalin fixation and sections were stained by H&E. Pathology reports were written according to international recommendations. Carcinomas were graded

using the Nottingham modification of the Bloom-Richardson system and staging was established according to the 2003 TNM classification. Detection of hormone receptors by immunohistochemistry was not available in Cambodia at this time.

Results: A total of 107 ladies from Phnom Penh and different provinces in Cambodia, aged 16 to 76, presented with malignant tumors of whom 12 (11.2%) had biopsy then surgery, 81 (75.7%) directly had surgery and 14 (13.1%) solely had biopsy. Lesions included 96 (89.7%) carcinomas, nine (8.4%) Phylloides tumors and two (1.9%) lymphomas. The mean age of patients with carcinoma was 48.2 (23-76). Among these patients, invasive ductal type was the most frequent, identified in 79 ladies (82.3%) and high histological grade was found in 74.7% of cases. Radical surgery was performed in 63 of the 79 affected patients and proportions of T1, T2, T3 and T4 were respectively 9.6%, 41.2%, 15.9% and 33.3%. Lymphadenectomy was performed in 31 of 63 patients (49.2%) and lymph node metastases were observed in 26 of 31 (83.9%). Medullary carcinomas accounted for 11.4% (11/96) of the ladies, whose mean age was 46.4 (40-59). Metaplastic, papillary, mucinous and mixed histological types of carcinoma were rare and no case of invasive lobular carcinoma was diagnosed.

Conclusion: Our findings demonstrated a high frequency of medullary carcinomas and malignant phylloides tumors in Cambodian ladies. In contrast, no case of lobular carcinoma was found. The fact that most of our cases were at late stage of the disease, may suggest that early detection of the disease should be strengthened in Cambodia. In addition, standardized surgical procedures should be established so that lymphadenectomy, for instance, is systematically performed at the time of tumor excision.

#### P 399

##### **TUMOUR GRADE AND TUMOUR MARKER EXPRESSION IN INVASIVE BREAST CARCINOMA WITH INTRA-DUCTAL COMPONENT.**

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Introduction: Ductal carcinoma in-situ is regarded as a precursor to invasive breast cancer. Trying to find steps in this progression, we compared the grade and expression of some tumour markers (ER, PR, c-erb-B2, MIB-1, P53 and Bcl-2) of both components in 74 cases of invasive carcinoma with an intra-ductal component. Materials and methods: Both components (intra-ductal and invasive) were classified independently according to the guidelines of the European Commission Working Group on Breast Screening Pathology. The results of the staining were analyzed in the intra-ductal and in the invasive components separately. We worked with the cut-off limits generally accepted.

Statistical analysis: For comparison between the two components the chi-square test was used. Statistically significant differences were assumed when  $P < 0.05$ .

Results: 1) Tumour grade - a): high grade of the intra-ductal and grade of the invasive component: in 28% cases the invasive component was grade 3; in 57% cases the invasive component was grade 2 and in 14,3% the invasive component was grade 1; b) intermediate grade plus low grade of the intra-ductal component: in 34% cases the invasive component was grade 2; in 56,6% the invasive component was grade 1. Only in 5 cases (9,4%) the invasive component was grade 3. 2) ER expression: in 62,2% cases both components were ER+ and in 27% cases both components were ER-. 3) PR expression: in 21,6% cases both components were RP+ and in 66,2% both components were RP-. 4) c-erb-B2: in 17,6% cases c-erb-B2 was over-expressed in both components; in 77% cases c-erb-B2 was negative in both components. In no one case we observed c-erb-B2 negative in the intra-ductal component and

over-expression in the invasive component. 5) MIB-1: in 12,2% cases MIB-1 was high in both components but in 18,9% cases its expression move on to low in the invasive component; in 64,9% cases MIB-1 was low in both components. 6) P53 expression: in 66,2% cases both components were P53+ and in 10,8% cases P53- in both components. 6) Bcl-2 expression: in 27% cases Bcl-2 both components were Bcl-2+ and in 63,5% cases both components were Bcl-2-.

Conclusion: These results allow us to conclude that the step between in-situ and invasive carcinoma, seems to occur independently of tumour grade. The tumour markers that we studied were not clearly associated with the progression from in-situ to invasive; the expression of these tumour markers are almost identical in the two components.

#### P 400

##### **ROUND/OVAL SHAPE IN BREAST LESIONS. A MARKER OF BENIGN LESION? CYTO-HISTOLOGICAL STUDY OF 544 CASES FROM THE VERONA BREAST SCREENING PROGRAM.**

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Introduction: The round/oval shape is a radiological feature suggesting a diagnosis of benign breast lesion.

Purpose: To evaluate the pathologic and mammographic correlation of round/oval-shaped breast lesions.

Methods: Between 1999 and 2004, we studied the cytological and histological features of breast lesions with ultrasonographic and/or mammographic circular shape within the Verona Breast Screening Program (BSP).

Results: Out of 544 round breast lesions, 125 (22,9%) were malignant and 419 (77,1%) benign. Malignant lesions were all invasive; histotype was ductal: 105 cases (84%), lobular: 13 cases (10,4%), mucinous: 4 cases (3,2%), medullary: 2 cases (1,6%), tubular: 1 case (0,8%). The TNM of malignancy was pT1: 102 cases (81,6%); pT2: 18 cases (14,4%); pT3: 2 cases (1,6%) and pT4: 3 cases (2,4%).

Nodal status, available in 98% of cases, was N+: 24.4% and N-: 75.6%. All benign lesions were investigated with cytology and so stratified: fibroadenoma: 188 (44,9%); cyst: 53 (12,6%); benign lesion, NAS: 54 (12,9%); fibrocystic change: 32 (7,6%); steatonecrosis: 17 (4%); adenosis: 15 (3,6%); adipose nodule: 12 (2,9%); papilloma: 7 (1,7%); intramammary lymph node: 5 (1,2%) and other (focal fibrosis, granulomas, abscess): 36 (8,6%). Surgery was performed in 14 cytological benign lesions; histology was benign in 9 cases, atypical in 5.

Conclusion: In our series, round/oval-shaped breast lesions were malignant in 23% of cases. The most frequent histotype was infiltrating ductal cell carcinoma. The most common benign lesion was fibroadenoma. From our data we conclude that follow-up is not adequate in breast lesions with round-oval imaging, but further investigations are necessary. (Supported by Fondazione Cassa di Risparmio VR-VI-BL-AN. Bando 2004. Progetto Carcinoma Mammella)

#### P 401

##### **BREAST SCREENING PROGRAM IN VERONA. CYTO-HISTOLOGICAL FINDINGS OF FIVE YEARS OF ACTIVITY AND COMPARISON WITH OTHER SCREENING PROGRAMS IN EUROPE.**

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Breast Screening Program (BSP) for women with age comprised between 50 and 69 years started in Verona in

1999. Compared with the "classic" screening, the Verona program has a different organization, similar to that of a Breast Clinic, and operates in a building exclusively devoted to the BSP. First level mammograms and all second level exams (US, FNAC, core biopsy, mammotome) are performed in the same structure by a staff composed of two radiologists, two cytopathologists, four technicians and two nurses. All women are submitted to a double projection mammography; the Radiologists perform real time interpretation of mammograms. Results are immediately communicated. Negative patients are dismissed after reassurances; suspicious patients are immediately submitted to second level examination. FNAC is evaluated for adequacy and a diagnostic report is immediately given to the Radiologist. Core-biopsy and mammotome specimens are centrally collected in Pathology Department of University of Verona. Result of histological exams are communicated within two days. Results: In the first five years of activity (1999-2004) 55.000 women have been evaluated by screening. The participation rate was 61% in initial screening, 89% in subsequent screening. Among 513 cases sent to surgery, 424(83%) were malignant, 24(5%) atypical hyperplasia, 65(12%) benign. The ratio benign vs. malignant was 0.18. The detection rate for cancer was 9.6% in the first round and 4.9% in the following rounds. The pre-operative diagnosis of malignancy was done in 85% of cases with FNAC and in 15% of cases with core biopsy/mammotome. The TNM of malignancy was pTis: 23.4%, pT1:63.9%, pT2:10.3%, pT3:0.9%, pT4:1.4%. Nodal status was N+:21% and N- :79%. Surgery was conservative in 80% with 20% mastectomy. TNM(M0) staging was St0:23.4%; StI:54.5%, StII:15.3%, StIII:6.8%, StIV:0. Interval cancers rate was 8.6%.Conclusions: Compared with other screening program, the results of the Verona screening program are all within the preferred results of the European guidelines. The peculiar organization of the program, with its centralized integrated radio-pathological activity seems a major factor for a successful screening program.(Supported by Fondazione Cassa Risparmio VR-VI-BL-AN.Bando 2004. Progetto Cr. Mammella)

#### P 402

##### MALE BREAST CARCINOMA : A CLINICO-PATHOLOGICAL STUDY OF 21 CASES

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##### Introduction:

Breast carcinoma rarely occurs in males and information regarding this pathology are limited. The aims of this study were to document epidemiological, clinico-pathological and immunocytochemical features of this cancer and to establish which factors would predict prognosis.

##### Materiels and Methods:

A retrospective study was performed of 21 cases of male breast carcinoma accessioned at the pathological anatomy and cytology laboratory of the University Teaching Hospital of Sfax between 1992 and 2003.

##### Results:

The patients' mean age was 65,4 years. The average duration of signs and symptoms before the diagnosis was 10,4 months. Presentation occurred most often (85,7%) with self-detected lump. Clinically the majority of the cases presented advanced cancers. Three patients had stage 0, 5 had stage II, 4 had stage III, 9 had stage IV disease. Histology found: 13 canalar infiltrating carcinoma, 3 neuroendocrin carcinoma, 1 papillary carcinoma and 4 ductal carcinoma in situ. Four Paget's disease were diagnosed. The histological grad of SBR was precised: 2 patients had grad I, 8 had grad II, 7 had grad III. Lymph node involvement was found in 52,3% of the cases. Immunohistochemically data were reviewed: 65% of tumors were oestrogen receptor positive, 80% were progesterone

receptor positive, 23,5% over expressed CerbB2, 40% over expressed Bcl2 and P53 and 15% exprimed vimentin. The overall 5 years corriged survival was 61,9%. The disease stage, high histological grad, lyph node involvement and the over expression of the biological biomarkers (receptor of oestrogen, P53, , CerbB2, vimentine) were correlated with poor prognosis.

##### Conclusion:

The poor prognosis of male breast cancer is related to older age at diagnosis in advanced stage of disease at presentation.We find that to get better prognosis, it is important to improve medical and technical means, to increase information and to promote early detection.

#### P 403

##### PRELIMINARY ANALYSIS OF THE HISTO-PROGNOSTIC POWER OF A HETEROGENEITY INDEX FOR BREAST CANCER

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In 1998, the Kananaskis Tumour Heterogeneity working group conjectured that there should be a correlation between the level of intratumoral heterogeneity and the seriousness of a carcinoma. This group emphasized the need for a reproducible and quantifiable measure of heterogeneity to test this hypothesis.

In this paper, we use a recently developed Intratumoral Spatial Heterogeneity Index (ISHI) complying with the Kananaskis group requirements. This index has been shown to be very useful in characterisation of breast cancer tumours. In particular it was shown that the ISHI for various blocks of a single tumour was invariable, regardless of the fact that various blocks differ in size and in number of marked cells. We present in this paper our preliminary results about the histo-prognostic ability of the ISHI.

The ISHI has been derived on a set of 78 blocks coming from 56 breast carcinoma patients and labelled by monoclonal anti-MIB-1 marker. A complete analysis of the ISHI and its correlation with SBR grade assigned by a pathologist has been conducted and some interesting results are obtained. The analysis shows clearly that blocks coming from patient with a SBR grade I, have also low level of heterogeneity (ISHI close to 1). ISHI separates well (as validated by a statistical test) SBR grade I from grade II and III, but grade II and III are not well separated.

We conducted a survival analysis of a sub-sample of 37 breast carcinoma patients with 10 to 12 years of follow-up, to evaluate the histo-pronostic value of the ISHI. For this purpose we divided the set of 37 patients into three equal size sets containing patients with decreasing value of ISHI; group 1 with low heterogeneity (ISHI between 1 and 0.9), group 2 with medium heterogeneity (ISHI between 0.9 and 0.82), group 3 with large heterogeneity (ISHI from 0.82 to 0.67). We therefore compared the survival curve of the 3 group. It appears that no mortality has been reported in group 1 (with low heterogeneity) and that the survival curve of the group 3 is clearly below the group 2 and 1 (as validated by a logrank statistical test). This could be seen as a preliminary validation of the histo-pronostic value of the ISHI.

Even if the number of samples used in this study is not enough to reach to a definitive answer, however this gives some indications that motivate us to conduct a larger scale survival study.

#### P 404

### **SPORADIC INVASIVE CARCINOMAS OF THE BREAST WITH MEDULLARY FEATURES SHOW BASAL-LIKE CHARACTERISTICS.**

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**INTRODUCTION:** Tumours from women with germline BRCA1 mutations have a basal-like phenotype: estrogen receptor (ER) negative, HER2 negative, cytokeratin 5 (CK5) and/or epidermal growth factor receptor (EGFR) positive. Moreover, they show high cyclin E expression and frequently show medullary features.

**AIMS:** To investigate whether or not atypical medullary sporadic breast carcinomas have a basal-like phenotype.

**MATERIAL and METHODS:** We studied a group of 39 sporadic invasive breast cancers that displayed features of atypical medullary carcinoma according to the 2003 WHO criteria, and a series of 76 consecutive sporadic breast tumors (as a control group). They were analysed immunohistochemically for ER, PR, p53, Her2neu, ki-67, cyclin E, CK5 and EGFR expression. Cyclin E alterations were also analysed by fluorescence in situ hybridization (FISH). Both studies were performed on paraffin-embedded tumours included in tissue-microarrays (TMA).

**RESULTS:** In the medullary carcinoma group, 100% and 92,3 % of the cases were negative for Her2neu and ER whereas 28,2%, 61,5% and 66,7% were positive for EGFR, CK5/6 and cyclin E, respectively. The three ER positive cases were negative for both CK5/6 and EGFR. Interestingly, we observed that cyclin E gene was amplified in 15,4% of the cases. On the other hand, in the control group 76,3% and 22,3% of the cases were negative for Her2neu and ER, respectively. We found positivity for CK 5/6 and EGFR in 8 (10,5%) and 11 (14,5%) cases, respectively but only 5 (6,6%) cases fulfilled all the criteria to be included in the basal-phenotype group. Cyclin E was overexpressed in 36,8% of the cases but no gene amplification was found.

**CONCLUSION:** These data indicate that as well as in BRCA1-mutated tumours, sporadic invasive breast cancers with medullary features show the characteristics of the basal phenotype. Interestingly, medullary breast tumours show a high percentage of cyclin E gene amplification, a fact not previously been reported in breast tumours.

#### **P 405**

### **WIDESPREAD BREAST CANCER METASTASES TO THE UTERUS AND LEIOMYOMA - REPORT OF TWO CASES**

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### **WIDESPREAD BREAST CANCER METASTASES TO THE UTERUS AND LEIOMYOMA - REPORT OF TWO CASES**

**Background:** Breast carcinoma is the most common malignancy in the female population. Frequent sites of metastases include lymph nodes, liver, lung and bone. Widespread gynecologic and leiomyoma metastases from breast carcinoma is a well-known but extremely rare phenomenon and had only been reported sporadically in the literature.

We present two cases of gynecologic and leiomyoma metastases from invasive ductal and lobular carcinoma of the breast respectively. Immunohistochemical studies including CEA, estrogen, progesteron, C-erb-2 and human milk fat globulin was performed for differential diagnosis.

**Case I:** An 62-year old multigravid woman with a history of breast cancer and tamoxifen therapy presented with abnormal uterine bleeding. After the diagnosis of malignancy was made on cervical smear, biopsies were taken from cervix and endometrium, which revealed an infiltrating moderately differentiated ductal adenocarcinoma consistent with breast metastasis. A total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymph node sampling were performed. The uterus measured 10x6x4 and it was markedly distorted with multiple gray-white cervical, intramural and endometrial tumor nodules with the maximum diameter of 3 cm. Final pathology examination revealed metastatic ductal carcinoma of the breast including vagina, cervix, endometrium, myometrium and fallopian tubae.

**Case II:** A 49-year-old multigravid woman was admitted to the hospital with the complaint of uterine bleeding and abdomino-pelvic pain. Her gynecologic history was unremarkable, however, 4 months previously, she was underwent a right sided modified radical mastectomy for invasive lobular carcinoma. At the time of admission, her physical and genital examinations showed no abnormalities except multiple leiomyomas. After the vaginal hysterectomy and bilateral salpingo-oophorectomy; microscopic examination revealed metastatic foci of invasive lobular carcinoma in the leiomyoma and ovary

**Conclusion:** Metastases to the female genital tract from extragenital cancers are uncommon. It should be kept in mind that abnormal uterine bleeding may be the first sign of metastatic disease in a woman with a history of malignant disease as in our cases.

#### **P 406**

### **IMMUNOHISTOCHEMICAL EXPRESSION OF P21 WAF1/CIP1, BAG-1, BAX, MCL-1 PROTEINS IN BREAST CARCINOMAS. CORRELATION WITH PROGNOSTIC FACTORS**

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**Introduction** Identification of apoptosis factors and cell proliferation carries valuable information about specific features of tumor clinics, treatment sensitivity and survival of patients. Cell cycle progression is governed by cell cycle regulators and inhibitors such as cyclin dependent kinases, p21 WAF1/Cip1, BAG-1, BAX, and Mcl-1.

**Material and methods** Immunohistochemical analysis for estrogen/progesterone receptors (ER/PR), c-erb B2, p21 WAF1/Cip1, BAG-1, BAX, Mcl-1 was performed on formalin-fixed infiltrating ductal (81%) and lobular (19%) breast carcinomas of 64 patients with

Ö1-2 N0 M0 G2-3. They were undergone mastectomy and chemotherapy therapy taking into account c-erb B2 expression. Two groups of patients were studied in terms of survival. The first group with more than 3 years survival (53), the second – less than 3 years (11).

Results The tumors of the first group of patients had positive reaction ER/PR in 83% cases, c-erb B2 in 18%, p21 WAF1/Cip1 – 75% (tumor having more than 20% staining cells were considered to be positive), BAG-1 and BAX in 100% in c-erb B2 positive tumors and 25% in c-erb B2 negative tumors, Mcl-1 in 20% in c-erb B2 positive tumors and 36% in c-erb B2 negative tumors. The tumors of the second group patients had positive reaction ER/PR in 38% cases, c-erb B2 in 25%, p21 WAF1/Cip1 – 83%, BAG-1 and BAX in 30%, Mcl-1 in 20%.

Conclusion Higher expression of BAG-1, BAX, Mcl-1, as well as ER/PR, indicates good prognostic factors of survival.

#### P 407

##### **INCREASED LEVEL OF FATTY ACID SYNTHASE AND PROGESTERONE RECEPTOR PROTEINS IN PREMALIGNANT MAMMARY LESIONS AND IN SITU CARCINOMA**

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While progestins increase the risk of breast cancer after menopause (the WHI trials 2002 and 2004), the mechanism of this effect is unclear. One approach is to estimate the level of proteins related to progestin action in different mammary preinvasive lesions compared to adjacent normal mammary glands, in an attempt to define at which stage of carcinogenesis progestin action could be increased. We previously found that Fatty acid synthase (FAS) was overexpressed in invasive breast cancer and induced by progestins and growth factors. FAS was then described by others as corresponding to a mammary and prostate nutritional oncogene.

We now estimate by immunohistochemistry, FAS and progesterone receptor (PgR) expression in “benign” breast disease (BBD) of increasing histological risk according to Dupont and Page (non proliferative, proliferative without atypia, proliferative with atypia) and in situ breast carcinomas (of lobular and ductal subtypes). This study was performed in archive paraffin embedded tissues from 116 patients with 164 BBD and in situ carcinomas, compared to their adjacent normal ducts and lobules, using a rabbit polyclonal FAS antibody (raised by D. Chalbos in our laboratory) and a PgR monoclonal antibody (clone PgR636-Dako). The expression level of these markers was quantified by computer image analyser. A statistical analysis was performed to compare staining level in normal glands to that in the different lesions and to correlate FAS and PgR levels with MIB-1 proliferation index and clinical parameters (age, menopausal status and hormone therapies).

Results will be given showing at which premalignant lesion the expression level of these two markers was found to be significantly increased.

#### P 408

##### **THE STATUS OF C-ERBB-2/HER-2/NEU AND EXPRESSION OF E-CADHERIN IN INVASIVE LOBULAR AND DUCTAL CARCINOMAS OF THE BREAST**

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Introduction. E-cadherin (E-CD) is considered to be the most important cell adhesion molecule in mammary gland. Some studies suggest that downregulation of E-CD and subsequent loss of cellular adhesiveness correlate with poor prognosis and metastasis but this is not confirmed by other studies. Many investigations suggest that E-CD protein is not expressed in invasive lobular (ILC) in comparison with invasive ductal carcinoma (IDC) of the breast. A few papers report that E-CD mediated cell adhesion system can be disrupted by oncoprotein c-erbB-2/HER-2/neu in c-erbB-2-positive breast carcinomas despite ductal or lobular type. Purpose of study. To evaluate the relationship between expression of HER-2/neu and E-CD in ILC and IDC and analyze an association with lymph node positivity. Methods. We reviewed 106 cases of breast cancer: 91 IDC and 15 ILC including 3 pleomorphic lobular carcinomas (PLC). All cases were examined in our laboratory for suspicion for c-erbB-2 overexpression. Nottingham histologic grade, immunohistochemical staining for estrogen and progesterone receptors (ER and PR), proliferating cell nuclear antigen (PCNA), E-CD; fluorescence in situ hybridization for HER-2/neu gene amplification; and lymph node positivity were evaluated. Results. HER-2/neu gene amplification was observed in 60.5% of IDC and 26.7% of ILC including one case of PLC and positively correlated with higher histological grade and lymph node positivity. Strong positivity for PCNA was observed in 84.2% of IDC and positively correlated with histological grade and HER-2/neu positivity. IDCs were negative for ER in 50%, and PR in 57.8% of cases. ILCs were negative for ER in 40%, PR in 46.7% of including one case of PLC. ER/PR-negativity was associated with histological grade, HER-2/neu gene amplification and lymph node positivity. Majority (80%) of ILC including two cases of PLC showed negative staining for E-CD. E-CD expression was lost in 10 cases (26.3%) of 38 IDCs and positively correlated with histological grade, HER-2/neu gene amplification and lymph node positivity. Conclusion. The loss of E-CD expression can be a feature of some typical invasive ductal carcinomas of the breast. E-CD negativity seems to be associated with higher histological grade, HER-2/neu gene amplification and lymph node positivity suggesting that c-erbB-2 may act as a regulator of E-CD expression in most human breast carcinomas in vivo.

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#### P 409

##### **PATHOLOGICAL AND IMMUNOPHENOTYPICAL RESPONSE TO TRASTUZUMAB THERAPY IN HER2 POSITIVE BREAST CANCER. ARGUMENTS IN FAVOR OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC).**

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Background: Treatment with trastuzumab (T) has shown excellent clinical response in patients with HER2-overexpressing breast cancer (BC). However, the exact mechanisms of action of T has not been fully characterized and appears to be complex. The objective of this study was to determine, in vivo, whether the immune response is a mechanism of action of T in human BC.

Material and Methods : 23 patients with BC characterized as 3+ HER2 by immunochemistry (IHC) were enrolled to receive T + docetaxel (D) primary systemic therapy (PST) as

part of the 'TAXHER01' trial. Following surgery, they were 9 (40%) pathological complete response (pCR). The remaining 14 BC displayed partial (N =13) or no (N=1) pathological response. Additionally, 23 BC treated with D-containing PST, and 23 BC treated with anthracycline-containing PST were analyzed as non-T groups. Each cases of the 2 control subgroups was chosen to be matched (same pathological response) with those of the case group. Of these 69 surgical specimens, residual tumors areas or pCR areas were blindly analyzed by morphology and IHC. B-lymphocytes were stained with anti-CD20. T-lymphocytes were stained with CD3, CD4 and with CD8. Macrophages were stained with CD68. Dendritic cells were stained with PS100 and CD1a. Natural Killer (NK) cells were stained with CD56 and with NK1. Potential cytolytic cells were stained with Granzyme B and with TIA-1. HER2 status was also analyzed by IHC on residual tumor cells. These analyses were performed by 2 independent pathologists.

**Results :** All residual tumor cells from the cases group were HER2 positive on IHC and there were no differences in HER2 status before and after T PST in the case group. The number, topography, and activation status of various type of immunocompetent cells were generally different between the case group and the two control groups. Significantly more NK cells were in contact with tumor cells in the case group than in controls. In addition, there was significantly greater expression of Granzyme B and Tia1 by lymphoid cells in the case group than in controls.

**Conclusion :** This study provides evidence in favor of the immune response as one of the mechanisms of action of T in BC. NK cells in particular, that are able to mediating antibody-dependent cell toxicity against antibody-coated cells via their expression of FcγRIII, seemed to play an important role. This finding may have further implications for the management of T treatment in BC.

**P 410**  
**IMMUNOHISTOCHEMICAL DETECTION OF ERBB RECEPTORS OVEREXPRESSION IN HUMAN BREAST CARCINOMAS**

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**Abstract**

ErbB receptors are expressed in various tissues, in which they are involved in the control of diverse biological processes such as proliferation, differentiation, migration and apoptosis. Cerbb-2 overexpression has been implicated in many types of human cancers and is associated with poor prognosis.

The aim of our study is to identify the expression of cerbB-3, cerbB-4 and cerbB-2 in human breast carcinomas.

**Materials and Methods:** The study comprised 47 breast cancer specimens of 47 women with follow up period 3-10 years. Histological examination showed 3 in situ ductal carcinomas and 44 ductal invasive carcinomas common type (11 grade I, 23 grade II and 10 grade III ). Eight out of 44 women with invasive carcinoma had a recurrent disease. Immunohistochemistry was carried out by using polyclonal antibodies against cerbB-3 and cerbB-4 (Santa Cruz Biotechnology) and a monoclonal antibody against cerbB-2 (Biogenex). Tumors were scored by the proportion of tumor cells stained.

**Results:** Expression of cerbB-3 was evident in all in situ and in 10 (22,72%) invasive carcinomas. One out of the 10 women with invasive carcinoma had a local recurrence (9%). CerbB-4 expression was evident in 1 in situ carcinoma and in 28 (63,6%) invasive carcinomas. Seven out of the 28 women with invasive carcinoma had a recurrent disease (24,13%).

CerbB-2 expression was evident in all in situ and in 7 invasive carcinomas (~15%). Two out of the seven women with invasive carcinoma had a recurrent disease (28,57%).

**Conclusions:** Our results demonstrate that erbB receptors are expressed early in human breast carcinoma. ErbB-2 and erbB-4 overexpression is correlated with recurrent disease.

**P 411**  
**CERB B2 OVEREXPRESSION BREAST CANCER CELLS REVEAL DISTINCTIVE NUCLEAR FEATURES BY IMAGE CYTOMETRY**

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**Background:** To understand if CerbB2 over-expressing breast cancer cells have distinctive phenotypic features.

**Design:** N0 Grade II twelve invasive breast cancer cells showing heterogenous CerbB2 protein over-expression were included in the study. For measurements, 100 CerbB2 immunoreactive tumor cells, and 100 non-reactive tumor cells were selected for each case from their paraffin embedded tumor tissue sections stained immunohistochemically with mouse antihuman CerbB2 antibody (BioGenex, CA, USA). Nuclear area, nuclear perimeter, and nuclear form factor of these selected cells were determined on SAMBA 4000 image analysis workstation. Discriminative analysis was performed whether measured parameters are accurate to identify CerbB2 positive and negative cellular phenotype.

**Results:** Nuclear area was found to be the most distinctive feature for CerbB2 over-expression second to the nuclear perimeter (p<0,05). CerbB2 expressing cells have bigger nuclear area and bigger nuclear perimeter significantly (p<0,05).

**Conclusion:** CerbB2 protein expression was found consistent with distinct nuclear features in invasive breast carcinoma cells that may be identified by image cytometry.

**P 412**  
**EXPRESSION OF HEDGEHOG SIGNALING COMPONENTS IN BREAST CANCER**

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The hedgehog signal transduction pathway plays an important instructional role during the development of vertebrates. This pathway includes hedgehog ligands (Sonic hedgehog, Shh; Desert hedgehog, Dhh; Indian hedgehog, Ihh) binding to the membrane receptor patched (ptch) and the trans-membrane protein smoothed with its intracellular effectors (Gli1, Gli2, Gli3). Mutations of the receptor ptch are known to induce human tumours, i.e. the basal cell carcinoma (BCC).

Lately, hedgehog has been described to play a crucial role in the carcinogenesis, differentiation and proliferation of breast cancer.

The aim of the present study has been to reveal the expression of single components of the hedgehog pathway in normal tissues, precancerous lesions, non-invasive and invasive cancer of the breast.

Formalin fixed and paraffin embedded human normal (n=3) tissue, precancerous lesions (atypical hyperplasia, DCIS; LCIS n=15) and invasive ductal and lobular carcinoma tissues (n=25) have been investigated. Additionally breast cancer tissue arrays and breast cancer cell lines were analyzed for the expression of components of the hedgehog pathway using immunohistochemistry. The ligands (Shh, Dhh, Ihh), the

receptor Patched and the transcription factors (Gli1, Gli2, Gli3) as main partners of an activated pathway were analyzed. Twelve breast cancer cell lines (MDA-231, SK-BR-3, BT-20, MDA-361, HS-578, T-47 D, ZR 751, MCF-7, HBL-100, 734B, MTSV 1-7, ALAB) have been examined by real-time polymerase chain reaction for the quantification of mRNA.

The results suggest a constitutively activated hedgehog pathway in all breast carcinoma tissues. Furthermore, there are differences in the expression of the hedgehog components. Invasive ductal carcinomas apparently show an increased expression of Shh, Gli1, Gli2, Gli3 and Ptch. In addition to the over-expressed components in invasive ductal carcinoma, ductal carcinomas in situ indicate an intensive staining of Ihh. Shh, Ihh, Gli1, Gli2, Gli3, and Ptch are expressed in invasive lobular carcinomas and in LCIS.

Elucidation of the role of hedgehog signalling pathway in breast cancer could contribute to a new therapeutic approach by obstructing the hedgehog pathway cascade with cyclopamine or antibody therapy.

#### P 413

##### **A MODIFIED APPROACH FOR EVALUATION OF ERBB2 (HER2) GENE COPY NUMBER IN MODERATELY AMPLIFIED INVASIVE DUCT CARCINOMAS (IDC) OF THE BREAST**

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**INTRODUCTION:** In IDC of the breast ERBB2 gene FISH status is reported as a ratio of a total number of ERBB2 signals and a total count of chromosome 17 centromere signals (ERB/CEP17). Using this system, interpretation of borderline findings may be difficult. **PURPOSE:** To introduce a more accurate approach to describe ERBB2 gene status in cases with a borderline ERBB2 gene amplification.

**METHODS:** We investigated ERBB2 gene status in tissue imprints (92 IDC) and histological sections (127 IDC) using FISH PathVysion (Abbott/Vysis). We evaluated 100 tumor cells in each case and classified the nuclei according to the number of ERBB2 signals in three categories: without the gene amplification, with a moderate (<10 signals) and with a strong (>10 signals) amplification. The evaluation of ERBB2 signals was performed relatively to the number of CEP17 signals in each counted cell. The IDCs were classified as strongly amplified if more than 10% of tumor cells had the strong amplification. A case with a moderate amplification was defined as having more than 10% of tumor cells with the gene moderately amplified. **SUMMARY OF THE RESULTS:**

A group of tumors which were by our system of evaluation strongly amplified had the ERB/CEP17 ratio >2 (101 IDC; 2,11-16,14; median 6,81), and a group of tumors without amplification had the ratio <2 (90 IDCs; 0,84-1,90; median 1,07). However, in 28 cases classified as moderately amplified by our system the ERB/CEP17 ratio varied from 1,18 to 4,77 (median 1,96) – 10 cases with imprints 1,18-2,96 (median 1,84), 18 patients with tissue sections 1,30-4,77 (median 2,12 – reflecting the cutting of the nuclei). We found that neither approach to FISH evaluation of borderline cases has a correlate to the protein status. **CONCLUSION:** In a majority of IDCs the ERB/CEP17 ratio describes ERBB2 gene status in a comparable way with our system. In cases with a borderline ERBB2 gene amplification ERB/CEP17 ratio may lead to misinterpretation of the result. Evaluation of histological sections should be done with a caution because centromeres may be cut off disproportionately to the ERBB2 signals resulting in an increase of ERB/CEP17 ratio. On the other hand, ERB/CEP17 ratio may hide a minor tumor cell clone with a strong amplification. From this reason it is more useful to report the percentage of tumor cells with a strong and with moderate amplification. The result must be carefully

confronted with the ERBB-2 protein status. FNM MZO 00064203

#### P 414

##### **ESTROGEN RECEPTOR BETA EXPRESSION IN HIGH GRADE BREAST CANCER PATIENTS MAY PREDICT METASTASIS AND MORTALITY**

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**Background:** The two isoforms of estrogen receptor (ER) alpha and beta are involved in breast tumorigenesis and progression. ER alpha is a well established prognostic and predictive factor for anti-estrogen therapies in breast cancer, and predicts a favorable disease outcome, ER beta potential as a clinical prognostic marker remains to be defined. The aim of this study was to identify the potential of ER beta as a predictor of metastases and mortality in high grade breast cancer patients.

**Methods:** In a group of 50 patients with confirmed diagnostic of ductal carcinoma and a SBR of 8-9, we assessed by immunoblotting and IHQ (using specific monoclonal antibodies directed against ER alpha and ER beta) the distinct expression pattern of this ER isoforms in primary tumor samples. Chi-square and log rank tests were used to determine differences between proportions of each ER isoform and mortality and survival distributions respectively. A P value <0.05 were considered significant.

**Results:** The median follow up of this case series was 80 months (2-107) months. Twenty-three (46%) of 50 patients developed metastases, and 25 of 50 (50%) were positive for expression of ER alpha and ER beta respectively. We found a high frequency of metastasis (13 of 25; 52%) among those cases that express ER beta. Similar to 17 of 25 (68%) observed in ER alpha negative samples. This relationship was very significant (P=0.0000) when compared to the rest of the group. We also found a statistically significant correlation between patients with positive ER beta and negative ER alpha and higher mortality (P=0.03 and P=0.0038 respectively).

**Conclusions:** These findings suggest that ER beta expression in high grade samples from ductal carcinoma histologic subtype may be used as a poor prognostic marker in breast cancer patients.

#### P 415

##### **NY-BR-1 REPRESENTS A NEW DIFFERENTIATION ANTIGEN IN INVASIVE BREAST CANCER, WHICH REMAINS STABLE DURING METASTATIC PROCESS.**

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NY-BR-1 is believed to represent a new differentiation antigen in breast cancer that represents a potential target antigen for antibody based therapies. We investigated immunohistochemical expression of NY-BR-1 in invasive breast cancer cases by using a monoclonal antibody. The retrospective study involved 124 invasive breast cancer cases comprising well (G1), moderately (G2), and poorly (G3) differentiated carcinomas. Lymph node metastases were examined in 38 cases. NY-BR-1 was detected in all normal breast samples and all ductal carcinoma in situ components (100%). Among invasive carcinomas, NY-BR-1 was detected in 76% of G1, 63% of G2 and 51% of G3 carcinomas. Expression profile of NY-BR-1 remained remarkably stable in the lymph node metastases. Our data could provide further evidence that NY-BR-1 is a differentiation antigen by being ubiquitously expressed in normal breast tissue and ductal carcinoma in situ, and its expression was decreasingly detected by dedifferentiation. NY-BR-1 might possibly play

an invasion suppression function at least in a subset of breast cancer cases. As NY-BR-1 exhibits organ specificity, it might be of practical use at routine practice by unknown primaries. Whether NY-BR-1 could be seen as a potential target for immunotherapy, needs to be investigated by further studies.

#### P 416

##### **THE MULTIFUNCTIONAL ROLE OF SMAD2 PROTEIN IMMUNOHISTOCHEMICAL EXPRESSION IN INVASIVE BREAST CARCINOMAS**

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**Introduction:** Smad2 participates in the Transforming Growth Factor- $\beta$  (TGF $\beta$ ) signaling pathway, where, in a complex with other Smad proteins, cooperates with transcription factors to regulate expression of defined genes. The purpose of the present study was to investigate the expression of phosphorylated smad2 (pSmad2) in association with clinicopathological parameters and various biological markers of proliferation and invasion.

**Methods:** A two step immunohistochemical method (Envision) was applied on paraffin embedded sections from 164 patients with invasive breast carcinomas to detect the expression of the proteins pSmad2, Ki-67, topoisomerase II alpha (topoII alpha), Extracellular Signal Regulated Kinase-2 (ERK2), catenin p120, Matrix Metalloproteinases (MMP)-14, -2 and Tissue Inhibitor of Metalloproteinase-2 (TIMP-2). The results were subjected to statistic analysis.

**Results:** pSmad2 protein was detected in the nuclei in the 68.1% of malignant cells and in the fibroblasts of the tumor (55.2%). Nuclear pSmad2 was inversely correlated with histological grade ( $p=0.047$ ) and the normal immunoeexpression of catenin p120 ( $p=0.028$ ). Both nuclear and stromal pSmad2 were inversely correlated with lymph nodal status ( $p=0.054$  and  $p=0.053$  respectively), Ki67 ( $p=0.003$  and  $p=0.043$  respectively) and topoIIa ( $p=0.021$  and  $p=0.035$  respectively), whereas their relation with MMP-14 of tumor cells ( $p=0.012$  and  $p=0.008$  respectively) and ERK2 of tumor fibroblasts ( $p=0.008$  and  $p<0.0001$  respectively) was found to be parallel. Stromal pSmad2 was further positively related with MMP-14 of stromal cells ( $p=0.006$ ) and TIMP-2 of cancer cells ( $p=0.022$ ) and nuclear pSmad2 with MMP-2 of stromal cells ( $p=0.038$ ).

**Conclusion:** In the present study, pSmad2 was found to play both tumor suppressive and invasive role, probably via its collaboration with MAP kinase pathway.

#### P 417

##### **PROGNOSTIC SIGNIFICANCE OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)-C AND ITS RECEPTOR, FLT-4, IN INVASIVE BREAST CARCINOMA**

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**Introduction:** The vascular endothelial growth factor (VEGF) family currently includes five members. Among them, VEGF-C is a ligand for VEGF receptor-3 (VEGFR-3 or flt-4). Both show some selectivity toward lymphatic endothelial cells but their role in cancer is largely unknown. The aim of the present study was to investigate the role of VEGF-C and its receptor, flt-4, in invasive breast carcinomas through their correlation with classic clinicopathological parameters, biological markers indicative of aggressive tumor phenotype, such as proliferation markers (Ki67, topoII alpha), invasion markers

[Metalloproteinase(MMP)-11 and abnormal e-cadherin], c-erbB-2 and patients' survival.

**Methods:** Immunohistochemistry was applied on paraffin embedded sections from 169 patients with invasive breast carcinomas to detect the expression of the proteins VEGF-C, flt-4, c-erbB-2, Ki67, topoisomerase II alpha (topoII alpha), (MMP-11) and e-cadherin. Results were subjected to statistic analysis.

**Results:** VEGF-C was detected in the cytoplasm and the membrane of malignant cells in 86% and 32% of the cases respectively, whereas flt-4 was found to be expressed in the nuclei of cancer cells (41.4 %) and tu-mor fibroblasts (65%). Membranous VEGF-C showed a parallel correlation with proliferation markers Ki67 and topoII alpha ( $p=0.006$  and  $p=0.026$  respectively), stromal MMP-11 ( $p=0.012$ ) and abnormal e-cadherin ( $p=0.035$ ) and it was further found to be an independent prognosticator of patients' shortened disease-free survival ( $p=0.03$ ), while cytoplasmic VEGF-C had an independent unfavorable effect on patients' overall survival ( $p=0.016$ ). Flt-4, on the other hand, when detected in the nuclei of malignant cells showed a parallel correlation with topoII alpha ( $p=0.039$ ), whereas stromal flt-4 was positively associated with c-erbB-2 ( $p=0.017$ ) and a shortened overall survival of postmenopausal patients ( $p=0.058$ ). Patients who expressed simultaneously membranous VEGF-C and stromal flt-4 were found to have the worst prognosis as far as both disease-free and overall survival is concerned ( $p=0.012$  and  $p=0.039$  respectively).

**Conclusion:** VEGF-C and its receptor, flt-4, were associated with biological markers indicative of aggressive tumor phenotype. Furthermore, VEGF-C seems to exert an unfavorable effect on patients' survival, suggesting that it may be an independent prognosticator for invasive breast carcinomas.

#### P 418

##### **HER2 OVEREXPRESSION PREDICTS SURVIVAL IN STAGE II AND III OF BREAST CANCER PATIENTS TREATED WITH NEOADJUVANT DOCETAXEL PLUS EPIRUBICIN**

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**Objective:** The study was designed to evaluate the importance of biological markers to predict response to neoadjuvant chemotherapy and the overall survival in stage II and III of breast cancer patients. **Patients and methods:** Fifty-two patients received preoperative docetaxel (75 mg/m<sup>2</sup>) in combination with epirubicin (50 mg/m<sup>2</sup>) in i.v. infusion in D1 every 3 weeks after incisional biopsy. They received adjuvant chemotherapy with CMF or FEC according the axillary status following definitive breast surgery. Clinical and pathologic response rates were determined after preoperative therapy. We evaluated the response rate to neoadjuvant chemotherapy and the prognostic significance of clinicopathologic and immunohistochemical parameters (p53, HER2, p21, RE and RP). Median patient age was 50 years with a median follow up of 46 months from time of diagnosis. **Results:** Preoperative treatment achieved clinical response in 73% and complete pathologic response in 5.7%. The p53 overexpression was associated with an increased rate of objective response ( $P=0.04$ ). Among all clinicopathologic and immunohistochemical parameters, only HER2 overexpression, the axillary status (>2 positive LN) and age (under 41 years old) were associated with decrease in disease free survival ( $P=0.004$ ,  $P=0.001$  and  $P<0.0001$ , respectively). The overall survival was reduced in HER2 overexpression patients ( $P=0.02$ ) and in patients with

more than seven axillary lymph node metastasis ( $P=0.03$ ).  
 Conclusions: p53 overexpression is associated with a higher objective response rate and HER2 overexpression is associated with poor prognostic in stage II and III of breast cancer patients treated with neoadjuvant taxanes/anthracycline based chemotherapy.

**P 419**  
**IMMUNOHISTOCHEMICAL ANALYSIS OF CHROMOGRANINE A, SYNAPTOPHYSINE AND KI67 IN PURE MUCINOUS BREAST CARCINOMA.**

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Introduction : Breast mucinous carcinoma (BMC) are characterized by their favourable prognosis but they are not completely homogenous at microscopic level.

Aim of the study : to analyse endocrine differentiation and ki67 in BMC.

Material and method : Only pure BMC are eligible for the study. They were classified as follows according to the proportion of tumor cells/the whole tumor surface : A:<20%, B: 20-50%, C>50% and the presence (1) or absence (2) of cytoplasmic mucus. The following parameters were analyzed: age, follow-up, tumor size, lymph node status, Elston and Ellis grade. Immunohistochemical study of the following antibodies (Dako, dilution : 1/50) was performed : chromogranine A, synaptophysine and Ki67 (clone KiS5).

Results : Endocrine markers are positive in 7 cases. Chromogranine A is positive in 4 cases and synaptophysine in 5 cases with co-expression of the two markers in 2 cases. Two phenotype groups are distinguished :

- group I : immunolabeling of more than 50% of the cells (1+ to 3+)
- group II : immunolabeling of a low number of cells (20% : 1 case, few cells : 2 cases) with high intensity (2+ and 3+).

Expression of neuroendocrine markers is more frequent when the stroma is less abundant (type B and C) and when the cells contain cytoplasmic mucous (type 1). There's no difference for other parameters (size, lymph node, Elston and Ellis grade, survival).

Rates of Ki67 expression was distributed as follows : <1% : 11 cases, <10% : 7 cases, >20% : 4 cases. The rate is not correlated to the cellular type, neither to the the proportion of tumor cells/tumor surface

Conclusion : Endocrine differentiation doesn't influence the outcome of the tumor. The low rate of Ki67 expression could explain the favourable course of the tumor.

**P 420**  
**AN IMMUNOHISTOCHEMICAL STUDY OF EXTRAMAMMARY PAGET'S DISEASE**

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Introduction: Extramammary Paget's disease (EPD) has diverse histogenetic pathways, some lesions originating primarily from cutaneous adnexal structures (primary EPD), while others resulting from secondary involvement of the skin by a subjacent carcinoma (secondary EPD). In primary EPD, most Paget cells seem to be cytokeratin (CK) 7+/CK 20-/gross cystic disease fluid protein (GCDFP-15)+, while CK20+/GCDFP-15- Paget cells suggests the presence of a subjacent cancer with a corresponding immunophenotype, most often a rectal carcinoma or a transitional cell carcinoma (TCC). Mammary and extramammary Paget cells share histopathological similarities. The receptor complex of

heregulin-alpha consisting of HER2/neu, HER3 and HER4, is present in almost all cases of mammary Paget's disease. Published studies report a variable (0-88%) overexpression of HER2/neu in EPD. Expression of HER3 and HER4 in EPD has not been studied before.

Aim: To immunophenotype 15 cases of EPD and evaluate the expression of HER2, HER3 and HER4.

Methods: Immunohistochemical stains were performed on formalin-fixed, paraffin-embedded tumour tissue from 15 patients with EPD, using antibodies to CK7, CK20, GCDFP-15, TAB250, CB11, HER3 and HER4. HER2 oncogene amplification was assessed by fluorescence in situ hybridization (FISH).

Results: Mean age at diagnosis was 68.3 years (range 38-84). The EPD involved the vulva and/or perineum or urethra in all females (n=9). In the males (n=6), the EPD was located in perianal, scrotal and/or inguinal regions and symphysis. Eleven tumours were intraepithelial and 4 were invasive. Four patients had a subjacent cancer: 2 rectal carcinomas, 1 in situ TCC and 1 mucinous/endometrioid carcinoma of the vagina. The CK7+/CK20-/GCDFP-15+ immunophenotype was found in 6 cases, all without an associated carcinoma, and CK20+/GCDFP-15- in another 6 cases, including 1 associated TCC and 2 associated rectal carcinomas. The CK7+/CK20-/GCDFP-15- immunophenotype was found in 3 cases, including 1 associated mucinous/endometrioid carcinoma. Three cases of EPD showed HER2/neu oncogene amplification. HER3 and HER4 was expressed in 7 and 9 cases respectively, pointing at a role for heregulin-alpha in the chemotaxis of extramammary Paget cells.

Conclusion: We confirm that immunophenotyping of EPD with CK7, CK20 and GCDFP-15 is valuable in predicting the risk of an associated cancer. New therapies targeting the frequently expressed HER3 and HER4 might be promising.

**P 421**  
**VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)-A IMMUNOHISTOCHEMICAL EXPRESSION IS ASSOCIATED WITH AN AGGRESSIVE**

**PHENOTYPE IN INVASIVE BREAST CARCINOMA**  
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Introduction: Angiogenesis is a complex process which includes the proteolysis of the basal vascular membrane and intercellular matrix, migration and proliferation of endothelial cells and the formation of tubular structures. Tumor growth is dependent on angiogenesis that is regulated by peptide growth factors of which vascular endothelial growth factor (VEGF)-A is one of the most selective and potent. In the present study we investigated the role of VEGF-A in invasive breast carcinoma through its correlation with classic clinicopathological parameters (menopausal status, tumor size, histologic type, grade, lymph nodal status, stage of the disease, ER, PR), biological markers indicative of aggressive tumor phenotype (p53, c-erbB-2) and invasiveness [Membrane type1- Matrix Metalloproteinase (MT1-MMP)] and patients' survival.

Methods: A three step immunohistochemical method (ABC/HRP) was applied on paraffin embedded sections from 162 patients with invasive breast carcinoma to detect the expression of the proteins VEGF-A, p53, c-erbB-2 and MT1-MMP. Results were subjected to statistic analysis.

Results: VEGF-A was detected in the cytoplasm of malignant cells and the tumor stroma in 49.6% and 48.4% of the cases respectively. Cytoplasmic VEGF-A showed a parallel correlation with accumulative, non functional p53 and c-erbB-2 ( $p=0.007$  and  $p=0.028$  respectively). Additionally, parallel was found to be the correlation between cytoplasmic VEGF-A and MT1-MMP in cancer and stromal cells

( $p=0.018$  and  $p=0.020$  respectively) as well as MT1-MMP of vascular endothelial cells ( $p=0.018$ ). VEGF-A was found to have no significant relation to the clinicopathological parameters and patients' disease free or overall survival.

Conclusion: VEGF-A expression seems to be associated with the emergence of an aggressive tumor phenotype through its correlation with p53 and c-erbB-2. Moreover, VEGF-A correlation with MT1-MMP indicates its participation in angiogenesis, which further supports its contribution to the aggressive behavior of breast carcinoma.

#### P 422

##### **NATURE OF C-KIT ALTERATIONS IN BREAST CANCER. A PUTATIVE NEW PROGNOSTIC AND THERAPEUTIC FACTOR**

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Mutations of protooncogen c-kit (CD117) are believed to have a pathogenetic role in several neoplasms. Detected in most gastrointestinal stromal tumors (GIST), are highly relevant for treatment, determining a target for tyrosine kinase receptors inhibitors as Imatinib. C-kit mediated signal pathway is important for the development of breast cancer cell lines and c-kit gene expression seems to be lost in most breast neoplasms. However, an increase of c-kit expression has been associated with aggressiveness and poor outcome. The mechanism of these changes of expression and its importance in breast cancer biology and treatment have not been determined.

In order to study the nature and relevance of c-kit alterations in breast cancer, we have analyzed 85 invasive ductal carcinoma for c-kit expression at protein level, by immunohistochemistry (IHC) using a polyclonal antibody from DAKO, and at mRNA level by Quantitative RT-PCR. We also analyzed DNA for point mutations in exons 9, 11, 13 and 17 of the gene.

Normal ductal epithelia was always positive for c-kit protein but most neoplastic samples lost this expression when analyzed by IHC. However, 11 (12.9%) tumors showed overexpression of the protein, most of them (7 out of 11) of high histologic grade (WHO grade III). None of these cases contained point mutations when DNA was analysed, and the single detected mutation was present in a case negative by IHC. mRNA expression of c-kit in tumor tissues did not correlate with protein expression patterns nor presence of point mutation, suggesting a different origin. The nature, mechanism and significance of c-kit overexpression in breast cancer will be discussed.

#### P 423

##### **PHYLLODES TUMOURS OF BREAST: A RETROSPECTIVE STUDY OF 18 CASES**

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Introduction:

Phyllodes tumours (PT) are rare accounting for 0,3 to 1% of all primitive tumours of the breast. They constitute a spectrum of tumours ranging from benign to malignant form.

Objective:

The purpose of this study is to discuss the clinic and pathologic characteristics, the course and the prognosis of PT of breast.

Material and methods:

We report a retrospective study of 18 cases of PT diagnosed in the pathology department of Habib Bourguiba Hospital (Sfax-Tunisia) over a five years period (1999 - 2003)

Results:

The mean age of patients was 40 years (extremes: 17 and 60 years). Most patients (72,3%) gave a history of a slowly enlarging mass. The mammographic aspect was in favour of a benign tumour in ??? cases (71,4%). Macroscopically, the tumour size ranged from 1,5 to 22 cm (mean size : 7,9 cm). 11 PT were benign (61,2%), 4 borderline (22,2%) and 3 malignant (16,6%). A tumorectomy was carried out in 17 cases followed by a mastectomy in 4 cases. One benign PT and one borderline PT recurred after a follow up of 12 and 24 months. Two borderline PT and one phyllode sarcoma metastasised after a mean follow up of 12 months. A complete remission was observed in 15 case (83,3%) with a mean follow up of 32 months (extremes : 2 and 60 months).

Conclusion:

PT are usually benign but relapses and metastases are not uncommon. The clinical course can not be predicted reliably from histologic grading.

#### P 424

##### **INDUCTION OF APOPTOSIS PREDICTS THE RESPONSE TO THE NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER**

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Objective: This study was designed to evaluate the relationship between the apoptosis induced by chemotherapy and the clinical response in breast cancer. Patients and methods: We studied the Apoptotic Index (AI) and the overexpression of the protein p53 in 44 breast tumors from patients submitted to neoadjuvant chemotherapy. Objective response (OR) to primary chemotherapy was observed in thirty-seven (84%) and no response (NR) in seven. The AI was measured by the rate of apoptotic cells identified by morphological criteria in hematoxylin and eosin stain. The p53 protein expression was evaluated by the immunoperoxidase technique. The cases were interpreted as positive if more than 2/3 of tumors cells exhibited a dark brown nuclear staining. Results: The median AI change (AI initial - AI post-chemotherapy) observed in the patients with objective response was 0.84 while in patients with no response the median AI change was -0.01 ( $r=0.40$ ;  $P=0.006$ ). P53 protein overexpression was observed in 16.6% of OR patients and 42.8% of NR patients ( $r=-0.32$ ;  $P=0.034$ ). The median AI change in p53-positive cases was 0.75, and in p53-negative cases, it was 1.72 ( $r=0.44$ ;  $P=0.0029$ ). Conclusion: We find a positive correlation between the increase in the AI and the clinical response and a negative correlation between the p53 expression and the clinical response, in patients with breast cancer submitted to neoadjuvant chemotherapy.

#### P 425

##### **ADHESIVENESS IN SHORT-TERM PRIMARY CULTURES DERIVED FROM INFILTRATING DUCTAL BREAST CANCER AND THEIR ASSOCIATION WITH CLINICOPATHOLOGICAL VARIABLES**

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INTRODUCTION: Breast cancer is the most commonly diagnosed non-skin malignancy in women, being its incidence

and death rates increasing world-wide. E-cadherin adhesion molecule is involved in morphogenesis, maintenance of tissue cytoarchitecture and cellular migration. Short-term primary cultures isolated from ductal breast tumors are more reliable as a tool to study the specific cellular behaviour than commercial cell lines.

**PURPOSE OF THE STUDY:** We have studied the possible correlation between E-cadherin expression and pathological variables (histologic grade, c-erbB2 amplification...), metastasis, disease-free and survival time periods. The role of E-cadherin and histologic grade in growth pattern and metastatic properties of short-term primary cultures were also evaluated.

**PATIENTS AND METHODS:** We studied 30 patients that were preoperatively diagnosed with infiltrating ductal breast cancer and were informed about the study and gave their consent. The infiltrating ductal breast cancer tumors (IDC) were classified according to TNM stage, Nottingham prognostic index combined histological grade (NPI) and histologic grade (G1, G2, G3). Cells fractions were isolated and maintained for a short-period of time in 19 viable epithelial cultures derived from breast tumors. Tumor cell invasion was evaluated using a modified Boyden chamber invasion assay. E-cadherin expression was evaluated by immunohistochemistry and Western Blotting.

**RESULTS:** The median follow-up value was 33.2 months. 63.3% of the tumors were cultured, and their growth rate is associated with G3 ( $P=0.009$ ). Lack of expression of E-cadherin by Western Blot is directly correlated to invasion properties ( $P=0.027$ ) and inversely correlated to c-erbB2 overexpression ( $P=0.008$ ). There is no association between cell invasion and metastasis in vivo ( $P=0.51$ ). E-cadherin expression did not associate with estrogen and progesterone receptor expression ( $P=0.35$ ,  $P=0.16$ , respectively) or growth rate ( $P=0.65$ ). Histological grade is closely associated with ER and PR status ( $P=0.003$ ,  $P=0.041$ , respectively), proliferative index ( $P=0.005$ ) and overall survival ( $P=0.009$ ).

**CONCLUSIONS:** 1. Viability of cultures is closely associated to unfavourable histological grade 2. E-cadherin expression is correlated with invasive properties in cell cultures.

#### **P 426**

### **CARDIOMEGALY AND MYOCARDIAL INFARCTION IN AN INFANT OF A DIABETIC MOTHER**

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#### **Introduction**

Pregnancy in diabetic women is a risk state that involves increased somatic size or macrosomia, an increased incidence of perinatal death and prematurity, hypertrophy of the islets of Langerhans with B-cell hyperplasia and hyperinsulinemia, and an increased frequency of congenital malformations. Today, diabetes is the most common chronic disease complicating pregnancy and its occurrence is especially high in Finland. The onset of diabetes occurs at an increasingly young age and vascular complications are thus becoming more common. The strain on maternal care services is also increasing. We describe an unhappy case with many complications of diabetic pregnancy.

#### **Case report**

The case consists of the second pregnancy and delivery of a 27-year-old female. The mother has had insulin-treated DM1 for 17 years. The first pregnancy in 2003 ended in birth at 38 + 2 weeks. The weight of the baby was 4435g. During the present pregnancy the patient's diabetes was in satisfactory control after the first trimester. In the third trimester, however, she developed pre-eclampsia. Emergency Caesarean section was done at 38 + 3 weeks because of CTG changes. The weight of the male baby was 5370g (+4SD) and length 59cm. Apgar score was 3/5/7 at 1/5/15 minutes. The infant's appearance was typical of diabetic macrosomia. Thorax X-

ray and ultrasonography showed a hypertrophic heart. The baby died at 13 hours of age. Autopsy revealed ventricular and septal myocardial hypertrophy and mild obstruction of the outflow canal. There was a recent infarction in the septal myocardium and disorganized structure. The islets of Langerhans in the pancreas were hyperplastic and increased.

#### **Conclusions**

In Finland insulin-treated diabetes complicates about 4-5/1000 births. Many pregnant women have had diabetes long enough to develop diabetic vascular complications because the onset of diabetes occurs at a young age. Although hypertrophic cardiomyopathy is uncommon, the findings and the outcome of the present case indicate the importance of third trimester echocardiographic monitoring of fetal heart function when the mother has diabetes.

#### **P 427**

### **RESPIRATORY CHAIN DYSFUNCTION IN "NON-MITOCHONDRIAL" GENETIC DISORDERS – THE ROLE OF PATHOLOGICAL EXAMINATION OF SKELETAL MUSCLE**

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Detection of respiratory chain (RC) complex deficits in spectrophotometric assay of muscle and fibroblasts consists an important contribution to diagnosis of mitochondrial disorders (MD). However RC complexes may show defective activity in several disorders not primarily related to genes/proteins involved in structure and function of the RC.

Out of 300 skeletal muscle and fibroblast samples obtained from children suspected for MD 130 showed RC dysfunction in spectrophotometric assay. In 51 patients eventually molecular genetic studies of mtDNA and nDNA confirmed the diagnosis of MD. In 27 patients with RC complex deficits other diagnosis was finally confirmed. The diagnoses include: Duchenne and Becker muscular dystrophy, spinal muscular atrophy, myotubular myopathy, glycogen storage disease type II, vanishing white matter leukodystrophy, Lowe syndrome, LCHAD deficiency, Fanconi Bickel syndrome, hereditary fructose intolerance and others. Histological and histochemical assessment of skeletal muscle in children with RC complex deficits revealed: diagnostic pattern of mitochondrial or other non-mitochondrial disease; non-specific lesions; or normal morphology. The mechanisms of RC deficits in patients with diagnoses other than MD are probably diverse and subject to further investigation.

**Conclusions:** Respiratory chain defects may be associated with various „non mitochondrial” disorders. Morphological examination remains useful in differential diagnosis of secondary respiratory chain defects. Disregard of morphological examination may lead to misdiagnosis.

#### **P 428**

### **DISEASE OF LETTERER-SIWE: A CASE REPORT CHEDDADI F.**

The Langerhans'cell histiocytosis has an incidence of 5 per million and often occurs in childhood with a male predominance.

Its clinical translation and its forecast are very variable and depend on the age of occurred (lower than two years) and of the organs reached. Pathologies of benign evolution and pathologies of bad forecast are thus defined.

The Langerhans' cell histiocytosis is characterized by a more or less polymorphic inflammatory infiltrate but always containing cells of Langerhans with notched nucleus which express the CD1a antigen intensely.

The diagnosis of LCH per defect is not posed any more with the advent of the antibody O10. It is necessary to avoid the diagnosis by excess met more and more in inflammatory pathologies or an increase in the histiocytes is observed.

It acts, in the observation which we report of a 4 month old nourrisson which presents:

- a cutaneous eruption generalized prevailing on the level of the folds of inflection,
  - three masses of three cm large axis each one, occupying the right and left skull area,
  - a hypertrophic gingivitis with early dental eruption,
  - a chronic diarrhea,
- and a multiple gaps of the dome of the skull with osseous lysis of the internal table.

The diagnosis of LCH was posed on fragments biopsic coming from the left skull mass, gum and the bone.

The cutaneous, oral and osseous attack concomitant realize the board of LCH in its traditional form multi focal and multi systemic and which returns within the framework of the clinical entities of bad forecast.

The marking of the molecule of adherence (E<sub>1</sub>A<sub>7</sub>) could not be done for a digestive attack search.

Conclusion: the disease of LETTERER - SIWE is rare. The majority of the LCH cases corresponds to a focal disease (eosinophilic granulom solitary). the role of the histopathology in the diagnosis of the LCH largely profited from the Ag CD1a.

Unfortunately there are not criteria histopathologic of bad forecast from where major importance of confrontation anatomo-clinic.

#### P 429

**OVERCOMING THE BLOOD BRAIN BARRIER WITH HIGH DOSE ENZYME REPLACEMENT THERAPY IN THE MURINE MUCOPOLYSACCHARIDOSIS VII MODEL OF LYSOSOMAL STORAGE DISEASE**  
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Enzyme replacement therapy (ERT) with recombinant enzyme is effective in reducing lysosomal storage in patients with several of the lysosomal storage diseases although improvement in CNS disease has been limited because the blood brain barrier restricts access of enzyme to the CNS. ERT with recombinant beta-glucuronidase (GUS) is not yet available for human MPS VII. However, there is a good murine model for this disorder in which glycosaminoglycan storage in the CNS produces severe cognitive disability. Studying this model, we found that the CNS is accessible to and improved by infused recombinant human GUS (rhGUS) in the newborn period but not improved by treatment begun after age 2 weeks. We recently reported that this enzyme delivery to brain in the newborn period is dependent on transcytosis of phosphorylated enzyme across the blood brain barrier by the mannose 6-phosphate/IGF-2 receptor. Down-regulation of this receptor mediated delivery system by age 2 weeks appears to explain the lack of response in the adult MPS VII mouse. In the current study we asked whether the obstacle to enzyme delivery to the CNS in the adult could be overcome by administering much higher doses of enzyme than

used in conventional trials. We measured reduction in lysosomal storage for doses of 0.3-40 mg/kg body weight of rhGUS administered i.v. for 1 - 13 weeks to MPS VII mice tolerant to GUS. Mice treated for only 3 weeks with 0.3-5 mg/kg enzyme weekly had a slight reduction in meningeal storage but no change in storage in neurons. When mice received doses of 1-4 mg/kg/week for 13 weeks, there was a dose-based therapeutic effect on meninges, parietal neocortical and hippocampal neurons and glia. Only mice treated with 4 mg/kg showed a response in both neocortex and hippocampus. However, doses of 20-40 mg/kg GUS given 3 times over 1 week failed to reduce storage in any area of the CNS except the meninges. On the other hand, mice given 20 mg/kg weekly for 4 weeks, showed a decrease in neuronal, glial and meningeal storage and, on average, 2.5% of wild type GUS activity in brain. Our results indicate therapeutic enzyme can be delivered across the blood brain barrier in the adult MPS VII mouse if 1) one administers higher doses of enzyme than used in conventional ERT trials and 2) if these high doses of enzyme are administered over a sufficient period of time. These results have important implications for ERT for human lysosomal storage diseases.

#### P 430

**SOLID PSEUDOPAPILLARY TUMOR OF THE PANCREAS IN CHILDREN**

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Introduction: Solid pseudopapillary tumors of the pancreas are rare and their origin is unknown. The aim of this work is to report a new case diagnosed at a child. Thus, we present the case of 14 years old girl hospitalised for complaining of severe epigastric pain. The radiological examination demonstrated a well-demarcated pancreatic mass. The surgeons decided to remove the tumor.

Macroscopically examination carried out the removed tumor showed a 5 cm diameter, well circumscribed and capsulated tumor with a gray-whitish colour on cut surface, soft consistency and haemorrhage zones.

Material and methods: Fragments of the tumor were fixed in formaldehyde 10% included in paraffin and the sections were stained with HE, VG and immunohistochemical markers like Vimentine (VIM), progesteron receptors (PR), neuron-specific enolase (NSE) and glucagone.

Results: The histological examination revealed proliferation of uniform cells with basophilic cytoplasm without atypia arranged in nests; between nests we observed giant multinucleated cells, cholesterol crystals within a haemorrhagic stroma and pseudopapillary features. Immunohistochemical analysis: VIM positives, PR diffuse positives, NSE diffuse positives, glucagone positives

Conclusions: All these findings plead for the diagnosis of solid pseudopapillary epithelial tumor of the pancreas.

#### P 431

**SOME IMMUNOPATHOLOGICAL CHARACTERISTICS OF EPITHELIAL CHANGES IN JUVENILE POLYPS OF COLON AND RECTUM IN CHILDREN**

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The aim of this study was to outline variants of morphological epithelial changes in solitary juvenile colorectal polyps. Material and methods: Formalin fixed paraffin embedded tissues were obtained from 30 patients with solitary juvenile polyps (JP) of colon and rectum. Patients were 6 months - 9

years old (median age 4.3 years). Localization of polyps was the rectum (n=22) and sigmoid colon (n=8). Size of JP varied from 0.5 to 5.0 cm (median 3.4 cm). H&E, histochemical (van Gieson, periodic acid Schiff (PAS) and Gomori's aldehyde fuschin - Alcian-blue (GAF-AB) stains and immunohistochemical (expression of Ki-67, p53 and carcinoembryonic antigen (CEA)) analyses were performed.

Results: The following morphological variants of epithelial changes were observed: in 25/30 JP comprised reactive regenerative changes within focal complex glandular structures, with high mitotic counts and expression of Ki-67. 22/30 cases showed hypertrophic epithelial changes, consisting of enlarged and pseudostratified goblet cells, suggestive of so called transitional mucosa. In 19/30 cases we observed focal cytoplasmic expression of CEA. 12/30 cases showed eosinophilic epithelial changes consisting of glands with abundant eosinophilic cytoplasm and centrally located small round nuclei. Expression of Ki-67 and CEA was negative in this group. In 7/30 cases we observed Paneth cell metaplasia, and in 5/30 cases, mild dysplasia with expression of p53 in less than 10% of nuclei in polyp glands.

Conclusion: JP showed a broad spectrum of epithelial changes that might represents degenerative, regenerative and metaplastic processes. The main diagnostic pitfall is early dysplasia. Level of expression of p53 in JP may aid in differential diagnosis between reactive, regenerative changes and dysplasia.

**P 432**  
**HETEROTOPIC ORAL GASTROINTESTINAL CYST :  
REPORT OF A CASE**

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Heterotopic gastrointestinal cyst of the oral cavity is an extremely rare lesion which fewer than 40 cases have been reported in the English language literature. They usually involve the soft tissue of the floor of the mouth and the tongue. The lining of these lesions resembles gastric or intestinal mucosa. In this report we describe an oral heterotopic gastrointestinal cyst of tongue in a 1-month-old boy and discuss its pathogenesis.

**P 433**  
**A CASE OF A COMPLETE PYLORIC PANCREATIC  
HETEROTOPIA AT CHILD CAUSING  
GASTROESOPHAGEAL REFLUX**

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Introduction and aim: Heterotopic pancreas is usually found incidentally. Despite many cases having been reported heterotopic pancreas causes diagnostic difficulties for clinicians. Authors report a case of 1 year 1 month baby boy hospitalized for a history of vomiting, bleeding, gastroesophageal reflux and weight loss.

Plain abdominal radiograph showed a "trumpeting elephant" image. Endosonographical examination revealed a pyloric mass and a surgery was conducted to explore the area for tumor ablation.

Macroscopic examination showed a small tumor with a diameter of 6mm, firm, yellow, sharply circumscribed from the surrounding tissues, located beneath a mucosa; central umbilication was present.

Material and methods: Fragments of the tumor were fixed in formalin 10%, embedded in paraffin and sections were stained

with HE. Immunohistochemical tests have been done: CD 56, Chromogranin, Glucagon, Insuline, Somatostatin, Sinaptophysin and VIP.

Results: Microscopic examination revealed a tumor proliferation consisting of acini's group, pancreatic ducts, Langerhans island on the pyloric sub- mucous and hypertrophic muscular layer. The tumor was positive for insuline, glucagone, VIP, CD 56

Conclusions: The morphological appearance and immunohistochemical results enabled us to the diagnosis of complet form of heterotopic pancreas. It is important to differentiate between a cancer, an ectopic pancreas, a metastasis of a tumour and a pancreatic metaplasia of gastric mucoasa. Thus, an ectopic pancreas must be considered in the differential diagnosis of gastroesophageal reflux.

**P 434**  
**IDENTIFICATION OF THE MOTILIN CELLS IN  
DUODENAL EPITHELIUM OF PREMATURE  
INFANTS**

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Introduction: Motilin is one of the gastrointestinal hormones, and originates in the endothelial cells of the upper small intestine. Motilin regulates the gastrointestinal motility via migrating motor complex (MMC) activity. Most preterm infants of <32 weeks gestation are considered to lack MMC activity.

Purpose: The aim of the present study was to examine the presence of motilin in the duodenal epithelial cells of premature infants of <32 weeks gestation.

Methods: Specimens from 10 deceased infants (gestational age: 26.4 ± 2.7 weeks and birthweight: 808 ± 303 g) were examined as subjects. All infants died of severe cardiopulmonary disorder or intraventricular hemorrhage (grade IV). The average survival period was 3.1 ± 1.9 days. Autopsies were performed and formalin-fixed duodenums were immunostained with rabbit antiserum to motilin by the labeled streptavidin-biotin (LSAB) method. An adult duodenum obtained by pancreatoduodenectomy was also examined for the presence of motilin as a positive control specimen. An absorption test using motilin peptide was performed to prove the specificity of the binding with rabbit antiserum to motilin.

Results: Motilin-containing cells were detected in the adult specimen, and the binding by rabbit antiserum to motilin was completely inhibited by excess amounts of motilin peptide, indicating that this binding was specific to motilin. All 10 infants had presence of motilin antigen in the epithelial cells of their duodenums.

Conclusion: This preliminary study indicates that the immunohistological analysis is specific to detect motilin-containing cells, and certifies the presence of motilin in duodenal epithelial cells of premature infants of <32 weeks gestation, including one at only 22 weeks gestation.

**P 435**  
**KARYOMETRIC ANALYSIS OF THYMOCYTES IN  
DOWN SYNDROME**

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**Introduction.** Down syndrome (trisomy 21) is the most common numeric aberration of autosomal chromosomes in man; the incidence in newborn is about 1 in 700. Intrauterine growth retardation is a consistent finding in infants with Down's syndrome. The common anomalies, in order of frequency, are cardiac defects, duodenal obstruction, talipes equinovarus, cataracts, imperforate anus, cleft lip or palate, congenital megacolon, and meningomyelocele. Endocrine disorders in patients with Down's syndrome include hypothyroidism, occasionally associated with precocious puberty or diabetes mellitus. T-cell immunodeficiency in Down syndrome has been well documented.

**Purpose of the study.** To estimate nuclear size and integrated optical density of thymic lymphocytes in patients with Down's syndrome and control group.

**Material and Methods.** During the years 1988 to 2000, fourteen cases of Down's syndrome were found (eight male and six female). Ten babies without congenital anomalies, died with respiratory distress syndrome, were analyzed as a control group. Karyotyping was performed in all cases during life. After standard pediatric autopsy, 5  $\mu$ m thick sections were stained with hematoxylin and eosin. Karyometric analysis was done using ImageJ software, NU-1 microscope (Carl Zeiss, Jena, Germany), at objective x63 (NA=0.65), after manual editing of a binary image.

Five nuclear variables were estimated: mean number of lymphocyte nuclei per visual field, area, perimeter, circularity, and integrated optical density (IOD), in both cortex and medulla.

**Results.** In the thymic cortex, the mean number of lymphocyte nuclei was significantly lower in patients with Down syndrome. Integrated optical density of thymocyte nuclei was significantly higher in Down syndrome patient, compared to control group. Thymocytes nuclei were smaller in Down syndrome patients, but this difference was not statistically significant.

**Conclusions.** In Down syndrome patients the thymus is usually small and may be dysplastic with lymphocyte depletion; a small, contracted cortex and loss of corticomedullary demarcation resemble thymic involution. Large Hassall's corpuscles with calcification and cystic change in the thymus has been suggested as a marker for Down syndrome. The present results suggest altered gene expression in excessive genetic material, disordered maturation of cortical lymphocytes, with decreased number and increased chromatin density.

**P 436**  
**MORPHOLOGIC AND ULTRASTRUCTURAL FEATURES OF SOFT PALATE AND PHARYNGEAL MUSCLES IN CHILDREN WITH CLEFT PALATE**

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**Introduction.** Palate and lip muscle alterations in patients with cleft are poorly known. Few studies have focused attention on lip muscle, while very rare data are reported on the pathologic features of palate muscles. The morphological alterations of palate and pharyngeal muscles in infants with cleft palate could be responsible for a persistent postsurgical velopharyngeal insufficiency with consequent inadequate functional recovery especially for speech and swallowing. **Purpose.** Based on this, we decided to analyze the histologic features of the superior pharyngeal constrictor muscle and

right and left palatopharyngeal muscles in a series of 11 patients affected by isolated cleft palate and 9 patients affected by unilateral or bilateral cleft lip and palate. In 4 cases ultrastructural analysis was also performed. **Methods.** A muscle biopsy was done during palatoplasty. Muscle sections were stained with hematoxylin-eosin, modified Gomori trichrome, PAS and NADH-TR. The analyzed parameters were: organization and type of muscle fibers, presence of ragged red fibers, characteristics of nuclei, degree of fibrosis, presence of adipose tissue and inflammatory infiltrate.

**Results.** Our results showed the presence in both type of muscle of dystrophic-like alterations such as variability of fiber size, increase of connective tissue and presence of adipose tissue. No ragged red fiber, inflammatory infiltrate or neurogenic atrophy was observed. Electron microscopy evidenced focal areas of disruption of myofibrils containing amorphous materials. **Conclusions.** The marked alterations of palate muscles in children with cleft suggest that muscle damage could represent a significant pathogenetic factor in this type of malformation. The degree of muscle alterations may be responsible for a persistent postsurgical velopharyngeal insufficiency despite successful surgical repair. Muscular biopsy during palatoplasty could offer useful functional prognostic information in patients with cleft lip and palate.

**P 437**  
**HYPOPHYSEAL DUPLICATION WITH FRONTONASAL AND CNS MALDEVELOPMENT**

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**INTRODUCTION:** Hypophyseal duplication is an uncommon malformation that appears isolated or associated with craniofacial malformations. About 24 cases have been reported since 1880.

**PURPOSE OF THE STUDY:** To present a new case and review the central nervous system (CNS) and craniofacial anomalies associated with hypophyseal duplication in the autopsy cases reported in literature.

**METHODS:** Clinical reports, scanner images, and autopsy study of a girl (46, XX) 2 months and 20 days old.

**RESULTS:** The propositus had frontonasal malformation (hypertelorism, broad nasal bridge, bifid nasal tip and bifid anterior cranium), macrostomia, retrognathia, and bifid tongue. She also presented maxilomandibular bony fusion, as well as fusion of the tongue to the palate and to the inferior lip by a hairy polyp, both corrected surgically.

In the cranium, the sella turcica was broadened, with little prominence of the clinoid apophyses, and with two cavities that contained two complete hypophyses with two infundibules. The three cranial fosae were narrowed antero-posteriorly.

The CNS had both olfactory bulbs and corpus callosum agenesis, anomalous morphology of the brain stem and spinal cord, and neuronal heterotopia in occipital white matter and leptomeningeal heterotopia.

**CONCLUSIONS:** There are seven previous autopsy cases of hypophyseal duplication that associate, in order of frequency, the following frontonasal malformations: hypertelorism (6 cases), an intraoral mass –epignatus- (4), bifid tongue (3), duplication of the anterior groove of the spinal cord (1), and “V” shaped hairline (1). The associated anomalies of the CNS reported were broadened sella turcica and hypothalamic anomalies (7); corpus callosum agenesis (5) and olfactory bulbs agenesis (2), and glial and/or neuronal heterotopias (1). The present case has several common features with the one described by Hori et al. in 1983, that, in addition, had cleft palate, abnormal circle of Willis, duplication of the spinal

cord groove extended to the thoracic level, but it had no olfactory bulbs agenesis.

There are several hypothesis about the origin of the hypophyseal duplication and the associated malformations, yet none of them is definitive.

#### P 438

##### **MELANOTIC NEUROECTODERMAL TUMOR OF INFANCY IN THE RETROAURICULAR REGION: A CASE REPORT.**

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EFSTRATIOU Ioannis, ZAVITSANAKIS Athanassios, NEOFYTOU Andreas, PETROPOULOS Anastassios.

Melanotic neuroectodermal tumor of infancy in the retroauricular region: a case report.

Melanotic neuroectodermal tumor of infancy (MNTI) is a very rare neoplasm arising in the first year of life and localized mainly in the craniofacial region. It grows rapidly and has a high rate of recurrence.

We report a case of MNTI because of its unusual extraosseous localization. The tumor has been investigated by means of histology and immunohistochemistry,

A 10 month-old male infant presented with a swelling of the left retroauricular region. Ultrasonography showed a soft tissue lesion 1 cm in diameter with poor vascularisation without destruction of the skull. A dermoid cyst was suspected.

The surgical specimen measured 1,5x1x0,5 cm and had a whitish focally black color with elastic consistency.

Histologically the tumor consisted of alveolar nests and tubules within fibroblastic tissue. These formations were composed of two cell populations, large melanin-containing cells around smaller neuroblast-like cells with smashed nuclei. Immunohistochemically the melanocyte-like cells expressed cytokeratin and HMB-45. The smaller 'blue cells' didn't stain for any marker due to the smudging artefact. Histological and immunohistochemical characteristics of the tumor confirm the diagnosis of MNTI. Six months later the patient has no evidence of recurrence.

MNTI is a dysembryogenetic tumor of neural crest occurring commonly in the maxilla and in the mandible. In our case the tumor had a very infrequent site. The vast majority of these tumors behave in a benign fashion after local excision. However follow-up for at least 2 years is necessary because of the high recurrence rate.

#### P 439

##### **DIFFERENTIATED MELANOTIC NEUROECTODERMAL TUMOR OF INFANCY**

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A three-month-old male infant was admitted due to left temporal mass. He was born at full term by vaginal delivery. His birth weight was 2.8 kg and his head circumference was within normal limits. A mass was noted on left temporal area at the age of one month, and the mass progressively increased in size. Brain CT revealed an extra- and intra-dural tumor with calcification and destruction of overlying calvaria. Temporoparietal craniotomy with near-total removal of the tumor was done. On operation, the tumor involved left temporal bone, periosteum, dura and cerebral cortex. Microscopically, the tumor consisted of lobules and solid clusters of monomorphous small cells admixed with gland-

like or tubular arrangements of pigmented epithelium. Both pigmented melanin-containing epithelial cells and a small-cell component resembling neuroblasts were present, and the diagnosis of melanotic neuroectodermal tumor of infancy (MNTI) was made. The patient received 18 cycles of  $\text{if}^8$  drugs in 1 day  $\pm$  chemotherapy for 15 months. Follow-up CT disclosed a residual mass attached to the petrous bone with a peritumoral cyst. The mass showed strong contrast enhancement. Second operation was done 11 years after the first operation, and the residual mass was excised. The surgical specimen of second operation was a 3 cm-sized, pigmented mass with focal calcification. Microscopically, the tumor consisted of two different components. In the center of the tumor, pigmented epithelial cells formed clusters, glands or tubules. In the peripheral region of the tumor, lobules of mature ganglion cells, immature ganglion cells and abundant neuropil were surrounded by pigmented epithelial cells and fibrovascular septa. Compared to the original tumor, the residual tumor revealed extensive maturation of neuroblast-like small cells. We diagnosed this case as a differentiated MNTI.

MNTI is a rare but well-documented lesion of neuroectodermal derivation. Maturation of the neural elements has been reported only occasionally. To the authors' knowledge, this case is the first one of MNTI in which neuronal differentiation of the neuroblastic cells is convincingly demonstrated in a residual tumor over 10 years, and in which chemotherapy might have induced differentiation of the neuroblastic cells.

#### P 440

##### **1H MRS STUDY OF THE BRAIN IN NORMAL CHILDREN AND IN CHILDREN WITH SEIZURE DISORDERS**

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Purpose: We propose the quantitative indicators for the characteristics of regional and age-related peculiarities of brain metabolism in normally developing children and in children with seizure disorders.

Materials and Methods: Two groups of children are examined by 1H MRS using 1.5T Magnetom Vision (SIEMENS). The 1st group (NG) consists of 10 healthy children (in the age from 1 months to 16 years). The 2nd group (PG) includes 24 developmentally delayed children with seizure disorders (in the age from 2 weeks to 10 years). In all children 12x12 spectral matrixes in supraventricular region parallel to the canthomeatal line are obtained. 2DCSI 1H spectra are recorded with: TR/TE=1500/135 ms, NS = 1.

Results: In each voxel of the spectral matrix we introduce two indicators: the metabolite content AM as the peak area and the metabolite concentration CM as the ratio of the peak area to the sum of all the peak areas S:  $CM=AM/S$ . We describe the metabolic state in each voxel by the triad  $T^* = \{A_{Cho}, A_{Cr}, A_{NAA}\}$ , where ACho, ACr, and ANAA are the peak areas of the signals from Cho, Cr and NAA. For each of the areas we assign three values: 1, 2 and 3, to obtain six symbolic spectral configurations:  $1^* = \{1,2,3\}$ ,  $2^* = \{2,1,3\}$ ,  $3^* = \{1,3,2\}$ ,  $4^* = \{3,2,1\}$ ,  $5^* = \{3,1,2\}$  and  $6^* = \{2,3,1\}$ . We analyzed the temporal alterations of the triad distributions during neurodevelopment. In the NG during infancy (from 1 month to 1 year) the most frequent configurations in gray and white matter are  $3^*$ ,  $4^*$  and  $5^*$ . During childhood (from 1 year to 11 years) in the NG triads  $1^*$  and  $2^*$  dominate in all voxels of VOI. In the PG in the electrically defined seizure focus the triads  $5^*$  and  $6^*$  are observed. Signal of lactate is elevated only in children of PG, who had seizures immediately before MRS-examination.

Conclusion: The triad distributions in developing brain give us a quantitative way for the estimation neurodevelopmentally abnormalities.

#### P 441

##### THE CHILD'S CEREBRAL TUMORS

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The child's cerebral tumors (CCT) are rare. They represent 20% of the child's malignant tumors. They constitute the second malignant tumors in pediatrics after the leukemias 34,5% and the first cancer strong tumor, just before the lymphoma 7-10%. The object is to value through a set of 52 cases of CCT the epidemiological and pathologic features of this pathology. It is a retrospective review (survey carrying) on 210 cases of cerebral tumors collected to the service of anatomy pathology between June 2001 and August 2004.

The CCT represent 24,76% of the cerebral tumors. The median age at diagnosis was 8.9 years (range 2-16 years). The sex-ratio was 2 with a masculine predominance. The cerebral tumors of the posterior fossa cerebral occupied the first rank with 68.18%. The médulloblastoma represents 51,9% followed of the astrocytoma 19,23%. The CCT are highly heterogeneous, so in every case the histological diagnosis should be obtained. They distinguish themselves to the one of the adult by a particular topographic and histological distribution: Big frequency of the tumors of the posterior fossa cerebral, clean predominance of the astrocytoma, médulloblastoma. Their diagnosis require an anatomo-clinical and radiological confrontation. Prognostic factors are the degree of histologic malignancy and the extent of surgical resection.

#### P 442

##### ENCEPHALOCELE ASSOCIATED TO AN AGENESIS AND A LIPOMA OF CORPUS CALLOSUM : A CASE REPORT.

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Lipomatous hamartomas are rare disorders affecting the central nervous system. Forty percent of all lipomas develop in the anterior third of the corpus callosum. In 48% of the cases, there is partial agenesis of the corpus callosum. In 50% of the cases, there is no clinical expression.

We report the case of a 30 months old boy with frontal tumefaction since his birth. The neurological examination was normal. There were no associated malformations. The brain computed tomography scanning showed a lipoma and an agenesis of the corpus callosum. An hydrocephaly of the temporal and occipital horns has been found. Surgery was indicated. The mass was composed of typical adipose tissue in which a large number of blood vessels were present. The evolution was marked by post-surgery meningitis.

Our purpose was to describe a very rare association of an encephalocele, agenesis and lipoma of the corpus callosum.

#### P 443

##### IMMUNOHISTOCHEMICAL EXPRESSION OF CELL CYCLE REGULATORS IN PEDIATRIC EMBRYONAL BRAIN TUMOURS

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Background: Embryonal brain tumours in children are aggressive neoplasms with overlapping clinical and histological features. The distinction between Medulloblastomas (MB) and Atypical Teratoid Rhabdoid tumours (ART) has prognostic and therapeutic value.

The purpose of this study was to investigate the immunophenotypic features and the expression of cell cycle regulators, p27, Cyclin D3, Cyclin E, in 35 pediatric embryonal brain tumours in correlation with Ki-67 and p53, bcl-2 proteins.

Our material comprised 29 MB and 6 ART. The Streptavidin-Biotin HRP method was performed on paraffin sections for the detection of Synaptophysin (SY), GFAP, Neurofilaments (NF), EMA, Cytokeratin AE1/AE3, Cytokeratin 8, Vimentin (VIM), Smooth muscle actin (SMA), Desmin, Ki-67/MIB-1, P53 (DO7), bcl-2, p27 (1B4), Cyclin D3, Cyclin E with monoclonal antibodies.

The immunophenotype of MB was: 29+/29 SY, 9+/29 NF, 5+/29 GFAP, 0/29 EMA and AE1/AE3, while the ART were: 6+/6 VIM, 5+/6 EMA, 6+/6 AE1/AE3 and Cytokeratin 8, 4+/6 NF, 5+/6 GFAP, 4+/6 SMA.

High expression of Ki-67 (>25%) and p27 (>50%) was observed in 89,6% (26/29) and 79% (23/29) of MB respectively with increasing p27 expression in areas of neuroblastic differentiation. Cyclin D3 and Cyclin E were detected in 25% (6/24) and 62,5% (15/24) of MB.

High Ki-67/high p27 was observed in 68,9% (20/29) of MB with detection of Cyclin E in 65% (13/20) and a parallel Cyclin D3 expression in 3 MB. P-53 and bcl-2 proteins were detected in 75% (22/29) and 27,5% (8/29) of MB respectively.

High Ki-67 (>25%) and p53 (>10%) expression was revealed in all ART, while high bcl-2 and p27 expression was observed in 5. Cyclin E and Cyclin D3 were detected in 6 and 1 ART respectively.

In conclusion, the complex immunophenotype including the detection of epithelial markers contributes significantly in the differential diagnosis of ART from MB.

The parallel high Ki-67/p27 expression in correlation with Cyclin E detection in a considerable number of MB and ART suggests that dysregulation of G1/S transition may play an important role in their pathogenesis.

#### P 444

##### CHILDREN'S KAPOSÍ'S SARCOMA IN CAMEROON (CENTRAL AFRICA) WITHIN THE ONGOING AIDS EPIDEMIC AN ENDEMIC AREA

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Background: Cameroon (Central Africa) was known to be an endemic area of Kaposi's sarcoma even before the onset of AIDS epidemic. Jensen and al reported 16 cases of Kaposi's sarcoma in Cameroonian children between 1968 and 1974. An increase in the incidence of Kaposi's sarcoma have been noted in AIDS patients

all over the world nowadays. The aim of this study was to show the anatomoclinical aspects of Kaposi's sarcoma within the ongoing AIDS epidemic in Cameroon.

Materials and methods: we carried out this retrospective study over a period of 18 years ( from 1987 to 2004).All cases of children Kaposi's sarcoma were retrieved. We noted:

- the total number of Kaposi's sarcoma within the period of study,
- the ages and gender of the patients,
- the clinical signs,
- the HIV status,
- the pathological aspects,
- the treatment received and the evolution under treatment.

Results: among the 575 cases of Kaposi's sarcoma noted, 4% were aged 0 to 15 years. The youngest patient was aged 3 months and was HIV positive. The male to female ratio was 3/1. The most common site of involvement was cervical lymph nodes. All the children presented with fever and weight loss. Skin and lymph nodes were simultaneously involved in 2 cases. Only 4 cases of HIV positive serology were observed in this serie of children and their mothers. The pathological aspect most frequently observed was advanced stage Kaposi's sarcoma. Less than 10 children received fulldose of chemotherapy made up of cyclophosphamide, vincristine and prednisone. Only one child survived 9 years after chemotherapy.

CONCLUSION: Children Kaposi's sarcoma is still a rare disease in Central Africa for the moment.

#### P 445

##### **INTRAMEDULLARY OSTEOID OSTEOMA: A CASE REPORT.**

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Osteoid osteoma is a benign bone tumour. It accounts for 2 to 3% of the whole of bones tumours. The intramedullary localization is rare and poses diagnosis problems at the radiological stage.

We report the case of an 8 years old boy without particular pathological antecedents. He has consulted for pains of the left leg appeared 6 months before. Plane radiographes objectified a medullary tibial hyperdensity and a cortical thickening. The computed tomography showed intramedullary calcifications. A biopsy exèrèse of the tumour was carried out. The anatomopathological examination confirmed the diagnosis of the osteoid osteoma.

Intramedullary osteoid osteoma is rare. It poses a real radiological diagnosis problem. Its final diagnosis is anatomo-pathological. The characteristics of our case are the unusual localization of the osteoid osteoma associated with intense reactive bone sclerosis.

#### P 446

##### **CONGENITAL BILATERAL ADRENAL NEUROBLASTOMA WITH MULTIPLE METASTASES: A CASE REPORT**

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We report a case of bilateral congenital neuroblastoma (NB) with multiple metastases found at autopsy.

The neonatus was born by normal vaginal delivery in a local hospital at 40 weeks of gestation and weighed 3000g. Maternal history was unremarkable. His abdomen was markedly distended, suspected to congenital malformation. Prenatal ultrasound was not performed. He was urgently transported to the Central Hospital but died on the way two hours after birth.

Postmortem examination, revealed a relatively well demarcated huge tumors in the both adrenal glands with necrosis and hemorrhage. The kidneys were not involved by tumors. The left tumor measured 4x4x2cm and weighed 120g and the right tumor measured 6x4x2cm, weighed 140g. The left tumor was ruptured with massive intraabdominal bleeding (300ml). Multiple metastatic nodules in the liver (500g), lung and in the brain were noted. The other organs were grossly unremarkable. Microscopically the tumors were composed of small poorly differentiated neuroblasts with minimal stroma and without fibrillary background. The diffuse pattern of growth was accompanied with necrosis, hemorrhage, angiomatoid and pseudovascular formations. The tumor cells were immunoreactive for neuron specific enolase, chromogranin and synaptophysin, while desmin, MIC2, and leucocyte common antigen were negative. The microscopical findings of the liver, lung and brain were similar. A diagnosis of NB with multiple metastases was made. The NB was classified on the basis of INPC (Shimada) classification as one of the stroma-poor tumors, poorly differentiated, with mitosis karyorrhexis index of less than 200 - favorable histology.

#### P 447

##### **RENAL DYSPLASIA. ANALYSIS AND CLASSIFICATION OF 16 POST-NATAL CASES.**

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##### **INTRODUCTION.**

Renal dysplasia (RD) is the major cause of renal failure in children. It consists of a heterogeneous group of disorders with different etiologies and it is classified according to well-recognised histopathologic criteria. RD may affect the whole kidney or may be focal or segmental and may have a broad spectrum of severity. RD shows usually unilateral involvement of a kidney. Most forms of dysplasia have associated cysts suggesting common etiopathogenetic mechanisms. One of them is the disruption of renal branching morphogenesis. The more frequent forms of RD include the isolated multicystic dysplastic kidney, the dysplastic kidney from early fetal ureteric obstruction and the dysplastic kidney associated with a genetic syndrome.

##### **PURPOSE OF THE STUDY.**

To evaluate the clinical data and the microscopic feature of the post-natal dysplastic kidneys submitted to the Pathology Department in the last ten years according to the most used classification (Osathanondh and Potter 1964) and using a semiquantitative evaluation, devised by Tarantal et al. in 2001 in their experimental model, to grade dysplasia.

##### **METHODS.**

Clinical data and pathological material of 16 patients with RD registered between 1995 and 2005 were retrieved and reviewed.

##### **RESULTS.**

The 16 patients included 11 males and 5 females. Their ages ranged from 1 to 29 months with a mean age of 10 months (ten patients were less than 12- months old). Only one patient had bilateral RD (postmortem case) and 7 out of 16 had segmental disease. The malformations most frequently associated were ureter duplication (9 cases) and ureteral stenosis or atresia (4 cases). The microscopic features of the renal parenchyma including renal architecture, cyst, alterations in collecting tubules, primitive ductal structures, fibromuscular rings, changes in the metanephric mesenchyme, growth of primitive glomeruli and tubules and evidence of islands of cartilage has been scored. Cysts were very scanty in 2 cases and cartilage was absent in 9 cases ( 5 females). The dysplasia was less severe in female patients. Chronic inflammation was usually present.

##### **CONCLUSION.**

The prevailing classification of RD takes into account the clinical manifestations and the pathological changes but a completely satisfying classification system, encompassing new data from molecular studies on morphogenesis and traditional histology, is still missing.

#### P 448

##### **EVALUATION OF PROGNOSTIC IMPORTANCE OF THE 2001 SIOP PROTOCOL IN CHILDREN WITH NEPHROBLASTOMAS. A PRELIMINARY STUDY.**

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The application of modern complex therapeutic protocols in children with Wilms' tumor caused a spectacular increase in the curing rate up to 85-90% cases. Histopathological classification is a vital part of these protocols and morphological diagnosis determines the schedule of subsequent treatment.

The new Revised SIOP Working Classification of renal Tumor of Childhood (2001) was introduced in our center.

For our research we used formalin-fixed and paraffin-embedded archival material from 48 patients with nephroblastomas cured in our Hospital.

For histopathological evaluation of tumors we applied the previously used system and also the new Revised SIOP Working Classification of renal Tumor of Childhood (2001). Statistical analysis was based on Kaplan-Meier survival method and Kruskal-Wallis as well as Chi2 tests.

The aim of our study was to correlate the prognostic importance of both classifications.

Results:

The presence of regular and irregular blastemal component in evaluated nephroblastomas was related to poor prognosis. Children with Wilms' tumor with low differentiated epithelial component did similarly as patients with nephroblastomas which contained regular and irregular blastemal component. The application of the former classification showed the correlation between the histological grade of the tumor and the presence of metastases and relapses as well as with the presence of deaths from the disease.

The use of new SIOP (2001) classification also showed a strong correlation between the histological grade of the tumor and the presence of deaths from the disease.

Conclusion:

The new SIOP classification is an excellent tool in both the diagnosis and treatment of children with Wilms' tumor. The subdivision of cases fulfilling criteria for intermediate-risk tumors depending on either high or low epithelial differentiation might be useful for further prognostic analysis.

#### P 449

##### **IMMUNOHISTOCHEMICAL ANALYSIS OF PROTEIN P16 IN WILMS TUMOR**

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**SUMMARY:** Cyclin-dependent kinase (CDK) inhibitors represented by the INK4 family (including p16(INK4a), p15(INK4b), p18(INK4c), and p19(INK4d) are regulators of the cell cycle shown to be aberrant in many types of human cancer. AIM: The aim of our study was to investigate p16

protein expression in Wilms tumor, to compare it with the expression in normal renal tissue as well as to see if there is a correlation between p16 expression in Wilms tumor with tumor stage, histological type and prognostic group.

**METHODS:** 28 cases of Wilms tumor (2 cases with metastasis) and 2 samples of normal kidney tissue were studied using streptavidin-biotin-complex technique. P16 expression levels were semiquantitatively scored. **RESULTS:** The expression of p16 was observed in the majority of cases (96%). There was statistically significant relationship between p16 expression and tumor stage ( $p=0.015$ ) that is, tumors of higher stage (III/IV/V) more often expressed p16 than tumors of lower stage (I/II). Expression of p16 was detected in various histological types of Wilms tumor, but there was no statistically significant association ( $p=0.82$ ). p16 was found more frequently in high risk tumors than in tumors with good prognosis ( $p=0.02$ ). P16 immunostaining was observed in two cases of metastasis. **CONCLUSION:** Although, p16 is inhibitor of CDK, the results of our investigation show that nephroblastoma cells may proliferate in the presence of elevated levels of p16.

#### P 450

##### **ROLE OF THE ? TYPE COLLAGEN IN FORMING OF CONNECTIVE TISSUE ELEMENTS OF HUMAN FETAL KIDNEYS AT IUGR CASES IN 20-22 WEEKS OF GESTATION**

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It is suggested that fetal intrauterine growth retardation (IUGR) may lead to hypertension in adults. Alteration of kidneys tissue maturation may play an important role in the pathogenesis of a hypertension too. The aim of present study was to determine the distribution of the III type collagen in forming of connecting tissue of the fetal kidneys in cases of IUGR. **Methods:** 20 pairs of fetal kidneys (including 10 pairs of kidneys in cases of IUGR) from the late medical abortions because of psychotherapeutic reasons at 20-22 weeks of gestation were weighted, studied macroscopically and microscopically, and then the distribution of the III type collagen with the help of antibodies was investigated. Results have shown that kidneys in IUGR group have macroscopic and histological signs of both organ and tissue growth restriction. The intensity of the III type collagen expression and the area of its distribution increased, mainly in the perivascular connective tissue, kidneys cortex and medulla's stroma. **Conclusion:** Fetal IUGR is accompanied with the lesion of renal tissue formation. The intensified synthesis of collagen ? may be a marker of tissue remodeling when connective tissue formation and sclerosis outrun the parenchyma elements growth and maturation.

#### P 451

##### **PENTALOGY OF CANTRELL : AN EARLY CASE REVEALED AT 12 W.G.**

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We present the case of a 39 years old female foetus with termination of pregnancy at 14 W.G. for a polymalformation syndrome with: a midline supra umbilical wall defect, ectopia cordis, anterior diaphragmatic defect, defect of the lower sternum and omphalocele.

The G4P1A1 woman (2 miscarriages), with no history of systemic illness or familial disease, showed at the first obstetric ultra sonogram (12 W.G.) an increased nuchal translucency (6 mm), omphalocele, wall defect with ectopia cordis evocative of the Pentalogy of Cantrell. The autopsy showed a normal growth male foetus (Karyotype: 46, XY), with the full spectrum of the Pentalogy and also club feet, nuchal oedema without cystic hygroma, persistent left vena cava and probably a septal ventricular defect. The X ray films were normal.

The Pentalogy was first described by Cantrell in 1958 with five cases. The full Pentalogy occurs rarely and such early cases are very rare. The pathogenesis has not been elucidated. Cases are generally sporadic even if some families, monozygotic twins and rare chromosomal abnormalities (triploidy and trisomy 18) have been described.

We present this case because of its rarity and to stress on the importance of the examination of the foetus even in very early termination of pregnancy for an appropriate genetic counseling.

#### P 452

##### **HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDY OF THE EFFECTS OF TEUCRIUM POLIUM ON THE KIDNEY IN RAT**

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Teucrium Polium is commonly used in Iran traditional medicine for decreasing of blood sugar level. The aim of this project was to study of the effects of Teucrium Polium water extract on histologic structure of kidney in male rats. To examine such effects, 50 Sprague-Dawley rats were divided randomly to four experimental and one control groups. Experimental groups were fed with Teucrium Polium water extract (4gr/kg, 3gr/kg, 2 gr/kg, 1gr/kg) for 14 days. The rats were scarified, their kidneys were removed, and fixed, histological sections were prepared and stained with PAS/alcian blue and toluidin methods.

The results indicated that histological structure did not change but the extract affected on extracellular matrix. There was a mild precipitate of metachromatic material around the renal tubules of the cortex. Increasing amount of acidic glycoconjugates and metachromatic components were observed in extracellular matrix of medulla and it was dose dependent.

It seems that this extract causes some kinds of changes in renal extracellular matrix, therefore it is recommended that this extract should be used cautiously and it needs more research to find its complications.

Keywords: Teucrium Polium, kidney

#### P 453

##### **ESTIMATION OF GLOMERULAR VOLUME ON GLYCEROL-INDUCED ACUTE RENAL FAILURE IN RAT**

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Background: The use of stereological methods in evaluation of volume changes in vital organs such as kidney is very important. In this study, the volume of glomeruli in glycerol-induced acute renal failure in rat was determined.

Methods: 48 Sprague-Dawley male rats were divided into 2 groups (1 control, 1 experiment). Experimental group 10ml/kg of 50% glycerol was injected intramuscularly. And control group the same volume of saline was injected. After 48h, animals were dissected under deep anesthesia, and right

kidneys were fixed by vascular perfusion. Microscopic slides were stained with H-E. Glomerular volumes were calculated using projecting microscope, point counting and cavalieri principal.

Finding: The results indicated that renal failure induced-glycerol didn't effect on glomerular volume

Conclusions: It seems that estimation glomerular volume can not be useful variable in identifying of acute renal failure induced glycerol, but further investigation is needed to clarify the issue.

Key Word: Kidney, Stereology, Mean Volume

#### P 454

##### **POLYOMA (BK) VIRUS INFECTION IN TRANSPLANTED PATIENTS.**

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INTRODUCTION: Polyoma (BK) virus nephropathy was first described in 1971. In immunocompetent patients it has a high prevalence but a low morbidity. Urinary tract is usually affected. Ureteric stenosis, hemorrhagic cystitis and interstitial nephritis had been described in renal graft before introducing cyclosporine A and Induction therapy. Afterwards BK infection was rarely reported for decades.

MATERIAL AND METHODS. From July 2001 to May 2003 132 adult patients underwent kidney transplantation at the University of Padua. Among these 7 patients developed BK infection with histological proven interstitial nephritis. The patients were 5 males and 2 females, mean age 42 years. Time interval between transplant and infection ranged from 6 to 79 months (mean 25,5). Triple therapy was performed in all cases : FK-506, Mycophenolate Mofetil and Steroids was performed in 5, Cyclosporine, Mycophenolate Mofetil and Steroids in 1 case and Cyclosporine, Rapamycin and Steroids in 1 case. Biopsy was performed in all cases due to increase in serum creatinine level. Diagnosis was suspected at histology on haematoxylin and eosin stain and confirmed by PCR method. Three patients experienced graft loss.

CONCLUSIONS. Polyoma (BK) infection is becoming more frequent in renal grafts. This may be related either to new immunosuppressive agents or to total level of immunosuppression. Reaching biopsy diagnosis following creatinine elevation, is usually too late in order to allow graft survival, because nephrotoxicity of antiviral therapy. Role of surveillance biopsies and PCR on biopsic material for earlier viral detection should be considered in heavily immunosuppressed transplanted patients.

#### P 455

##### **TWO CASES OF CRYPTOCOCCOSIS DIAGNOSED ON KIDNEY GRAFT BIOPSY**

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Cryptococcus neoformans accounts for 4% of the fungal infections after renal transplantation, and mortality remains high despite therapy (30 to 60%). Diagnosis relies on isolation of the yeast or its capsular antigen in biological samples. We report on two cases of neuromeningeal cryptococcosis that have been diagnosed on histologic study of renal graft biopsy.

Mrs D, 62-year-old, had received a kidney graft for polycystic disease. Immunosuppression associated thymoglobulins, mycophenolate mofetil, tacrolimus and steroids. Two years after transplantation, a graft biopsy performed for nephrotic syndrome and progressive renal failure revealed an allograft glomerulopathy, with arterial fibrosis and membranoproliferative pattern. Immunofluorescence was positive for IgM deposits. Hepatitis serologies were negative. Suspecting chronic rejection, she received anti CD20 antibody therapy. Two months later, she died from a terrifying (foudroyant?) meningoencephalitis of unknown origin. Diagnosis was made on post-mortem graft analysis, which revealed cryptococcal yeasts in small renal vessels. It was secondary confirmed by blood cultures.

Mr S, 46-year-old, had chronic replicative hepatitis C, and type III cryoglobulinemia. Twenty years prior before, he had been transplanted for childhood malformative uropathy, and was treated by azathioprine, ciclosporine and steroids. In 2003, azathioprine was replaced by mycophenolate mofetil because of chronic allograft nephropathy. One year later, he presented a neuromeningeal cryptococcosis, diagnosed on lumbar puncture. The same month, immunosuppression discontinuation and end-stage graft failure led to transplantectomy. Endovascular cryptococcal yeasts were noted on transplant biopsy. Antifungal therapy led to complete recovery.

Histologic diagnosis of *C. Neoformans* infection must be evoked in front of capsulated yeast that are strongly Periodic Schiff Acid positive. They are typically stained by Grocott coloration and Alcian Blue staining.

Cryptococcal infection occurs in cell immunity deficient patients, such as AIDS, prolonged steroid therapy, malignancies, sarcoidosis and transplantation, especially under heavy immunosuppressive therapy. Because of its systemic spreading, the pathologist must be able to recognize such yeasts in peripheral organs, such as kidney graft in transplanted patients.

#### P 456

##### UNUSUAL DIFFERENTIAL DIAGNOSIS FOR VASCULAR ACUTE REJECTION : EARLY RENAL GRAFT PYELONEPHRITIS

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Accelerated acute rejection occurs within a few days after renal transplantation, due to preformed cytotoxic antibodies. It is often responsible for early graft loss. Histologic studies show evidence of vascular lesions with thrombosis, and polymorphonuclear leukocytes infiltrating glomeruli. We report on a case of acute graft pyelonephritis that mimicked an accelerated acute rejection.

Mrs B., a 34-year-old woman, developed 32% panel-reactive antibodies to HLA class I after a prior kidney transplantation for lupic nephropathy. Fourteen years later, she underwent a second renal allograft, with a pre-transplantation negative crossmatch, but a low positive crossmatch against B lymphocytes, on a one-year-old serum. The patient received anti-thymocyte globulins, mycophenolate mofetil, tacrolimus and steroids. After initial normalization of renal function, she became anuric on the 5th day. Clinical examination and Doppler ultrasound of the graft were normal. There was no fever, nor biological markers of inflammation or haemolysis. Bacteriological screening returned negative. The patient

received two steroid pulses and two plasma exchanges for suspected accelerated acute rejection.

Graft biopsy, performed on the 8th day, showed major polymorphonuclear (PN) cell infiltration of glomeruli, invading urinary space, as well as numerous PN casts stating the diagnosis of pyelonephritis. Interstitium examination revealed diffuse oedema, but only moderate PN leukocytes infiltrate. Rare glomerular thrombi were noted. The following day, urine and blood cultures returned positive for *Escherichia Coli*, and inflammatory markers elevated. Infection and renal function improved under antibiotherapy. Urinary tract infections are the most frequent infections in renal transplant recipients, favored by immunosuppression and urologic surgery. Although most of them are benign, they can sometimes threaten graft function, especially in early post-transplant period. In such cases, the differential diagnosis with acute rejection can be difficult, because of attenuation of physical and biological signs by steroid therapy, and then relies on kidney graft biopsy.

#### P 457

##### RELATIONSHIP BETWEEN POLY (ADP-RIBOSE) POLYMERASE WITH COLD ISCHEMIA AND DELAYED RENAL FUNCTION IN TRANSPLANT. DEMONSTRATION IN A PARP-1 KNOCKOUT MOUSE MODEL.

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Kidney allografts undergo pre-transplant cold ischemia and consequent ischemia-reperfusion injury (IR). Poly (ADP-Ribose) Polymerase (PARP-1) overactivation leads to massive NAD<sup>+</sup> consumption and ATP depletion with induction of cellular necrosis under ischemic conditions, which may lead to an increase in acute tubular necrosis (ATN) and a delay in total recovery of renal function (RFR) of the transplanted organ. Objective: Our aim was to demonstrate that the expression of PARP-1 are relationship with ischemia, develop of acute tubular necrosis and delayed of renal function.

Material and Methods: We studied the semiquantitative nuclear expression of PARP-1 in tubular cells by immunohistochemistry with the monoclonal antibody PAR01 in kidney transplant biopsies from expanded-criteria donors (ECD) (N=166), and allografts with ATN (N=94), and by immunohistochemical and western blot expression of parp-1 in knockout mice ischemia/[45 min.]/reperfusion [6 and 48 hours] model.

Results: In 50% of ATN biopsies, more than 50% of tubular nuclei were immunostained for PARP-1. PARP-1 expression was higher in ATN biopsies than in those from ECD ( $2.40 \pm 0.74$  vs  $0.92 \pm 1.13$ ,  $p=0.0001$  Mann-Whitney). PARP-1 showed a statistically significant relationship with the time required to achieve effective diuresis (Rho:0.608,  $P=0.0001$  Spearman test), with serum creatinine, and with duration of cold ischemia (Rho:0.650,  $P<0.0001$  Spearman test); these relationships were stronger in the biopsies with ATN (Rho: 0.774; Rho:0.806,  $P=0.0001$  Spearman test, respectively). In IR experimental model we induced tubular lesion in PARP-1 wild-type mice and we reduced the severity of renal injury in PARP-1 knockout mice and in PARP-1 wild-type mice chemical blocks with PARP inhibitor.

In conclusion, multivariate analysis demonstrated that PARP-1 expression and cold ischemia duration in kidney biopsies with ATN predicted the short-term RFR (delay in total recovery of renal function, and serum creatinine in the first

month) It is proposed that PARP-1 plays a pivotal role in the pathophysiology of I/R injury of the human kidney.

#### P 458

##### **BK –VIRUS NEPHROPATHY FREQUENCY IN RENAL TRANSPLANT BIOPSIES.**

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BK virus nephropathy is the most common type of viral disease following renal transplantations. Although the frequency is differs according to the transplantation centres, the rate is between 1.5% and 20%. The diagnosis can be established histologically by renal allograft biopsy.

The aim of this study is to search the frequency rate of the BK-virus nephropathy which causes the graft rejection in our cases.

The material of this study consists of the graft rejection cases that we received in last two years. The biopsies are diagnosed routinely according to the Banff criterias. We have found 5 cases (15.6%) of BK virus in 32 cases. All of the cases have been treated by immunosuppressive drugs following the transplantation surgery. Biopsies have been performed because of the disturbance of the renal functions. Only one case have had urinary examination.

Morphological diagnosis of BK virus nephropathy has been followed by serological examination.

The most frequent morphological changes with our BK virus infection are basophilia, nuclear inclusions, nuclear enlargement, on the tubul epithelium. Decoy cell has not been found in the only case who had urinary examination.

The treatment of immunosuppression of the cases with BK virus nephropathy has been reduced and antiviral treatment has been started.

BK virus nephropathy should be kept in mind in the differential diagnosis of transplant rejection and drug toxicity.

#### P 459

##### **IGNORED ALPORT SYNDROME REVEALING BY QUICKLY PROGRESSIVE GLOMERULONEPHRITIS ON RENAL BIOPSY OF TRANSPLANT**

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Miss KA, born in 1983, eldest child of six with consanguineous parents of first degree followed on hemodialysis since 2000 for chronic renal insufficiency developed after ignored initial nephropathy: (focal segmental glomerulosclerosis ?). Family history: \* a sister dead at 4years ago in a swollen syndrome, \* another 20 years ago sister has renal insufficiency and \* a brother of 16years ago has proteinuria higher than 3g/24h. TRANSPLANTATION of KA in June 2003 with the kidney of her mother of 43years ago. Good evolution under immunosuppressor therapy. EVOLUTION of the transplant :\* At 6 months (01/01/04) after surgery, occurrence of a arterial stenosis of the renal graft successfully cured. \*At 18 months (01/01/05) after transplantation, occurrence of h maturia with progressive increased creatinin and serum urea suggesting acute rejection or toxicity in Cyclosporine? Two biopsy were performed Renal biopsy 1: extra-capillary diffuse glomerulonephritis with linear IgG and C3deposits , leading to diagnosis of a quickly progressive glomerulonephritis by anti-glomerular basement membrane (GBM) antibody developed in ignored

Autosomal Recessive Alport's Syndrome . No recovery even after plasmatic exchanges and bolus of solumedrol and endoxan .Renal Biopsy 2 performed one month and half after the first one showed important lesions of chronicity. Patient given in hemodialysis. Discussion: For this rare complication the diagnosis is against all new transplantation and must lead to the meeting of a genetic council

#### P 460

##### **TYPE III COLLAGENOFIBROTIC GLOMERULOPATHY ASSOCIATED WITH IGA MESANGIAL DEPOSITS**

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The collagenofibrotic glomerulopathy is a recently described entity that is characterized by massive accumulation of banded collagen fibers in glomerular mesangial and subendothelial zones. Abnormal accumulations of banded collagen are an ultrastructural pathologic hallmark of nail-patella glomerulopathy. In this disease the banded collagen occur predominantly within the glomerular basement membranes, whereas in the collagenofibrotic glomerulopathy the abnormal collagen fibers occur predominantly in the subendothelial zone and mesangium.

Collagenofibrotic glomerulopathy is a rare disease that usually presents with nephrotic syndrome and renal insufficiency. It has been first reported in 1979 by Masaaki Arakawa. In 1993 M-C. Gubler et al. reported 10 children with collagenofibrotic glomerulopathy who had evidence for familial renal disease with an autosomal recessive transmission.

The pathogenesis of this disease is unknown. Some patients have elevated serum levels of type III procollagen suggesting up regulation of collagen synthesis.

A case of collagenofibrotic glomerulopathy has been diagnosed in a series of over 500 renal biopsies. This is the first internationally reported association of type III collagenofibrotic glomerulopathy and IgA mesangial deposits. A 22-year-old male was admitted with recurrent macroscopic hematuria. Physical examination revealed normal blood pressure and no edema. Laboratory values - normal blood tests. Hematuria: 30-60/mm<sup>3</sup>. Proteinuria: 250 mg/24h

The diagnostic was established after performing immunohistochemistry on frozen sections, light microscopy and a thorough electron-microscopic investigation. The light microscopy showed slight mesangial proliferation. The immunofluorescence emphasized IgA mesangial deposits. These IgA deposits have been clearly identified as dense paramesangial spots in electron microscopy too. In electron microscopy, type III collagen fibers, having a diameter of about 100 nm and a standard periodicity, were found arranged as clusters in the mesangial matrix and less in the sub endothelial area. Only a few fibers were found in the glomerular capillary basement membrane and in the capsular basement membrane. Not all the glomeruli showed type III collagen fibers. In the renal interstitial area, especially around the glomeruli, the strips of collagen fibers were more conspicuous than usual.

#### P 461

##### **THE MORPHOMETRIC STUDY OF IMMUNOFLUORESCENT C3D INTERSTITIAL DEPOSITS IN IGA GLOMERULONEPHRITIS**

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The IgA glomerulonephritis (IgAGN) is one of the most common primary glomerulonephritis and has a variable and difficult to predict evolution toward the end-stage nephrosclerosis. The deposition of C3d complement component in peritubular capillaries (PTCs) indicates a variant type of acute rejection while C3d deposition in primary glomerulonephritis (GN) is poorly documented.

The aim of this study is to examine C3d expression in peritubular capillaries (PTCs) and its correlation with the severity of renal injury in IgA glomerulonephritis. Since these interstitial, immunofluorescent deposits have tiny and poorly structured shapes our investigation applied some computerized morphometry programs. Thus we could demonstrate the occurrence of the C3c IF deposits in strict connection with the peritubular capillaries in either cross or longitudinal section. Polyclonal FITC conjugated rabbit anti-human C3c and C3d antibodies were used for direct immunofluorescent evaluation of the C3c and C3d deposits in 24 kidney biopsies with IgA glomerulonephritis.

The study revealed that the C3d deposits in peritubular capillaries were associated with known predictor aspects for rapid progression of IgAGN such as: glomerular sclerosis (63,6%), atrophic tubules (90,9%) and interstitial sclerosis (81,8%). The intensity of the C3c glomerular immunofluorescent deposits was related with active lesions. Thus, the predictive value of C3d deposition on PTCs in IgAGN is worth to be taken into consideration as an unfavorable outcome of the disease and request further long run investigations.

#### P 462

##### **MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS COMPLICATING DIABETIC NEPHROPATHY**

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Renal diseases other than diabetic nephropathy (DN) can be found in diabetic patients. Various forms of primary and secondary glomerular diseases were reported, but membranoproliferative glomerulonephritis (MPGN) is rare. We noted 4 patients with MPGN associated with DN at our Department for the past 3 years.

Two of the patients had enlarged lobular glomeruli due to mesangial and endocapillary hypercellularity. Intraglomerular cells consisted of monocytes, rare neutrophils, mesangial and endothelial cells. Mesangial matrix was enlarged with focal hyaline-sclerotic areas. The silver methenamine staining showed duplication of glomerular basement membrane (GBM). Visceral epithelium was hypertrophic and adherent to parietal epithelium. Interstitial edema with slight fibrosis was visible. Intrarenal blood vessels presented hyaline insudative changes. This was followed with deposition on immunofluorescence of granular deposits of IgA, IgM, IgG, C3, C4 and Fibrinogen. The whole histopathology in these two patients suggested nodular form of DN with diffuse MPGN.

The other two patients presented diffuse diabetic glomerulosclerosis. Inflammatory cellular mesangial proliferation with intracapillary influx of neutrophils was also present, with duplication and partial destruction of GBM. Part of the glomeruli presented diffuse changes, and the rest presented segmental changes. Tubular epithelium showed degenerative changes and the interstitium was edematous with diffuse mononuclear cell infiltration. Changes of intrarenal

blood vessels affected only the vascular poles. The immunofluorescent analyses showed granular deposits for the above mentioned antibodies.

The renal structure and function in both diseases are similar. Pathological changes in DN include thickening of all renal extracellular basement membranes and the mesangial matrix. Mesangial expansion is related to proteinuria, hypertension and declining GFR. Interstitial volume may be increased in insulin-dependent diabetes mellitus particularly in areas containing sclerotic glomeruli or marked tubular atrophy. Parallel findings were documented for type I MPGN in which the increased mesangial volume was related to decreased GFR, increased glomerular permeability to protein and hypertension. The cortical interstitial volume is correlated with functional abnormalities both in DN and MPGN. Besides these similarities, hyperperfusion injury characteristic for DN, can also be found in MPGN.

#### P 463

##### **THE ROLE OF VIRAL INFECTION IN ORIGIN AND PROGRESSION OF PRIMARY GLOMERULONEPHRITIS AND SOME NEPHROPATHIES**

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It is known that viral infection has a high profile in origin and progression of glomerulonephritis and noninflammatory nephropathies in children and adults.

The aim of our study was to determine the role of different viruses in development of glomerulonephritis and some nephropathies.

Methods and materials. 136 biopsies from patients (aged from 1,5 to 58 years) with unspecified viral presence (group I) and 68 biopsies from patients with proven viral infection (group II) were studied. Biopsies were examined by means of light microscopy, immunohistochemistry and electron microscopy. Viral typing was performed by means of PCR and IFA.

Results and discussion. Electron microscopy has revealed viral inclusions in 70 of 136 cases from group I. 12 types of viruses were determined in 68 cases of group II. It was revealed that single viruses as well as viral associations (from two to three viral types) play a role in progression of glomerulonephritis and noninflammatory nephropathies. The most commonly encountered were Herpes simplex virus (HSV), adenoviruses, hantaviruses and their associations. The correlation analysis between type of virus and morphology of nephropathy was performed. The most common morphological form of glomerulonephritis was mesangioproliferative glomerulonephritis (31 cases): in 30 cases single-type virus was revealed (adenovirus type 8 or 11, HSV type 1 or 2, Coxsackie-virus, RS-virus, hantaviruses (Hantaan and Puumala viruses), and cytomegalovirus). Membranoproliferative glomerulonephritis was revealed in 15 cases: membranoproliferative glomerulonephritis type I with 2 virus type combination was diagnosed in 7 most serious cases. Parvovirus B19 was revealed exceptionally in segmental-focal sclerosing glomerulonephritis and arteriolo-hyalinosis (6 cases). Herpes simplex virus type II was found in 1 case of segmental-focal sclerosing glomerulonephritis. Antiviral therapy resulted in strongly beneficial effect.

Conclusion. As a result of our study we confirmed viral infection participation in development and/or progression of glomerulonephritis and some nephropathies. However further investigations are needed in order to determine the role of viruses as an etiological factor, or as disease progression factor originated from corticosteroid therapy of.

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**DYNAMIC GD-ENHANCED MRI, IN VIVO  
<sup>1</sup>H MRS AND T<sub>2</sub>****RELAXOMETRY STUDY OF HUMAN KIDNEYS**

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**Purpose:**We propose quantitative analysis of Gd-enhanced MRI (time-intensity curves), in vivo <sup>1</sup>H MRS, and T<sub>2</sub>-relaxation time for differential diagnosis of renal tumors.

**Materials and Methods:** 44 patients are examined by 1.5 T Magnetom Vision System (SIEMENS). MRI are obtained after i.v. bolus of 0.1 mM/kg body weight of Gadovist 1.0 (Schering) with breath-hold sequence: FLASH-2D sequence:FA=70<sup>o</sup>,TR/TE=160/6ms,TA=22s which was repeated every minute for 10 minutes. <sup>1</sup>H MR-spectra are recorded with SVS-STEAM sequence:TR/TE = 1500/60 ms. In T<sub>2</sub>-relaxation time measurement the <sup>1</sup>H MR-spectra are recorded in and outside of the tumors with SVS-STEAM sequence:TR/TE = 1500/60,80,100,140ms.

**Results:**The following enhancement patterns were established for the normal renal medulla (NRM, maximal signal intensity (mSI) in the 2-4 min after Gadovist 1.0 injection), renal cell carcinoma (RCC, mSI in the 1min), and the transitional cell carcinoma (TCC, flat curve, enhancement weaker than in the NRM). In the case of angiomyolipoma the enhancement was not different from the RCC. From <sup>1</sup>H MR spectra the peak areas of main metabolite signals: water (4.7ppm), lipids(1.5,2.3-2.5,5.4ppm),

creatine+creatinine(Cr,3.0ppm), choline-containing compounds(Cho), and betaine (3.25-3.9ppm) are obtained. For NRM the following mean values of T<sub>2</sub> are calculated: T<sub>2</sub><sup>NRM</sup>(Wat)=(114.3+-9.6)ms before and (92.4+-3.5)ms after Gadovist 1.0 injection. The following mean values of T<sub>2</sub> are obtained: for RCC the mean values of T<sub>2</sub><sup>RCC</sup>(Wat)=(83.2+-4.7)ms before and (61.7+-5.2)ms after Gadovist 1.0 injection, and for TCC T<sub>2</sub><sup>TCC</sup>(Wat)=(72.6+-4.3)ms before and (53.7+-4.2)ms after Gadovist 1.0 injection.

**Conclusion:** This work is very helpful for study of kidney metabolism and for differential diagnosis of renal tumors.

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**TWO FAMILIAL CASES OF ANCA-ASSOCIATED  
VASCULITIS**

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Antineutrophil cytoplasmic autoantibodies (ANCA) directed to proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) are closely associated with systemic necrotizing vasculitides (AAV). However, ANCA alone are not sufficient, as based on clinical and experimental data, and other, probably exogenous factors, seem necessary for disease induction. Besides, various genetic factors are most likely involved in disease susceptibility.

**Case Reports**

We report on two familial cases of AAV. In the first family, the father had presented with an acute renal failure due to PR3-ANCA-associated disease three years before his daughter had exactly the same manifestation. However, at the time of his admission to the hospital she had already had E.N.T. involvement, which was later attributed to Wegener's granulomatosis. There were also very similar morphological features in their kidney biopsies. The second family involved two sisters, who were diagnosed with microscopic polyangiitis (MPO-ANCA associated) with a more gradual decline of renal function within four months of each other.

The younger sister actually suggested the diagnosis of her sibling based on her symptoms and probably saved her a significant amount of renal function.

**Conclusion**

As in other autoimmune diseases, the etiology of AAV is heterogenous and basically unknown. Different predisposing factors probably play differential etiopathogenic roles in various groups of AAV. The two familial cases support the hypothesis that genetic predisposition plays an important, although not yet fully understood role.

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**FREQUENCY OF DETECTION OF EPSTEIN-BARR  
VIRUSES (EBV) IN THE RENAL BIOPSIES: VALUE  
OF ULTRASTRUCTURAL FINDINGS AND  
POLIMERASE CHAIN REACTION.**

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**Introduction.** Concomitant viral infection exerts negative influence on the basic process and treatment of chronic renal pathologies. Intranuclear virion-like inclusions are often seen during diagnostic electron microscopy of renal biopsies, but it is difficult to identify these unusual inclusions for lack of specificity of its ultrastructure. We used within one year not only electron microscopy, but also polimerase chain reaction (PCR) and immunohistochemistry for analysis of all renal biopsies.

**Aim.** The purpose of the present study was the revelation and exact identification of viruses in the renal biopsies and also characteristic of accompanying alterations.

**Methods.** Renal biopsies with preliminary diagnosis chronic glomerulonephritis (n=145) were the subjects of the study. The material was prepared by the standard methods for light microscopy, immunohistochemistry and electron microscopy (EM-208/Philips). Identification of EBV was confirmed by PCR, we isolated DNA from non-fixed or formalin-fixed, paraffin-embedded tissue.

**Results.** In the biopsy samples DNA of EBV was detected by PCR most frequently (16 positive reactions, 10% of cases). For the same period DNA of a Herpes-virus (1-2 types) was found out in one case, and in one case DNA of cytomegalovirus. Ultrastructural analysis of these samples showed the specific alterations of nucleus - small or large electron dense chromatin formations with a light halo, numerous nuclear bodies, inner nuclear membranes thickening. The same abnormal nucleus ultrastructure was in the mesangial and visceral epithelial cells and in the interstitial monocytes. Light microscopic examination has detected presence of numerous limphocytes in the inflammatory infiltrate of the interstitium. Immunohistochemical examination of EBV antigen (Dako anti-EBV primary antibody, clone LMP) has demonstrated distribution of LMP in the cytoplasm of the glomerular podocytes, rarely in the infiltrating cells and in the tubular epithelial cells. The most frequent forms of basic pathology - amyloidosis, minimal change glomerulopathy and membranous glomerulonephropathy, are accompanied by tubulointerstitial alterations.

**Conclusion.** Our results show the high level of concomitant EBV infection in the renal biopsy samples from patients with chronic renal pathologies. Ultrastructural identification of intranuclear inclusions is helpful for early determination of EBV and other Herpesviruses in the renal tissue.

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**COMPUTERIZED ANALYSIS OF RENAL  
CORPUSCLE MORPHOLOGY IN RAPIDLY  
PROGRESSIVE GLOMERULONEPHRITIS**

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**Purpose:** The evaluation by computerized morphometry of the morphologic elements characterizing the renal corpuscles in rapidly progressive glomerulonephritis (RPGN), pointing out several gravity stages that induce a quantitative-type classification and justify distinct attitudes in the clinical approach.

**Material and methods:** We analyzed 10 cases diagnosed by renal biopsy as RPGN. The measurements were automatically performed on 100 renal corpuscles (10 per biopsy), relying on the procedure COREN, developed by us for running under KS400 (Zeiss) software. COREN exploits the RGB segmentation to accurately individualize the corpuscle (cor) and its entities presenting interest for the quantitative analysis: capillaries (cap), nuclei (nuc), connective tissue of the mesangium and crescent (cot), Bowman space (bos). For each considered corpuscle ( $i=1$  to 100), COREN measures the areas (square microns) of the corpuscle (denoted by  $A(i)_{cor}$ ) and of the aforementioned entities (denoted by  $A(i)_x$ , where  $x=cap, nuc, cot, bos$ ), computes the corresponding percentages relative to  $A(i)_{cor}$  (denoted by  $P(i)_x=A(i)_x/A(i)_{cor}$ ) and stores the results in a data base. Next, for each case ( $c=1$  to 10), COREN extracts the adequate information from the data base and calculates the mean values per case denoted by  $A(c)_{cor}$ ,  $A(c)_x$  and  $P(c)_x=A(c)_x/A(c)_{cor}$ . Finally, COREN provides the mean values characterizing the entire lot of biopsies, denoted by  $A_{cor}$ ,  $A_x$  and  $P_x=A_x/A_{cor}$ .

**Results:** For the analyzed biopsies we have obtained the following mean areas:  $A_{cor}=3485$ ,  $A_{cap}=460$ ,  $A_{nuc}=355$ ,  $A_{cot}=2140$ ,  $A_{bos}=260$ , which, in percentage terms, yield  $P_{cap}=13,2\%$ ,  $P_{nuc}=10,2\%$ ,  $P_{cot}=61,4\%$ ,  $P_{bos}=7,4\%$  and  $7,7\%$  for other elements (i.e. erythrocytes). Based on appropriate comparisons between the above values and the values corresponding to the 10 reported cases (not reproduced here for brevity reasons), we have studied the role of  $P(c)_x$  in the classification of the cases, reflecting different gravity degrees, which cannot be rigorously evaluated by the usual microscopic exam. Further evolutions of our cases have shown that the proposed separation in classes is fully motivated from the point of view of the medical practice.

**Conclusions:** The morphometric approach opens new perspectives for the analysis of the renal biopsy in the sense of refining the diagnosis and, consequently, the therapy. Future researches should focus on the general validity of the classification criteria, by enlarging the number of studied cases.

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##### **EXERCISED-INDUCED APOPTOSIS IS MEDIATED BY BOTH ANGIOTENSIN II AT1 AND AT2 RECEPTORS IN RATS KIDNEY**

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Excessive physical exercise may lead to disturbance of the entire homeostasis in the body, including the damage not only in skeletal muscles but also in many distant organs. Among

the mechanisms responsible for the exercised-induced changes could be an oxidative stress or angiotensin II. We previously showed that acute exercise led to apoptosis in kidney but not as a result of oxidative stress. In this study, we examined the role of angiotensin II and its receptors AT1 and AT2 in mediating exercised-induced apoptosis in kidney. We clearly demonstrated that an acute physical exercise induced apoptosis in renal distal tubular cells. Moreover, those cells displayed an increased expression of both AT1 and AT2 angiotensin II receptors and p53 protein. These results suggest that angiotensin II could upregulate p53 expression via both AT1 and AT2 receptors in renal distal tubular cells what might be a crucial mediating mechanism of apoptosis occurrence in kidney after excessive exercise.

#### P 469

##### **ROLE OF INDUCED NITRIC OXIDE SYNTHASE (i-NOS) IN UNILATERAL OBSTRUCTIVE NEPHROPATHY**

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**Background:** Obstructive nephropathy leads to tubulointerstitial fibrosis and loss of function. Nitric oxide has been shown to have antifibrotic properties. We explored induced nitric oxide synthase (i-NOS) expression in kidneys from rats underwent obstruction- release of unilateral ureteral obstruction.

**Methods:** Experiments were performed using a total 63 male Sprague-Dawley rats. The rats were either subjected to left proximal unilateral ureteric obstruction (UUO) or sham operation. The duration of UUO differed according to the group (1 day, 7 days, or 14 days obstruction). At each time point (1, 7 and 14 days) groups of rats ( $n=5$ ) were sacrificed without relieving the obstruction to evaluate the severity of the obstructive injury. Other rats had their obstruction relieved and were left for another 15 days before sacrifice to evaluate the extent of structural recovery. Controls animals ( $n=5$ ) were sacrificed in parallel at each time point. Paraffin sections were stained CD68 and iNOS expression and assessed by immunohistochemistry.

**Results:** A significant increase in cortical interstitium macrophage number on CD68 immunolabelling was shown between obstruction- release and control group. By contrast no differences were observed on renal cortical and medullar interstitium in control groups. A significant difference in macrophage infiltration in cortex and medulla interstitium was found between obstruction-release group and control group. ( $p<0.05$ )

Significant increase on iNOS expression in cortex and medulla tissue of obstruction release group compared to control group was observed. ( $p<0.05$ ) On the contrary, difference on i-NOS expression in cortex of control group compared to medulla of control group was found. But a significant increase on iNOS expression in medulla tissue of obstruction release group compared to cortex at the same group was detected.

**Conclusion:** In kidneys with UUO an expression of iNOS in medulla and cortex was increased. These results provide evidence for the iNOS expression in the renal cortex induced by inflammatory cytokines at medulla tissue of UUO kidneys. It is suggested a possible link in the pathogenesis of post "Cobstruction renal fibrosis.

#### P 470

##### **A RENAL INTRAVASCULAR LARGE B- CELL LYMPHOMA REVEALED BY PROTEINURIA.**

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Intravascular lymphoma is a rare subtype of large B-cell lymphoma. The lumina of small vessels are plugged with large lymphoid cells, especially in the brain, skin or lungs, without tumor mass or leukemia. Symptoms are non specific, related to vascular occlusion, and clinical diagnosis is difficult unless a microscopic examination of an affected organ is performed. This lymphoma has a propensity to rapid systemic dissemination and many cases have been diagnosed only at autopsy.

Case report : a 73-year-old male with a medical history of hypertension, repeated pulmonary embolism, heart deficiency and mellitus diabetes was hospitalized for a 38°C fever and weight loss without adenopathy, neither hepatosplenomegaly or neurological symptoms. Laboratory investigation disclosed proteinuria near the nephrotic range (2.2 to 3.3 g/24 hours), a severe inflammatory syndrome (C-reactive protein 115 mg/l, fibrinogen 7.3 g/l) and a mild elevation of serum lactate dehydrogenase (LDH) (473 UI/l). Immunoserology was negative (ANCA, antinuclear antibody). A kidney biopsy showed large atypical lymphoid cells only localized in glomerular capillaries, without other glomerular changes and a mild chronic interstitial nephritis. Tumor cells were immunostained by the CD45, CD20 and CD79a antibodies but negative for CD5 and CD10. There was no other tumor localization on brain, spine, skeletal, thorax and abdominal imaging as well as on bone marrow aspiration and biopsy. Laboratory tests quite suddenly worsened with a high LDH titer (> 1000 UI/l), hemoglobin concentration at 7 g/dL, and signs of microangiopathic haemolysis. Combination chemotherapy was initiated and after six cycles, the patient was alive and well, with normal laboratory tests. Nine months after the diagnosis, a second renal biopsy showed focal hyaline scars in glomeruli with no residual lymphoma cells. Comments: this case of intravascular large B-cell lymphoma appeared limited to the kidney, and, as in a few other reported cases, revealed by proteinuria. An early diagnosis by renal biopsy was followed by combination chemotherapy usually effective in high-grade non-Hodgkin's lymphoma and leading to complete remission.

#### P 471

##### **CONTRIBUTIONS TO THE KNOWLEDGE OF THE EVOLUTIVE POTENTIAL OF THE METANEFROGEN MESENCHYMA**

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Introduction. Oscar Hertwig (1881) proposed the term of mesenchyma as a morphogenetic entity instead of mezoblast. Today, it must be defined as an ancestral system endowed with variable differentiation potentialities in fundamental structures (cardiac, angiogenetic, nefrogenetic).

Purpose of the study. The purpose of this paper is to submit anatomic arguments for the knowledge of the differentiation ability of the metanefrogen mesenchyma.

Materials and methods. Our research has been achieved on human material (6 embryos, 4 fetus, 5 new born). We used classical micro anatomical staining techniques (Hematoxilin Eosine, Van Gieson, Gomori, PAS, Giemsa).

Results. The knowledge of the evolutive potentialities of the metanefrogen mesenchyma has been achieved due to our efforts in understanding the genesis and the evolution of the tubular and vascular nefron structures. By analyzing the serried section through tissue fragments in different stages of metanefrogenesis, we observed the existence of the dual differentiation potentiality of the metanefrogen mesenchyma – a part generated the walls of the Muller-Bowman capsule and

of the contort and Henle renal tubes; another part formed the glomerular capillaries.

Conclusions. We consider that the differentiation of the metanefrogen mesenchyma is determined by the inductive action of the urethral bud for the genesis of the nefron and tubular structures and by the neural crest migrated cells for the formation of the nefron vascular structures.

Key words: metanefrogen mesenchyma, Bowman capsule, nefron

#### P 472

##### **PROSPECTIVE STUDY OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AND C-KIT EXPRESSION IN NON SMALL CELL LUNG CANCER (NSCLC) AND NEURO-ENDOCRINE CARCINOMAS (NEC)**

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The new target therapies are designed molecular missiles, aimed at specific cancer cellular command centers. Kit (CD117), the product of the proto-oncogene c-kit, is a tyrosine-kinase transmembrane receptor. Kit positivity is in fact a definitive feature of GIST. EGFR is another important cellular receptor, with EGFR-targeted agents such as gefinitib, OSI-774, and cetuximab in current development.

We decided to analyse the CD117 and EGFR immunohistochemical profiles of majors types of lung tumors on surgical specimens or lung biopsies. This prospective study of expression of EGFR and c-kit was assessed on 49 adenocarcinomas, 8 squamous tumors and 8 small cell lung carcinomas (SCLC) or neuro-endocrine carcinomas (NEC). Material was formalin-fixed, paraffin-embedded tissue, obtained by thoracoscopic wedge biopsies, bronchial biopsies or resection specimens.

44/49 adenocarcinomas, 6/8 squamous epitheliomas, and 1/8 SCLC or NEC, revealed increased expression of EGFR. 5/49 adenocarcinomas, 1/8 squamous epitheliomas, and 5/8 SCLC or NEC, revealed increased expression of c-kit CD117 immunoreactivity was also more frequent for the patients with SCLC or NEC lung carcinomas (p=0.001). For 46 adenocarcinomas, 6 squamous carcinomas and 6 SCLC or NEC, we evaluated if the expression of EGFR or CD117 immunoreactivity was correlated with differents variables as clinicopathological characteristics (gender, tobacco's status), therapy (surgery or chemotherapy) or response to chemotherapy. Statistical analysis used a Fisher's exact test. None of the tested parameters was significative for the squamous carcinomas or adenocarcinomas. There was no statistical link between tobacco's status and EGFR overexpression. CD117 immunoreactivity was correlated with a good response to chemotherapy and a lack of progression for the patients with SCLC or NEC (p=0.07).

The success of imatinib in the treatment of metastatic GIST has stimulated interest in understanding the functional sence of kit immunoexpression in others tumors. This therapeutic's way could be attractive for the SCLC or the NEC, as they often overexpress c-kit. Although a recent study with a little number of patients with SCLC was negative, it should be interesting to assess this molecule's efficacy in the other NEC.

#### P 473

##### **PROGNOSTIC VALUE HER-2/NEU ONCOGENE AND ESTROGENE RECEPTOR EXPRESSION IN NSCLC PATIENTS**

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**Introduction:** Lung cancer is the leading cause of cancer mortality worldwide. Patients prognosis partially depends on some traditional pathohistological parameters and clinical stage, but in recent time many studies try to find some additional risk factors which would be able to divide patients with better disease-free and overall survival from those who will have early tumor recurrence and metastatic disease.

**Patients and methods:** Clinical information and pathological slides and paraffin blocks from 132 consecutive patients with non-small cell lung carcinoma (NSCLC) were collected to evaluate HER-2/neu oncogene and oestrogen receptor (ER) expression, as well as their correlation with patients survival. All tumors were reviewed to verify histological parameters as histological type, tumor and nuclear grade, quantity of lymphocytic infiltrates, mitotic rate, and degree of tumor necrosis. Additionally, clinical data as sex, smoking habits, preoperative clinical stage, and overall survival were collected. HER-2/neu and ER status were determined immunohistochemically and correlated with clinical and pathohistological data.

**Results:** The results showed statistically significant correlation between HER-2/neu and nuclear grade ( $p=0,010$ ). Additionally correlation between ER expression and histological type of tumor ( $p=0,043$ ), and mitotic rate ( $p=0,008$ ) was found. Kaplan-Meier analysis showed statistically significant correlation of patients overall survival with TNM status ( $p<0,0001$ ) and degree of tumor necrosis ( $p=0,0153$ ). Cox proportional hazards regression analysis showed that male gender ( $p=0.01214$ ), histological type ( $p=0.0303$ ), high degree of necrosis ( $p=0.0064$ ), and higher histological grade ( $p=0.0370$ ) had significant influence on patient's survival. Relative risk of death was 2.7 times higher for male gender, 2.2 times for adenocarcinomas in comparison with squamous carcinoma, and 3.4 times higher for tumors with prominent necrosis in comparison with tumors without necrosis.

**Conclusion:** Our findings indicate that some traditional histological parameters are important for determination of NSCLC patient's prognosis, but that ER and HER-2/neu expression might be of potential usage in clinical practice for assessment of certain group of patients who might benefit of specific treatment.

#### P 474

#### EVALUATION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND C-ERBB2 IN DIFFERENT TYPE OF NON SMALL CELL CARCINOMA OF LUNG FAHKRJOU ASHRAF M.D.,MONTAZERI VAHID M.D.

**INTRODUCTOIN:**The aim of this study was to evaluate the effects of EGFR(epidermal growth factor receptor) and c-erbB2 in the different type of non small cell lung cancer ,and to compare the results with normal lung tissue and different stages of tumor.

**METHOD:** For this study ,79 paraffin blocks were obtained for 64 patients having a confirmed pathological report of non small cell lung cancer and 15 normal lung tissue as a control group.All of specimens were stained with monoclonal antibodies against EGFR and c-erbB2.

**Results:**In this study 30 patients with SCC(squamous cell carcinoma) , 30 patients with AC(adenocarcinoma),2 cases of large cell cacinoma and 2 patients with carcinoid tumorwere selected.In 27(90%) patients with SCC ,EGFR was positive and in 20(66%) patients with adenocarcinoma,c-erbB2 was positive.None of large cell carcinoma and carcinoid tumor were positive for these factors.

**CONCLUSION:**According to the results of this research there is sinificant difference between SCC and AC of lung that can help for adjuvant treatment after surgery of tumor.

**key words:**Lung cancer,non small cell cancer,c-erbB2,EGFR.

#### P 475

#### PROGNOSTIC SIGNIFICANCE OF KI67, CD44,AND CD 105 TUMOUR MARKERS IN SURGICALLY TREATED NON-SMALL CELL LUNG CANCER (NSCLC)

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**Introduction:**Given the fact of an ominous prognosis in surgically treated non-small cell lung cancer (NSCLC), even in early stage cases, more specific prognostic factors could be of help in the preoperative categorisation of these neoplasms.

**Aim of the study:**We examined the expression of Ki67 (nuclear proliferation index), CD44 (adhesive molecule) and CD105 (vascular index)in surgically treated NSCLC and correlated these markers with histological type, stage and size of the tumour.

**Material and methods:**41 specimens of surgically treated NSCLC were included in this study, 23 adenocarcinomas (56.1%),13 squamus (31.7%)and undifferentiated (12.2%).22 patients were staged as Ia-Ib (61.1%)and 14 as IIa-IIb (38.95%)

Immunohistochemical staining was performed on paraffin sections.Our results were correlated to the clinical and pathological parametres(size,histology,stage).The evaluation of CD105 vessels positivity was based on microvessel density technique (MVD).

**Results:**All results were statistically assessed using Pearson chi2 score.Statistical significance was observed in Ki67 expression in adenocarcinomas ( $p<0.014$ )which could be explained by an increased agressiveness of this type of cancer.CD105 was also overexpressed and correlated to a more advanced stage of disease which apparently seems to be related to a worse disease prognosis.CD44 did not present with significant statistical advantage and awaits further investigation considering the fact that a larger number of cases may be apparently required to provide us with more conclusive results.

#### P 476

#### PROGNOSTIC SIGNIFICANCE OF NK CELLS AND TNFA IN SURGICALLY TREATED NON-SMALL CELL LUNG CARCINOMAS

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**Aim:** To assess the prognostic significance of NK cells (CD56) and TNFa in biopsy specimens with non-small cell lung cancer (NSCLC).

**Materials and methods:**104 patients who underwent surgery because of NSCLC were investigated as follows: tumors were classified as squamous cell carcinoma (n=53) or adenocarcinoma (n=51), and age, tumor stage, and tumor size were assessed. Expression of TNFa in lung cancer cells and the NK cells were estimated with the use of monoclonal antibodies. Samples showing >10% of TNFa positive tumor cells were considered positive. Samples with 20-30% infiltration with CD56 were considered positive and >30% strongly positive for the NK cells.Results were assessed

statistically using Pearson chi square. Life tables were estimated by Kaplan-Meier statistics and survival curves were compared using the long-rank test (SPSS 10.0).

Results: 28 samples (26.9%) were TNF $\alpha$  positive and 76 samples (73.1%) were TNF $\alpha$  negative. The survival for negative samples was 56,97 months and 37,52 for positive (p=0.01). There was also a positive correlation between the TNF $\alpha$  expression and more advanced stages IIIA and IIIB (p=0.01). 43 (43%) specimens were CD56 negative, 44 (44%) CD56 positive and 13 (13%) were strongly positive. There was no correlation of CD56 positive expression with the stage and survival (p=0,1000/p=0,2392 respectively). No relation was found with the size of the tumour, the histological type and the age.

Conclusion: The current study could support a role for TNF $\alpha$  as a prognostic factor for patients with non-small cell lung cancer.

#### P 477

### EXPRESSION OF MATRIX METALLOPROTEINASES 7 AND 9 IN NON-SMALL CELL LUNG CANCER; RELATION TO CLINICOPATHOLOGICAL FACTORS, PROGNOSIS AND BETA-CATENIN

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**INTRODUCTION:** Lung tumour aggressiveness is based on its invasive and metastatic properties probably regulated by different cell-cell and cell-matrix interactions. Matrix metalloproteinases have an important role in many physiological processes and cancer cell behaviour.

**PURPOSE OF THE STUDY:** The present study was undertaken to analyze the expression and prognostic value of MMP-7 and MMP-9 in non-small cell lung cancer (NSCLC). The relationship of MMP-7 with beta-catenin was also evaluated.

**MATERIAL AND METHODS:** The study consists of 212 patients with resected NSCLC. The representative tumour samples were collected and stained immunohistochemically. The expression of MMP-7 and MMP-9 was evaluated in both tumour cells and peritumoural stromal tissue. Results were compared to beta-catenin expression and clinicopathological factors of the patients.

**RESULTS:** The normal bronchial epithelium was either MMP-7 negative or showed weak positivity mainly in apical cells. In MMP-9 stainings the positive staining signal was noted through the whole bronchial epithelium. The dysplastic epithelium was seen in 11 cases. Three out of 11 cases were weakly positive for MMP-7, whereas high MMP-9 staining was seen in all cases throughout the dysplastic epithelium. A high staining of MMP-7 and MMP-9 in tumour cells was noted in 62 (30%) and 113 (57%) cases, respectively. Expression of MMP-7 in cancer cells was associated with histological type of tumour (p=0.022). High cancer cell associated MMP-7 was related to less advanced tumour (p=0.037) and better tumour differentiation (p=0.005). High MMP-7 was also correlated with normal beta-catenin expression in tumour cells (p=0.001). High MMP-9 expression in tumour cells was related to poor tumour differentiation (p=0.016). The stromal signal for MMP-9 was observed in 58 (32%) cases and was linked with higher tumour grade (p=0.031). In survival analyses the significant predictors of survival were histological type of tumour and tumour stage (p=0.0009 and 0.0012, respectively). MMP-7 or MMP-9 expressions were not related to patient's outcome.

**CONCLUSION:** The results indicate that in NSCLC high MMP-9 expression both in cancer cells and tumour stroma is

a sign of more aggressive tumour, while high MMP-7 expression correlates with less advanced disease.

#### P 478

### MORPHOMETRIC INTRATUMORAL MICROVESSEL AREA EVALUATION: USEFUL INDICATOR FOR COADJUVANT THERAPY IN RESECTED NSCLC

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**Introduction:** Despite improvements on diagnosis and treatment, the prognosis of non small cell lung cancer (NSCLC) still is reserved. There has been much research on new biological parameters and prognostic markers for innumerable malignancies. Studies have demonstrated that angiogenesis can be considered as an independent prognostic factor in several neoplasias including those from lung. Computed assisted analysis of angiogenesis has been performed in some types of tumours by assessment of the number of tumour vessels. This study, however, includes also the area of tumour vessels with image analysis in NSCLC as well as survival rates in Brazilian patients. **Purpose:** To accomplish the tumour vascular area measurement and the microvessel count with computed assistance through microscopic images from NSCLC, and verify the correlation between these measurements and patient survival in a 5-year period. **Material and methods:** Paraffin blocks from 107 surgical specimens for NSCLC from Pavilhao Pereira Filho - Santa Casa (Porto Alegre, Brazil). Immunohistochemistry with anti-CD34 monoclonal antibody (QB-End10; Dako Corporation) was performed on paraffin-embedded tissue sections in order to highlight the vessels. The tumour vascular area and the microvessel count were obtained through Image Pro Plus - 3.0 program. 6 images (200x) from 3 hot spots have been taken from each case, totalling 18 images per case. With this software, the vascular area and the number of tumour vessels images have been obtained. The vascular area of each case was assumed as the mean value of its all 18 images. The cases were then separated into high or low vascular area groups, the cut-off being the mean from all 107 cases. Similar process has been made to the microvessel count method. **Results:** the 5-year survival rate of high vascular area group was 21.7 months, b 2.5, significantly lower (P<0.0001) than the low vascular area cases, 38.9 months, b 3.0. The correlation coefficient between the microvessel area and the microvessel count was 0.5 (P=0.001). The tumour vascular area showed higher correlation to survival (correlation coefficient: 0.48 / P= 0.001) than the tumour microvessel count (correlation coefficient:0.2 / P=0.03). **Conclusion** The tumour vascular area measurement with computed assistance can be used as a prognostic marker in NSCLC.

#### P 479

### THE IMPACT OF IMMUNOHISTOCHEMICAL DETECTION OF POSITIVE LYMPH NODES ON STAGING AND PROGNOSIS IN EARLY LUNG CANCER

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Detection of micrometastatic disease is an interesting topic about lung cancer. In early stage, some researchers showed its prognostic impact on survival. Therefore we conducted a study to determine whether the detection of mediastinal lymph node spread by immunohistochemical analysis affects the risk of recurrence and metastasis.

Between 1997 and 2003 21 patients underwent complete resection with mediastinoscopy and systemic nodal dissection for early stage (Stage I and II) lung cancer in our clinic. 426 lymph nodes from 21 patients were analysed. Epithelial specific antigen Ab-9 and Keratin Pan Ab-1 were used as immunohistochemical markers. Based on nodal spread four of the 21 patients (19.04%) were upstaged after the immunohistochemical analysis. One patient with stage IB (T2N0) were upstaged to IIIA (T2N2); two patients staged as IIB (T2N1) were upstaged to IIIA (T2N2). only one of the 11 patients who had pN0(i) developed local recurrence. On the other hand three of four patients who had both pN1(i) and pN2(i) disease, developed either local recurrence or metastatic disease. Statistical analysis showed that the lymphatic dissemination detected with immunohistochemical analysis was associated with reduced disease-free-survival.

Our study provides some verification that patients with lymphatic micrometastasis have a reduced disease free survival. These results suggest that adjuvant therapies may have a role in early stage lung cancer. Before the new TNM staging system will be commonly used more information is needed to understand the prognostic impact of micrometastatic dissemination.

#### P 480

##### DIFFERENTIAL DIAGNOSTIC OF SMALL-CELLED LUNG CANCER USING PLOIDOMETRY

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**Background.** The problem of early differential diagnostics of small-celled and non-small-celled lung cancer is particularly important in the light of different approaches to clinical management depending on a tumor type.

**Aim:** to use ploidometry research for studying the epithelial cell clones in the lung tumors to determine objective criteria of diagnostics of small-celled and non-small-celled lung cancers.

**Methods.** Computer ploidometry test was carried out for nuclei of epithelial cells in bronchial biopsy specimens, taken from 50 patients with small-celled and squamous cells carcinomas in different stages of cancerogenesis. Bronchial epithelium nuclei taken from 10 healthy individuals were used as control. Histological preparations of 8 micron thickness, stained by Feulgen's technique, were subject of the research. Nuclei's ploidy was defined by means of the computer image analyzer "Imager-CH" ("Avtan-San" software version). The "tissue ploidy standart" (2c) was used for obtaining the data of 1856 bronchial epithelium nuclei. Inter-phase cell nuclei of clones of normal bronchi epithelium, small-celled and squamous cells carcinomas in different stages of differentiation were the subjects of investigation.

**Results:** In comparison with the average ploidy value of the normal bronchial epithelial cells nuclei, the average ploidy index increases 1,7 times in small-celled cancers. 2 times in well differentiated squamous cells carcinomas and 2,5 times in poorly differentiated squamous cells carcinomas. The study results showed that tumor cells proliferative activity and nuclei ploidy increase in parallel with the increased disdifferentiation of squamous cells carcinomas. In routine diagnostics of bronchial biopsy specimens morphological identification between small-celled and poorly differentiated squamous cells carcinomas is particularly complicated and

equally important for further clinical management. The average ploidy of nuclei significantly differs between clones of small-celled and squamous cells cancers (4.2 c versus > 6.3 c) which makes the ploidy index a useful differential diagnostic criteria.

**Conclusions:** Computer ploidometry may be used in differential diagnostics of small-celled and squamous cells lung cancers. Use of ploidometry data in morphological diagnostics of lung tumors can be an objective tool for taking justified clinical decisions.

#### P 481

##### THYMUS TUMORS ON OPEARTIVE TISSUE

##### MATERIAL

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**Background:** Thymus tumors are the most frequent tumors of anterior mediastinum. It is a great challenge for surgical pathologist to estimate a precise type of thymus tumor.

**Methods:** We analysed 75 operated tumors of anterior mediastinum of thymus origin in last fourteen years. Thymus tumors were classified and reclassified according to the most recent classification. Thymus cysts were included in a group of anterior medisatinum operated tumors of thymus origin.

**Results:** Only on 14 (18.6%) preoperative percutaneous needle biopsies pathohistological diagnosis of thymoma was established. They measured approximatively 79mm in the greatest diameter. Operated tumors were mostly encapsulated by smooth capsule, lobulated by wide septas, mostly solid or partly cystic. Eleven of them penetrated capsula and involved surrounding tissues. The most frequent was thymoma of A type - 18 (24%), then of AB type - 12 (16%), of B1 - 8 (10.66%), of B2 - 4 (5.33%) and B3 - 4 (5.33%), of C type 6 (8%). Also 3 Hodgkin lymphoma and 3 germ cell were diagnosed, each of 4%. One thymolipoma and one carcinoid tumor of thymus were also estimated - each of 1.33%. Very frequent were diagnosed thymus cysts - 15 (20%).

**Conclusion:** Preoperative percutaneous needle biopsies is helpful to distinguish thymus tumors from to other tumors of anterior mediastinum, especially mesenchimal tumors and Hodgkin and B and T non Hodgkin lymphoma. According to their morphology and immunophenotype the precise pathohistological diagnosis could be established and adequate therapy, operated or else oncologic, applied. On small tissue samples the tumor biological behaviour, penetration of thymus capsula and involving of surrounding tissues, can not be estimated. It is only possible on operated thymus tumors.

#### P 482

##### MUCINOUS CYSTIC TUMOR OF THE THYMUS: A DOCUMENT OF AN EXTREMELY UNCOMMON CASE

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Pure epithelial benign neoplasms without lymphoid element are extremely rare in the thymus, although several examples of thymic adenocarcinoma have been described in the literature. We recently encountered a cystic tumor almost purely composed of goblet cell-like mucus-producing cells of the thymus arising in a middle aged Japanese male and describe herein its histological features with brief discussion regarding to the histogenesis. The patient was a 54-year-old male, who was pointed out of enlargement of the

mediastinum in 2004. But he had no complaint such as pain and remained untreated approximately for a year. At the initial presentation to our hospital in early 2005, computed tomography revealed a tumor of the mediastinum and surgical removal of the thymus was performed. Gross specimen presented a unilocular cystic lesion containing abundant mucin, which was surrounded by thymic tissue and measured 8x5.5x4.5cm. Dense fibrous tissue occasionally associated with calcification intervened between the lesion and the thymic tissue. Microscopic examination revealed a cystic tumor consisting of bland mucus-producing cells resembling goblet cells and forming tiny daughter cysts within the dense fibrous capsule. In some areas, pseudostratified columnar cells were recognized as well as flattened cells showing slight atypia and nuclear enlargement in the basal layer. No destructive growth or infiltration into surrounding thymic tissue was observed. Thus, the tumor should be regarded as a mucinous cystic tumor of the thymus, probably • gmucinous cyst adenoma• h. Immunohistochemically, tumor cells showed positive reaction for AE1/AE3, CAM 5.2, but negative for 34betaE12 and involucrin. Only a few cells were reactive for p63. Two hypotheses are to be discussed on histogenesis; this tumor is likely considered to have developed from a thymic cyst on basis of pseudostratified columnar cells occasionally identified among mucinous cells. Otherwise, it might have a close relationship to mucoepidermoid carcinoma rarely found also in the thymus, although definite evidence of squamous differentiation was not confirmed. Anyway, the present case is an extremely common neoplasm which has not been described.

#### P 483

#### EXPRESSION OF OCT4 AND CELL CYCLE-REGULATORY PROTEINS IN SEMINOMA AND EMBRYONAL CARCINOMA ORIGIN IN THE MEDIASTIUM

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**Background.** Primary germ cell tumor is a relatively rare tumor usually located in the anterior mediastinum. A previous study has postulated that OCT4 is a nuclear transcription factor that is expressed in pluripotent embryonic germ cells. The cell cycles in the germ cell tumors have not been well documented. This study attempted to identify and characterizations of OCT4 expression and cell cycles in the specific germ cell tumors, seminoma and embryonal carcinoma.

**Methods.** A retrospective study conducted between 1983 and 2005 included 10 consecutive patients with seminoma and embryonal carcinoma in the mediastinum whose tumors had been surgically excised. Overexpressions of OCT4, Ki-67, p16, p53 and p63 genes were studied by immunohistochemistry.

**Results.** Of the 10 seminoma and embryonal carcinoma in the mediastinum patients, all were men (age 26.8  $\pm$  15.5 y). Clinical presentations included asymptomatic (38%), chest pain (49%), and dyspnea (13%). The study group contained 10 (100%) men, with a mean age of 26.8  $\pm$  15.5 years and follow-up 37.2  $\pm$  18 months. Over-expression of the OCT4 (66.7;  $\pm$ 38.3%), p53 (68.6;  $\pm$ 31.3%), and p16 (55.5;  $\pm$ 34.7%) were considerably higher than Ki-67 (31.4;  $\pm$ 27.6%).

**Conclusions.** This work first examined the immunohistochemical over-expression of OCT4 and cell cycle (Ki-67, p16 and p53) in seminoma and embryonal carcinoma in the mediastinum. This approach demonstrates OCT4 is highly sensitive and specific for the diagnosis; and distinguishes the potential roles of cell cycle in the development of seminoma and embryonal carcinoma.

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#### P 484

#### CLINICOPATHOLOGIC STUDY OF THYMOMAS (ABOUT 40 CASES)

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#### INTRODUCTION

Thymomas are primary tumors that arise from epithelial cells of the thymus. They account for more than 50% of anterior mediastinal masses and approximately 20% of all mediastinal tumours. These neoplasms are most commonly associated with parathymic syndromes, especially myasthenia gravis.

#### PURPOSE:

We present the clinical and histologic features of thymomas and review the literature.

#### METHODS

From January 1994 until December 2004, microscopic slides of 40 cases of thymomas were reviewed retrospectively and reclassified according to the new World Health Organization histologic classification. The stage was determined according to Masaoka's classification. Clinical records were collected from medical folders.

#### RESULTS

There were 23 females and 17 males. Mean age was 51,05 years (range14-76). At presentation 4 patients were asymptomatic and 7 presented with myasthenia gravis. Diagnosis was achieved on surgical specimen in 29 cases and on biopsy in 11 cases .The distribution of histologic subtype was type A (n=1), type AB (n=7), type B1 (n=6), type B2 (n=17), type B3 (n=6), micronodular thymoma (n=2), metaplastic thymoma (n=1). 10 patients were in stage I, 10 in stage II, 8 in stage IIIa, 5 in stage IIIb, 5 in stage IVa and 1 in stage IVb.

#### CONCLUSION

The predominant histological type in our study was B2 and half of patients were stage I and II of Masaoka. Surgery was the mainstay of treatment, and complete resection with clinical stage seem the most important prognostic factors in this disease.

#### P 485

#### MEDIASTINAL GANGLIONEUROMAS: A REPORT OF 7 CASES

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#### INTRODUCTION

Peripheral neuroblastic tumors include neuroblastoma, ganglioneuroblastoma and ganglioneuroma. These tumors were classified on depending of neuroblastic differentiation and the degree of schwannian stroma. Ganglioneuroma was a mature and benign form of them. It was rare slow-growing tumor, localized commonly in the posterior mediastinum and retroperitoneum

#### PURPOSE

The aim of the study is to describe the clinical and pathological characteristics of mediastinal ganglioneuroma in our institution.

#### METHODS

We retrospectively analyzed seven cases of mediastinal ganglioneuroma diagnosed between January 1993 and December 2004. Medical records and microscopic slides were reviewed.

#### RESULTS

Patients were 2 males and 7 females. Mean age was 22.14 years (4-84 years) and there were 4 pediatric cases. The most common symptoms were cough and chest pain. One patient presented with Claude-Bernard-Horner's syndrome. Two presented supraclavicular lump and one patient was fortuitously diagnosed. CT scan showed posterior mediastinal mass with dumbbell type in one case. Six patients underwent total tumorectomy. In macroscopy, 5 tumors were firm in consistency, one was soft and other was cystic with calcifications. The mean size was 5.9 cm (3.5 – 9 cm). In histological examination, tumors were composed of nerve fibers and mature ganglion cells. Lymphocytes were observed in 4 cases, fibrous capsule in three cases and calcifications in one case.

#### CONCLUSION

Ganglioneuromas tend to develop in children and young adults. They are asymptomatic in 50% of cases and symptoms are related to the mass effect. The mean tumor size reported in literature is 8 cm. In our study, tumors were litter and paradoxically the most of them were symptomatic (6 cases). The definitive diagnosis is histological and the presence of neuroblasts excludes ganglioneuroma. So, complete surgical resection is necessary for ultimate diagnosis and it' is the best chance of cure.

#### P 486

##### NEUROGENIC TUMORS OF THE MEDIASTINUM IN ADULTS: A REPORT OF 44 CASES.

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#### INTRODUCTION

Neurogenic tumors originate in the embryonic neural crest cells, which constitute the ganglia, paraganglionic and parasympatic systems. In mediastinum, they are relatively uncommon, though they represent 20% of all primary mediastinal tumors in adults and occur almost exclusively in the middle and posterior compartments.

#### PURPOSE

We retrospectively reviewed our institutional experience of mediastinal neurogenic tumors in adults with emphasis on the clinical and pathologic characteristics.

#### METHODS

Between January 1993 and January 2005, 44 mediastinal neurogenic tumors were diagnosed in patients older than 14 years. Clinical records ( demographic data, clinical presentation, diagnostic investigations, operative procedures and follow up) were analysed and all microscopic slides were re-examined. Comparison for parameters to distinguish malignant forms to benign, was made by X2 test.

#### RESULTS

Patients were 16 males and 28 females and ranged from 14 to 84 years of age. 9 patients were asymptomatic, otherwise, chest pain was the main symptom. 4 patients presented a thoracic lump and three a Recklinghausen disease. The tumors had the following characteristics: 38 of them were located in the post mediastinum and 3 tumors showed an intraspinal extension, the so called dumbbell-type. They were 36 benign and 8 malignant tumors with 21 schwannomas, 10 neurofibromas, 7 malignant peripheral nerve tumors, 3 ganglioneuromas, one ganglioneuroblastoma and 2 paragangliomas. Resected was performed for all benign tumors and 4 cases of malignant tumors.

With a X2 test, we founded that tumor size;Ý 8 cm, extension in TDM, dyspnea, thoracic lump and general status alteration were the predictive factors for malignancy.

#### CONCLUSION

Most studies of mediastinal neurogenic tumors compared pediatric and adult forms and age seem the most important clinical parameter for distinguishing between histological type and rate of malignancy for these tumors. In adult population, it appears that tumor size and some symptoms were the predictive factors of malignancy, especially general status alteration that seem the only independant factor.

#### P 487

##### EFFECTS OF MELATONIN ON HISTOPATHOLOGICAL CHANGES AND OXIDANT-ANTIOXIDANT STATUS IN RABBITS EXPOSED TO SMOKE

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#### ABSTRACT:

Objectives: To show the effects of smoke exposure in rabbits to the histopathological changes in lungs, oxidant-antioxidant status, and the effects of melatonin on these changes.

Methods: Four groups each with five rabbits are formed. A glass box (80x80x80 cm) is used. Group 1 (control) is exposed to cigarette smoke, group 2 (melatonin) is given melatonin only, group 3 (smoke+melatonin) is both exposed to smoke and given melatonin in a duration of 1 month for 1 hour daily and group 4 (control) is the control group. Then lung tissues are examined histopathologically . Protein sulfhydryls (SH), carbonyl, prostaglandin F2á (PG F2á), melandialdehyde (MDA), glutathion peroxidase (GPX), superoxide dismutase (SOM) levels in blood are measured.

Results: Histopathologically intraparenchimal vascular congestion and thrombosis, intraparenchimal hemorrhage, respiratory epithelial proliferation, macrophages in alveolar and bronchial lumens, and bronchoalveolar hemorrhage scores were significantly higher in group 1(smoke) than group 4(control). The scores of bronchoalveolar hemorrhage is significantly decreased in group 3(smoke+melatonin) than group 1 (smoke). Levels of SH and PG F2á were significantly higher and levels of SOD were significantly lower in group 1(smoke) than group 4(control). Carbonyl levels were significantly lower in group 3(smoke+melatonin) than group 1(smoke).

Conclusion: Exposure to cigarette smoke causes severe histopathological changes and negatively effects the oxidant-antioxidant status on rabbits. Melatonin treatment has no healing effect on histopathological changes and oxidant-antioxidant status.

#### P 488

##### TRANSTHORACIC FINE NEEDLE BIOPSY OF THE LUNG

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BACKGROUND AND AIMS: Transthoracic fine needle biopsy of the lung is one of the basic invasive diagnostic procedures in patients with lung diseases. It is safe, cost effective and successful, especially in detecting of malignant diseases. Our objective was to assess the correlation between cytologic and histologic diagnoses.

**MATERIAL AND METHODS:** We analysed cytologic and histologic diagnoses of 39 patients in whom transthoracic fine needle biopsy of the lung was performed between January 2002 and December 2004.

**RESULTS:** The patients were 31 – 84 years old (mean 59). The cell samples were insufficient for cytologic diagnosis in 8 cases (20,5%), 5 of them were insufficient also for histologic diagnosis. In 13 out of 31 representative cases cytologic diagnoses were correctly interpreted as benign changes (41,9%). There were two cases of hamartoma and two cases of abscedent inflammation among benign lesions, remaining 9 were reported as non-specific benign changes. In 5 patients cytologic diagnoses were suspicious for carcinoma. In three of them histologic diagnosis confirmed epidermoid carcinoma, in one mesotelioma. One of cytologically suspicious cases was suspicious also after histologic examination. 13 out of 31 representative cases were correctly diagnosed as malignant. Among malignant lesions 4 were adenocarcinomas, 3 epidermoid carcinomas, 2 non-small cell carcinomas and 2 small cell carcinomas. Histologic examination of the two malignant tumors with very unusual cytomorphologic picture revealed synovial sarcoma in one case and PNET in the other.

**CONCLUSION:** Transthoracic fine needle biopsy of the lung and cytologic examination is a reliable method in distinguishing benign or malignant nature of lung lesions. It is successful also in differentiation of malignant tumors. However, histologic examination is more reliable in further classification of benign changes.

#### P 489

##### **DIFFUSE PULMONARY OSSIFICATION: A FORGOTTEN ENTITY IN CHRONIC LUNG DISEASE**

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**INTRODUCTION:** Diffuse pulmonary ossification (DPO) is an uncommon chronic process characterized by progressive metaplastic lung parenchima ossification. Clinical course is usually asymptomatic, although mild symptoms and restrictive pulmonary physiology have been described in some cases.

**OBJECTIVE:** To present two cases of DPO and correlate the clinical, radiographical, and pathological features of this entity.

**METHODS:** Lung biopsies and adult autopsies performed at the University Hospital of La Paz from 1966 to 2004 were reviewed to identify the cases of DPO. One case of DPO was identified out of 9,964 lung biopsies and the other out of 4,051 adult autopsies.

**RESULTS:** The biopsy was obtained from a 72 year-old man clinically diagnosed of pulmonary restrictive disease. The other case was a 81 year-old man with biventricular hipertrophy and a peacemaker who died of bilateral bronchoneumonia. Radiographically, both patients presented signs that could suggest DPO. Histologically, lungs from both patients presented a nodular pattern of DPO (lamellar deposits of calcified osteoid material located within the alveolar spaces). Even though, the underlying pathology of the first patient has been associated to the dendriform or branching pattern of DPO.

**CONCLUSIONS:** DPO is an unusual entity seen incidentally at autopsy. Since it is hardly ever considered by clinical physicians, it is rarely diagnosed. A better understanding of the process of DPO formation, its associated diseases, and radiographical appearance is important in order to avoid misdiagnosis with other types of calcium accumulation in the lungs.

#### P 490

##### **CENTRAL AND PERIPHERAL AIRWAYS EXPRESSION OF NF-KBP65 AND HDAC2 IN NONSMOKERS, ASYMPTOMATIC SMOKERS AND COPD**

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**Introduction.** Airways inflammation and oxidant/antioxidant imbalance is a major cause of lung damage in COPD. An oxidative stress lead to redox-sensitive transcription factor nuclear factor-kB (NF-kB) activation, which regulates the transcription of inflammatory mediators. The molecular mechanism by which inflammatory genes are switched off is mediated by chromatin deacetylation by histone deacetylase (HDAC2).

**Purpose of the study.** The aim of our research was to compare inflammatory changes in central and peripheral airways in nonsmokers, asymptomatic smokers and in smokers with COPD.

**Patients and methods.** The study population was composed of 17 subjects undergoing lung resection for a solitary peripheral carcinoma. They have been subdivided into three groups: 5 subjects was nonsmokers and had normal lung function, 5 subjects were smokers with normal lung function and 7 subjects had COPD (FEV1/VC<70%). Immunohistochemical methods were used to identify NF-kB p65 and HDAC2 positive cells. The alveolar destruction index was calculated by formula DI (destruction index)={D/(D+N)}\*100%, where D-is destroyed, but N-normal alveolar walls.

**Results and discussion.** Asymptomatic smokers had more NF-kBp65 positive cells in central compared to peripheral airways and alveolar walls. Subjects with COPD had more NF-kBp65 positive cells in alveolar walls and peripheral airways compared to central airways. The NF-kBp65 was activated both in macrophages and bronchial epithelial cells. In nonsmokers NF-kBp65 expression was found mostly in cytoplasm, but in smokers in cell nucleus. In central airways of asymptomatic smokers there was a positive correlation between the number of NF-kBp65 positive cells (p=0,04; r=0,65) and smoked pack-years. In patients of all groups there was a positive correlation between macrophages in alveolar walls and destruction index (p=0.04; r=0,68). HDAC2 was expressed in all inflammatory cells. HDAC2 expression was suppressed in smokers.

**Conclusion.** It has been suggested that NF-kB was activated both in asymptomatic smokers and in smokers with COPD. In COPD NF-kBp65 positive cells mostly infiltrate alveolar walls and peripheral airways. The HDAC2 activity was suppressed in smokers.

#### P 491

##### **PARTICULARITY OF CHRONIC DIFFUSE LUNG DISEASE IN PATIENTS LIVED AT RADIOACTIV-POLLUTED TERRITORIES OF SEMIPALATINSK REGION**

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**Aim.** To study diseases, mortality and morphological peculiarities of chronic diffusion diseases of lung in persons lived at radioactive-polluted territories of Semipalatinsk region over a long period of time.

**Materials and methods.** The analysis of 631 postmortem examination reports with the diagnosis chronic diffusion disease of lungs and archival paraffin autopsy, surgical and biopsy material from 300 patients with CDLD who lived near to polygon and were exposed to the radiation over a long period of time since childhood up to 2003 (annual dose of the irradiation exceeded 0,1 bar). Light microscopy with painting by haemotoxilin and eosin; Van-Gison were performed;

chromogranin A (DAKO) were studied by immunohistochemically.

Results. In the presence of CDLD except known morphology particularities we can point to: sclerosis of walls of bronchus and interstitial; vessel pathology with development of hemosiderosis; accumulation of dust as an indirect index of deposit of radionuclide; hyperplasia of neuroendocrine cells; displace of bronchus epithelium.

Conclusion. The analysis of diseases, mortality, and decrease of these indexes after closing of the polygon and morphology data and information from the literature showed that radiation has a very high mean in pathogenesis of CDLD.

#### P 492

#### THE FIRST YEAR OF TRANSBRONCHIAL BIOPSY (TBB) AFTER LUNG TRANSPLANTATION (LT). THIRTEEN YEARS EXPERIENCE.

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Objective.

To analyze the evolution of the mean, indications and diagnostics of 700 TBB performed in the first year from 241 lung transplants performed between 1990 and 2003

Design.

We divided our TBB database for LT into two groups, the historical group (HG) (80 LT & 345 TBB, between 1990 to 1997), and the recent group (RG) (161 LT & 355 TBB, between 1998 to 2003). Each of them were divided into early posttransplant period (EPT) (<45 days), and late posttransplant period (LPT) (45 to 365 days). We also considered whether the biopsy was clinically indicated (Bcli) or it was a surveillance biopsy (Bsurv)

Results.

There is a current decrease in the mean TBBs, principally in the LPT, in both Bsurv and Bcli compared the HG and RG. The frequency of TBB in EPT were similar in Bsurv and Bcli in the HG and RG

Historic Group: TBB\* 345 (I:4.3); EPT 114 (I:1.42); LTP\* 231 (I:2.88); B sur\* 163 (I:2.06); B sur EPT 45 (I:0.56); B sur LTP\* 118 (I:1.47); B clin 182 (I:2.27); B clin EPT 69 (I:0.86); B clin LPT\* 113 (I:1.41).

Recent Group: TBB\* 355 (I:2.2); EPT 214 (I:1.32); LTP\* 141 (I:0.85); B sur\* 150 (I:0.93); B sur EPT 88 (I:0.54); B sur LTP\* 62 (I:0.38); B clin 205 (I:1.27); B clin EPT 126 (I:0.78); B clin LPT\* 79 (I:0.49).

\* p<0.05

In Bcli, in TBB from the recent group we can see an increase of diagnostic biopsies of acute rejection (AR) from 32 to 61 cases (17.6% in HG vs 29.8% in RG, p<0.05) and bronchitis/bronchiolitis from 3 to 17 cases (1.6 in HG vs 8.3% in RG, p<0.05) and decrease of non-diagnostic biopsies (ND) from 38 to 26 cases (20.9% in HG vs 12.7% in RG, p<0.05).

In Bsurv observed an increase of AR diagnostics from 32 to 48 cases (19.6% in HG vs 32% in RG, p<0.05) and bronchitis/bronchiolitis from 4 to 20 cases (2.5% in HG vs 13.3% in RG, p<0.05)

Conclusions.

- 1) We are currently managing LT with less TBB before.
- 2) Present better definitions of clinical settings (i.e. chronic airway rejection) see to improve the percentage of diagnostics in B cli
- 3) Better recent biopsy protocolization provides Bsurv with some worthwhile indications, not only in the early PT period, but also in late non far PT period

#### P 493

#### DISCREPANCIES OF CLINICAL AND POSTMORTEM DIAGNOSIS IN PULMONARY CASES.

Kleina Regina, Aboltins Arnis

The aim of our study is to analyze frequency and reasons of undiagnosed lung diseases in hospitals of Riga (Latvia) during 1999-2004.

Material and methods. We have evaluated autopsy protocols, histological specimens, cases reports of 51 patients with nonspecific inflammation processes and tuberculosis. All dates were evaluated with SPSS 10 program.

Results. In State Center of Pathology (Riga) in six years were made 3600 necropsies. The level of uncoincidence between clinical and pathological diagnosis was 10, 6 %. The comparison of clinical cause of death and autopsy diagnosis showed misdiagnosis of lung diseases in 16, 1 % of cases. Between undiagnosed lung diseases were: 64 % with lobar pneumonia (LP), 19 % -bronchopneumonia (BP), 5 % -chronic bronchitis (CHB), 12 % -tuberculosis (T). Hospitalization time of patients with LB. was 5 days. These were cases with such complications as empyema and purulent meningitis. BP. cases were mainly with abscesses. Persons dead due to CHB were very old people. In misdiagnosis of T. clinical doctors ignored bone tuberculosis in anamnesis and T. was not proved by X-ray examinations.

Instead of lung diseases clinical doctors had diagnosed pathologies of CNS (33, 3 %), coronary heart disease (12%), gastrointestinal system 18, 5% and other pulmonary problems (28%). Duration of patient's hospitalization was average 2, 6 days; ratio male to female was 1, 9:1, average age of men -49, female 60 years. Diagnostic difficulties were also due to severe concomitant diseases of liver and cardiovascular system.

Conclusions. Misdiagnoses of lung diseases were mainly due to short hospitalization time of patient. Doctors ignored patients complains and overlooked X-ray findings, leukocytosis, high ESR, coughing, dyspnoe.

#### P 494

#### EXPRESSION OF EXTRACELLULAR MATRIX METALLOPROTEINASE INDUCER (EMMPRIN) IN INTERSTITIAL LUNG DISEASES.

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Background : Extracellular matrix metalloproteinase inducer (EMMPRIN) is a transmembrane glycoprotein, member of the immunoglobulin superfamily, which stimulates the production of various matrix metalloproteinases (MMPs) from mesenchymal cells. Several MMPs (MMP-1, MMP-2, MMP-9, MMP-14) are involved in lung tissue remodeling and fibrotic processes in experimental and human interstitial lung diseases, as Usual Interstitial Pneumonia (UIP), Non Specific Interstitial pneumonia (NSIP), Chronic Organizing Pneumonia (COP or BOOP), diffuse alveolar damage (DAD), or sarcoidosis.

Purpose of the study : This study aimed to analyse the EMMPRIN expression in various human interstitial lung inflammatory diseases, with or without fibrosis, to precise the cellular source and relationships between EMMPRIN, inflammation and fibrosis.

Methods : The expression was evaluated by immunohistochemistry on surgical lung biopsies in 14 UIP (7 idiopathic or IPF and 7 with associated disease), 4 other interstitial pneumonia (1 NSIP, 1 COP, 2 without specific histologic pattern), 5 DAD, 5 sarcoidosis, 5 pneumoconiosis at early stages, 5 localized fibro-bullous disease and 4 histological normal tissues.

Results : In normal lung, EMMPRIN was slightly expressed in alveolar macrophages and in bronchiolar epithelial cells. In all cases, EMMPRIN was mainly localized in alveolar and

tissue macrophages and bronchiolar cells, with an increased cytoplasmic staining in interstitial pneumonia. In UIP and DAD, pneumocytes of type II present in areas of fibrosis (honeycomb pattern or fibroblastic foci) expressed EMMPRIN according a membranous pattern, along the basement membrane and the cellular interfaces. Pneumocytes of type I, observed in preserved areas of UIP or in all other diseases, were never stained.

Conclusion : EMMPRIN, as an inducer of MMPs, is implicated in the migration/proliferation and phenotype change of the fibroblasts to myofibroblasts with subsequent accumulation and remodeling of extracellular matrix. In UIP and DAD, the increase and peculiar expression of EMMPRIN demonstrated that both the inflammatory (macrophages with activation) and epithelial (alveolar epithelial cells with injury/activation) pathways play a major role in the development of fibrosis. This could be underlined in these two interstitial pneumonia for therapeutical research, because their dramatic evolution towards fatal fibrosis with no or limited response to classical anti-inflammatory drugs.

#### P 495

##### **METAPLASTIC CHANGES IN BRONCHIAL GLANDS ON THE PLACE OF THE PREVIOUS BIOPSY**

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INTRODUCTION: In the bronchi on the place where the previous biopsy had been done, we noticed presence of regenerative changes, while in the bronchial glands we discovered changes with necrotizing sialometaplasia characteristic, which can be wrong diagnostic like mucoepidermoid or squamous cell carcinoma.

MATERIAL AND METHODS: Research includes 141 biopsy samples, which was get by repeated biopsy (rebiopsy). From 141 biopsy samples, changes in bronchial glands are noticed in 44 biopsy samples. The main goal of this research is discovered morphological changes in bronchial glands at the place of previous biopsy.

RESULT: Morphological changes which are discovered on the place of previous biopsy in bronchial glands are: lobular look of changes (59,09% all cases), lesion on the surface ( or mucous membrane)(86,36%), fibrin (90,91%), granulations tissue (100%), squamous cells in fibrin (75%), reparative defect in the cartilage (66,67%), granulocytes in the epithelium (93,18%), cylindrical epithelial hyperplasia (93,02%), mucus in the interstitial tissue (77,27%), cystic formation (70,45%), dyskeratotic changes (70,45%), nuclear polymorphism (56,81%) and hyperchromatism (36,36%), multinuclear cells (15,91%), emphasized nucleolus (90,91%), mitosis (47,73%), necrosis (13,64%), 'capture' squamous cells in the granulation tissue (90,91%).

CONCLUSIONS: The characteristic changes in the bronchial glands on the place of previous biopsy are: lobular look of lesion with defect on the surface field up with fibrin or granulation tissue inside who accumulated islands squamous cells with cellular polymorphism, emphasized nuclei who are mitotic active, intraepithelial and stromal granulocytes, reparative defect in cartilage, goblet and cylindrical cells, mucus in the interstitium and cystic formation. The above facts could be wrong diagnostic like mucoepidermoid or squamous cell carcinoma.

KEY WORDS: bronchi, rebiopsy, bronchial glands, regeneration

#### P 496

##### **INTERACTIVE TELEPATHOLOGY WITH THE COOLSCOPE, IN CONSULTATIVE DIAGNOSTICS OF THE DISPERSED LUNG LESIONS.**

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The term „dispersed lung lesions” [DLL] is a common preliminary clinical diagnosis accompanying the lung biopsy specimen for the histological examination. Actually, DLL is a radiological term used for an undefined lung lesions, does not correlate with their extension nor anatomic distribution. The clinical interpretation of DLL includes: diffuse interstitial lung diseases [DILD], infections, neoplasms (primary or secondary), thromboembolic and cardiovascular diseases, ARDS. Many diseases (especially DILD) have various manners of manifestations and histologic patterns; need complex analysis of the patient data and create the diagnostic problems for the pathologist who will need the second opinion. As our previous studies showed the static telepathology has limited value in the teleconsultations of DILD.

The study was performed to assess the usability of a new generation of telemicroscopy - the coolscope system (NIKON, Japan) for the interactive teleconsultations of DLL.

40 cases of lung biopsy with the preliminary diagnosis “DLL” entered the interactive teleconsultations between KPCP and NTB&LDRI. The coolscope was installed at the workstation with 10 MB network transfer capacity; the viewing workstation used 2 MB transfer capacity (17 inches flat panel monitor with 1,3 mln pixels). For the voice Internet communication the Windows Messenger software was used. The telediagnoses were verified with the light microscope.

The viewing time of a complete microscopic slide ranged from 10 to 60 minutes/case and was depended on the case difficulty and time of image downloading (30' - 7'). The main diagnostic problems encompassed: classification of idiopathic interstitial pneumonias (usual v. non-specific interstitial pneumonia; mixed multilobular patterns; honeycomb stage) - 20 cases; granulomatous inflammatory pattern (extrinsic allergic alveolitis, sarcoidosis, aspergillosis) - 9; occupational diseases - 4; lymphoproliferative diseases - 2; infections - 2; thromboembolic disease - 2; bronchiolitis - 1. An overall diagnostic accuracy was 95% (38/40), 2 cases needed additional immunostaining.

The benefit from this technical development is a very high diagnostic accuracy in DLL teleconsultations combined with the high efficiency of the system (the standard Internet connection, free time access to the system, independent platforms, cheap and good quality of the service).

#### P 497

##### **ISOLATED AMYLOIDOSIS OF THE TRACHEA**

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Introduction: Respiratory amyloidosis is a rare disease that occurs in three forms: tracheobronchial, nodular parenchymal and diffuse parenchymal involvement. Tracheobronchial amyloidosis is characterized by focal or diffuse deposition of amyloid in the submucosa of the trachea and proximal bronchi.

Clinical and laboratory features: A female 55-year-old patient presented in the Department of Thoracic Surgery with symptoms of dyspnea on exertion the last 2 years. She reported previous hospitalization for sangeinous sputum and bronchiectasis. On physical examination she had rales and

prolongation of the expiratory phase. Her chest radiography was negative and the CT scan showed thickness of the posterior wall of trachea. On bronchoscopy, soft, whitish, spontaneously bleeding patches were discovered and biopsied. One of the lesions was obstructing the opening of the left main bronchus. Histological examination showed isolated amyloidosis of the trachea, without any involvement of the larynx or the bronchi. The diagnosis was confirmed by the Congo Red histochemical stain. The patient was initially treated with endoscopic laser surgery. Given that obstruction of the left main bronchus persists, the patient is a candidate for stent placement.

Conclusion: Primary isolated amyloidosis of trachea is a rare and probably recurrent disease. Airway compromise can be a persistent problem. Repetitive laser or cryotherapy interventions and use of stent is a palliative therapy for this disease with poor prognosis.

#### P 498

##### **NON AMYLOÏDOTIC BRONCHOPULMONARY DEPOSITS OF IMMUNOGLOBULIN IN WALDENSTRÖM'S MACROGLOBULINEMIA**

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We report the case of a 49 years old woman, followed up since 2001 for Immunoglobulin M (IgM) kappa monoclonal gammopathy of undetermined significance and presenting for 4 years evolutive dyspnea with bronchorrhea.

Paraclinical evaluation has shown non-infectious proximal diffuse bronchiectasis associated with parenchymatous destruction by chest tomodensitometry; a decrease of CO diffusion; « candle stains » lesions of bronchi by endoscopy. A high rate of monoclonal IgM, previously undervalued because of a cryoglobulinic pattern, was found. There wasn't Bence Jones proteinuria. A bone marrow biopsy led to the diagnosis of Waldenström's macroglobulinemia. (WM)

##### **MATERIAL AND METHODS :**

The bronchic and pulmonary biopsies sampled by endoscopy and thoracotomy were fixed or frozen for optical microscopy, electronic microscopy and immunofluorescence.

##### **RESULTS :**

Under optical microscopy, bronchic submucosa exhibited deposits of a perivascular and interstitial amorphous eosinophilic material. Pulmonary tissue showed proximal acinar emphysema and bronchiolar dilatation. Congo red and T thioflavin stains showed these deposits to be non amyloidotic. Immunofluorescence of the deposits were positive for anti kappa and anti mu immunoglobulin chains antibodies. They were granular under electronic microscopy. Similar but smaller deposits were found in alveolar wall.

##### **DISCUSSION :**

There are two classical types of lung involvement in WM : AL amyloidosis and primary lymphomatous invasion. The reported case is original because :

the bronchopulmonary presentation ( proximal bronchiectasis ) is exceptional

the nature of the deposits made of complete or fragmentary light chain and heavy chain of IgM is non amyloidotic

##### **CONCLUSION :**

This case represents an original anatomo-clinical syndrom.

The pathological mechanism which explains that the monoclonal IgM kappa cryoglobulin forms deposits at bronchopulmonary level and leads to these clinical manifestations is still undetermined.

#### P 499

##### **MUCORMYCOSIS MIMICING LUNG CANCER ( CASE REPORT)**

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The authors introduce two cases, whose history includes diabetes mellitus. Either of them after ketoacidic coma were found to have unilateral pulmonary infiltration. Bronchoscopy showed cone shape stricture of the right main bronchus. The surface of the mucus membrane was rough and looked like a tumor. Histology did not find anything other than fungal infection.

In one case the regulary done investigation found a tumor, suspected, infiltration of the left lower lobe. The resected lobe has undergone histopathological investigation and showed infection of mucormycosis.

In the literature mucormycosis stands in forth place among opportunistic pulmonary fungal infection. Background of the recent years recognised growing incidence of well known immunodeficiency and increasing number of diabetes mellitus.

#### P 500

##### **IMPROVED PCR-BASED DETECTION AND DIFFERENTIATION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX IN PARAFFIN-EMBEDDED TISSUES BY APPLICATION OF THE HOPE\*-TECHNIQUE**

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Introduction: Detection of mycobacterial DNA by PCR in formalin fixed, paraffin-embedded specimens may lack sensitivity due to degradation of nucleic acids.

Purpose of the study: To improve the results we set up an approach by application of the novel HOPE-fixative in PCR-detection of mycobacteria in paraffin-embedded tissues. Methods used: PCR, Real time PCR, Spoligotyping.

Summary of the results: Comparison of PCR-results using DNA extracted either from HOPE- or formalin-fixed specimens in BCG-infected SCID-mice revealed a more than 100fold enhanced sensitivity for the HOPE-fixed material. Due to the preservation of DNA from degradation in HOPE-fixed tissues even differentiation within the M. tuberculosis complex was possible by spoligotyping.

Conclusions reached: We demonstrate that the HOPE-technique is a useful tool for molecular pathology, which enhances the sensitivity of PCR-based methods for detection of pathogens in paraffin embedded tissues compared to formalin-fixation. Due to the better preserved DNA improved differentiation of mycobacteria from archived materials is possible. These results promise new and substantially enlarged possibilities in the field of molecular pathology.

\*(Hepes-Glutamic acid buffer mediated Organic solvent Protection Effect

#### P 501

##### **HISTOLOGICAL DIAGNOSIS OF LUNG BACTERIAL NECROTIZING INFECTION: A STUDY OF 4 FULMINANT CLINICAL CASES**

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We report the clinical and histological findings of four cases of pulmonary infection due to bacteria which were associated with a lung extensive necrosis. These infections were fulminant with a fatal course within a few hours. Histological findings were very similar in these 4 cases and were characteristics of these infections by associating an extensive hemorrhagic tissue necrosis, large layers of bacteria observed inside the alveola and/or in the connective tissue, and most often, a very weak or no inflammatory infiltrates. These infections were due to group A Streptococcus in two cases, Staphylococcus aureus in one case, and Haemophilus influenzae in one case. The histological features observed in these infections are due to the production of large amounts of different necrotizing toxins. These dramatic infections are extremely rare and can occur, both in immunodeficient and in immunocompetent patients without any apparent underlying disease. The differential diagnosis includes a viral infection which can be very difficult to be eliminated histologically. The precise diagnosis is always based on a correlation between the histological and the microbiological results. These data confirm the reappearance of some highly invasive strains, such as strain of group A streptococci, capable of producing a variety of fulminant diseases, including shock and diffuse pulmonary necrosis. The nonspecific presentation and fulminant progression require that the treating physician, the microbiologist, and the pathologist be both knowledgeable and vigilant.

#### P 502

#### LOH AT 9P21 (P16 LOCUS) AND 17Q13 (P53 LOCUS) CAN BE DETERMINED IN ORAL EXFOLIATIVE CYTOLOGY FROM NON-SMALL-CELL-LUNG-CANCER PATIENTS.

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Background: LOH at different loci is a frequent genetic alteration detected in non-small-cell lung cancer. Identical allelic deletions were found in most cases in normal bronchial cells adjacent to tumor. Smoking exposure has been involved with these genetic changes in oral, larynx and lung tumorigenesis. Therefore, demonstration of LOH in any of these locations may detect patients with a higher risk of cancer. Design: Oral exfoliative cytology was obtained from 64 patients divided in 3 groups: 23 current smokers in which NSCLC was detected, 24 current smokers with no evidences of malignancies (3-5 years follow-up), and 17 non-smokers control population. Results: We found LOH at 17q13 in 3/15 (20%) and LOH at 9p21 in 2/12 (16.6%) of the informative tumor cases. In total, 5 out of 18 (27.5%) patients that were informative for either alleles showed deletions. LOH was not found at any locus in oral mucosae from any non-tumoral case, smoker or non-smoker.

Conclusion: LOH at 9p21 (p16) and 17q13 (p53) can be determined not only in normal bronchial cells adjacent to NSCLC but also in oral exfoliative cytology. In our series these somatic genetic changes were unusual in patients with similar age and sex distribution with no evidences of malignancies. LOH assessment in oral exfoliative cytology may be useful in screening to detect smoker patients at a higher risk of malignant transformation.

#### P 503

#### THE POTENTIAL ROLE OF INCREASED CD68 POSITIVE MACROPHAGES AND MACROPHAGE MICROACCUMULATIONS PRESENCE, IN CASES OF CROHN'S COLITIS AND THEIR CONTRIBUTION IN ITS DISTINCTION FROM ULCERATIVE COLITIS

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Background: Previous studies have demonstrated that macrophage microaccumulations (MA) are present in gastric and small bowel mucosal biopsies from patients with Crohn's disease (CD). However, their presence in inflammatory diseases of the colon has not been fully elucidated. In this study we investigated whether CD68 immunostaining and MA can be used to distinguish CD from ulcerative colitis (UC).

Design: The study included 123 routinely processed colonic mucosal biopsies, including 72 specimens obtained from 30 patients with CD (mean age: 47 yrs, M/F: 10/20), 33 specimens from 20 patients with UC (mean age: 33 yrs, M/F: 5/15) and 18 specimens from 18 normal controls (mean age: 63 yrs, M/F: 8/10). On paraffin sections a standard streptavidin-biotin peroxidase method was applied using the anti-CD68 antibody. The total number of CD68(+) cells in 10 consecutive intercrypt regions (ICR) of the lamina propria was recorded in each biopsy specimen. The number of MA, defined as loose collections of at least 5 macrophages identifiable only by CD68 immunopositivity, and not by H&E staining, present in 10 consecutive ICRs was also recorded. Finally we employed statistical analysis in order to compare the results from the different patient groups.

Results: There were no significant differences in the number of subepithelial or basal lamina propria CD68(+) cells between the three patient groups. However, the mean number of CD68(+) macrophages in the mid lamina propria was higher in CD (29±4) than UC (19±8, p<0.05) and normal controls (23±3, p>0.05). MA were present in 24 biopsies from 19 (63%) CD patients, compared to 6 biopsies from 5 (25%) UC patients (p<0.01) and none (0%) of the normal controls (p<0.0001). Furthermore, only CD cases contained >1 MA (9 biopsies from 6 patients, p<0.01). None of the UC or control patients had >1 MA.

Conclusions: The study demonstrates that small aggregates of CD68(+) macrophages are more frequently present in CD than in UC and multiple MA are found exclusively in CD. Thus, the presence of multiple MA may contribute to the distinction of CD from UC in colonic biopsies.

#### P 504

#### AN ANALYSIS OF THE RELIABILITY OF DETECTION AND DIAGNOSTIC VALUE OF VARIOUS PATHOLOGICAL FEATURES IN CROHN'S DISEASE

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Introduction. Due to the relatively high frequency of intestinal tuberculosis (IT) and intestinal involvement of Behçet's disease (BD) in Turkey, the differential diagnosis of Crohn's disease (CD) is difficult. This difficulty is valid for the pathologic investigation of both endoscopic and surgical GIT material. The aim of this study is to specify the pathological features of CD that will differentiate from the other conditions, and to analyze the reliability and diagnostic value of these features.

Material and Methods. In this study, 26 standardized patients, that have been diagnosed as CD through standard clinical, laboratory, radiological, endoscopic, microbiological procedures and further operated, have been investigated. The preoperative endoscopic biopsies, the surgical specimens and the immediate mucosal punch biopsies of the resected intestine have also been revised.

Results. In the resection specimens of 23 patients, fissure formation, transmural lymphoid follicle infiltration, submucosal fibrosis, neuronal hyperplasia, subserosal

fibrosis, which are the classical findings of CD were observed. The striking point in this regard was the infrequent presence of granuloma formation in these patients (30%). In the remaining 3 patients, even though some of the above findings exist, the classical Crohn's picture was not established. In the endoscopic biopsies the most presenting finding was antral metaplasia, and the most interesting finding was increase in eosinophils. In 2 of the patients the coexistence of BD and CD was observed.

Conclusion. Granuloma formation which a finding of CD is not observed too frequently among the patients in Turkey. The presence of antral metaplasia and increased eosinophils in the endoscopic biopsies of the chronic patients may be useful for diagnostic approach when combined with other histological findings. Due to the incidence of coexistence of BD and CD, the investigation of standardized BD and IT groups, are subjects of our ongoing studies.

#### P 505

##### A NEW SIMPLIFIED HISTOLOGICAL CLASSIFICATION OF COELIAC DISEASE

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The diagnosis of Coeliac disease (CD) is still based on the biopsy-proven presence of duodenal-jejunal mucosal lesions which improve after gluten-free diet.

It is believed that the currently used criteria are often the source of disagreement between pathologists and clinicians. Marsh proposed a four stage grading: type 1 infiltrative lesion, normal mucosal architecture with an increased number of intraepithelial lymphocytes (IELs), type 2 hyperplastic lesion, an increase in crypt depth without villous flattening, type 3 destructive lesion, villous atrophy. Oberhuber et al subsequently proposed a new standardized report scheme, based on the Marsh classification, in which stage 3 was split further into 3a, 3b and 3c, characterised respectively by mild villous flattening, marked villous flattening and completely flat mucosa. How can the Marsh-Oberhuber classification be simplified to improve its reproducibility without reducing its diagnostic accuracy with respect to CD? Obviously, type 1 infiltrative lesion – cannot be put aside. This lesion can in fact be the only evidence of a gluten-sensitive enteropathy in dermatitis herpetiformis and a sensitive, marker of latent CD. More recently, the upper limit of the normal range has been established as being 25 IELs/100 epithelial cells and we therefore feel that IELs counts above this figure allow the diagnosis of a type 1 infiltrative lesion. As regards type 2 hyperplastic lesion, we doubt its usefulness in the diagnosis of new patients – who would, in any case, already be identified by the increased IELs. As regards the substages 3a and 3b characterised by mild and marked villous flattening but not yet by flat mucosa, we suggest that these should be grouped in a single stage. Finally, as regards type 3c there is no doubt that this stage should be maintained. A simplification of the current histological classifications of CD is necessary in order to make the work of pathologists more uniform and to facilitate the relationship between pathologists and clinicians. We therefore propose that the lesions characterising celiac disease be divided into non-atrophic (grade A) and atrophic (grade B) and that the latter be further split into grade B1, in which the villus/crypt ratio is less than 3/1 with still detectable villi, and grade B2 in which the villi are no longer detectable.

#### P 506

##### ROLE OF OXYGEN FREE RADICALS ON THE MOTILITY OF RAT ILEUM:

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#### ABSTRACT

The major objective of the present study was to characterize the effects of oxidants which generated by xanthine (X) plus xanthine oxidase (XO) on isolated rat ileum motility. The effects of three concentrations of X/XO on the basal tone of the rat ileum preparation were studied for 20 minutes. Developed tensions were measured in mg/mg tissues and then expressed as percentage of baseline tension. Also the effects of 2X concentration of X/XO in the presence and absence of Superoxide dismutase, catalase, mannitol, histidine, and deferoxamine were evaluated. The result were expressed as mean  $\pm$  SE. Xanthine plus xanthine oxidase produced relaxation of ileum. Superoxide dismutase (a superoxide anion metabolizer) and catalase (a hydrogen peroxide scavenger) did not protect ileum from effects of X/XO, suggesting that neither superoxide anion nor hydrogen peroxide involve in X/XO-induced relaxation of ileum. The results of this study suggest that hydrogen peroxide formed extracellularly by X/XO may enter the cells and interact with intracellular iron to form a highly reactive oxidant, hydroxyl radical. The finding that two powerful hydroxyl radical scavengers, dimethylthiourea (DMTU) and mannitol offered protection against X/XO-induced relaxation of ileum suggest formation of hydroxyl radical within the cells. Pretreatment with deferoxamine, a potent iron chelator, reduced the relaxation of ileum, indicating that hydroxyl radical plays an important role in mediating the X/XO-induced relaxation of ileum. In addition, the ability of exogenously administered histidine to reduce relaxation suggests that singlet oxygen is another oxygen derivatives which is responsible for relaxation of ileum-induced by X/XO.

Key Words: Ileum Strips; Xanthine; Xanthine oxidase; Hydroxyl radical; Singlet oxygen; Superoxide dismutase; Catalase; Dimethylthiourea; Mannitol; Histidine; Deferoxamine

#### P 507

##### THE EFFECT OF GINGER ON SMALL INTESTINE CHANGES INDUCED BY DIABETES IN RATS

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In the present study, we investigated the effect of ginger consumption on the morphological and histological changes in the small intestine of diabetized rats. Eighteen male wistar rats with average body weight 250 gr and 8 months old were chosen to do the study. They were divided into three equal groups (n= 6): the first group rats were nondiabetic; the second group rats were diabetized by streptozocine (60 mg/kg body weight) which received normal diet, and the third group rats were also diabetized which received normal diet and ginger (6 percent of daily diet weight). After 6 weeks abdominal cavity was opened and mesentery was separated carefully by using scissors. Then the small intestine was taken from pyloric sphincter and ileocecal sphincter. After weighing and measuring the length of small intestine, from three parts of small intestine including duodenum, jejunum and ileum 5

cm long samples were taken. After filling the lumen with 10% formalin buffer and ligation of it, they were put into formalin. After sectioning and staining, villi length, crypt depth and muscle thickness of samples was measured. The weight and length of small intestine, length of villi, depth of crypt and muscle thickness increased in non-treated rats comparing to those of nondiabetic rats. The mentioned parameters did not show any significant difference comparing to two groups of ginger-treated and healthy rats. The study showed that ginger as an antioxidant probably prevented changes caused by diabetes in small intestine.

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##### **MUCOSAL LYMPHATIC VESSELS IN IDIOPATHIC BOWEL DISEASE**

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##### Introduction

The lamina propria of normal colorectal mucosa contains only some small lymphatic vessels near the muscularis mucosae. New monoclonal antibodies (MoAbs) facilitate their differentiation from blood capillaries.

##### Purpose of study

Chronic inflammations induce lymphatic angiogenesis in different organs. We investigated number and localisation of intramucosal lymphatic vessels in chronic idiopathic bowel disease (IBD), in respect of their contribution to the pathogenesis of colitis ulcerosa (C.u.) and Crohn's disease (CD)

##### Materials and Methods

MoAb D2-40 (reacting with lymphatic endothelium specific O-linked sialoglycoprotein MW 40K; DCS Hamburg; RTU) and LYVE (reacting with a lymphatic hyaluronanreceptor; DCS Hamburg; RTU) were used as markers for lymphatic vessels. Proliferation was quantified by nuclear Ki-67 (DCS Hamburg; Clone SP6).

##### Results

##### Colitis ulcerosa

In the interface of mucosa and muscularis mucosae, there are dense networks of thinwalled lymphatic vessels; they are oriented parallel to the circumference of the colon; their endothelial cells have activated nuclei, though they are negative for proliferation marker Ki-67. Numerous lymphatic vessels are found in the lower third of the mucosa; the middle third of the lamina propria contains less lymphatic channels; the upper third is free of lymphatics. Only rarely, there are perpendicularly oriented lymphatic vessels (mostly near lymphatic follicles).

The density of lymphatic vessels correlates well with the duration of C.u. and the proliferative activity of crypt epithelia; the extension of inflammation and proliferative activity of lymphoid cells in the mucosa does not influence the density of lymphatic vessels.

Carcinoma in C.u. shows high density of lymphatic vessels in between of the neoplastic glands, correlating perhaps to its aggressive course.

##### Crohn's Disease

In contrast to C.u., the density of lymphatic vessels is augmented not only in the lower third of the lamina propria, but also in the middle and upper parts of the mucosa. High density is seen in fibrotic areas and in parts with enteral metaplasia. Their number correlates to the intensity of inflammation.

##### Conclusions and hypothesis

Proliferation of mucosal lymphatic vessels, consequence of elevated levels of VEGF in IBD, contributes to the differences in pathogenesis of late phases of C.u. and MC.

#### P 509

##### **BCL-2 PROTO-ONCOGEN EXPRESSION IN ISCHEMIC MAMMALIAN SMALL BOWEL MUCOSA**

##### **IN RELATION TO THE DURATION OF ISCHEMIA. (PRELIMINARY REPORT).**

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Introduction. Bcl-2 proto-oncogen is a key factor in the prevention of apoptosis in lymphoid and epithelial tissues. The aim of this study is to evaluate the role of Bcl-2 expression in ischemia-induced apoptosis of small intestinal mucosal cells.

We investigated the appearance of apoptosis at various time points of ischemia in comparison to the expression of the Bcl-2 protein.

Materials and methods. We examined samples obtained from mammalian small intestine (New Zealand White Rabbits) following ischemia induced by strangulation-obstruction of 5cm of jejunum. Samples were taken preoperatively and progressively at 30, 180 and 360 min from the onset of strangulation. Tissue sections were stained for H&E for the estimation of mucosal ischemic damage. Tissue sections taken from the same samples were stained immunohistochemically for Bcl-2 protein.

Observations from both H&E and Bcl-2 staining were compared to estimate the correlation of Bcl-2 expression with the severity of the ischemic injury.

Results. Histological changes were observed in the jejunum concerning the upper third, the upper two thirds and the whole length of the villi respectively at 30, 180 and 360 min from the onset of ischemia. Cytoplasmic expression (++++) of Bcl-2 protein was observed at villous epithelial cells, while it was barely obvious at the crypts at the onset of ischemia. Minimal reduction (++) of Bcl-2 expression at the apical third of the villi at 30 min and increased reduction concerning the two thirds of the villi at 180 min after the induction of ischemia was observed. Only scattered apoptotic epithelial cells were observed expressing the protein after 360 min of ischemia.

Discussion. Progressive suppression of the Bcl-2 proto-oncogen expression on ischemic injured small intestinal mucosal tissue suggests a key role of the Bcl-2 protein in the apoptotic pathway induced by ischemia. The correlation of this phenomenon to the severity of ischemic injury at consequent time intervals leads to the assumption that this is not a triggered effect but a dynamic process aggravated by the progression of ischemia.

#### P 510

##### **INTESTINAL VASCULITIS MIMICKING A COLONIC NEOPLASM. REPORTING OF A CASE.**

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##### INTRODUCCION

Vasculitis of the gastrointestinal (GI) tract is known to occur as a part of a systemic process such as Panarteriitis Nodosa, Churg-Strauss angiitis, Behcet's disease, giant-cell arteritis, Buerger's disease, systemic Eritematous Lupus, Henoch-Schölein purpura and rheumatoid arthritis. Systemic and isolated vasculitis classification is based on the predominant type and size of the vessel involvement. Polyangiitis overlap syndrome proposed by Leavitt and Fauci is defined as systemic vasculitis that does not fit precisely into a single category of classical vasculitis or overlaps several of the categories.

##### CASE REPORT

A 77-year-old woman presented with 2-days history of abdominal pain, bowel obstruction and abdominal distention. An abdominal computed tomography scan was performed and showed swelling and bowel wall thickening of the sigmoid colon. With the clinical diagnosis of a malignant tumor, the sigmoid colon was resected. Pathological examination of the resected colon segment revealed a stenotic pseudotumoral lesion (3-4 cm.), mucosal ulceration and severe obstruction that compromise 70% of the circumference. Microscope examination showed severe mucosal ischemic necrosis associated with necrotizing vasculitis involving medium and small-sized arteries and heavy infiltration of eosinophils. No granuloma formation was observed.

#### DISCUSSION

The main differential diagnosis has to be made within the group of vasculitis involving medium and small sized arteries. Wegener's granulomatosis is distinguished by its predilection to affect the upper and lower respiratory tract and kidneys, positive C-ANCA titer and GI involvement is uncommon. Churg-Strauss syndrome is a disorder characterized by clinical features such as asthma, allergic rhinitis, hypereosinophilia and histological features of necrotizing vasculitis, eosinophilic infiltration and granulomas. GI involvement is about 50%. PAN is a systemic disease that showed acute arteritis with fibrinoid necrosis of the vessel wall. GI involvement is relatively frequent (40-70%).

In our case, according to the clinical and pathological findings, we suggest the diagnosis of a polyangiitis overlap syndrome with some features of allergic granulomatous angiitis and Polyarteritis nodosa.

#### P 511

##### **PRIMARY RHABDOID CARCINOMA OF THE ILEUM: REPORT OF THE SECOND CASE IN THE LITERATURE WITH EXTENSIVE IMMUNOHISTOCHEMICAL (IHC) WORK-UP AND OVERVIEW OF DIFFERENTIAL DIAGNOSIS.**

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**Introduction:** Primary rhabdoid tumors of the small intestine are very rare but do occur as we demonstrate herewith.

**Purpose:** To describe the second case of primary rhabdoid carcinoma of the ileum with multiple recurrences.

**Material and methods:** The 81-y-old male patient had been treated for ischemic heart disease, arteriosclerosis and a benign papilloma of the urinary bladder. In November 2004 he was admitted and urgently operated on because of melena and progressive ileus. Abdominal examination revealed a firm, left parumbilical mass, easily palpable that was cca. 8 cm in diameter. A 20 cm long ileum-segment was removed. Its wall was widely infiltrated by a cauliflower-like, extensively hemorrhagic, ulcerated tumor. The invaded segment firmly adhered to the parietal peritoneum hence the resection included that part as well. Since the 1st operation the tumor has recurred twice (in 01/05 and in 03/05), each time breaking through the wall of the small intestine from the peritoneal surface and causing mucosal ulceration. These tumors were removed and re-excision of the surgical wound was also necessitated by local neoplastic re-growth. Presently tumorous infiltration along the surgical incision is evident again, but the patient refuses further surgery. The formalin-fixed, paraffin embedded tumors comprised large necrotic-hemorrhagic areas and solid, highly cellular, solid sheets with focal loss of cellular cohesion. The round or polygonal cells were intermingled with bizarre, pleomorphic, often

multinucleated forms. The nuclei were large, often polymorphic and eccentric with prominent nucleoli and numerous abnormal mitoses. The Mib-1 labeling index was 50-75%. Characteristic, pathognomonic, homogeneous eosinophilic cytoplasmic inclusions ("hyalin-globules") were seen. IHC revealed diffuse, strong vimentin, panCK and focal EMA positivity. CD30, Bcl-6, S100, HMB45, c-kit, CK7, CK20, p27-kip1, E-cadherin, SM actin, desmin, caldesmon and CD34 were negative. CD3, CD20 and LCA decorated the reactive lymphoid elements. Monoclonal p53 was diffuse positive. Some areas reacted with neu oncoprotein (++) . All lymphnodes were reactive-hyperplastic but tumor-free. So far no distant metastases occurred.

**Discussion:** IHC and light microscopic morphology prove rhabdoid cancer excluding lymphoma, adenocarcinoma or leiomyosarcoma. EM and molecular tumor-analysis are in progress, the results will be presented. Literature-search disclosed only 1 earlier jejunal case by 04/05.

#### P 512

##### **APPENDICEAL MUCINOUS NEOPLASMS: A ROLE IN PSEUDOMYXOMA PERITONEI**

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Mucinous neoplasms of the appendix are rare and associated with colonic or ovarian neoplasms, and pseudomyxoma peritonei (PMP). PMP is a rare form of dissecting gelatinous ascites associated with multifocal peritoneal and omental implants that secrete copious globules of extracellular mucin. Recent immunohistochemical and molecular genetic evidence suggests the appendix to be the primary site. We analyzed 30 cases of appendiceal mucinous tumors (22, mucinous cystadenoma; 4, borderline mucinous tumor; 4, malignant mucinous tumor), seven of which were accompanied by PMP. An immunohistochemical study for MUC2, MUC5AC, CK7, and CK20 was also performed to confirm the origin of PMP. Among 30 appendiceal mucinous tumor cases, four cases had right colon cancer, one case exhibited Peutz-Jeghers syndrome, one case endometriosis, and one diverticulosis of the right colon. Seven PMP cases showed both appendiceal and ovarian mucinous tumors. The appendiceal, peritoneal, and ovarian mucinous tumors showed the same immunoprofile (MUC2+/MUC5AC+/CK7-/CK20+). One case of appendiceal mucinous cystadenoma was associated with ovarian mucinous cystadenoma of borderline malignancy with no evidence of PMP. The appendiceal lesion was MUC2+/MUC5AC-/CK7-/CK20+. In contrast, the ovarian lesion was MUC2-/MUC5AC+/CK7+/CK20-. Therefore, these lesions are considered to be synchronous. In conclusion, the MUC2+/CK7-/CK20+ expression profile supports an appendiceal rather than ovarian origin for PMP.

#### P 513

##### **ARE GOBLET CELL CARCINOIDS OF THE VERMIFORM APPENDIX CRYPT CELL CARCINOMAS?**

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**Introduction and aim of the study:**

Goblet cell carcinoids (GCC) of the vermiform appendix are rare neoplasms of uncertain histogenesis, with features reminiscent of both classical carcinoids (CC) and conventional colonic adenocarcinomas (CAC). This study was performed to investigate whether GCCs should be regarded as a separate entity or as a variant of CCs, of CACs or of other primary appendiceal tumors, i.e. mucinous cystadenocarcinomas (MCA).

#### Material and Methods:

The immunohistochemical expression pattern of 19 different markers in 16 GCCs was compared with the expression pattern in 14 CCs, 19 CACs and 10 MCAs. The mutation status of KRas codon 12 was determined in all tumors by a mutant-enriched PCR method. The results were subjected to a stepwise linear discriminant analysis.

#### Results:

GCCs could be distinguished from the control groups and were characterized by diffuse positivity for CAM5.2, CDX2 and CEA, frequent coexpression of Ck7 and Ck20, scattered positivity for endocrine markers in up to 50% of the cells and a proliferative activity (Ki67 positivity) intermediate between that of CCs and CACs. Alterations in KRas codon 12, TP53, DPC4 and in the Wnt-signaling pathway were rare or absent in GCCs. Histologically, GCCs showed a typical growth pattern and contained different cell types reminiscent of the neoplastic counterparts of normal crypt cells.

#### Conclusion:

The results suggest, that GCCs should be regarded as a separate entity, probably arising from a crypt progenitor cell as previously suggested. Therefore, the formerly used term 'crypt cell carcinoma' may be more appropriate than GCC and this diagnosis also better reflects the more aggressive clinical behaviour of these tumors.

#### P 514

##### **TOXIC EFFECTS OF WATER EXTRACT OF TEUCRIUM POLIUM ON THE RAT INTESTINE**

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Teucrium Polium is traditionally used to lower blood sugar in diabetic patients in Iran and some other parts of the world. However, some gastro-intestinal side effects have been reported by the patients who have used this remedy. The aim of this investigation was to study of the effects of Teucrium Polium water extract on the structure of the small intestine in male rats. Fifty wistar rats were randomly divided to four experimental and one control groups. The experimental groups were fed different doses (4 g/kg, 3 g/kg, 2 g/kg, 1 g/kg) of the Teucrium Polium extract by oral route for 14 days. The rats were then sacrificed and histological sections were prepared. The result indicated that the extract could cause vascular delatation as well as lymphoid cell, macrophage and neutrophile infiltration in the intestinal tissues. Apoptosis was also observed in liver tissue. It seems that this extract may cause damage and changes to the small intestine, therefore, it is recommended that the extract of Teucrium Polium should be used cautiously. More investigation is needed to find its complications and mechanisms of damages induced by this extract.

#### P 515

##### **LACK OF CLAUDIN 4 EXPRESSION AND HIGH EXPRESSION OF CLAUDIN 3 AND CLAUDIN 7 DIFFERENTIATE PANCREATIC ENDOCRINE TUMORS FROM PANCREATIC DUCTAL CARCINOMA**

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**Introduction:** A new family of tight junction (TJ) proteins called claudins was discovered in 1998. These transmembrane proteins are responsible for cell adhesion, polarity and paracellular permeability. Although changes in permeability of TJs were noted in several cancers, the role of claudins in carcinogenesis is controversial. Claudin 4 overexpression was reported in primary and metastatic pancreatic adenocarcinomas. These findings support use of claudin 4 as target for novel therapeutics of pancreatic cancers.

**Purpose:** To analyse different claudin expressions in human pancreatic endocrine tumors and ductal carcinomas using a panel of polyclonal (claudins 1, 3, 7) and monoclonal (claudins 2, 4) antibodies.

**Methods:** Twenty formalin fixed, paraffin embedded pancreatic endocrine tumors and ten pancreatic ductal carcinomas were studied, and immunohistochemical evaluation performed to analyse claudin 1, 2, 3, 4 and 7 expressions. Semiquantitative evaluation was used for the area of extension (0-5) and intensity (0-3) of reaction.

**Results:** Claudins 1, 2 proved negative in all endocrine tumors. The majority (7/10) of ductal carcinomas was positive for claudin 4, while endocrine tumors were negative. In contrast, claudin 7 showed high expression in all endocrine tumors, however, ductal carcinomas did not express the protein. Claudin 3 was detected in 80% (16/20) of endocrine tumors, while ductal carcinomas were negative. Claudin 7 expression was significantly higher in endocrine tumor cells, compared with claudin 3 ( $p < 0.0032$ ).

**Conclusion:** Our findings support that claudins 3 and 7 are specific marker for endocrine pancreatic tumors in contrast to ductal carcinomas. Further studies are necessary to assess the biological role of these proteins in pancreatic carcinogenesis.

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#### P 516

##### **GASTROENTEROPANCREATIC ENDOCRINE TUMORS: HISTOPATHOLOGIC FEATURES AND CATEGORIZATION ACCORDING TO WHO CLASSIFICATION**

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**INTRODUCTION:** Although gastrointestinal and pancreatic endocrine tumors (GEPs) are relatively rare they represent a complex tumor entity. The aim of this study was to determine the histopathological features and immunohistochemical profiles of GEPs diagnosed in our department.

**METHODS:** Of the 125 cases of GEP diagnosed between the years of 1995 and 2004, we reviewed cases based both on histologic patterns common to GEP and immunohistochemical results. Immune antibody panel included Chromogranin A, Synaptophysin, NSE, PGP 9.5 to all, Serotonin, Gastrin, Somatostatin, Glucagon, Insulin, VIP and some other peptides like ACTH, GRH to some sporadic cases. Ki67 expression was investigated to determine the proliferation index. Due to results of examination all cases were categorized according to new WHO classification.

**RESULTS:** 125 cases were collected from the archives of our department with a male to female ratio of 1.21 and a mean age of 61 years. The most common location was large intestine (43.2 %) followed by stomach, small intestine, pancreas and appendix (39%, 7.2%, 6.4%, 4.0% respectively). 64.8% of the cases were pure GEP whereas others were mixed endocrine- exocrine tumors. Among the pure GEP group, 11.1% were benign, 6.2% uncertain behavior tumors,

13.6% well differentiated and 69.1% poorly differentiated carcinomas. Synaptophysin was the most common immune marker observed and Ki67 indexes were correlated significantly with the biologic behavior categories ( $p < 0.05$ )

**CONCLUSIONS:** The results of this study demonstrated that the distribution of our GEP cases in gut and the biologic behavior categories varied however malignancy –predictive factors including Ki67 index and immune profile were in accordance with previous reports.

#### P 517

##### **IMMUNOHISTOCHEMICAL STUDY OF 29 SOLID PSEUDOPAPILLARY NEOPLASMS OF THE PANCREAS ON TISSUE MICROARRAYS**

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**Background :** Solid pseudopapillary neoplasm of the pancreas is a rare tumour of uncertain histogenesis, that may raise difficult diagnostic issues.

**Aim:** To examine in a series of solid pseudopapillary neoplasm the expression of proteins of potential diagnostic and histogenetic interest by immunohistochemistry on tissue microarrays.

**Methods :** 29 solid pseudopapillary neoplasms of the pancreas were analysed. The expression of the following proteins was studied: CD10, oestrogen receptor, progesterone receptor, vimentin, cytokeratin (KL1), chromogranin A, synaptophysin, Mismatch Repair proteins (hMLH1, hMSH2, hMSH6), p53, b-catenin, E-cadherin.

**Results :** Tumours expressed the various antibodies with the following frequency: CD10 100%, progesterone receptor 100%, vimentin 93%, cytokeratin 72%, synaptophysin 46%, chromogranin A 0%, oestrogen receptor 0%. None of the cases presented a loss of expression of hMLH1, hMSH2 or hMSH6. There was no overexpression of p53 protein. In all cases a strong nuclear immunostaining of b-catenin was present in most tumour cells, with a “dot-like” cytoplasmic expression of E-cadherin that showed no membranous expression.

**Conclusion:** CD10, progesterone receptor, vimentin and b-catenin are expressed in solid pseudopapillary neoplasms of the pancreas, and are useful diagnostic markers for this rare tumour type. The pattern of b-catenin and E-cadherin expression that we observed suggests a major role for these proteins in the development of this tumour. The particular localisation of E-cadherin could be due to an abnormal trafficking of the protein to the membrane and/or to an endocytosis of this protein. Although solid pseudopapillary neoplasm develops in young patients and has a good prognosis, there is no immunohistochemical argument for the presence of microsatellite instability in these tumours.

#### P 518

##### **SOLID AND CYSTIC PAPILLARY NEOPLASM OF THE PANCREAS**

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**OBJECTIVE:** Solid and papillary cystic neoplasm of the pancreas (SCPN) is a rare primary pancreatic neoplasm of unknown etiology, occurring most commonly in young women. Four patients with solid and cystic papillary tumor of the pancreas are evaluated to discuss the histopathological and

immunohistochemical findings with the clinical course and surgical treatment.

**PATIENTS AND METHODS:** Three cases of SCPN located in the tail of the pancreas and one located in the head of the pancreas are presented. In two patients distal pancreatectomy with splenectomy were performed. Spleen was preserved in one of the patients. Whipple’s operation was performed for the patient with the tumor at the head of pancreas. The cases located in the tail of the pancreas were a 14 year old female, a 73-year-old female and 69-year-old male with the diameter of the masses as 6 cm, 2,5 cm and 6,5 cm respectively. The fourth case was a 17-year-old woman with a 5 cm mass in the head of the pancreas. In all patients the pancreatic lesions were shown by ultrasonography and a computed tomography scan. Histological diagnosis was made by performing haemotoxylene-eosin and PAS staining, immunohistochemical staining for progesteron receptors, vimentin, chromogranin A, EMA, NSE, cytokeratin, synaptophysin, CEA, Ki-67, CD10 and CD56.

**RESULTS:** The histological findings showed large encapsulated tumors with the section of the tumors surrounded by hemorrhagic and necrotic areas. The solid areas were composed of small and medium size tumor cells, which had no obvious atypia. Pseudopapillary structures were found in the cystic degeneration areas. Immunohistochemically, all of the tumors were positive for CD56, vimentin and progesterone receptor, negative for EMA, CEA. Two tumors stained for chromogranin or synaptophysin and one tumor stained for cytokeratin and synaptophysin. One tumor stained for CD10. Three tumors (Case 1,2,3) were, 1% nuclear positive and one of the tumors (Case 4) was, 5% nuclear positive for Ki-67. Case 4 showed an infiltrative growth into the surrounding pancreatic parenchyma. In the follow-up period (1-5 years) all patients are alive with no evidence of recurrence.

**CONCLUSIONS:** Solid and cystic papillary neoplasm of the pancreas (SCPN) is a neoplasm with a low malignancy and show favorable prognosis after surgical removal. In spite of the characteristic macroscopic and microscopic aspects, the immunohistochemical profile is nonspecific.

#### P 519

##### **PANCREATIC SOLID AND CYSTIC HAMARTOMA IN ADULTS: CHARACTERIZATION OF A NEW TUMOROUS LESION**

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**Introduction:** Nonneoplastic tumor-forming lesions in the pancreas are uncommon. They include cystic and noncystic varieties that have to be clearly distinguished from malignancies.

**Patients, Materials and Methods:** We report on a solid and cystic tumor-like lesion of the pancreas that occurred in 4 adult patients. Because of the unclear nature of these lesions, resection was performed. Formalin-fixed, paraffin-embedded tissues from the specimens were investigated by means of immunohistochemistry.

**Results:** The four resection specimens each contained a firm, gray, well demarcated nodule with focal cystic changes. The size ranged from 2 to 7 cm in diameter. The surrounding pancreatic tissue was unaffected. Histologically, the tumors showed a disorderly arrangement of well differentiated endocrine and exocrine pancreatic tissue that was embedded in a fibrous stroma. The cystic structures were lined by duct-

like cells. Immunohistochemically CD34 and c-kit coexpression was seen in the spindle cell component in two cases. Scattered insulin-producing cells were detected between acinar and ductal cells. Islets were lacking.

Discussion: The four pancreatic tumors showed a histologic pattern compatible with that of a hamartoma of the pancreas.

#### P 520

##### **ECHO ENDOSCOPIC ULTRASONOGRAPHY FINE NEEDLE ASPIRATION (EUS-FNA) IN PANCREATIC LESIONS. EXPERIENCE AT HOPITAL SAINT-JOSEPH.**

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Background: Endoscopic ultrasonography (EUS) fine needle aspiration (FNA) is a new safe procedure used to explore non accessible pancreatic lesions.

Objectives: Evaluation of our early experience of EUS-FNA in pancreatic lesions.

Methods: The study was performed until 2003, by an experimented operator (OM), using a ultrasound transducer echoendoscope, with microbiopsy (FNA 22 G), without antibiotics prophylaxy (expected in cystic lesions). For each case 1 to 4 FNA was performed and pull in 1 or 2 AFA containers. For each case the reporting data consisted in : clinical setting with echographic results, endoscopic procedure (exact position of the trasducer, and transfixed organ) and site of biopsy. The pathological procedure consisted in paraffin inclusion, after centrifugation. For histologic analysis a set of 5 slides were obtained with a single 3 HES staining sections, and 5 slides for histochemistry (PAS or Alcian Blue) or immunohistochemistry study.

Results: 50 EUS-FNA were performed in 30 patients (12 M/ 18 F) 62 years old mean (40 - 80). Echoendoscopic results were : 5 cystic lesions, 5 single masses, 16 unresectable tumors. The maximal diameter was 26 mm (7-42), and 2 patients had a pancreatitis history. The site of biopsy was : body 3, head-isthmus 18, tail 9. The transfixed organ was stomach 52%, duodenum 48%. The number of biopsy studied was 1.8 (1-4). A non concluent diagnosis was performed in only 1 case (cystic lesion). The diagnoses were : malignant in 22 cases (Pancreatic carcinoma 16, Pancreatic endocrine tumor 5, lymphoproliferation 1) and benign in 7 cases (Absces 1, Tuberculosis 1, Normal pancreas 2, Pancreatitis 2, Mucinous cystadenoma 1).

Conclusion: The fiability of EUS-FNA in pancreatic lesion in our experience is 94%, similar to the fiability reported in the literature. In our experience, EUS-FNA is a vailable method even in small tumors, and specially in pancreatic adenocarcinoma. Cooperation between pathologist and gastroenterologist is necessary to obtain the best organization for the best diagnosis.

#### P 521

##### **ADENOCARCINOMAS OF THE AMPULLA OF VATER AND OF THE HEAD OF PANCREAS HAVE DIFFERENT EXPRESSION OF MUC1, MUC2, MUC5AC, AND MUC6 APOMUCINES**

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Background: Adenocarcinomas (AC) arising from the ampulla of Vater (AV) and the head of pancreas (HP) have similar morphologic features, clinical presentation and a common surgical treatment (Whipple procedure) but a different outcome. AV-AC are histologically classified as intestinal type (IT) and biliopancreatic type (BPT) ACs. The

classification is based on the histological pattern, Cdx-2 expression and/or cytokeratin 20 positivity for IT ACs and cytokeratin 7 positivity for BPT ACs. Apomucines have different expression according to the site of the tumor or to the phenotypic differentiation. The de novo expression of MUC1 is indicated as a negative prognostic marker, while MUC2 seems to be involved in the tumor suppression of colorectal cancer.

The aim of this study was: a) to investigate the expression of MUC1, MUC2, MUC5AC and MUC6 and b) to explore the utility of apomucines in differentiating the HP ACs from the AV ACs.

Design: Forty four surgically resected invasive AV-ACs, previously classified as IT (28) and as BPT (16) by using Cdx2 immunoreactivity, were immunostained for MUC1, MUC2, MUC5AC and MUC6 using avidin-biotin-complex method, including positive and negative controls.

Results: Twenty-seven out of 44 AC of AV (61%) were positive for MUC1. MUC5AC was present in 15/44 (34%), MUC6 in 13/44 (29%) and MUC2 in 11/44 (25%) cases. MUC1 was present in 14/28 (50%) IT AC and in 13/16 (81%) BPT AC. MUC2 was present in 8 AV AC classified as IT and in 3 cases classified as BPT. MUC5AC and MUC6 had no difference in their distribution in relationship to IT or BPT phenotype. In HP ACs we found 30/35 (86%) cases expressing MUC1, 25/35 (71%) MUC5AC, 7/35 (20%) MUC6 and 2/35 (6%) cases expressing MUC2. Follow up was available for 32 patients, 13 of which alive with a mean survival of 58 months and 19 dead of the disease after a mean survival of 20 months. Twelve of 13 alive patients express one or both intestinal markers.

Conclusions: The adenocarcinomas of AV and HP have different pattern of MUC expression. MUC1 and MUC5AC are much more expressed in HP ACs, in association with the poor prognosis of pancreatic cancer. AV-BPT adenocarcinomas show a pattern very similar to HP ACs. Only MUC2 is preferentially observed in AV-AC and it can be useful as marker of intestinal type differentiation of AV AC, related to a better prognosis of this type of cancers.

#### P 522

##### **DIFFERENTIATION PATHWAYS IN AMPULLARY CARCINOMAS: COMPARATIVE STUDY ON MUCIN PEPTIDE CORE ANTIGENS (MUC1, MUC2, MUC5AC) AND CDX2 EXPRESSION, INCLUDING GASTRIC AND COLON CARCINOMAS.**

Gursan N1, Gursan MS2,

A broad histomorphologic spectrum of ampullary carcinomas of Vater make a reproducible histologic classification difficult. The aims of this study were to determine histologic subtype of ampullary carcinomas kullanarak mucin peptide core antigens and CDX2 in comparison with gastric and colorectal carcinomas. We examined immunohistochemically for expression of MUC1, MUC2, MUC5AC, and CDX2 in ampullary carcinoma (30), gastric carcinomas (30) and colon carcinomas (30). sixty-four percent of the gastric carcinomas and seventy-five of the ampullary carcinoma expressed MUC1, whereas none of the colorectal carcinomas did. MUC2 mucin was highly expressed in colorectal carcinomas. In ampullary carcinoma, gastric carcinomas expressed MUC2 89%, 83%, respectively. Immunohistochemically, MUC5AC were frequently expressed in gastric carcinoma. fifty-five of the ampullary carcinoma expressed MUC5A. The nuclear expression of CDX2 was closely associated with the expression of MUC2 in colon carcinomas. In ampullary carcinoma, gastric carcinomas expressed CDX2 75%, 62%, respectively. Moreover, the expression of MUC1, MUC2 MUC5A and CDX2 was a useful to determine histologic subtype of tumors derived from the ampulla of Vater.

#### P 523

### HEP PAR 1 IMMUNOEXPRESSION IN THE TUMOURS OF PERIAMPULLARY REGION AND HEAD OF THE PANCREAS

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Ampullary carcinomas may arise from two different types of mucosa and comprised of two different histologic types; intestinal and pancreatobiliary. It is important to distinguish them from each other since they have different therapeutic and prognostic implications. Monoclonal antibody Hep Par 1 which shows hepatocytic differentiation is generally used in the differential diagnosis of hepatocellular carcinomas. Some authors advocate that this antibody indirectly shows small intestinal differentiation. The aim of this study is to evaluate the Hep Par 1 immunopexpression in pancreatic, duodenal and extrahepatic biliary tract carcinomas including periampullary tumours and to explore the utility of this antibody in differentiating pancreatic ductal adenocarcinoma and ampullary adenocarcinoma of pancreatobiliary type from the intestinal type ampullary carcinoma.

We studied Hep Par 1 immunopexpression in 61 cases of periampullary (n=40), pancreatic (n=16), duodenal (n=4) and mid-choleductal (n=1) carcinomas. Twentytwo (55%) periampullary carcinomas, 14 (87.5%) of the tumours originating from head of the pancreas and two (50%) of the duodenal tumours were negative for Hep Par 1 (p<0.05). Fourteen of the 18 intestinal type periampullary tumours (77.7%) were positive for Hep Par 1 whereas only three of the 21 pancreatobiliary type tumours (14%) showed focal immunoreactivity (p<0.0001). There was no significant difference between the Hep Par 1 expressions of the pancreatic carcinomas and the ampullary carcinomas of pancreatobiliary origin.

In conclusion, we suggest that the ampullary tumours of intestinal origin can be separated from the tumours of pancreatobiliary origin either ampullary or pancreatic by Hep Par 1 immunohistochemistry.

#### P 524

### PROGNOSTIC SIGNIFICANCE OF EGFR AND HER2/NEU(C-ERB2) IN PANCREATIC CANCER

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Pancreatic duct carcinoma is a malignant tumor with an extremely poor prognosis. The mechanisms of the aggressive growth and metastasis are not completely understood.

Recent studies have shown that some growth factor receptors with tyrosine-kinase activity, e.g., the epidermal growth factor receptor (EGFr) and the Her2/neu(c-erb2) oncoprotein, are associated with aggressive biologic behaviour in a variety of tumors.

The aim of this study was to detect the relation of EGFr and c-erb2 to the progression and metastasis of pancreatic duct carcinoma and analyze the correlation between EGFr and c-erb2 expression pattern

and clinicopathological factors.

**MATERIAL and METHODS:** We examined the immunohistochemical expression of c-erb2 and EGFr in formalin-fixed paraffin embedded tissue samples from 42 patients with pancreatic ductal adenocarcinoma, 4 patients

with carcinoma of Vater's ampulla and 10 patients with chronic pancreatitis.

**RESULTS:** 19 of 42(43.6%) samples of pancreatic cancer stained positive for EGFr and 23 of 42(53%) for c-erb2. 3 of 4 samples of carcinoma of Vater's ampulla expressed EGFr and 2 of 4 expressed c-erb2. 1 of 10 samples with chronic pancreatitis expressed EGFr and 1 of 10 c-erb2. The coexpression rate of the two proteins was 28%(16/42). This coexpression in the pancreatic duct adenocarcinoma groups was correlated with histopathological grades and metastatic status of TNM classification (p<0.01).

**CONCLUSIONS:** The immunohistochemical expression of EGFr and c-erb2 can be used as a marker of aggressive biological behaviour including recurrence and metastasis of pancreatic duct adenocarcinoma.

#### P 525

### IS THE PERITUMORAL REGION IN EXOCRINE PANCREAS CARCINOMA DIFFERENT FROM NORMAL PARENCHYMA?

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**Background:** Ductal adenocarcinoma and its variants make up over 90% of all pancreatic tumours. Adenocarcinoma of the pancreas is characterized by local tumor growth that infiltrates vascular structures, nerves and lymphatics, and causes early hematogenous spread to the liver. Studies of pathologic characteristics of the primary or metastatic tumor have failed to define high-low risk subgroups of clinical significance, mainly because most patients experience rapid disease progression. **Purpose:** We conducted a study to identify histological quantitative and qualitative modifications in the region surrounding exocrine pancreas carcinomas (peritumoral region) compared with normal parenchyma. **Methods:** We have studied 28 cases of ductal adenocarcinoma localized in the head, body, and tail of pancreas who underwent surgical resection. We defined the peritumoral region, as the portion of apparently not affected parenchyma in 1 centimeter surrounding the tumor. In this region we have been analyzed the inflammatory infiltrate (using an immunohistochemical technique with the antibody for CD 68) and angiogenesis (with antibody for CD 34) comparative within the tumor and to the parenchyma from distance. **Results:** In the studied cases, the inflammatory infiltrate was slight increased in the peritumoral region than the tumoral and normal, and there was a significant positive staining for CD68 (expressed on macrophages and monocytes). The expression of CD34 was not significant modified. Various pathological parameters have been evaluated (size, mitotic rate, vascular -perineural invasion, lymph node metastasis, necrosis, surgical margins, location). **Conclusion:** Although the cell kinetics of the pancreas are not understood, it seems likely that there is a continuous slow turnover of cells, fed from a stem cells population in the ducts, and that the controls on the production rate of each cell type are local rather than systemic. The surrounding inflammatory infiltrate (especially macrophages) in the exocrine pancreas tumors is important in prognosis. This parameter can be important for tumoral behavior and therapeutic strategies.

#### P 526

### MIXED DUCTAL ENDOCRINE CARCINOMA OF THE COMMON BILE DUCT

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The mixed bile duct endocrine/ductal carcinoma is a rare neoplasm and only a few cases have been reported in the literature reviewed. We present a case of a carcinoma of the common bile duct with mixed histological features in a 71 years old man who presented with jaundice and a cholestatic biochemical profile. The carcinoma measuring 2,2 x 1 cm, arose in the wall of the common bile duct and invaded the gallbladder neck and the pancreas forming an intrapancreatic tumor mass of 3 cm in diameter. Histologically the tumor tissue consisted of a poorly differentiated endocrine carcinoma (small cell carcinoma) and a carcinomatous component with features of ductal adenocarcinoma. The two neoplastic elements showed a separate or merging growth pattern while biphasic structures exhibiting both cell types were also encountered. Interestingly, the pancreas was infiltrated exclusively by the small cell carcinoma. The endocrine immunophenotype was confirmed by the expression of chromogranin, NSE, synaptophysin and CD56 while the ductal adenocarcinoma showed a positive reaction to EMA, CEA, CD15 and cytokeratin 19. Regarding histogenesis the intimate topographical coexistence of the heterogeneous cell populations is very likely to represent their histogenetic association with a multipotential precursor cell.

**P 527**

**IMMUNOHISTOCHEMICAL STUDY OF GENETIC ALTERATIONS, MUC1 AND MUC2 EXPRESSION IN INTRADUCTAL AND INVASIVE DUCTAL TUMORS OF THE PANCREAS**

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Pancreatic carcinoma is a highly malignant neoplasm that still carries a very poor prognosis. Majority of pancreatic carcinomas are malignant. So the classification has a clinical and prognostic significance. The ductal adenocarcinomas (DA) arising from Intraductal Papillary Mucinous Neoplasia (IPMN) are said to have a much better prognosis than the classical adenocarcinoma. Also the pancreatic intraductal lesions, named as Pancreatic Intraepithelial Neoplasia (PanIN) have been classified by WHO. Multiple genetic alterations are involved in development of pancreatic neoplasm. In this study, pancreatic tissue obtained from 135 operations were examined for PanIN lesions. The frequency of PanIN in tumoral and nontumoral pancreata were compared and DA cases were evaluated for presence of IPMN. Then we investigated expression of p21, p53 and DPC4, the major apomucins (MUC1, MUC2) in PanIN lesions and tumors of the IPMN related carcinomas and the classical DA by immunohistochemical method. p21 and p53 expression increased progressively with the severity of the lesion. p21 expression was (-) in normal pancreatic tissue, PanIN1a, PanIN1b, PanIN2 lesions and was (+) in 2 PanIN3 lesions both of which were sampled from IPMN(-) DA (2/20) and 10 invasive carcinoma area most of which belonged to IPMN (-) DA. p53 expression was (-) in normal pancreata, PanIN1a, PanIN1b, PanIN2 lesions and was (+) in most of PanIN3 and invasive carcinoma samples. DPC4 was highly expressed in normal pancreata, PanIN1a, PanIN1b lesions and DPC4 expression decreased with the severity of lesions ( PanIN2: %61, PanIN3: %57, TM: %43). IPMN(-) tumors highly express MUC1 and only 1 case was (+) for MUC2. On the other hand most of IPMN (+) cases were (+) for both MUC1 and MUC2. The most frequent PanIN lesion was PanIN3 in tumor cases. A positive correlation between PanIN1a, PanIN1b, PanIN2 and chronic pancreatitis was found. There was no correlation between chronic pancreatitis in DA and high grade PanIN lesions. Vascular invasion and lymph node metastasis were significantly less in IPMN(+ ) DA compared to IPMN (-) DA. This finding supports the

hypothesis that IPMN(+) adenocarcinomas have a better prognosis than a classical DA. DPC4 expression is especially present in low grade carcinomas in IPMN(-) tumors and loss of DPC4 expression increases with the grade of IPMN(+) tumors. p21 and p53 expression were both found to be late events in development of pancreatic neoplasia according to our results.

**P 528**

**NON-INVASIVE INTRADUCTAL PAPILLARY MUCINOUS TUMORS OF THE PANCREAS. EXPERIENCE OF THE HOSPITAL CLINIC OF BARCELONA.**

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**Introduction.** Intraductal Papillary Mucinous Neoplasms (IPMN) of the pancreas are intraductal tumors characterized by variable amount of mucin production, cystic dilatation and papillary growth, that have increased its frequency in the last past years. They display a wide spectrum of cytologic and architectural atypia, from benign adenomatous lesions to invasive carcinomas.

**Objective.** The purpose of these study was to report the clinicopathological features of non-invasive IPMN in our hospital.

**Material and Methods.** We reviewed the histologic material from surgical pancreatic resections performed between 1995 and April 2005 at the Hospital Clinic of Barcelona in which the words mucinous, cystic or papillary formation were identified in the pathology reports, in order to identify and classify IPMN according the current WHO definition of these neoplasms. The clinical history and image studies of each patient were also reviewed.

**Results.** A total of 27 IPMN were identified of which 14 (52%) were non-invasive. Ten of the 14 patients were man and 4 female, with a mean age of 72 years. In two patients the lesion was discovered because of abdominal pain and 1 for a constitutional syndrome. In 5 patients (36%) IPMN were discovered incidentally whereas a history of recurrent acute pancreatitis was found in 6 patients (43%). Most of the lesions were localized at the pancreatic head (10 71%) and 3 at the body/tail. The mean size was of 3 cm (1.3 to 5.8). In 9/14 cases USE-FNA was performed previous to surgery, obtaining mucin and ductal cells with or without atypia. Histologically, 10 cases displayed features of IPM adenoma, 2 moderate dysplasia and 2 high grade dysplasia/in situ carcinoma. Branch-type was the most frequent IPMN type found (11/14 79%) and in the other 3 cases the main pancreatic duct and its branches were affected. None of the patients had recurrence or died of the disease after a median follow-up of 22 months (from 1 to 88).

**Conclusion.** IPMN represents a group of pancreatic neoplasms that have increased its frequency in the recent last past years, mostly due to their recognition in early phases as incidental lesions by the improvement and the most extended use of image techniques, specially in asymptomatic patients, as has been observed in 36% of the patients of our series of non-invasive IPMN.

**P 529**

**MORPHOLOGY OF THE GALLBLADDER MUCOSA IN VARIOUS TYPES OF CHRONIC CHOLECYSTITIS**

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**Background:** Chronic inflammation of gallbladder comprises simple form with mucosa with slight atrophy and rarely chronic hyperplastic form with arboriform hyperplasia of mucosa, suggesting neoplasia.

**Aim:** The aim of this study was the comparison of the morphology, proliferative activity and karyometry of mucosa cells in both types of cholecystitides.

**Material and methods:** 25 cases of simple chronic cholecystitis and 30 of chronic hyperplastic cholecystitis were collected. The morphological analysis of mucosa architecture was done using confocal laser scanning microscopy, proliferative activity was assessed with Ki-67 reaction, karyometric measurements included: nuclear area, length, breadth, perimeter, roundness, aspect and mean grey level.

**Results:** Simple chronic cholecystitis was characterized by monolayered glandular epithelium, in cases of hyperplastic chronic inflammation the multilayered epithelium was observed moreover, Ki-67 was positive in almost all epithelial cells, whereas in simple chronic inflammation only single cells were Ki-67-positive.

**Conclusion:** Chronic hyperplastic cholecystitis shows the different architecture of epithelium, increased proliferative activity and different karyometric characteristics, including rounding and widened nuclei with predominance of euchromatin.

#### P 530

##### **LIVER CELL DYSPLASIA IN PERCUTANEOUS LIVER BIOPSY IN CHRONIC HBV OR HCV HEPATITIS**

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The role of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in the etiopathogeny of the hepatocellular carcinoma (HCC) is well established.

**Material and methods**

We studied the presence of large cell changes (LCC) and small cell dysplasia (SCD) according to the etiology, age, necroinflammatory activity and fibrosis in a group of 1173 percutaneous liver biopsies from patients with chronic viral hepatitis B, C and D. The necroinflammatory activity and fibrosis were assessed based on Ishak semiquantitative score. A statistical analysis was performed using CHITEST (X 2) with a level of statistic significance (P) of 0.05.

**Results**

LCD was exclusively revealed in HBV+/-HDV infection (P=1x10<sup>-54</sup>); SCD was more frequent in HCV infection (P=9x10<sup>-6</sup>).

There were significant association between high necroinflammatory activity and LCC in HBV infection (P=2x10<sup>-27</sup>) and HBV+HDV infection (P=1x10<sup>-5</sup>); also, SCD and low necroinflammatory activity were associated with HCV infection (P=2x10<sup>-6</sup>) and HCV+HBV infection (P=6x10<sup>-6</sup>).

Advanced stages of fibrosis were associated with LCC and HBV infection (P=4x10<sup>-31</sup>), LCC and HBV+HDV infection (P=2x10<sup>-17</sup>), SCD and HBV infection (P=1x10<sup>-18</sup>), SCD and VHC infection (P=0.01) and SCD and VHC+VHB infection (P=1x10<sup>-212</sup>).

#### Conclusions

LCC appear in chronic hepatitis with high grades of necroinflammatory activity and advanced stages of fibrosis (they need a long time to develop – important fibrosis – but it seems they are elicited by the hepatocytic lesions – important activity). SCD also need advanced stages of fibrosis (long time to evolve) but low grades of necroinflammatory activity (SCD are caused by accumulations of genetic alterations in the hepatocytes)

#### P 531

##### **USEFULNESS OF FINE NEEDLE ASPIRATION BIOPSY (FNA) AND THIN NEEDLE CORE BIOPSY (NB) FOR THE DIAGNOSIS OF HEPATIC NODULES LESS THAN 2 CM IN CIRRHOTIC PATIENTS**

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**Introduction:** Systematic screening of cirrhotic patients for the early diagnosis of hepatocellular carcinoma (HCC) results in the detection of nodules less than 2 cm that cannot be fully characterized by imaging techniques. The role of biopsy in this setting remains controversial, due to the risk of sampling error and to the assumed very well differentiated nature of the tumors.

**Purpose:** To evaluate the efficacy of a prospective diagnostic approach based on the combination of FNA and NB for the diagnosis of small (<2cm) nodules in cirrhotic patients.

**Material and methods:** 45 cirrhotic patients undergoing systematic ultrasonographic (US) screening in which a nodule less than 2 cm (17 out of them 1cm or less) was detected. FNA (45 cases) with cell block when enough sample was obtained (43 cases) and NB with a 20G cutting needle when allowed by the location of the lesion (16 cases) were performed. Both cytologic and histologic diagnoses were based on previously published criteria. Cases were classified in five categories: 1. Negative 2. Benign nodule (regenerative vs. low grade dysplastic) 3. Suspicious (high grade dysplastic vs well differentiated HCC) 4. Malignant 5. Insufficient. Cases in the 3 and 4 categories were treated accordingly. Nondiagnostic and benign cases were followed-up for a period ranging from 3 to 12 months and rebiopsied according to the same protocol when indicated by the imaging features (US-Sonovue, computed tomography and magnetic resonance) or nodule growth (21 rebiopsies in 17 patients).

**Results:** 22 patients were classified in the categories 3 or 4 after the first biopsy, and other 7 after a second (6 cases) or a third biopsy (sensitivity for the first biopsy 75,7%). 3 out of the 7 false negative cases were classified in category 5 in the first biopsy, and the other 4 in category 1. 7 cases diagnosed as category 3 or 4 measured 1cm or less; 6 were diagnosed at the first biopsy and the other in the second biopsy. FNA (cytology + cell block) failed to diagnose 4 out of 29 lesions classified as 3 or 4, whereas NB missed 4 out of 12 lesions in which this procedure was performed.

**Conclusions:** FNA combined with NB is an efficient method for the diagnosis of small nodules in cirrhotic patients.

Failures in diagnoses seem to be related to sampling rather than to diagnostic difficulties by the pathologist. FNA comprising cytology and microhistology of cell blocks is more sensitive than NB, probably due to better sampling.

#### P 532

##### **FINE NEEDLE ASPIRATION BIOPSY OF FOCAL LIVER LESIONS – THE EXPERIENCE OF 2111 CASES**

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Introduction: Ultrasound (US)-guided fine needle aspiration biopsy (FNAB) of focal liver lesions is a cost effective diagnostic method, by which the nature of the lesion (benign / malignant, primary / metastatic) is easily diagnosed in most cases. Clinicians and pathologists of those centers, that lack an experienced team needed for these examinations usually deny the efficacy of cytological examinations, and prefer thick needle – core- biopsies.

Our purpose was to show our data, demonstrate the most relevant, most frequently occurring technical and diagnostic difficulties, and to show the role of FNAB in the diagnostic armory.

Method: In the past 20 years, 2111 US guided liver FNABs were performed in the neighbouring US laboratories, that were examined by our cytological team. The smears were wet fixed with ethanol, and stained with H&E.

The results are the following: Benign lesion (cyst, adenoma, FNH, etc) 405 cases. Malignant lesion: 1046 - in this group 206 hepatocellular carcinomas (HCC) 719 metastatic diseases were found. In further 121 cases in this group malignant disease was diagnosed without comment, whether the lesion was primary or secondary. In the next category, „suspicious for malignancy” (122 cases). Malignancy could not be proven in 290 cases. In 145 cases, the smears were unsatisfactory for any diagnosis or opinion. In 60 % of these cases clinical follow up and/or histology or autopsy confirmed the nature of the hepatic lesion. No false positive case was found. In four cases metastatic disease was suspected, that turned out to be HCC, or mixed HCC-cholangiocarcinoma.

Conclusion: Our data may persuade those colleagues, who deny or haven't yet experienced the usefulness of liver FNAB-s, to use this easy method as a diagnostic modality, of which we do believe the patients benefit the most. We would like to share some of our „favourite cases” with the audience.

#### P 533

##### **REGRESSION OF LIVER ADENOMATOSIS AFTER WITHDRAWAL OF ORAL HORMONAL CONTRACEPTIVE : A CASE REPORT**

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Liver adenomatosis (LA) is a rare disease characterized by the presence of multiple hepatocellular adenomas. Initially, LA was considered as a separate clinical entity from isolated hepatic adenoma by no relation with oral contraceptive use. However, recently, frequent biallelic inactivation of TCF1 gene encoding Hepatocyte Nuclear Factor -1fN̄ (HNF-1fN̄) was identified in both hepatocellular adenomas and LA, suggesting a possible overlap of these two entities. Pathogenesis of LA is unclear. One case of LA regression is described in the literature after hormonal therapy withdrawal, but without any information concerning the TCF1 gene status. We report the second case of regressive LA after withdrawal of hormonal therapy. In this case, TCF1 was not inactivated.

A 48-year-old woman was admitted to our hospital in April 1990 because of a polynodular liver. Ultrasonography (US), computed tomography (CT) and magnetic resonance imaging showed at least eight masses in the liver: the largest one (6 cm) was located within segments VIII-VI. One of these masses, in the left lobe, was typical of angioma. Histological diagnosis of hepatocellular adenoma was made on laparoscopic liver biopsy performed on 2 nodules.

The patient had no diabetes and had been taking oral contraceptives for 10 years at the time of diagnosis. After withdrawal of hormonal therapy, she was examined regularly once a year by US and CT over a period of 10 years, and partial regression of the adenomas was documented. She died in June 2000 because of a pancreatic cancer. Necropsy showed a pancreatic cancer corresponding to an anaplastic carcinoma. Macroscopic and microscopic examination of the liver showed an hemangioma in the left lobe and revealed only one hepatocellular adenoma in the right lobe measuring 3 cm with large areas of ischemic necrosis, haemorrhage and fibrosis. A few microadenomas with steatotic hepatocytes were also present.

The immunohistochemical analysis for estrogen receptors and progesterone receptors performed on the partially regressive adenoma was negative. Neither somatic nor germline inactivation or mutation of TCF1 was found in the hepatocellular adenoma and the normal liver tissue.

With this case report, we demonstrate that LA, without inactivation of TCF1 gene, may almost completely regress after hormonal therapy withdrawal. Exogenous hormonal therapy probably has an important role in some cases of this disease and it seems indicated to avoid it in these patients.

#### P 534

##### **ASSESSMENT OF IMMUNOHISTOCHEMICAL PROFILE IN HUMAN HEPATOCARCINOMAS**

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The immunoprofile of human hepatocarcinoma (HCC) has a more or less diagnostic value, doing difficult the differential diagnosis with cholangiocarcinoma or metastasis of adenocarcinoma.

Our aim was to investigate and to test the efficiency of a selected panel of antibodies for assessing the immunohistochemical profile (IHC) of HCC for positive diagnosis.

We performed the indirect triserial ABC method of IHC for 7 antibodies: CK 7, CK 8, CK 18, CK 19, AFP, CD 34, OCH1E5 on formalin fixed paraffin embedded tissues taken by biopsies from 33 cases with HCC.

Hepatocarcinomas were immunoreactive to CK 8 and 18 in 65% cases, the tumors being positive especially to CK 18; the immunoreaction intensity was higher to CK 18 than CK 8 in tumor and peritumoral hepatic tissue. Alpha fetoprotein was positive in 85% of cases, showing a great sensibility in all tumoral types. Well differentiated tumors stained strongly to CD34, with a perisinusoidal pattern of vessels; low differentiated tumors with compact pattern stained weakly to CD34 (with random distribution of vessels) or negative. OCH1E5 is a liver specific antibody (positive in over 75% of tumors, and in 100% of peritumoral hepatic tissue), but it is pretentious, requiring special technical pretreatments.

Despite of tumoral immunoreaction variability, this 7 antibodies panel proved to be a useful tool with significant diagnostic value of human hepatocarcinomas.

#### P 535

##### **HEPATOCYTE GROWTH FACTOR AND ITS RECEPTORS C-MET AND C-MYC IN HEPATOCYLLULAR CARCINOMA**

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Despite a variety of therapeutic strategies, HCC remains a significant cause of cancer death. Therefore, to study the HCC pathogenesis is of the utmost importance for the prevention and treatment of this disease. In the present study markers known of regeneration (HGF/c-met system) and cell proliferation (c-myc oncogene) were studied in a series of 20 HCC cases. HGF concentration was estimated in serum by ELISA & in tumor tissues by immunohistochemistry, c-myc & c-met mRNA were detected by RT-PCR. HGF concentration in sera of HCC cases was higher than normal controls (mean value 52.2 versus 20.7 pg/ml). However it was not detected by immunohistochemistry in any of tumor tissues. The elevated levels of HGF had significant correlation with less differentiated and bigger size tumor tissue. c-met was detected in 45% of cases and c-myc in 30% of cases. There was a significant correlation between HGF & c-met & tumor size while no significant correlation between HGF/c-met system and c-myc.

#### P 536

**THE POTENTIAL ROLE OF ACTIVATED CASPASE-3 EXPRESSION IN HEPATOCELLULAR CARCINOMA**  
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Background: Caspase 3 is a downstream effector cysteine protease in the apoptotic pathway. Previous studies have shown that caspase-3 is expressed in 52% of hepatocellular carcinoma (HCC) by immunohistochemistry. Nuclear survivin, present in 42% HCC, correlated with poor prognostic survival parameters. In this study we investigated caspase 3 expression in HCC and related it to survivin overexpression, prognostic parameters, and outcome.

Patients and Methods: Formalin-fixed, paraffin-embedded 4mm sections obtained from 76 HCC hepatectomy specimens, were subjected to immunohistochemistry (streptavidin-biotin peroxidase) using polyclonal antibodies for activated caspase-3 and survivin (SantaCruz, USA). Human tonsils were used as positive controls. Immunostaining was evaluated in the cytoplasm and nucleus of hepatocytes and Kupffer cells. Results followed morphometric analysis and were expressed as % of positive cells. Mean follow up time was 27.19 months (range 1.5-83 months).

Results: In HCC, activated caspase 3 was expressed within hepatocytes in 69/76 (90.7%) and within Kupffer cells in 52/76 (68.4%) cases. The stain was cytoplasmic and nuclear. Survivin was present in 34/76 (44.7%) and the stain was cytoplasmic and nuclear. Caspase expression was correlated with lower grade ( $p < 0.05$ ), higher disease free ( $p < 0.01$ ) and higher total survival ( $p < 0.01$ ). Caspase 3 expression was reversely correlated with cytoplasmic survivin expression ( $p = 0.024$ ).

Conclusions: Activated caspase 3 expression is correlated with higher disease free survival and higher total survival; in addition it displays a reversed correlation with survivin expression which is considered as a poor prognostic parameters given the fact that suppresses apoptosis. The higher caspase-3 expression probably reflects the fact that apoptosis is associated with good prognosis in cases of HCC.

#### P 537

**GLUTATHIONE S- TRANSFERASE M1 (GSTM1) NULL GENOTYPE AND RISK OF HEPATOCELLULAR CARCINOMA IN HCV CIRRHOTIC PATIENTS.**

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Glutathione S-transferase M1 (GSTM1) gene has a polymorphism and there are large differences in the prevalence of its null genotype among ethnic groups. Patients with GSTM1 null genotype are more susceptible to some cancers and other diseases. Although a few publications have assessed the link between GSTM1 null genotype and hepatocellular carcinoma (HCC), to our knowledge no one has assessed the impact of this polymorphism on cirrhosis due to HCV, the main etiological factor for HCC. The aim of this study was to investigate the modifying effect of GSTM1 null genotype on hepatocarcinogenesis in patients with cirrhosis secondary to HCV infection. Forty-three patients with cirrhosis secondary to hepatitis C virus infection were evaluated. Of these patients, 16 had HCCs (37.2%) and three had high-grade dysplastic nodules (HGDN). Age, gender, prior alcohol intake, grade of necroinflammatory activity and genetic polymorphism of GSTM1 were studied. DNA was extracted from paraffin blocks of explanted cirrhotic livers and their respective gallbladders. GSTM1 polymorphism was determined by PCR technique. The prevalence of GSTM1 null genotype was 13/43 (30.23%). Only GSTM1 null genotype was associated with HCC/HGDN in explanted HCV-related cirrhotic livers (RR: 4.49; 95% CI, 1.108-18.266;  $P = 0.046$ ). In conclusion, this association of GSTM1 null genotype with HCC/HGDN in patients with cirrhosis secondary to HCV might represent the identification of a subgroup with a high risk of HCC development.

#### P 538

**THE GLUTAMINE SYNTHETASE GENE IS A RELIABLE MARKERS OF MUTATED- $\beta$ -CATENIN HUMAN HEPATOCELLULAR CARCINOMAS**

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Activation of Wnt/  $\beta$ -catenin signalling is a frequent event in human hepatocellular carcinomas (HCC). We evaluated various  $\beta$ -catenin target genes as molecular markers of aberrant activation of the  $\beta$ -catenin pathway in  $\beta$ -catenin and Axin1 mutated HCC. A collection of 45 HCC was characterized for activating  $\beta$ -catenin and inactivating Axin1 mutations and analyzed for expression of the glutamine synthetase (GS), glutamate transporter (GLT-1), ornitho-aminotransferase (OAT), Gpr49, LECT2, c-myc and cyclinD1 genes by real-time RT-PCR. A subset of HCC was analyzed for  $\beta$ -catenin and GS by immunohistochemistry. We found a significant correlation between  $\beta$ -catenin mutations and over-abundance of mRNA for GS, GPR49 and GLT-1 ( $p = 0.0001$ ), but not for OAT, LECT2, c-myc or cyclin D1.

GS appears to have the best predictive value for  $\beta$ -catenin activation and is a faithful immunohistochemical marker of  $\beta$ -catenin activation in human HCC. Interestingly, in Axin1-mutated tumors, none of the liver  $\beta$ -catenin target genes validated in human HCC was overexpressed and accordingly, no aberrant accumulation of  $\beta$ -catenin was observed. In conclusion, our results show that overexpression of each GS and GPR49 are reliable markers of HCC carrying an activated  $\beta$ -catenin mutation. Surprisingly, HCC presenting inactivated Axin1 mutation do not show anomalous expression of the same genes.

#### P 539

##### **THE VALUE OF IMMUNOHISTOCHEMICAL MARKERS (IMS) FOR THE DIAGNOSIS OF LIVER TUMORS IN CT-GUIDED LIVER BIOPSY**

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**Introduction:** Guided and not blind liver biopsy is essential for diagnosing primary or metastatic liver tumors. Combination of IMs are useful for detection of specific antigens indicative of cell differentiation/maturation/function and their selective use is mandatory for a correct diagnosis.

**Purpose of the study:** To evaluate the usefulness of IM panels designed according to histological patterns and clinical history for the diagnosis of liver tumors.

**Materials-Methods:** 50 cases of CT-guided liver biopsies (Bs) were studied (43 males, 7 females). 10 patients had suffered from another primary malignancy, hepatocellular carcinoma was suspected in 9 cases of chronic hepatitis B and 40 were investigated for unknown primary tumor. Formalin fixed Bs were diagnosed with H+E, PAS, PAS-D, Masson Trichrome and Gordon's and Sweet's reticulin stain. The immunohistochemical method Envision-HRP was used for detection of mixed cytokeratins, CK7, 8,18,19, 20, CEA, Ca19-9, Ca125, c-erb-B2, TTF1, chromogranin A, oestrogen/progesterone receptors and the HepPar1 antibody which is normally expressed in hepatocytes.

**Results:** In 10/50 cases with another primary malignancy the selective use of IMs confirmed liver metastasis from the primary tumor. 9 hepatocellular carcinomas were diagnosed but attention should be paid to the focal or absent expression of HepPar1 in high grade tumors. Primary site was suggested in 40 cases and confirmation of a correct liver biopsy diagnosis was available in 16/40 surgical specimens. 24 patients with proposed diagnosis were not traced as they were treated in other hospitals. Discordance was observed in two adenocarcinomas with immunophenotype (Ca19-9 +, CK7 +, CK20 -). The upper GI tract was proposed as primary site but colonic adenocarcinoma was diagnosed in another hospital. Comparison of liver and colonic tumors was unavailable.

**Conclusion:** CT-guided liver biopsy is a useful method for distinguishing primary from metastatic liver tumours and for the recognition of primary site. As the pool of IMs increases, a logical approach is needed for achieving high specificity and sensitivity

#### P 540

##### **EVALUATION OF APOPTOTIC CELL TURNOVER AND P62 EXPRESSION IN THE PROCESS OF NEOPLASTIC TRANSFORMATION BEGINNING FROM CHRONIC HEPATITIS TO HEPATOCELLULAR CARCINOMA**

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**Background and Aim:** It is well known that reduced apoptosis plays a significant role in hepatic carcinogenesis. Actin fragment, which is produced by caspase 3 during apoptosis, can be recognized by fractin and it has been demonstrated that fractin expression in tissues has a place in the evaluation of apoptosis. p62 is a tumor-related insulin-like growth factor which is shown that approximately one-third of hepatocellular carcinomas exhibited high expression of p62 protein immunohistochemically, whereas adjacent non-malignant parenchymal liver cells had no detectable staining. The aim of this study is to evaluate fractin and p62 expressions in cases of chronic viral hepatitis, cirrhosis and hepatocellular carcinoma to reveal out the possible impact of these proteins in hepatocellular carcinogenesis.

**Material and Methods:** Fractin expression has been evaluated in paraffin embedded sections by immunohistochemistry in cases of 13 mild, 11 moderates and 3 severe chronic hepatitis, 8 cirrhosis and 7 hepatocellular carcinoma. Nuclear and cytoplasmic staining percentage was used for statistical comparisons

**Results:** Although fractin expression did not differ between groups of different degree of hepatitis, the difference was significant between cases of chronic hepatitis, cirrhosis and hepatocellular carcinomas. While the apoptotic rate was highest in chronic hepatitis, it was lower in hepatocellular carcinomas. In contrast to the literature, we could not detect p62 in hepatocellular carcinoma cases. However, it was found weakly positive in 1 hepatitis B and 1 cirrhosis case.

**Conclusion:** It has been suggested that apoptosis that evaluated by fractin antibody could be more helpful for defining preneoplastic lesions during hepatic carcinogenesis than p62 expression.

#### P 541

##### **TGF-BETA PATHWAY CORRELATES WITH APOPTOTIC INDEX IN NEOPLASTIC HUMAN LIVERS**

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**INTRODUCTION** TGF-beta1 plays a central role in regulation of cell growth and differentiation. The signalling pathway is complex and its effect is dependent by the activation of specific transduction factors. **PURPOSE OF THE STUDY** The aim of the present study was to investigate the expression of TGF-beta pathway (TGF-beta1, SMAD 1-2 and TGF-beta type II receptor) in neoplastic human livers. **METHODS** 43 HCC tissue samples obtained from 12 liver biopsies and 31 surgical resections, 20 normal liver tissues, 6 cirrhotic and 20 viral hepatitis were investigated for the TGF-beta1 and its signalling pathway (TGF-beta1 type II receptor and SMAD) expression. Semi-quantitative RT-PCR for the detection of TGF-beta1 was performed on all frozen liver tissues. Apoptotic index was evaluated by TUNEL technique and proliferation index was analysed with MIB1 (Ki-67 antigen). **Results** TGF-beta expression (mRNA and protein) was higher in HCC compared with normal liver but it was significantly lower than cirrhosis and hepatitis. **RESULTS** A concordant expression of TGF-beta1 with RII and SMAD 1-2 was observed in the majority of cases ( $p < 0.05$ ). A positive correlation was observed between apoptotic index and TGF-beta pathway. No correlation was observed between TGF-beta pathway and other clinical and pathological parameters. **CONCLUSIONS** TGF-beta is markedly low expressed in HCC compared to inflammatory and degenerative processes. TGF-beta pathway plays an important role in the arrest of cell growth in HCC.

#### P 542

### SCREENING OF SERUM BIOMARKERS IN HEPATOCELLULAR CARCINOMA BY SELDI TECHNIQUE

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**Objective:** Liver cancer is one of the diseases with higher incidence and mortality. New technologies for the earlier detection of liver cancer are urgently needed. The aim of this study was to screen serum biomarkers in patients with hepatocellular carcinoma by using surface-enhanced laser desorption and ionization time-of-flight mass spectrometry (SELDI-TOF-MS) **Methods:** Proteomic spectra were generated by mass spectroscopy in 88 cases, including 34 cases of hepatocellular carcinoma that had been pathologically confirmed with aged 45 to 94 years, and 34 cases of healthy control aged 20 to 78 years. 68 spectra obtained were used to train and develop a decision tree classification algorithm.

**Results:** A total of 17 distinguished proteomic peaks were detected, two of which were used to build a proteomic pattern. The results yielded a sensitivity of 91.18% (33/34), specificity of 97.06% (32/34), and positive predictive value of 96.88%.

**Conclusion:** SELDI-TOF-MS offers a unique platform for the earlier detection of tumor. It also offers a noninvasive method to study the local microenvironment associated with the development and progression of liver cancer.

#### P 543

### THE MESENCHYMAL HAMARTOMA OF THE LIVER IN ADULTHOOD: REPORT OF TWO CASES WITH IMMUNOHISTOCHEMICAL PROPHYLE, CLINICAL AND HISTOPATHOLOGICAL FEATURES

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**INTRODUCTION:** Mesenchymal hamartoma is an uncommon cystic mass of the liver occur primarily in children. However there are a few reports of adulthood mesenchymal hamartoma of the liver (MHL).

**CASE REPORTS:** In this paper we present two cases of MHL in two female patients 54 and 51 years old. In radiological examination of both of the cases the patients had multiple cystic lesions in the liver accompanied a kidney cyst. In microscopic examination a multilocular cyst lined by flattened epithelium surrounded by a mesenchymal component composed of mature connective tissue, arterial and venous vascular structures, peripheral nerve bundles, and ductal structures was observed. An immunohistochemical panel composed of desmin, smooth muscle actin, S-100, vimentin, CD34, CEA, Pan cytokeratin, cytokeratin 7 (CK7), CK8, CK17, and CK20 was applied to the paraffin sections. CK7 and CK19 immunoreactivity was observed in cystic epithelium and in ductal structures. Focal and patchy desmin immunoreactivity was observed in connecting tissue. S-100 was positive only in peripheral nerve bundles.

**CONCLUSION:** In conclusion MHL of adulthood is a localized tumoral abnormality that precedes birth which had delayed clinical presentation. These lesions seem to be in a maturation process just like the hosts carrying them. The stromal component consists of immature embryonic spindle cells stroma rich in mucopolysaccharides convert to mature paucicellular hyalinized connective tissue. This maturation process may be also related with loss of precancerous potential of these tumors observed in juvenile forms.

#### P 544

### HIGH EXPRESSION OF CLAUDIN-4 IN BILIARY CARCINOMAS

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**BACKGROUND/AIMS:** Cell adhesion molecules play an important role in carcinogenesis, especially claudins, being part of tight junctions. It has been shown the importance of certain claudins in development of several tumors, and some of them even suggested as a future therapeutic target. For this reason, the aim of our study was to analyse the expression of claudins in biliary carcinomas (BCs).

**METHODS:** A tissue microarrays comprising 24 biliary carcinomas, arising from different localisations (gallbladder, common bile duct, hepatic ducts, intrahepatic bile ducts) were constructed to investigate claudin-1,2,3,4,7 expression by immunohistochemistry on formalin fixed, paraffin embedded slides. Claudin-4 was further investigated by Western blot analysis on 5 human surgically resected, snap frozen BC specimens. Subcellular localisation of claudin-4 was detected by confocal laser scanning microscopy.

**RESULTS:** Intensive membranous immunolabeling was found for claudin-4 in BCs of different origin. A single band of the expected size was detected on Western immunoblots and revealed a strong expression of claudin-4. Confocal microscopy confirmed the increased expression of claudin-4 on the membrane of BC cells.

**CONCLUSIONS:** Our data report the first time a significantly increased claudin-4 expression in BCs. The results represent a novel feature of BCs and claudin-4 protein could well become a potential diagnostic marker and therapeutic target for biliary carcinomas, as it has been suggested for pancreatic and ovarian cancers.

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#### P 545

### DETECTION OF RESPONSE-PREDICTING MUTATIONS IN THE KINASE DOMAIN OF THE EPIDERMAL GROWTH FACTOR RECEPTOR GENE IN CHOLANGIOCARCINOMAS

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**Purpose:** Epidermal growth factor receptor (EGFR) signalings have recently been implicated in the genesis and progression of cholangiocarcinomas. Thus, the EGFR kinase inhibitor appears to be promising in the treatment of this cancer. The response-predicting mutations in the tyrosine kinase domain of EGFR gene have recently been detected in non-small cell lung cancers. This study was, therefore, to investigate if these mutations are also found in cholangiocarcinomas. **Methods:** Twenty-two consecutive cholangiocarcinoma patients who underwent surgical resection were enrolled. Their resected paraffin-embedded cholangiocarcinoma specimens were used for mutation analysis, which was performed by DNA sequencing of exons 18, 19 and 21 in the EGFR gene. Clinical characteristics were compared between each group

according to the presence or absence of mutations. Results: Three patients (13.6 %) harbored EGFR mutations. All the mutations found were deletions in exon 19. Mutations were more common in intra-hepatic or poorly differentiated tumors. Differences in age, sex, stage at diagnosis and survival were not observed between mutation-positive and -negative patients. Conclusions: This study, for the first time, demonstrates that a subset of cholangiocarcinoma patients has response-predicting EGFR mutations. Therefore, a highly selected application of the EGFR kinase inhibitor would be therapeutically effective in these patients.

#### P 546

##### **HEREDITARY HEMOCROMATOSIS ASSOCIATED WITH A FULL SPECTRUM OF BILE DUCT LESIONS: CHOLANGIOCARCINOMAS, BILE DUCT ADENOMAS AND BILIARY MICROHAMARTOMAS.**

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Genetic hemochromatosis (GH) is a relatively common inherited disorder associated with chronic liver damage and cirrhosis. Iron overload has been also associated with an increased risk for the development of primary liver tumours. Although hepatocellular carcinoma is the malignant tumor most frequently found in these patients, a wide spectrum of biliary lesions and cholangiocarcinoma have also been described. Von Meyenburg complexes and bile duct adenomas are two well known lesions associated with cholangiocarcinomas and considered putative precursor lesions.

We present a case report of a 64-years-old man with liver cirrhosis associated with GH, homozygotic for the C282Y mutation, and treated for the last 4 years with venesections. The patient was admitted to our hospital for the study of two hepatic focal lesions suspicious of metastasis. After the diagnosis of a well differentiated adenocarcinoma by a fine-needle aspiration of one of the nodules, and exclusion of a primary tumor in other localization, the patient was submitted for hepatic resection. A segmentectomy II-III, V and a percutaneous liver biopsy of the left lobe were performed. Macroscopically five white nodules were observed, from 2.5 to 0.2 cm, in a micronodular background. The non-tumoral liver parenchyma showed cirrhosis with a moderate iron deposition. Microscopically the two largest nodules were cholangiocarcinomas. A total of 7 bile duct adenomas and numerous von Meyenburg complexes were also identified in the adjacent liver tissue of all the specimens. No morphological transition between benign and malignant lesions was observed. Immunohistochemical studies using cytokeratin 7 and 20, EMA, CEA, p53, Mucins and Ki-67 antibodies were performed.

In GH individuals, primary liver tumors may present with a wide morphological spectrum, from hepatocyte-derived malignancies to cholangiocyte-derived lesions. A carcinogenic effect has been proposed for iron overload by both direct or indirect pathways. The unusual case we present shows a full spectrum of biliary lesions with biliary microhamartomas, bile duct adenomas and cholangiocarcinomas.

#### P 547

##### **SOLITARY FIBROUS TUMOR OF THE LIVER: REPORT OF THREE CASES ONE OF THEM SHOWING MALIGNANT FEATURES**

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Solitary fibrous tumors (SFT) are rare neoplasm arising from mesenchymal cells, which are difficult to diagnose. They have been reported in a wide range of anatomical sites usually benign, but malignant transformation has been described. SFT of the liver are exceptionally described and always benign.

We report three cases of SFT of the liver diagnosed during last ten years in our department. All were located in the right liver and surgically resected. Case 1 and case 2 were benign SFT diagnosed in 69 and 57 year-old women. The main clinical manifestation was abdominal pain with weight loss for case 1. Tumor size varied from 9 to 10.5 cm in diameter. The external surface was typically smooth, of firm consistency and well defined. The cut surface was light tan to gray-white with whorled texture with foci of cystic degeneration. Histologically, tumors showed alternating cellular and paucicellular areas. The former included spindle-shaped cells with finely disperse chromatin and uniform nuclei. The latter were made of abundant hyalinization and collagen deposits. The tumor vascular supply was well developed. Mitotic activity did not go beyond 4 mitoses/10 HPF. All tumor cells expressed CD34 and vimentin whereas cytokeratins, alpha smooth muscle actin, S100 protein and c-kit were negative. Proliferative index was low. The patients were alive without recurrence since 9 years and five months for cases 1 and 2 respectively.

Case 3 concerned a 70 year-old woman presenting with persistent abdominal pain. Tumor size was 16 cm with similar macroscopical characteristics as previous cases. Histologically the aspects were identical to previous tumor at one exception. An expansive and basophilic nodule was found in the cellular area with dense cellularity, fascicular pattern and, high mitotic activity (8 mitoses/10 HPF) and proliferative index. No necrosis nor lymph node metastasis were observed. The immunophenotype of tumor cells was identical as previous cases even in the atypical nodule. The patient is still alive without recurrence since three months. Solitary fibrous tumor of the liver are exceptional. They are usually benign, but tumor with malignant features can occur with unknown prognosis.

#### P 548

##### **SYNCHRONOUS PERIVASCULAR EPITHELIOID CELL TUMORS (PECOMA) INTRAHEPATIC, AND OF THE ROUND LIGAMENT OF THE LIVER.**

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Perivascular Epithelioid tumors (PEComas) is a recently introduced concept to describe a family of lesions sharing peculiar features: co-expression for muscular and melanocytic markers, spindle or epithelioid cell shapes, pale or clear cytoplasm, arrangement around blood vessels, melanosomes or premelanosomes in its ultrastructure, and absence of a normal precursor cell.

We present a case of double PEComa developed in left hepatic lobe, and round ligament, in a 24-year-old woman. She suffered acne, in treatment with oral contraceptives, and came to the hospital with slight dyspepsia. On the echographic exploration two hepatic lesion were seen. Magnetic resonance confirmed a 8 cm lesion dependent of umbilical vein, and a second intrahepatic 3.5 cm lesion, located in segment IVb. No signs of tuberous sclerosis were found. Both lesions were resected, and showed on microscopical examination identical appearance. The tumors were composed of uniform spindle to round cells, with clear cytoplasm, and slightly pleomorphic nuclei. A prominent vascular component subdivided the cells in fascicles and nests. Mitotic activity was lower than 1/10 high power fields. Some areas had cells with prominent cytoplasmic melanic pigmentation.

Immunohistochemically the cells in both tumors strongly expressed HMB-45, smooth muscle actin and Melan-A. S-100 protein, keratin and desmin were all negative. C-kit, and CD99 were positive in more than 80% of tumor cells; and Ki-67 showed a proliferation index lesser than 1%. Electron microscopy study revealed oval cytoplasmic inclusions with dark staining granules, and some with cross striated structures, characteristic of melanosomes.

These kind of lesions had been suffering a controversy regarding its histogenesis and even its real existence a clinicopathological entity. Generally considered as benign lesions, some cases have been described with malignant evolution or sarcomatous transformation. Our case raised also the possibility of a metastatic intrahepatic lesion from and initial ligamentous tumor. However and, in spite of the exceptionality of these lesion and especially for multiples ones, they are usually considered as multicentric processes and not true metastatic lesions.

#### P 549

### **TWO CASES OF BILIARY ADENOFIBROMA OCCURRING IN PATIENTS WITH HOMOZYGOUS HEMOCHROMATOSIS, ASSOCIATED WITH PRIMARY LIVER CARCINOMA AND OTHER BILIARY LESIONS.**

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Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) are reported as complications of genetic hemochromatosis (GH). We report two cases of GH with several benign and malignant lesions, including biliary adenofibroma (BAF).

For the first patient, of 68 years old, followed and treated by phlebotomy for 30 years, two hepatic nodules were found. Left hepatectomy was performed. Four nodules were macroscopically identified, measuring 0.5, 1.5, 1.5 and 5 cm. They corresponded, respectively, to bile duct adenoma, focal nodular hyperplasia, BAF and HCC. For the second patient, of 72 years old, GH and hepatic nodules were diagnosed at the same time. Abdominal imaging revealed four nodules in the right hepatic lobe. Histologically, 3 of them were HCCs and the fourth was BAF. In both patients, non neoplastic liver showed extensive fibrosis but no cirrhosis and multiple von Meyenburg complex (VMC). In the second case, the parenchyma showed severe siderosis with numeral iron free foci.

BAF is a rare benign neoplasm with only three previously reported cases in the literature. It is characterized by a microcystic and tubuloglandular architecture composed of a biliary epithelium supported by a fibroblastic stroma scaffolding. Here we report the 2 first cases of BAF in GH patients. Existence of several biliary lesions, developed in a non cirrhotic liver in two patients with GH, suggest a relationship between iron overload and these lesions. BAF, as suggested Varnholt et al, could be an intermediate step in biliary carcinogenesis from VMC to CC. In this setting, occurrence of CC in GH have been already reported in association with VMC. Morcos et al, studying liver cancer in GH, have shown a wide histologic spectrum including VMC and CC.

#### P 550

### **HISTOLOGICAL CHANGES OF NON TUMORAL LIVER IN RIGHT HEPATECTOMY FOR**

### **METASTATIC COLORECTAL CANCERS.**

#### **CORRELATIONS WITH PREOPERATIVE IMAGING.**

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**BACKGROUND :** Neo-adjuvant systemic chemotherapy is largely used in patients with liver metastatic colorectal cancers. The impact of such treatment on non-tumoral liver tissue is still poorly investigated. The aim of this study was to (1) describe the morphological changes of non-tumoral liver tissue on surgical specimens, (2) correlate the microscopic findings with the preoperative radiological constatations in a prospective cohort of patients with right hepatectomy.

**PATIENTS AND METHODS:** From March 2002 to February 2004, 58 consecutive patients with liver metastases had a right hepatectomy. Among them, 43 had chemotherapy, associated with a right portal vein embolization in 8 patients. Liver specimens were reviewed by 2 pathologists and the following features were semi-quantified: steatosis (0: absent; 1 <30%, 2 > 30%), sinusoidal dilatation (0: absent; 1: few foci, 2: numerous foci involving 2/3 of the lobular surface, and 3: extensive areas associated with regenerative nodular hyperplasia), portal fibrosis (0: absent or mild: portal tracts normal or minimally enlarged, 1: extensive fibrosis). Preoperative imaging included CT and MRI in all patients. The following signs were analysed in consensus by 2 radiologists: morphologic changes of the liver, steatosis, abnormal enhancement patterns and signs of portal hypertension.

**RESULTS:** At pathology, 52 out of 58 (90%) post-chemotherapeutic liver specimens displayed significant liver changes. Steatosis was observed in 35 cases (60 %), mild and moderate in 29 and 6 cases, respectively. Vascular changes were present in 28 cases (48 %), mild and moderate in 18 and 4 cases, respectively. In 6 patients (10%), regenerative nodular hyperplasia was diagnosed. CT and MRI had a sensitivity to diagnose steatosis of > 30 % and between < 30% of 67% and 7%, respectively with a specificity of 100%. Among patients with microscopic vascular abnormalities, only 3 had abnormal enhancement at imaging (30 %).

**CONCLUSION:** This study shows that significant morphological changes are observed in the context of neo-adjuvant systemic chemotherapy given in most patients for liver metastatic colorectal cancers, with steatosis and vascular changes the most prevalent lesions. Among them, regenerative nodular hyperplasia was frequent in this series and may have an important clinical impact on postoperative outcome. The accuracy of combined CT and MRI to detect steatosis was high whereas it was low for the detection of vascular lesions.

#### P 551

### **EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION IN COLORRECTAL CANCER LIVER METASTASES**

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EGFR represents a molecular binding for several antineoplastic agents. Results of treatment are not always related to the level of EGFR expression. This could be related to a different expression of EGFR in the primary tumor (PT) and in liver metastatic disease (LM).

Of the 48 cases 36 were EGFR positive in the PT and 12 were negative. Of the 36 positive patients in the PT 24 were also positive in the LM but in 12 LM EGFR were negative. From the 12 primary tumors EGFR negative 7 had EGFR negative LM and in 5 EGFR were positive in the LM. Besides the 48 cases 3 developed a second PT and in 2 of them EGFR expression of the second primary was the contrary of the first tumor. Seven of the 48 cases presented a second LM after a time (14.28 months), of these 4 presented a level of expression contrary to the first metastases.

In conclusion, EGFR expression in the colon PT and LM can be a prognostic factor for recurrence and response to anti-EGFR therapy.

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##### EXPRESSION OF CLAUDINS IN HUMAN HEPATOBLASTOMA

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#### P 553

##### METASTATIC RENAL CELL CARCINOMA IN THE SINONASAL TRACT-REPORT OF A CASE

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**Aim.** Few sinonasal tumours can manifest, histologically, as clear cell neoplasia, the most frequent encountered being metastatic renal cell carcinoma.

**Material and methods.** We present a case of a 63 years old men presented with a six-month history of right-sided epistaxis associated with nasal obstruction. Previous medical history was significant for renal cell carcinoma. The clinical examination revealed a tumor mass localized in the anterior right nasal cavity. A craniofacial resection was performed for tumor extirpation. The tumor was fixed in formaline, embedded in paraffin and the sections were stained with H-E, PAS-ALCIAN blue. Immunohistochemical examinations were performed for Cytokeratin, Vimentin, S-100, EMA, CEA and HMB 45.

**Results.** Histologically, the tumor was represented nests and tubular structures of tumor cells and little stroma with prominent vascularity. The cells were large, with abundant clear cytoplasm and distinct cytoplasm membranes, the nuclei were rounded, centrally located with reduced nuclear pleomorphism. The tumor was ulcerated on the surface. Histochemistry confirmed the presence of tumor glycogen but no mucin. Immunohistochemistry confirmed strong expression for Cytokeratin, Vimentin and EMA.

**Conclusions.** Sinonasal tumors that can appear as clear cell malignancies include primary squamous cell carcinoma, salivary tumors (such as mucoepidermoid carcinoma, acinic cell carcinoma, clear cell carcinoma, epithelial myoepithelial carcinoma), metastatic tumors (from renal cell carcinoma and follicular thyroid carcinoma) and recently described entity as "sinonasal renal cell like carcinoma". The differential diagnosis is made on histochemical, immunohistochemical and ultrastructural features.

#### P 554

##### THYROID METASTASIS FROM UNDIFFERENTIATED NASOPHARYNGEAL CARCINOMA: A CASE REPORT

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##### Introduction:

Metastatic lesions in the thyroid gland are rarely reported; they account less than 4% of malignant thyroid tumors ; but the number of cases seems to be increased in recent years, with routine use of fine-needle aspiration biopsy. The metastatic involvement of the thyroid gland does not appear to be dependent on age but is more common in females. The most common site of origin was the kidney closely followed by breast and lung. Thyroid metastasis from undifferentiated nasopharyngeal carcinoma ( UCNT) has not been reported in the literature;

##### Objective:

The purpose of this work is to report the clinical and morphological characteristics of this unusual metastatic lesion in the thyroid gland.

##### Case report:

we report an original case of metastasis to the thyroid from UCNT occurring in a 47-year-old man, 8 years after the diagnosis of the original primary nasopharyngeal tumor treated by chemoradiotherapy. Clinical examination revealed a multinodular goiter. Thyroid gland was voluminous with intact capsule and showed many nodules of different size, witch were diagnosed as metastasis from UCNT. On immunohistochemistry study, the neoplastic cell showed a focally positive immunoreactivity for LMP1 and were negatives for thyroglobulin.

##### Conclusion:

The thyroid metastasis from UCNT are not always easily identified on routine stained sections and their diagnosis need clinical findings and even immunohistochemical analysis.

#### P 555

##### EXPRESSION OF MLH1, MSH2 AND EGFR IN 35 INTESTINAL-TYPE SINONASAL ADENOCARCINOMAS

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**Introduction :** Intestinal-type sinonasal adenocarcinomas (ITAC) are malignant epithelial tumors that show histological, and immunochemical features reminiscent of colorectal adenocarcinomas. Unlike ITAC, colorectal adenocarcinoma is one of the most widely studied human malignancies. We have worked on the hypothesis that similarities between ITAC and colorectal adenocarcinoma might reflect equivalent genetic alterations and carcinogenesis.

**Purpose of the study :** The phenomenon of microsatellite instability (MSI) caused by mutation or methylation in the MLH1 and MSH2 mismatch repair (MMR) genes is a way of pathogenesis involved in 10-15% of sporadic colorectal adenocarcinoma. The aim of our study was to assess MSI status in ITAC by standardised immunochemistry in order to determine if the similarities between ITAC and colorectal adenocarcinoma extend beyond the light microscopic level to an eventual identical way of carcinogenesis.

Futhermore, overexpression of Epithelial Growth Factor Receptor (EGFR) is a common event in colorectal adenocarcinoma. We attempted to determine if such consistent expression of EGFR was also observed in ITAC. **Methods :** MLH1, MSH2 and EGFR expression were

investigated in paraffine sections of resection specimens of 35 ITAC using standardised immunochemistry with antigen retrieval.

The expression of MLH1 and MSH2 was considered positive if there was definitive nuclear staining in any of the tumoral cells.

Membrane staining of EGFR was evaluated in the neoplastic cells and graded using a semiquantitative score (0-3+).

Results : All of the 35 carcinomas examined showed preserved nuclear expression of MLH1 and MSH2 with most cases showing strong nuclear staining.

There was no staining of neoplastic cells for EGFR in 8 cases (23%, score 0). Staining was weak in 9 cases (25%, score 1+), moderate in 10 (29%, score 2+), and strong in 8 (23%, score 3+).

Conclusions : First, these results suggest that defective DNA mismatch repair does not play an important role in the pathogenesis of ITAC and confirms the previous investigation of Perez and coll. in 10 patients.

Secondly, we show for the first time that most of the ITAC (77%) stained positively for EGFR with a frequency quite similar to colorectal adenocarcinoma (74 to 97%). On the basis of the already proven antitumoral effect of agents acting as EGFR inhibitors, it is suggested that patients with ITAC might benefit from these agents.

#### P 556

#### **A MORPHOLOGIC AND IMMUNOHISTOCHEMICAL STUDY OF NASAL MUCOSA IN LEATHERWORKERS OF THE PROVINCE OF FLORENCE AND PISA, ITALY**

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Aims: An increased risk for developing sinonasal intestinal type adenocarcinoma has been ascertained in individuals occupationally exposed to leather manufacturing, but no conclusive data have been reported concerning the morphological precursors of these tumors. The purpose of this study is to investigate early histologic changes and modification in the phenotype of epithelial cells in nasal mucosa of leatherworkers, with specific reference to the presence of intestinal metaplasia.

Subjects and methods: 75 male subjects (age 38-75 years) who had a long-time exposure to leather manufacturing (range 7-48 years) underwent biopsy of the nasal mucosa of the lower turbinate. Samples were fixed in formalin and routine processed for histology. Serial sections were stained with haematoxylin and eosin, PAS diastase and immunostained for cytokeratin 20, CDX-2 and MUC-2.

Results: In the majority of the biopsies (66.6%) the normal ciliated epithelium showed areas of squamous metaplasia, which was associated with mild to moderate dysplasia in 24 cases (32%). Another frequent histopathologic finding was the presence of a chronic inflammatory infiltrate (90.6%). In 13 biopsies (17.3%) we observed an increased number of goblet cells, which showed an intense cytoplasmic positivity for MUC-2, while no immunostaining was observed for cytokeratin 20 and CDX-2 in any epithelial cell of surface or glandular epithelium.

Conclusions: As for woodworkers, the most common epithelial changes in the nasal mucosa of leatherworkers are squamous metaplasia (with or without dysplasia) and goblet cell hyperplasia. Intestinal metaplasia, with acquisition of

CDX-2 and cytokeratin 20 expression, is not observed in nasal mucosa of leatherworkers, and may be a late event in the pathway leading to development of sinonasal intestinal type adenocarcinoma.

#### P 557

#### **NASOPHARYNGEAL PAPILLARY ADENOCARCINOMAS. INCLUDING TWO CASE OF THYROID LIKE CARCINOMAS EXPRESSING TTF-1**

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Introduction: Nasopharyngeal papillary adenocarcinomas (NPPAs) are uncommon neoplasms with different histogenesis and histomorphology.

Purpose: To characterize the behavior, and IHC of NPPAs.

Design: 19 neoplasms coded as NPPAs were identified in our files. Demographic data and histomorphology were reviewed. IHC for CKs 5/6, 7 and 19, S-100 protein, thyroglobulin (THYG) and TTF-1 were performed.

Results: 11 females and eight males ranged in age from 9 to 79 years (mean 47.5 yrs). NPPAs were reclassified as: 8 primary low grade nasopharyngeal adenocarcinomas (LGNPA), 7 polymorphous low grade adenocarcinomas (PLGA), and two each thyroid-like papillary carcinomas and metastatic papillary thyroid carcinomas (MPTC). The only children in the study had TLPC. S-100 protein was expressed only in PLGA, CK 5/6 was positive only in LGNPA. TLPC and MPTC immunoreacted to CK 7, CK 19 and TTF-1, whereas only MPTC expressed THYG. All patients were treated surgically, the pts with MPTC in addition received postoperative radiotherapy. All pts are alive without tumor. Pts with MPTC are alive with residual or recurrent tumor.

Conclusions: Differentiating these adenocarcinomas has therapeutic and prognostic implications. Our findings document a strong correlation between histomorphology and IHC expression, allowing this distinction. Since THYG may not be expressed in metastatic papillary thyroid carcinomas, the diagnosis of TLPC is based in the absence of carcinoma in the thyroid gland

#### P 558

#### **SEBACEOUS CARCINOMA OF THE NASAL VESTIBULE: A CASE REPORT**

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#### Abstract

Background: Sebaceous carcinomas are cutaneous appendageal tumors that may be extremely rare in the nasal vestibule. These lesions are relatively rare, but some have a predilection for the head and neck region and can easily be misdiagnosed as more common lesions. To our knowledge, only 19 examples of nasal sebaceous carcinomas have been reported in the literature.

Case report: Here the authors described a case of a 90-year-old man who presented with an ulcerated mass in the right nasal vestibule with a history of basal cell carcinoma of the same area. The patient stated that the lesion had been present for 18 months. The examination revealed a destructive, irregularly shaped mass measuring 1.5 cm in maximum diameter. At follow-up, 12 months later, a new lesion of 0.5

cm in diameter had appeared in the same area. Histologic examination of the mass was composed of small basaloid cells and large foamy cells. Sebaceous differentiation was evident in some tumor islands. The sebaceous cells were well defined and variably sized and contained minimally pleomorphic, centrally located, vesicular-shaped nuclei with prominent nucleoli, and had poorly defined cellular outlines. A lot of smaller, darkly staining, basaloid cells with oval shaped nuclei and prominent nucleoli are also frequently identified. The basaloid cells exhibited strong reactivity with 34beta12 and sebaceous cells in tumoral islands stained positive with CEA and EMA. No staining was observed with vimentin, actin and S-100. Mucicarmine stain and periodic acid Schiff stains with and without diastase were negative, confirming that the vacuolated clear cells were neither mucus cells nor glycogen-rich squamous cells. As a result histology revealed a sebaceous carcinoma.

Conclusion: Sebaceous carcinoma can be highly malignant, due to its histological differentiation. It is usually a slowly growing tumor, which is locally aggressive and capable of metastatic spread to regional lymph nodes and distant sites. Historical data indicate a nearly 30% local recurrence rate with standard surgical excision. Careful pathological examination with clinical correlation is essential in the diagnosis and the differential diagnosis of common tumors of the face should include this rare malignancy.

#### P 559

##### SPERM PROTEIN 17 EXPRESSION DEFINES TWO SUBSETS OF PRIMARY ESTHESIONEUROBLASTOMA

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Introduction. Esthesioneuroblastomas (ENBs) are rare malignant tumors of the nasal vault whose origin, diagnosis and management are still subjects of discussion. The fact that there is no related prognostic factor or generally recognized therapeutic regimen highlights the need for further analyses of its underlying biological features and investigations of new marker proteins that allow more reliable clinical testing. Purpose. To investigate whether Sperm protein 17 (Sp17) is expressed in ENBs and to compare whether Sp17 expression is correlated with other well characterized ENBs markers. Methods: The analyzed tissues came from 42 patients (23 males and 19 females with a median age of 54.5 years; range: 10-84) treated at the University of Erlangen-Nuremberg and the University of Southern California. Two-micrometer thick sections were cut and processed for immunohistochemistry. The immunohistochemical expression of the antibodies (Sp17, Ki-67, chromogranin, S100, synaptophysin, neurofilament) was evaluated by means of light microscopy of all of the available tissues for each patient at 20 x and 40x objective magnifications. Results. We here show that Sp17 is expressed in the ciliated cells of the normal olfactory epithelium and in a proportion of primary ENB lesions. We found an association between Sp17 expression and metastases at relapse ( $p = 0.035$ ), chromogranin expression ( $p = 0,014$ ), and female

gender prevalence. A statistically non-significative relation was also found between Sp17 and S-100, synaptophysin, and neurofilament expression. No relation was also found between Sp17 expression and the proliferative capacity of the lesion that was evaluated with the Ki-67 immunohistochemistry. Conclusion. Our results extend previous findings showing that the ciliated cells of the respiratory system and male and female reproductive systems are immunopositive for Sp17, and suggest that Sp17 may be a useful new protein marker for distinguishing two subsets of ENB lesions. Furthermore, they may represent a first step towards the characterization of this histologically heterogeneous family of tumors going under the same name.

#### P 560

##### EXPRESSION AND DISTRIBUTION OF MASPIN PROTEIN IN POLYMORPHOUS LOW-GRADE ADENOCARCINOMA

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Introduction. Polymorphous low-grade adenocarcinoma (PLGA) is a malignant neoplasm that affects almost exclusively minor salivary gland, representing, in these sites, the second or third most frequent malignant tumour, according to the published series. Despite the recognised clinical importance of the PLGA little is known about its biology. Maspin is a member of the serpin family of serine protease inhibitors with a peculiar role as tumour suppressor. It inhibits cell motility, invasion and metastasis and loss or reduction in maspin expression has been associated with tumoral progression. Maspin is mainly associated with myoepithelial cell and it was already demonstrated, by our group, to be present in a series of salivary gland tumours with myoepithelial cell participation. The aim of this study was to investigate the presence and distribution of the maspin protein among the different histological patterns of PLGA.

Material and methods: Immunohistochemistry for maspin was performed on archival formalin-fixed, paraffin-embedded samples of 19 cases of PLGA, using the labelled-polymer method.

Results: All cases studied were positive for maspin but the pattern and extent of staining varied among the histological patterns. Maspin was abundant in the lobular, more densely cellularized areas, while cribriform, tubular and trabecular showed part of the cells positive and papillary and single file pattern showed only occasional cells positive for maspin. It was also possible to notice, among the usually described medium-sized cell, with nuclei revealing fine chromatin and inconspicuous nucleoli, the scattered presence of a smaller cell, which showed hyperchromatic nucleus. This cell was always negative for maspin. When visible, the periphery of the lesions showed no positivity.

Conclusions: Maspin expression was more abundant in highly cellular areas suggesting that maspin needs cellular contact to be expressed. Although the well-recognized cytological uniformity it seems that there is possibly less differentiated cell in PLGA which does not express maspin. In salivary gland, maspin is not exclusive of cells with myoepithelial differentiation.

#### P 561

##### IN SITU HYBRIDIZATION SIGNALING OF HOXA7 AND TGIF TRANSCRIPTS IN ORAL SQUAMOUS CELL CARCINOMA.

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Oral cancer is one of the most frequent cancers worldwide. Although different types of cancers can affect the oral cavity, approximately 90% are squamous cell carcinoma (OSCC). The literature shows an extensive search for biomarkers which could predict OSCC behavior. Homeobox genes comprise a family of developmental regulators that are vital for several aspects of growth and differentiation. Recent studies demonstrated genetic alterations in homeobox genes or its expression in many cancers including homeobox HOXA7 and TGIF. HOXA7 may act as a repressor of transcription and down-regulates multiple differentiation specific genes during keratinocyte proliferation. HOXA7 was correlated with leukemia, epithelial ovarian tumors and mouse skin carcinoma. TGIF encodes a protein that is a transcription regulator. TGIF amplification and over-expression was observed in esophageal SCC. The involvement of HOXA7 and TGIF in OSCC has not been demonstrated yet. The purpose of this study was to verify the presence of HOXA7 and TGIF transcripts in OSCC and adjacent non-tumoral tissues (NT), and to see if there is a relation with the cells morphology. Biopsy specimens of OSCC were obtained from Hospital das Clínicas of São Paulo, Brazil. Samples of NT and OSCC tissues were collected and divided in two parts. One half was used for RNA extraction and transcripts amplification by RT-PCR. The other half was submitted to in situ hybridization (ISH) with sense and antisense digoxigenin labelled riboprobes on frozen tissues sections placed in silanized slides. Amplification of HOXA7 was seen in 72.73% of cases, 13.64% only in NT tissues, 40.91% only in OSCC samples and 18.18% in both tissues. TGIF was amplified in 81.81% of cases, 4.54% only in NT tissues, 18.18% only in OSCC samples and 59.09% in both tissues. ISH signal was similar for both transcripts, but more intense for TGIF. In NT tissues there was an intense expression in the epithelium, either in basal and suprabasal layers or disperse and more intense in the spinous layer. In OSCC transcripts were localized in all tumoral cells, but in poorly differentiated areas the signal was less intense and in a few cases it was negative. These results show that OSCC express HOXA7 and TGIF transcripts, mostly in well differentiated regions, suggesting a participation of these genes in OSCC development or progression.

#### P 562

##### **ORAL SQUAMOUS CELL CARCINOMA: AN IMMUNOHISTOCHEMISTRY COMPARATIVE STUDY BETWEEN THE TUMOR AND THE PERILESIONAL MUCOSA**

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Oral squamous cell carcinoma (OSCC) is the most frequent malignant neoplasm of the head and neck region. Conversion of normal cells to cancer cells is achieved through a multi-step process that is closely associated with the accumulation of multiple gene changes including both oncogenes and tumour suppressor genes. This work aims to study the relationship between the immunoprofile of the OSCC and the corresponding perilesional normal tissue.

Immunohistochemistry with the antibodies p53, p63, p16, p21, p27, Ki-67, cyclin D1, BAG-1, matrix metalloproteinase 1 (MMP-1), MMP-2, CD34, vascular endothelial growth factor (VEGF), Checkpoint kinase 2 (CHK2), telomerase, epithelial growth factor receptor (EGFR), c-erbB-2, c-erbB-3, and Bcl-2 was performed in a formalin-fixed paraffin-embedded sample of human OSCC and the adjacent non

tumoral mucosa. The immunostaining was evaluated as negative (-), weak to moderate/focal (+), or strong/diffuse (++) . In the perilesional mucosa there was no reaction for CD34, EGFR, c-erbB-2, c-erbB-3, VEGF, Bcl-2, p21, p16, MMP-1 e MMP-2. The positivity for cyclin D1, BAG-1, p53 e CHK2 was graded as + while the positivity for Ki-67, p27, telomerase, and p63 was graded as ++. The carcinoma was negative for BAG-1, CD34, c-erbB-2, c-erbB-3, Bcl-2, p16, MMP-1, and MMP-2. None markers in carcinoma were classified as +. The positivity for Ki-67, cyclin D1, p21, p27, p53, p63, telomerase, EGFR, VEGF e CHK2 was graded as ++. These data indicate that the expression of p53, p21, EGF, VEGF, CHK2, BAG-1 e cyclin D1 changed from perilesional mucosa to the carcinoma. P21, p53 and CHK2 modulate multiple cellular functions, such as gene transcription, DNA synthesis and repair, cell cycle arrest, senescence and apoptosis. Mutations in these genes can abrogate their functions, leading to genetic instability and progression to cancer. Cyclin D1 overexpression plays a role in the carcinogenesis. BAG-1 has an anti-apoptotic effect, so its downexpression is a stimulus for the cellular proliferation. EGFR is a transmembrane protein, which is implicated in the progression of many epithelial cancer types. VEGF play a critical role in cancer progression by promoting new blood vessel formation. A better understanding of the molecular biology involved in the progression of OSCC is essential for new therapeutic approaches.

#### P 563

##### **SQUAMOUS CELL CARCINOMA OF THE SUPRAGLOTTIC LARYNX.SURVIVAL RATE-FIVE YEAR STUDY**

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Having in mind dissimilarity of scientific reports regarding the survival rate the patients with squamous cell carcinoma of the supraglottic region,the aim of our research was to present our 5 year experiance in radiotherapy of this neoplasm. Our study includes 171 patients with squamous cell carcinoma of the supraglottic larynx with various degree of the pathohistological differenciation and stage of illness(T1-T4).

Patients irradiated after surgical treatment from 1994-1998 have been analised at the Oncology Clinic University Medical School-Nis.Megavoltage therapy with x-rays of 10 MEV and electrontherapy of 6-14 MEV were utilized for the radiotherapy treatments.

In the group of patients irradiated after surgical treatment 3 years survival rate vary from 100%-12,5%,depending on the illness stage.In the same group of patients 5 years survival rate was recorded within 80%-12,5% cases.

Patients who were radically irradiated(irradiation alone)have had 3 years survival rate,recorded among 64%-8% and 5 years survival rate among 57%-4%.

The study discuss the influence of degree of tumor differentiation on the choise of the radiotherapy plan and the effects of side factors on radiotherapy (in)efficiency.

#### P 564

##### **LYMPHANGIOGENESIS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: EVIDENCE AND PROGNOSTIC IMPLICATIONS.**

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#### ABSTRACT

**Purpose:** Exploring the mechanisms of tumor lymphangiogenesis is necessary for understanding the metastatic behavior of epithelial neoplasms. The aim of this study was to investigate this phenomenon in head and neck squamous cell carcinoma (HNSCC) and address its possible clinical implications.

**Methods:** We examined 81 tissue specimens of patients with HNSCC using immunostaining for the specific lymphatic endothelium marker podoplanin. We assessed intratumoral lymphatic density (ILD) and peritumoral lymphatic density (PLD). We also quantified lymphatic invasion and examined the possible associations of all the above parameters with clinicopathological features and outcome. Finally, we used double staining with podoplanin and the cell proliferation marker ki-67 in order to evaluate intratumoral lymphangiogenesis.

**Results:** Both high ILD and high PLD were significantly associated with the presence of lymph node metastasis at the time of diagnosis ( $p < 0.001$  and  $p = 0.007$  respectively). We also noticed a significant correlation between ILD and lymphatic invasion. Patients with higher ILD exhibited shorter overall survival (OS, log rank  $p < 0.001$ ), whilst PLD had no influence on outcome. The correlation remained significant after multivariate analysis (Cox  $p = 0.04$ ) indicating that ILD is an independent prognostic factor for OS. Double staining revealed the existence of proliferating intratumoral lymphatics, in which tumor emboli were occasionally observed.

**Conclusions:** The above results indicate that lymphangiogenesis indeed occurs in HNSCC; that newly formed vessels are targets of invasion by cancer cells; and that ILD might be used as a criterion to separate patients at higher risk.

#### P 565

##### PROGNOSTIC FACTORS FOR SQUAMOUS CELL CARCINOMA IN ORAL CAVITY WITH EMPHASIS ON P53 PROTEIN EVALUATION

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**INTRODUCTION** An alteration in the p53 tumor suppressor gene is the most frequent genetic abnormality in human cancers. The aim of the present study was to evaluate the overexpression of p53 protein, histological grade, stage of the disease and tobacco habit in oral squamous carcinomas in order to relate them with progression and prognosis of these tumors.

**MATERIAL AND METHODS** Primary tumors were studied on a series of 30 patients. Immunohistochemistry has been applied on paraffin sections of oral squamous carcinomas using DO7 antibody.

**RESULTS** Overexpression of the p53 protein has been found in 40% of patients. Overexpression of the p53 protein has been correlated with histological grade of the tumor ( $p < 0.05$ ) and lymph node metastases ( $p < 0.05$ ). Overexpression of p53 protein in well differentiated carcinomas (33,3%) was

significantly higher than in poorly differentiated tumors (0%). Relaps of the disease and stage of the TNM has been correlated with prognosis in patients with this tumor. The higher TNM stage correlates with shorter survival of patients ( $p < 0.05$ ). No significant correlations were found between overexpression of p53 protein with stage of disease, tobacco habit and survival of patients.

**CONCLUSIONS** Study showed that immunohistochemical examination of p53 has no prognostic significance in oral squamous carcinomas. Overexpression of p53 protein correlates with histological grade and lymph node metastases.

#### P 566

##### HEAD AND NECK SQUAMOUS CELL CARCINOMAS (HNSCC): EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) PROTEIN EXPRESSION AND EGFR GENE COPY NUMBERS

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**INTRODUCTION:** EGFR protein is expressed on membranes of squamous cells and it is overexpressed in HNSCC. The protein is coded by EGFR gene at 7p12. An antibody against the EGFR protein (cetuximab) may be used therapeutically in patients overexpressing the protein. **PURPOSE OF THE STUDY:** To introduce the treatment with cetuximab it may be important to establish the extent and type of the EGFR protein expression, and to find out whether the overexpression of the EGFR protein is caused by the EGFR gene amplification and/or by increased copy numbers of chromosome 7 or not. **METHODS:** We used tissue sections from 33 patients with HNSCC. The EGFR protein expression was tested immunohistochemically (IHC) using EGFR pharmDx (DakoCytomation). FISH to determine EGFR gene and chromosome 7 copy numbers (Abbott/Vysis) was applied. **SUMMARY OF THE RESULTS:** We found the EGFR protein expression in 31 of 33 patients. The EGFR protein expression had a variable pattern. In one case there were only scattered positive individual tumor cells. In 12 cases we found a variable intensity of the expression in the tumor cells (from 1+ to 3+). In 5 cases the tumor cells were 3+ positive at the periphery of the tumor nests. In 13 cases all tumor cells overexpressed the EGFR protein strongly (3+). The investigation of the EGFR gene revealed its amplification in 7 of 33 patients. The amplification was present in up to 50% of the tumor cells in an individual patient (median 33%). Increased numbers of chromosome 7 centromere signals were found in 23 of 33 patients. We did not find any correlation between the subtypes of the EGFR protein expression and amplification of the EGFR gene and/or increased copy numbers of chromosome 7. **CONCLUSION:** In patients with HNSCC the overexpression of the EGFR protein was found in a significant number of cases. This phenomenon is not caused by the EGFR gene amplification or by increased numbers of chromosome 7. Hence, the evaluation of the EGFR gene copy number and the number of chromosome 7 signals does not contribute to the decision whether to put these patients on cetuximab treatment or not. The evaluation of the EGFR protein expression by IHC testing appears satisfactory for this purpose. The type of the EGFR protein expression should be taken into consideration when evaluating the responsiveness of patients treated with cetuximab in future studies. Research Project FNM MZO 00064203

**P 567****EXPRESSION OF C-ERBB-2 IN LARYNGEAL CARCINOMAS OF MALAYSIAN PATIENTS**

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**Introduction:** The c-erbB-2 (HER-2/neu) oncogene encodes for a 185-KD cell-membrane glycoprotein receptor sharing homologies with growth factor receptor. Overexpression of c-erbB-2 protein is a frequent and prognostically relevant event in a variety of human cancers. The aim of this study was to evaluate c-erbB-2 expression in laryngeal lesions and correlate the carcinomas with other available clinicopathological parameters.

**Materials & Methods:** We examined a total of 89 cases consisting of 47 cases of carcinomas (median 64.0; range 47-79 years), 10 papillomas, and 21 nodules or polyps diagnosed between 1995 and 2003 in Hospital Universiti Kebangsaan Malaysia. The specimens were obtained by laryngectomy or biopsies. Cases of the laryngeal carcinomas (44,[93.6%]males) included 45 squamous cell carcinomas (SCC), 1 sarcomatoid carcinoma and 1 spindle cell carcinoma. Immunohistochemistry was performed in 89 formalin-fixed, paraffin-embedded specimens using c-erbB-2 protein (DAKO A0485,1:300) by the streptavidin-biotin method. Membrane c-erbB-2 immunostaining was used and scoring was according to standard criteria, 0,1+,2+or3+. In addition, the 2+ case was evaluated using fluorescent in-situ hybridization (FISH) for HER-2 gene amplification. Clinical information was obtained from the medical records.

**Results:**Of the 89 patients, only 1(1.1%)case of SCC was c-erbB-2 protein-positive (2+) while the rest of the specimens were negative. This positive case showed gene amplification for HER-2. Clinical follow-up information was available for 35 patients, ranging from 2 months to 77 months (mean 31.2 months). Five (15.6%) of these patients died of the disease. There was no correlation between c-erbB-2 expression with histopathological grade of differentiation of laryngeal carcinoma, age, sex, lymph node metastases and stage of the patients.

**Conclusion:**c-erbB-2 protein is rarely expressed in laryngeal lesions and may not play a prominent role in laryngeal carcinomas.

**P 568****LOCALIZATION OF PMX1 TRANSCRIPTS IN ORAL SQUAMOUS CELL CARCINOMA BY IN SITU HYBRIDIZATION.**

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Oral squamous cell carcinoma (OSCC) is the most frequent cancer of the oral cavity being responsible for 95% of all oral malignancies and is the eighth most common cancer worldwide. Several studies have demonstrated that cancer and development share common features since both exhibit shifts between cell proliferation and differentiation. The homeobox gene family is responsible for encoding transcriptional factors during development bringing our attention for its potential role during carcinogenesis. Paired Mesoderm Homeobox 1 (PMX1, PRX1 or PHOX1) is a non-clustered homeobox gene and as all other members of this family has a homeodomain motif which is responsible for DNA binding. This gene has been related with mesenchyma throughout development such cardiovascular system and skeletal elements. The relation between PMX1 and tumor is not well established yet. The purpose of this study was to verify the presence of PMX1 transcripts in OSCC and adjacent non-tumoral tissues. For

such study, tissues were obtained from Hospital das Clínicas (HCFMUSP), with patients' agreement, and divided in two halves. One was used for RNA extraction and transcripts amplification by RT-PCR. Sense and antisense probes were generated by in vitro transcription and labeled with non-radioactive digoxigenin. The other part was fixed in 4% paraformaldehyde, treated with 1X PBS and 30% sucrose before freezing. For in situ hybridization (ISH), serial sections were placed in silanized slides. In the adjacent non-tumoral tissues ISH signaling were detected at the basal and parabasal layers. In the OSCC the signaling was spread all over the tissue becoming more intense in areas with isolated carcinoma cells. Our findings suggest a participation of PMX1 in oral carcinogenesis and its expression in poorly differentiated carcinomas is being analyzed in detail. It is known that PMX1 is mainly expressed in mesenchyme and it is responsible for the maintenance of appropriate level of gene expression in the epithelium. For the first time PMX1 transcripts are detected in oral epithelium and in oral squamous cell carcinoma.

**P 569****CORRELATION OF TSG101 AND E-CADHERIN EXPRESSIONS WITH TUMOR BEHAVIOR AMONG SQUAMOUS CELL CARCINOMA OF HEAD AND NECK**

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Tumor susceptibility gene TSG101 plays an important role to maintain genomic stability and cell cycle regulation. Aberrant expression of TSG101 has been associated with tumorigenesis in some carcinomas. Down regulation of E-cadherin has been known to be related to tumor invasion and metastasis. To study the clinical significance of TSG101 and E-cadherin expression among squamous cell carcinoma of head and neck, we retrospectively performed the immunohistochemical study on the surgical specimens of squamous cell carcinoma among head and neck region from 65 patients who had received primary surgical treatment from 1986 to 1997. In the mean time, we used TSG101 antisense probe to study mRNA expression of these tumors by in situ hybridization. We found that TSG101 together with E-cadherin homogenously expressed over the suprabasal, granular and cornified layers but not the basal layer among the normal squamous epithelium and the well differentiated squamous cell carcinomas. The expression of TSG101 significantly correlated with that of E-cadherin ( $p < 0.001$ ). Besides, down regulation of either TSG101 or E-cadherin expression among tumor cells was significantly associated with poor differentiation of the tumor ( $p < 0.001$ ). Moreover, both TSG101 and E-cadherin expression significantly correlated with nodal status ( $p < 0.001$ ) but not tumor stage. The mRNA expression detected by in situ hybridization well correlated with protein expression by the immunohistochemical study. In conclusion, studies on TSG101 and E-cadherin expressions among squamous cell carcinoma of head and neck may be useful for the evaluation of the tumor differentiation and the risk of nodal metastasis. Further study on the close relationship between TSG101 and E-cadherin expressions in squamous cell carcinoma would be of interest and would potentially disclose their role to control cell differentiation and behavior.

**P 570****B-CATENIN/CYCLIN D1 PATHWAY IS CRUCIAL FOR ORAL SQUAMOUS CELL CARCINOMA**

## **GROWTH AND SUSCEPTIBLE TO WORTMANNIN ACTION**

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B-catenin and cyclin D1 are crucial proteins related to tumor proliferation and progression. The complex composed by free cytoplasmic b-catenin and transcription factor LEF/TCF migrates to nucleus, activating several target genes such as cyclin D1. In order to evaluate possible correlations between b-catenin and cyclin D1 in oral squamous cell carcinoma (OSCC), the expression of these proteins was analyzed by immunohistochemistry on tissue sections and in OSCC cells lines treated with 2nM wortmannin for 10min. Protein expression was assessed in five cell lineages, HN6, HN19, HN30, HN31 and HaCat as control keratinocytes, analyzed by immunofluorescence and western-blot. Immunohistochemical results showed a strong correlation between cyclin D1 overexpression and b-catenin cytosolic and/or nuclear presence. No significant alterations in b-catenin expression in treated and control cells were observed by immunofluorescence. Wortmannin promoted cyclin D1 fast relocation to the cytosol. Western blot analysis confirmed the decrease of cyclin D1 levels after wortmannin treatment. This last molecule is able to simulate the PTEN pathway, blocking PI3K and consequent activation of proliferation pathways. Taken together, these results point out the crucial role that b-catenin/cyclin D1 and PTEN pathways might play together in OSCC, and may provide the development of more effective therapeutical approaches regarding this frequent neoplasm.

### **P 571**

#### **PSEUDOASCULAR ADENOID SQUAMOUS CELL CARCINOMA OF THE ORAL CAVITY**

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Pseudovascular adenoid squamous cell carcinoma (PASCC) is an uncommon histopathologic variant of squamous cell carcinoma characterised by acantholysis of the tumour cells, creating anastomosing spaces and channels, thus mimicking an angiosarcoma. PASCC has been reported in the skin of the head and neck as well as in other organs, such as the breast, lungs, vulva, uterine cervix and urinary bladder. It has been suggested that PASCC has a more aggressive behaviour and a worse prognosis than conventional squamous cell carcinoma, but the number of patients reported so far is too small to draw firm conclusions.

We report two cases of PASCC in the oral cavity. One occurred on the buccal mucosa in a 59-year-old man who was a heavy smoker but denied alcohol abuse. The other occurred on the floor of the mouth in a 79-year-old woman who denied smoking and alcohol abuse. In both patients, radical tumour excision and neck dissection were performed. The bulk of both tumours was histologically consistent with PASCC, and contained foci of squamous cell carcinoma suggesting the correct diagnosis. In one patient, they consisted of conventional squamous cell carcinoma, and in the other, they consisted of a spindle cell carcinoma. Immunohistochemically, the tumour cells expressed keratin and vimentin; they did not express CD31 and the vast majority of the tumour cells did not express E-cadherin. Both patients had no metastases at the time of diagnosis, and are alive and well 2 years later.

Pathogenesis of PASCC is not completely understood. It has been suggested that acantholysis is the underlying pathogenetic mechanism. Consistent with this suggestion, we observed in our cases loss of immunohistochemical expression of E-cadherin, one of the major adhesion molecules of epithelial cells. E-cadherin was expressed only in areas of both tumours which exhibited the morphology of

conventional squamous cell carcinoma or spindle cell carcinoma. We speculate that loss of E-cadherin expression might lead to the loss of the tumour cell-cell adhesion and acantholysis characteristic for this particular type of squamous cell carcinoma.

In conclusion, eventhough the prognostic significance of PASCC in the head and neck is at present unknown, its recognition is important because it may mimic angiosarcoma which may result in erroneous treatment. The study of our two cases suggest that PASCC might be pathogenetically related to the loss of E-cadherin expression.

### **P 572**

#### **LARYNGEAL SQUAMOUS CELL CARCINOMAS AND THEIR LYMPH NODES METASTASES COMPARED BY MULTIVARIATE STATISTICAL ANALYSIS OF DIFFERENT HISTOLOGICAL AND IMMUNOHISTOCHEMICAL PARAMETERS – RAISING THE QUESTION OF THEIR PRACTICAL VALUE.**

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Aims. Some of the histological and immunohistochemical parameters of laryngeal squamous cell carcinomas are frequently quantified for each patient but some of them raise more and more the question of their practical value in the prognostic and treatment of the patients.

Methods. A number of 40 primary tumors of squamous laryngeal carcinomas with and without lymph nodes metastases (20 cases of each one) were investigated by different histological and immunohistochemical parameters: grade of differentiation - G OMS, morphometric grade - MG (the percentage of suprabasal keratinocytic cells versus spinous and fully differentiated keratinocytes), type of tumor cells cohesion at the invasion front - IF, mitotic index corrected per volume - M/V (volume of tumor occupied by tumor cells and not connective tissue), MIB-1 proliferation index (percentage of MIB-1 positive tumor cells), p53 index, microvascular density index - MVD (the number of CD-31 positive microvascular endothelial cells) and the grade of peritumoral inflammatory infiltrate - ii. Results. The multivariate statistical analysis of these parameters suggests that the more elevated metastasizing potential have the tumors with the combination of high MIB-1, IF, M/V, MG, ii and G OMS. The most important indicators were the number of cells in cell cycle and the lack of cohesion between the tumors cells at the invasion front (both were three times higher than in the tumors without metastases). The MG was more important than G OMS suggesting that the differentiation pattern of tumor cells (number of spinous cells and the keratin production) may overshadow the real proliferating suprabasal keratinocytes. The p53 index did not affect the process of metastasis but the cells selected in this process were those with high p53 index (in the lymph nodes the p53 index was much higher than in the primary tumors). The high MVD influenced the metastasis, but MVD had much lower values in the lymph nodes than in the primary tumors. The metastasizing potential depends on the neoangiogenesis by the propensity of the tumor cells to create their minimal new ecosystem in the lymph nodes and not for other reasons.

### **P 573**

#### **MOLECULAR TARGETS FOR THERAPY IN NEOPLASTIC AND NEOPLASTIC LESIONS OF OROPHARYNX AND LARYNX**

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**Introduction:** EGFR, COX-2 and cerbB2 are known as molecular targets for antitumoral and antiangiogenic therapy. The aim of our study was to evaluate by immunohistochemical(IHC) method the expression of EGFR, COX2, and cerbB2 in preneoplastic and neoplastic lesions of larynx and oropharynx.

**Materials and methods:** We performed an IHC study on 30 biopsies from patients with preneoplastic and neoplastic lesions of the larynx and 20 squamous cell carcinomas of the oropharynx (ABC triserial IHC method; EGFR from SIGMA-USA, COX-2 from Novocastra, cerbB2 from DAKO)  
**Results:** In larynx neoplastic lesions we found EGFR expression in small vessels at the invasion front of the tumor, and lack of positivity in neoplastic cells; in premalignant lesions of the larynx EGFR expression decrease with the degree of dysplasia; the same expression we found for COX-2; for cerbB2 the positivity was inconstant; in neoplastic lesions of oropharynx we founded positive correlation between the expression of EGFR and COX-2; for cerbB2 the expression was focal and inconstant

We concluded that EGFR and COX-2 could be considered as valuable targets for antitumoral therapies in preneoplastic and neoplastic lesions of larynx and oropharynx; cerbB2 is expressed but require further investigation.

**P 574**  
**RETINOBLASTOMA GENE AND PROTEIN EXPRESSION IN EVALUATION OF LARYNGEAL SQUAMOUS CELL CARCINOMA\***

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The identification of possible genetic alternations in cancer involves inactivation of tumor suppressor genes or activation of proto-oncogenes. In cell cycle, most of the regulatory actions occur at the so-called restriction point (R) in the late G1 phase. Tumor suppressor genes; Rb, p53 and p21 are among the most important of the agents suppressing transition through R point. Changes in the expression of Rb (retinoblastoma) gene which is localized in 13q14, correlate with the presence of Rb protein and they are believed to be an early event in carcinogenesis. This issue seems to be not plainly defined in laryngeal cancer. Our purpose was to investigate 13q14 LOH and expression of Rb protein and their possible prognostic value in laryngeal cancer. Surgical specimens were obtained from 41 patients with 5-year time observation who underwent surgical treatment at the Department of Otolaryngology Medical University of Lodz. Paraffin-embedded tissue sections were immunohistochemically stained with a monoclonal antibody raised against Rb protein. PCR-based techniques were used for investigating 13q14 LOH. The products of PCR reaction were fractionated on acrylamide gel. There were assessed correlation between the expression of Rb gene and Rb protein presence and clinical and histological implications (patients age, localisation of tumour origin, tumour size and nodal status based on TNM system, local and nodal recurrences, overall and disease free survival rate after surgical treatment and histological grading). Based on this findings it can be deduced that investigation of Rb gene expression and its protein product makes it easier to understand the process of cancerogenesis in laryngeal cancer and to establish its prognostic value further research and observations need to be attempted.

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**P 575**  
**THE EXPRESSION OF P53 PROTEIN AND HUMAN PAPILLOMAVIRUS INFECTION IN CONJUNCTIVAL AND EYELID BENIGN AND MALIGNANT LESIONS**  
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**Purpose:** Squamous cell papillomas of eyelid and conjunctiva are common benign neoplasm of ophthalmological interest. Squamous cell and basal cell carcinomas are respectively the most common malignancy of conjunctiva and eyelid skin. Human Papillomavirus (HPV) infection as well as P53 protein function lost has been identified as frequent events in varies of human tumors.

The aim of our study was the evaluation of P53 protein expression and HPV infection in 45 benign (papillomas) and 38 malignant conjunctival and eyelid lesions (27 cases of basal cell carcinomas and 11 cases of squamous cell carcinomas).

**Methods:** Using monoclonal antibody for P53 protein (DAKO) performed the immunohistochemical reaction; LSAB technique and DAB detect the complex antigen-antibody. To detect HPV infection PCR-RFLP technique was performed, using specific set of primers (TaKaRa) and for identification of HPV types 16,18, 33 the PCR products were enzymatic digested at 37°C.

**Results:** P53 protein expression was observed in 30 out of 45 (66,6%) squamous cell papillomas. In SCC and BCC groups P53 positivity was noticed in 31 out of 38 carcinomas and there was a statistically significant correlation between histological type of tumor and P53 protein expression. Malignant type HPV 16 and HPV 18 were detected in 3 squamous cell papillomas, 2 cases of BCC and 1 of SCC. However we observed P53 protein expression only in 2 HPV positive papillomas and 1 infiltrative type of BCC.

**Conclusions:** The results suggested P53 protein role in development of conjunctival and eyelid tumors, however the role of P53 protein in malignant transformation should be carefully investigated in the future. HPV seems to occur rarely, although in some cases it's role in pathogenesis of conjunctival and eyelid tumorigenesis should be considered.

**P 576**  
**STUDY OF KIT (CD 117) EXPRESSION IN PRIMARY UVEAL MELANOMAS AND METASTASES OF UVEAL MELANOMAS.**

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Nevertheless, uveal melanoma (UM) rate is about 1% of all human cancers, UM mortality rate is about 18% from dissemination. Comparison of these indexes let us think that UM is most malignant tumor. In the recent years with the help of immunohistochemistry it was achieved a great progress in investigation of tumor progression mechanisms. The KIT-gene encodes proto-oncogene c-kit, that has tyrosine kinase activity. Mutation in any exones of c-kit gene in tumor cells activates oncogene and stimulates production of mutant protein KIT in the lack of ligand. The result of all these mutans is activation of mitotic activity of cells and tumor progression. Hyper expression of CD 117 detected in UM is the way of application of inhibitors of tyrosine kinases in the treatment of these tumors.

**Purpose:** To study CD 117 expression in primary uveal melanomas and metastases of UM.

**Materials and methods:** Expression of c-kit was analysed in 39 paraffin-embedded sections of primary UM and 4 paraffin-embedded sections of UM metastases. Immunohistochemistry

was performed using the polyclonal anti-CD117 antibody from Dako.

Results: Immunohistochemistry for CD 117 was negative in 6 cases (15%), weakly positive in 11 cases (28%), moderately positive in 10 cases (26%) and strongly positive in 12 cases (31%) of primary UM. In 4 metastases (100%) of UM the reaction for CD 117 was strongly positive.

Conclusion: It appears justified to investigate the utility of imatinib mesylate in the treatment of these patients in clinical researches.

#### P 577

##### **CENTRAL FACIAL DESTRUCTIVE GRANULOMA FOLLOWING COCAINE ABUSE**

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**Introduction** The use and consumption of cocaine by snorting and sniffing exploded into a full blown epidemic. The adverse effects of cocaine nasal passages have long been recognized. Only recently a cocaine complex mimicking the rare condition of massive facial midline necrosis is described.

**Methods** Four patients referred to us with extensive necrotizing lesions of naso-sinusal tract and midface are described. Diagnostic and treatment are discussed with a literature review.

**Results** On physical examination all patients had a severe crusting, granulation tissue, purulent discharge and necrosis of nasal septum, lateral cartilages and hard palate. None other medical conditions were associated. Biopsies of nasal tissue revealed chronic inflammation without evidence of vasculitis or granulomatosis. There were onion skin deposits of perivascular fibrosis with numerous eosinophils. Plasma cells and lymphocytes primary T cells were present.

**Conclusion** Massive facial midline necrosis continues to be a diagnostic challenge. The aetiology comprises autoimmune disorders and certain tropical diseases. We underline as a possible cause the cocaine abuse. Ischemic necrosis due to vasoconstriction and prolonged anaesthesia may be factors causing tissue destruction.

#### P 578

##### **A CASE OF : NASAL GLIAL HETEROTOPIA**

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We observed a baby born at terms with an apgar of 10, presenting an inspiration dyspnea on its second day of life . Endoscopy discovered a tissular mass filling the right sinus. The imagery made favoured a teratoma.Under microscopy, areas of mature glial tissue of variable cellularity were embedded in the nasal mucous membrane. The cells of this cellularity, sometimes multi nucleic or of dystrophic aspect showed immunochemistry which varied depending on the areas of glial and neuron markers. There were not noticed numerous mitosis nor atypical cyto nuclears. The diagnosis of glial nose heterotopia was proposed.

Median congenital face malformations occur every 20000 to 40000 births. Among the most frequent, we note hemangioma, dermoid cysts, and encephaloceles. Nasal glial heterotopia is less frequent with only 250 cases observed. This pathology only concerns babies, mostly during the first month of life. After an asymptomatic period of time, the children had a weight loss which was due to alimentation problems because of nose obstruction. The location of 60 per cent of the lesions

is extra nasal, 30 per cent of them is intra nasal and the remaining 10 per cent are mixed. They are probably due to confinement of an encephalocele during fetal life which sometimes leaves a fibrous cord. Microscopically, these lesions are composed of glial tissue areas of variable cellularity made of non atypical and little proliferative astrocytes. A association is that of a neuron component.

In presence of congenital median facial lesions, establishing diagnosis through biopsies is not indicated. Indeed in the case of encephaloceles this might open a (meningo) gap which could be responsible for a iatrogenic meningitis.

An MRA examining possible communication between intra cranial structures and lesions is compulsory before Therapeutic treatment. This consists presently of an endoscopy which avoids the complications of surgery needed by craniotomy.

#### P 579

##### **CYTOGENETIC ANALYSIS OF ORAL LICHEN PLANUS**

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**Introduction:** oral lichen planus (OL) is an inflammatory lesion having controversial malignant potential, sometimes evolving to squamous cell carcinoma (SCC). SCC develops along a multistep pathway and thus accumulate genetic hits in functional targets relevant to tumour evolution. Recent studies suggest that chromosome instability increases with the degree of histologic changes from normal tissues to carcinomatous lesions. To date, there are no classic cytogenetic data on OL. Purpose of the present study is to describe chromosomal alterations of a series of OL studied with classic cytogenetic technique.

**Material and methods:** fresh biopsies were divided in two specimens: one was formalin-fixed and paraffin embedded for histological and immunohistochemical analysis for p53 (DAKO, clone p53, diluted 1: 50) and Ki 67 (Dako, clone MIB-1, diluted 1:100) . The other was used for classic G-banding cytogenetic analysis on short term cultures.

20 cases having typical clinical and histological features of OL were obtained. No dysplasia was present in the lesions. Results were compared with 9 cases of normal gingival mucosa from tooth extraction.

On histology, all the cases showed OL typical inflammatory infiltration with basal membrane disruption, vacuolization and apoptosis of the basal cheratinocytes. Immunohistochemistry: p53 and Ki67 were expressed in 15 to 40% of basal and suprabasal cheratinocytes in OL, while they stained no more than 15% of basal cheratinocytes in normal gingival mucosa. Cytogenetic results: clonal alterations were found in 3/20 cases (gain of a chromosome 18 copy was present in two cases), while Non Clonal Alterations (NCA) were detected in 14/20 cases. The most frequent NCA were: losses of chromosomes 21, 14, 9 and gains of chromosome 18. Alterations (i.e. gains and losses) involved also chromosomes 6 and 3. No clonal nor NCA alterations were found in cultures from normal gingival mucosa.

**Discussion:** present histological and immunohistochemical results show that, in cases of OL, oral mucosa shows a high proliferation activity. Cytogenetic analysis shows aneuploidy as the more frequent alteration, suggesting presence of chromosomal instability in these lesions. Although we cannot identify, to date, the meaning of single gains or losses, we suggest that the chromosome instability in OL might indicate a risk of malignant transformation.

#### P 580

### IMMUNOHISTOCHEMICAL ASSESSMENT OF HEAT SHOCK PROTEIN 70 IN ADENOID TISSUE

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**Objectives:** To evaluate the expression of heat shock protein 70 (HSP70) and its relation to histopathologic parameters in adenoid hypertrophy. In addition, HSP70 expression in adenoid tissue was compared with in adult and childhood.

**Materials and Methods:** Parafin-embedded adenoid tissue sections were obtained from 17 childhood and 17 adult patients. Expression of HSP70 was evaluated by immunohistochemical staining using anti-HSP70 monoclonal antibody and correlated with histopathologic parameters.

**Results:** Positive HSP70 expression was observed mainly in the mucosal epithelium, lymphocytes in germinal centers, interfollicular lymphocytes, subepithelial plasma cells and vascular endothelium. HSP70 immunoreactivity in the metaplastic mucosal epithelium with severe intraepithelial lymphocytic infiltration was higher than in the respiratory epithelium. The decrease in plasma cells showing HSP70 positivity was correlated with age.

**Conclusions:** These results suggest that intraepithelial lymphocytic infiltration is induce HSP70 expression in mucosal epithelium of adenoid.

### P 581

#### SEVERITY OF EPITHELIAL DYSPLASIA IS ASSOCIATED WITH LOSS OF MASPIN EXPRESSION IN ACTINIC CHEILITIS

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**Introduction:** Maspin is a member of the serpin superfamily of protease inhibitors and it is known to have tumour suppressor functions. It was demonstrated that maspin is expressed in normal cells, down-regulated in neoplastic cells and absent in metastatic cells. It was also inversely associated with increased dysplasia degree in mammary gland and gastric epithelium. Maspin is expressed in oral squamous cell carcinoma associated with a favourable prognosis. Actinic cheilitis is a potentially malignant lesion that is caused by long exposure to sunlight. Many authors consider that lip squamous cell carcinoma is always preceded by actinic cheilitis. Histologically this lesion is characterized by different degrees of epithelial dysplasia.

This study had the aim of evaluating the maspin expression in actinic cheilitis and comparing the findings to the degree of epithelial dysplasia.

**Material and methods.** Twenty nine cases histologically diagnosed as actinic cheilitis were retrieved from our files. The cases were represented by 13 cases with mild epithelial dysplasia, 9 moderate, and 7 intense dysplasia. The sections were submitted to the anti-maspin antibody (BD Pharmingen, 1:100) using the streptavidin-biotin method of immunohistochemistry. Immunostaining was semi-quantitatively evaluated and graduated: 0 (no staining), 1 (<5% of cells positive), 2 (5 to 50% of cells positive), 3 (<50% of cells positive).

**Results:** All the cases with mild dysplasia were graduated 3. In moderate dysplasia 2: 66.6% were graduated 2 and 33.3% received 3. All the cases with intense dysplasia were graduated 1. When the extent of staining was evaluated in the different epithelial layers, it was possible to notice that basal layer was abundantly stained only in 5 cases of mild epithelial dysplasia. In the other cases this layer showed scattered or no cells positive for maspin. In general, positivity in the layers decreased with the increase of atypias, from the basal to the surface.

**Conclusions:** Maspin expression was very abundant in actinic cheilitis with mild dysplasia and this expression decreased with the increase of the dysplasia degree. The first layer to lose maspin positivity was basal layer and successively upper layers lost positivity with the increased degree of dysplasia. In conclusion, this data suggests that in actinic cheilitis maspin expression is inversely related with progression of the lesion towards malignancy.

### P 582

#### EFFECT OF IRON REPLETION AND CORRECTION OF IRON DEFICIENCY ON THYROID FUNCTION TESTS IN IRON-DEFICIENT IRANIAN ADOLESCENT GIRLS

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**Study objective:** To investigate whether iron supplementation can improve thyroid hormone function in iron-deficient adolescent girls.

**Design:** A double-blind intervention study.

**Setting:** The study was performed in 2002 through 2003 in a region in southern I.R.Iran.

**Subjects:** A total of 103 iron deficient subjects that fulfilled all of the inclusion criteria were chosen. In all 94 subjects successfully completed this study.

**Interventions:** Patients were randomly assigned to one of two groups and treated with a 300 mg ferrous sulfate 5 times/week (n=47) and placebo 5 times/week (n=47) for 12 weeks.

**Main outcome measures:** At the beginning and at the end of study blood samples were collected and assayed for hemoglobin, hematocrit, serum ferritin, iron, total iron binding capacity (TIBC), Thyroid stimulating hormone (TSH), total thyroxine (TT4), total triiodothyronine (TT3), free thyroid hormones (FT4 and FT3), triiodothyronine resin uptake (T3RU), reverse triiodothyronine (RT3), selenium and albumin concentrations.

**Results:** There was a significant increase in iron indices in iron treated group after intervention. Indices of thyroid at the end of study revealed a significant increase in TT4, TT3, T3RU, and a significant decrease in rT3 concentration in comparison to initial values in iron treated group, and a significant difference when compared to placebo group. Alterations in FT3 and TSH concentration were not significant, but concentration of FT4 revealed a significant difference between beginning and end of the study in iron treated group.

**Conclusions:** Our results indicate that improvement of iron status was accompanied with improve in some indices of thyroid hormones.

### P 583

#### ANALYSIS OF PARAFOLLICULAR-C CELLS IN POLYNODOSE COLOID GOITRE OF THYROID GLAND

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**INTRODUCTION:** C cells of thyroid gland belong to a diffuse neuroendocrine system of the organism. There are different data in literature about usual normal number of C cells in thyroid gland. Colloid goitre is an after effect of an insufficient synthesis of thyroid hormones, and it results with assembling of great quantities of inactive colloid in follicles. Diameter of follicles becomes larger and due to the pressure, the height of follicular cells decreased. As the illness progresses, the thyroid gland are enlarged at first diffusing and then due to the increase of connective tissue, a nodes is created.

**PURPOSE:** A connection between a number of C cells and height of follicular cells, size of follicles and quantity of colloid in polynodose colloid goitre of thyroid gland were analysed.

**MATERIALS AND METHODS:** There were analysed 96 polynodose colloid goitres, which were operated from year 2001 to 2004. The slides were fixed in formaldehyde and formed in paraffin blocks, and then they were colored with hematoxylin-eosin and immunohistochemical coloring on calcitonin.

**RESULTS:** The sample was divided into groups, according to C cells and height of follicular cells, size of follicles and quantity of colloid, and all the results are semiquantitative. The results were statistically elaborated and they show high statistical significance of C cells connection with height of follicular cells, although there is no significant connection between C cells and follicles size and quantity of colloid in observed sample.

**DISCUSSION AND CONCLUSIONS:** It is possible to conclude, according to the final data, there is a connection between C cells and height of follicular cells in polynodose colloid goitre of thyroid gland, but there is no connection with size of follicles and quantity of colloid.

#### P 584

##### **CONCOMITANT USE OF FINE-NEEDLE BIOPSY AND LARGE-NEEDLE BIOPSY IN THE DIAGNOSIS OF THYROID NODULES**

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Fine-needle aspiration biopsy (FNA) and large-needle (cutting) biopsy (LNB) were performed on 109 patients with thyroid nodule or goiter who later underwent thyroidectomy. FNA alone provided a correct diagnosis in 84 (77%) of the 109 cases and LNB alone in 84 (77%) of the 109 cases, with the two methods yielding the same accuracy rate. Ninety-nine (91%) of the 109 cases were diagnosed correctly when both results were considered together. As to the causes for the discrepancies between the FNA or LNB results and the corresponding surgical diagnoses, the most common one was cytologic interpretive errors and, in descending order, insufficient material and sampling errors in FNA. On the other hand, in LNB, histologic interpretive errors were less but sampling errors were more frequently found compared with FNA. The present study indicated a need to concomitantly perform FNA and LNB in the diagnosis of thyroid nodules. Careful attention is required to distinguish between the follicular variant of papillary carcinoma and follicular adenoma, between encapsulated follicular carcinoma and follicular adenoma, and between malignant lymphoma and chronic thyroiditis at FNA as well as LNB diagnosis.

#### P 585

##### **COINCIDENCE OF HYPERPARATHYROIDISM AND THYROID CANCER FOUND AT THE TIME OF PARATHYROIDECTOMY**

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**INTRODUCTION:** A few reports have suggested an association between hyperparathyroidism (HPT) with thyroid cancer (TC) previous to surgery. Synchronous thyroid disease was found in 7% to 18% of primary HPT patients undergoing surgery, while 12% of thyroid lesions were found to be malignant. Association between non-medullary TC and primary HPT has been frequently reported. There is also some evidence for an association between secondary and tertiary HPT and thyroid cancer.

**METHODS:** This is a retrospective study of all cases of thyroid cancer in association with parathyroid disease treated surgically in our hospital from January 2000 to January 2004.

**RESULTS:** There were 280 surgical procedures for HPT in 274 patients. All patients had an elevated preoperative serum level of parathyroid hormone and serum calcium levels. Intraoperatively, 4 parathyroid adenomas and 270 parathyroid hyperplasia were diagnosed. In 50 patients (18.2%) thyroid resection was performed simultaneously. Three patients (1.1%) were found to have papillary thyroid cancer at the time of parathyroidectomy (PTX): one patient had primary HPT, one patient had secondary HPT, and one patient had tertiary HPT. Two cases were associated with metastatic spread to local lymph nodes. In those cases, total thyroidectomy was performed, and frozen section revealed a small papillary microcarcinoma (diameter < 1cm in size). None of the patients with thyroid cancer had a prior history of head and neck irradiation.

**CONCLUSION:** These data suggest that the association between thyroid cancer and HPT is rare and coincidental. The association of simultaneous pathology in the two glands justifies preoperative thyroid imaging and fine-needle aspiration (FNA) biopsy to determine the best surgical approach for patients with HPT.

#### P 586

##### **AN UNUSUAL NODULE OF THE THYROID: ECTOPIC MICROINVASIVE THYROID THYMOMA OF AB TYPE (MIXTE)**

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We report a rare case of ectopic thyroid thymoma of AB type. A 56 years old patient presented a left sided thyroid nodule discovered by palpation. Scintigraphic examination displayed a cold nodule, which measured 38 mm. Strong adhesion with the left lobe was observed; the nodule predominated in the inferior pole of thyroid gland. Histologically the proliferation corresponded to a thymoma of AB type with neoplastic emboli and extension to the adjacent adipous tissue. The jugulo-carotidic pick-up did not find any metastatic lymph nodes.

Ectopic cervical thymoma is a part of epithelial tumors with thymic differentiation of the neck. Incomplete descending or persistence of the cervical part of thymus is considered as the origin of ectopic thymus. These tumors are revealed by more or less compressive cervical masses without paraneoplastic syndromes. They present the same histological sub-types as their mediastinal homologue, but do not show any genetic anomaly. Differential diagnostic problems might be seen by extension of a mediastinal thymoma to cervical region, with an undifferentiated carcinoma, squamous cell carcinoma or

with lymphoma. Evolution remains local; relapses are never seen if surgery is complete. No prognostic factor is known nowadays.

**P 587**

**CONNECTION OF THE LYMPHOCYTES PHENOTYPE AND PROTEIN OF APOPTOSIS IN LYMPHOCYTIC THYROIDITIS**

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In persons diseased on thyroiditis the number and function of CD8+ lymphocytes have been normal. It has been shown that iodide induces apoptosis of thyroid cells in culture and demonstrated that KI can do the same to rats with induced goiter. Impairment Bcl-2 and Bax genes on thyrocytes, have been detected as a direct consequence of administration of iodine in potassium iodide-KI. Wistar rats having been receiving KI, KCl, NaI or NaCl. The Determinations of Peripheral Blood and Thymocyte Subpopulations of Rats have been done by FACScan. The double staining used FITC (CD8) and SA-PE (CD4). Immunohistochemical staining have been done with DAB on avidin-biotin and peroxidase using ARK, stained them with antibodies to: Bcl2 oncoprotein and Bax. We evaluated the intensity and distribution of positive DAB staining on a scale of 0 to 4. We perform statistic analysis by ANOVA and Student T-test. The histological analyses of thymus, spleen and thyroid have shown the changes in the thyroid as a diffuse mononuclear cell infiltration in the follicles and in the spaces between destruction of the acini, proliferation of connective tissue have been seen began between 5th and 10th day. So KI induced the symptomless LT in rats. Significant differences between individual groups ( $p > 0.05$ ) nor in comparison to the non-treated controls ( $p > 0.05$ ) has not been presented. Bcl-2 and Bax gene expression was significantly higher in rats with LT iodine induced and correlated with the extent of lymphocytic infiltratio. Bcl-2-expression was constantly seen in regular thyrocytes and in the mantle-zone of lymphofollicular infiltrates. Bcl-2 and Bax gene expression were not in connection with lymphocyte subgroups in peripheral blood. This work was partiali supported by MNS of Serbia, grant 1997

**P 588**

**ABSENCE OF BRAF MUTATIONS IN HYALINIZING TRABECULAR TUMORS OF THE THYROID**

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Hyalinizing trabecular tumor (HTT) of the thyroid is a rare tumor of follicular cell origin with a trabecular pattern of growth and marked intratrabecular hyalinization. The etiology of HTT is unknown but nuclear features, association with conventional papillary carcinoma, and RET/PTC rearrangements in some of these tumors suggest a relationship with papillary thyroid carcinoma. The majority of studies find that RET/PTC rearrangements and BRAF mutations are mutually exclusive in papillary carcinomas; however, one study considered that BRAF mutations may cooperate with RET/PTC rearrangements in the development of papillary carcinoma. RET/PTC rearrangements were detected previously in 28.6-75% of HTT. We examined six hyalinizing trabecular adenomas and two hyalinizing trabecular carcinomas for BRAF mutations. All tumors showed positivity

for thyroglobulin and TTF-1 and lacked immunoreactivity for calcitonin, chromogranin A, and synaptophysin. DNA was isolated from paraffin-embedded tumor tissues and the exon 15 of the BRAF gene was sequenced directly. No mutations were found in any tumor samples analyzed. Our results show that BRAF mutation do not seem to play, at variance with RET/PTC, a role in the etiopathogenesis of HTT (neither the typical V600E mutation nor the mutation associated to the follicular variant of PTC-K600E, was detected in any HTT). This work was supported by research grant (PGIDE 99PXI 90201B) sponsored by Xunta de Galicia, Spain.

**P 589**

**THYROID FNA – QUALITY CONTROL**

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Introduction: Thyroid FNA is a widely available diagnostic method with different results according to different series.

Aim: To evaluate thyroid FNA diagnostic accuracy (DA), sensitivity (S), specificity (Sp), false negative (FN) and false positive (FP) values and predictive values, positive (PPV) and negative (NPV), in the present series.

Material and methods: All thyroid FNA performed between April 2000 and December 2004 (n=1044) were reviewed and compared with histological diagnosis in 148 cases. Patients age varied between 11 and 85 years old, 87.74% were females.

Results: FNA were classified as diagnostic in 70.2%: 707 benign (goitre and thyroiditis) and 26 malignant (papillary carcinoma, medullar carcinoma and lymphoma); 29.79% were non diagnostic: 36 undetermined (follicular tumour and suspicious) and 12 insufficient. In 148 cases histological diagnosis was available: 83 corresponding to negative FNA, 17 to malignant FNA, 36 to undetermined FNA and 12 to insufficient FNA. FN (n=5) and FP (n=1) were 23.8% e 1.3%, respectively. The PPV was 94.1%, NPV was 93.9%, S was 76.2%, Sp was 98.7% and DA was 94.0%.

Conclusions: Our findings are in the range reported in literature. FNA is a good diagnostic tool in our hospital.

**P 590**

**THYREOIDECTOMY INDUCED MORPHOLOGICAL ALTERATIONS IN MAMMARY GLAND OF THE RATS**

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The mechanism of the influence of lower level of thyroid hormones on breast tissue is not enough explored. There is a suggestion in literature that the deficit of thyreiodaea can predispose the development of the breast tumor.

The aim of our work is the influence of partial thyreidectomy on mammary glands of the rat.

We used for experiment virgo female albino Wistar rats, weight 120±10gr., 45 days old. The animals were treated with ether narcosis during the partial thyreidectomy. There were 18 rats as well as in the control group. The animals were sacrificed after 70 days and there was used a tissue of the breast for PH analysis.

All specimens were fixed in Bouins solution. The tissue was embedded in paraffin wax, and sections were cut at 4-5 micrometers and stained with HE and immunocytochemical ABC-method with antibodies against beta TSH, EGF and PNA-lectin.

The significant hyperplasia of the acinus and ductulus was observed with PH analysis until they looked like microadenoma. In cytoplasm of the cell there are present a large number of vesicles which are often confluent, forming a macrodroplet aspect. Mastocysts, hypergranulated and polymorph, are present in the lipomatous stroma around ductulus and acinus.

In comparison to normal mammary gland, in epithel of hyperplastic acinuses and ductuluses was found beta TSH, PNA-lectin and EGF.

Except in epithel of the hyperplastic acinuses and ductuluses, EGF is found also in fibroblasts and fibrocytes and in the epithel of the acinuses and ductuluses in mammary glands of control group of rats.

In this work we discuss about the mechanism of the influence of the deficit of thyroidal hormones on the mammary gland of the rats, as well as the influence of breast carcinogenesis.

#### P 591

##### **OVEREXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN THE SOLID CELL NESTS OF THE THYROID AND THE CYSTIC TUMOR OF THE ATRIOVENTRICULAR NODE OF THE HEART** CAMESELLE-TEIJEIRO José<sup>1</sup>, ALFONSIN-BARREIRO Natividad<sup>2</sup>, ABDULKADER Ihab<sup>1</sup>, PRETO Ana<sup>3</sup>, SOARES Paula<sup>3</sup>, REYES-SANTÍAS Rosa<sup>1</sup>, SOBRINHO-SIMÕES Manuel<sup>3</sup>

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Solid cell nests (SCNs) are a normal component of the human thyroid gland that play a putative stem cell role. The cystic tumor of the atrioventricular node (CTAVN) of the heart is a benign cystic mass located at the base of the atrial septum of the heart, in the region of the atrioventricular node. Recently, we proposed that CTAVN of the heart is the cardiac equivalent of the SCNs (ultimobranchial rests) of the thyroid. Both entities are identical and basically composed of solid and/or cystic nests of squamoid cells (main cells) with a minor population of neuroendocrine cells (C cells). Epidermal growth factor (EGF) has widespread growth effects, and overexpression of EGF and epidermal growth factor receptor (EGFR) were found in dysmorphogenetic goiters, follicular adenomas, and well differentiated and undifferentiated (anaplastic) carcinomas of the thyroid. For this reason we investigated a series of seven cases of SCNs and one of CTAVN immunohistochemically with a mouse monoclonal antibody directed against EGFR (EGFR pharmDX kit, DAKO). In all cases, the main cells of SCNs and CTAVN revealed a distinct membranous staining pattern with weak cytoplasmic reactivity. Normal follicular cells were totally negative for this staining. The overexpression of EGFR fits with our previous findings of a higher proliferative index in main cells and supports the idea of these structures having an active biological role. This work was supported by a research grant (PGIDE 99PXI 90201B) sponsored by Xunta de Galicia, Spain.

#### P 592

##### **COX2 AND MULTIDRUG RESISTANCE PROTEIN 2 (MRP2) IN MEDULLARY THYROID CARCINOMAS (MTC).**

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The characterization of the mechanisms regulating the expression of multidrug-resistance proteins (MRPs) is crucial to plan an adequate treatment of cancers that display different degrees of resistance to chemotherapeutic drugs. COX2, a key enzyme in the conversion of arachidonic acid to prostaglandins, is an up-regulator of a MRPs member, namely, MRP-1. To determine whether COX2 is a potential regulator of MRP2 in MTC we evaluated the expression of MRP2 and COX2 in MTC.

We investigated the expression of MRP2 and COX2 in 12 MTC nodules, 10 colloid nodules and 6 normal thyroids by immunohistochemistry. The 22 nodules were studied paired with the adjacent non-nodular (normal) tissue.

Only MTC nodules showed immunoreaction: 9/12 (75%) were positive for MRP2 and 12/12 (100%) for COX2. The proportion of MRP2 positive cells (10% to 40%, mean 25%) was inversely related with the proportion of COX2 positive cells (10 to 100%, mean 54%);  $r = -0.96$ ,  $P < 0.05$ . Both immunoreactions exhibited levels of intensity from moderate to strong and were localized on the apical side pertaining to plasma membrane (MRP2) and to cytoplasm (COX2).

The results indicate that COX2 is a possible regulator of MRP2 expression in MTC and suggest its role in the limited response to chemotherapeutic agents.

#### P 593

##### **THE SURVEY OF PATHOLOGICAL LESIONS IN THE AUTOPSY OF THYROID GLAND, ISFAHAN, IRAN.** REISIFAR Malek, NAJAFI Farideh, NAJAFI Mohammad Reza

Background; thyroid gland autopsy is the suitable and exact way to consider the morphology of thyroid gland.

methods; this study is descriptive-analytic type. The thyroid gland were dissected carefully, weighed and measured and fixed in 5% formalin for 72h and sliced for microscopic examination and histopathologic study by two pathologists.

Results; 251 whole of thyroid glands were collected at autopsy who had no known clinical history of thyroid disease (208 m; 43 f, ratio 4:84) and intend in anatomic study and 202 whole thyroid glands (159 m and 43 f, ratio 3.7) were collected in pathologic study. normal histopathology in 125 cases (4.85%) goiter 41 cases (20.3%; nodular, diffuse) thyroiditis 22; neoplasia 15 cases (12 papillary carcinoma and 3 follicular adenoma).

conclusion; normal thyroid gland can be lead clinically to the great pathological finding in microscopic study in consideration of high malignant incidence and occult carcinoma. We should pay more attention to the smallest findings during clinical examinations in Isfahan city toward the other parts of the world such as Brazil.

#### P 594

##### **PATTERN OF THYROID CARCINOMA AMONG UNITED ARAB EMIRATES NATIONALS A CLINICOPATHOLOGIC STUDY**

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#### Introduction

The United Arab Emirates (UAE) is a union of seven sovereign sheikhdoms of the Arabian Gulf, which was formed

in 1971. These sheikhdoms or emirates include Abu Dhabi (the largest), Ajman, Dubai, Fujairah, Ras AlKhaima, Sharjah and Um Al Quwain. Although the total population is estimated to be 4 millions, only about 600,000 people are 'nationals' or natives of the country, the rest representing an extensive expatriate population. The national or indigenous population is composed of Emirate Arabs who, historically, were registered into tribes that included pastoral nomads and small agricultural settlements. Thyroid cancer is the most common endocrine malignancy. In the UAE thyroid cancer is the sixth most common cancer among nationals and the second among females.

#### Objective:

The aim of this study is to analyze the distribution of various pathologic types of thyroid carcinomas in a native Arab population of the United Arab Emirates (UAE) at Twam hospital in AlAin.

#### Materials and methods:

Forty-nine patients with thyroid carcinoma diagnosed over a 14-year period (1991-2004) at Tawam Hospital; the main oncology center in UAE were retrospectively studied clinically and morphologically and classified according to the World Health Organization (WHO) histological classification.

#### Results

Forty-four patients (90%) were diagnosed before the age of 45 years with a mean age of 35.5 years. The overall peak incidence was in the 3rd decade. There was a high female to male ratio comprising 11:1. Of the 49 patients in the study, 86% had papillary thyroid carcinoma (PTC), 12% had follicular thyroid carcinoma (FTC) and 2% had anaplastic thyroid carcinoma.

#### Conclusions

Thyroid carcinomas in the UAE are more common among females than males with a very high female to male ratio suggesting that female gender is a possible risk factor in their development. Since most of our cases were diagnosed before the age of 45 years; with expected good prognosis, the age before 45 years can be considered as an important prognostic factor as well as a possible risk factor. PTC predominates the histologic pattern of thyroid carcinoma in UAE, which is usually associated with iodide sufficient area.

#### P 595

##### **ANAPLASTIC THYROID CARCINOMA IN A 14-YEAR-OLD CHILD. CASE REPORT.**

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Anaplastic thyroid carcinoma is a highly malignant tumor with peak incidence during the sixth and seventh decade of life. The youngest patient described so far was 22 years old. A 14-year-old boy was admitted to the hospital in Kielce. Physical examination revealed in the right thyroid lobe a painless resilient tumor of 1.5 cm in diameter. A FNA biopsy was performed revealing the presence of atypical pleomorphic cells, arousing suspicion of medullary or papillary carcinoma. Chest X-ray showed numerous oval metastatic foci as well as enhanced stroma architecture. The patient had been referred to the Institute of Oncology in Gliwice. The patient underwent ADM+CDDP chemotherapy. Because of epilepsy onset, magnetic resonance was performed revealing numerous metastatic foci in the brain. Despite further chemotherapy the metastases in the central nervous system continuously progressed. The patient died on November 24, 2001, 17 months after initial diagnosis. Pathologic evaluation. In cross-sections of the right lobe a weakly separated tumor was found (2.0 cm in diameter). In microscopic assessment the majority of tumor texture was formed by anaplastic spindle-shaped cells with pleomorphic hyperchromatic nuclei. Numerous mitotic figures were seen. Foci of extensive necrosis and extravasated blood were noticeable in regions with anaplastic

texture. Neoplastic texture fully occupied by anaplastic cancer with extensive necrosis was present in 4 lymph nodes measuring from 0.5 to 1.0 cm in diameter.

Immunohistochemical study. In a few tumor cells showing features of papillary cancer thyroglobulin immunoassay was positive. The same tumor areas showed also expression of cytokeratin AE1/AE3 and cytokeratin 19. A weak reaction with cytokeratin was observed in some anaplastic cells both in the primary tumor and in node metastases. The majority of cancer cells present in metastases did not show, however the presence of thyroglobulin. The fields of anaplastic, spindle-shaped cancer cells showed expression of p53 protein in ca. 50% of cell nuclei. Anaplastic histological features of the discussed neoplasm have been confirmed by the lack of reaction with TTF-1, a minimal reaction with thyroglobulin antibody and a very high, above 70% MIB-1 index in anaplastic fields of tumor. It appears symbolic that the birth date of a first child who died from anaplastic thyroid cancer is the same as the date of the Chernobyl disaster.

#### P 596

##### **INSULAR CARCINOMA OF THE THYROID GLAND IN CHILDREN.**

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**INTRODUCTION:** Insular carcinoma of the thyroid gland represents poorly differentiated tumors that are relatively infrequent. It is even rarer tumor in pediatric patients. It is written that this neoplasm occurs predominantly in patients elder than 50-60 years old. Currently, insular carcinoma is considered to be a tumor with very poor and unfavorable prognosis.

**MATERIALS AND METHODS:** We collected and investigated surgical thyroid specimens from 5 patients (age 9 to 15) with insular carcinoma. These specimens were routinely processed, stained with HE, PAS, van Gieson. Ultrastructural and immunohistochemical investigations with monoclonal antibodies to vimentin, cytokeratins, chromogranin A, thyroglobulin, calcitonin and Ki-67 were also performed. All case reports and follow-up were carefully evaluated.

**RESULTS:** The careful histological examination revealed the presence of insular pattern mixed with more differentiated (either papillary or follicular) thyroid carcinoma. We found no significant areas of necrosis in all cases, so peculiar and usual for this tumor. Ultrastructurally, all the features of follicular-cell differentiation were found. Immunohistochemically, all cases were positive for thyroglobulin, vimentin and cytokeratins. In majority of tumors the activity of proliferation, evaluated by means of Ki-67 expression, was moderate to high. All these tumors were negative for calcitonin, chromogranin A. Clinically, no evidence either of distant metastasis or of local recurrence was seen in all 5 cases. Whole this group of patients is still alive, showing post-operative survival from 2 to 5 years.

**CONCLUSION:** Insular carcinoma of the thyroid gland is very rare neoplasm in children and adolescence. In this age group it tends to have more favorable prognosis than it does in adults, where it often shows the evidence of distant metastasis and recurrence. In other words, the biological behavior of insular carcinoma in pediatric patients tends to have better than it seems by means of its histological appearance.

#### P 597

##### **CYCLOOXYGENASE-2 IN NORMAL, HYPERPLASTIC, AND NEOPLASTIC FOLLICULAR CELLS OF THE HUMAN THYROID GLAND**

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This study was undertaken to investigate cyclooxygenase-2 (COX-2) expression in follicular cells of the human thyroid. COX-2 expression was studied immunohistochemically in a total of 174 samples. COX-2 immunoreactivity was confined to the cell cytoplasm with the nuclei remaining unlabelled. COX-2 expression was observed in 5 cases (17.2%) of normal follicular cells and in one case (16.6%) of solid cell nests. Follicular carcinoma expressed COX-2 more frequently than follicular adenoma (93.4% vs. 21.1%) ( $p < 0.001$ ). A higher percentage of cases of papillary microcarcinomas up-regulated COX-2 in comparison with all papillary carcinomas ( $p < 0.05$ ). However, we could not establish any relationships between COX-2, patients' ages or lymph node metastases in papillary carcinomas. COX-2 expression was found in 12 (92.3%) poorly differentiated carcinomas and in 13 (92.8%) undifferentiated carcinomas. We found that COX-2 is not always useful as a marker of malignancy. Our results suggests that COX-2 plays a role in progression of all thyroid carcinomas but, in papillary carcinomas, seems more important only in the early stages. COX-2 expression in the undifferentiated carcinoma deserves special consideration due to its prognosis and to the fact that selective COX-2 inhibitors were found to enhance tumour response to radiation in some studies. This work was supported by research grant (PGIDE 99PXI 90201B) sponsored by Xunta de Galicia, Spain.

#### P 598

##### CONCOMITANT GLOBAL DNA METHYLATION EVALUATION AND GALECTIN-3: POTENTIAL TOOL IN DIFFERENTIAL DIAGNOSIS OF THYROID NEOPLASIA

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**Introduction.** The implications of global DNA hypomethylation were lately reported in several models of tumorigenesis. Little is known about this epigenetic event in thyroid neoplasia.

Over the last few years galectin-3 was promoted as an overall differentiating solution of thyroid nodules but it was also described limits of this marker regarding the follicular neoplasia differentiation.

This study aimed to evaluate concomitantly the status of global DNA methylation and galectin-3 in several types of thyroid tumors in order to define the diagnosis potential of this combination.

**Materials and methods.** 5-mc and galectin-3 immunostaining scores were evaluated by computerized image analysis in 17 papillary thyroid carcinomas (PTC), 6 follicular thyroid carcinomas (FTC), 16 follicular adenomas (FA), 19 nodular goiters (NG) and 10 Hürthle cells adenomas (HCA).

**Results.** We found a significant lower level of 5-mc immunostaining in thyroid carcinoma when compared with benign tumors or adjacent normal thyroid parenchyma ( $p < 0.0001$ ). Overall, 5-mc accuracy to distinguish malign from benign thyroid tumors was similar to that of galectin-3 (89% vs. 87%,  $p > 0.05$ ). The combination of 5-mc with galectin-3 lead to an excellent accuracy level of 96%. Among follicular neoplasia 5-mc accuracy to differentiate malign tumors trends to be higher than galectin-3 one (90% vs 66%,  $p = 0.06$ ).

**Conclusions.** These data stress out the necessity of epigenetic events evaluation among thyroid nodules and propose global DNA methylation assessment as a complementary diagnostic tool.

#### P 599

##### HBME - 1 MONOCLONAL ANTIBODY IN DIFFERENTIATION OF BENIGN AND MALIGNANT THYROID LESIONS

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**Abstract:**

**Introduction:**

Monoclonal antibody HBME-1 was prepared using cells from an epithelial malignant mesothelioma as immunogen. It is an antimesothelial monoclonal antibody that recognized an unknown antigen on microvilli of mesothelial cells. The antibody is only relative specific for mesothelium and is helpful in the differential diagnosis between mesothelioma and adenocarcinoma, within the context of an appropriate immunohistochemical panel. Thyroid lesions have high incidence in human and distinction between benign and malignant lesions is very important for surgical approach. In the past the only reliable method for diagnosis was H&E staining.

**Purpose of the study:**

The purpose of this study was to evaluate HBME1 monoclonal antibody staining for diagnosis of benign and malignant thyroid lesions.

**Methods used:**

This is an analytical descriptive study. 90 formalin fixed and paraffin embedded thyroid lesions (45 benign and 45 malignant) were examined by immunostaining and light microscopy. Data were analysed with chi-square test and P value lower than 0.05 was determined significant.

**Summary:**

We found strong positive staining in the majority of papillary carcinoma (28/31), in some of follicular carcinoma (4/6), in a few follicular adenoma (2/17) and negative staining in oxiphilic cell adenoma (0/4), nodular goiter (0/13) and undifferentiated carcinoma (0/8) and thyroiditis (0/11).

**Conclusion:**

The results suggest that monoclonal antibody HBME-1 is useful in differentiating papillary and follicular carcinoma of the thyroid from benign lesions especially in more differentiated lesions. Strong and generalized immunoreactivity for HBME-1 in a follicular lesion should raise the suspicion of malignancy, but negative staining specially in poorly differentiated lesion does not rule out malignancy.

#### P 600

##### DIAGNOSTIC VALUE OF GALECTIN-3, HBME-1, P27, CYCLIN D1, CYTOKERATIN19, AND HIGH MOLECULAR WEIGHT CYTOKERATIN IN THE DIFFERENTIAL DIAGNOSIS OF THYROID TUMORS

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The distinction of benign and malignant thyroid tumor is critical for the management of patients with thyroid nodules. Immunohistochemical markers, such as galectin-3, HBME-1, p27, cyclin D1, cytokeratin19 (CK19) and high molecular weight cytokeratin (HMWCK) have been suggested to be helpful in the distinction of thyroid malignancy and papillary

thyroid carcinoma. We applied immunohistochemical staining in 262 surgically resected thyroid nodules including 181 papillary carcinomas (PC; 147 conventional papillary carcinomas [CPC] and 34 follicular variant of papillary carcinomas [FVPC]), 22 follicular carcinomas (FC), 25 follicular adenomas, and 34 nodular hyperplasias, to determine the diagnostic accuracy of these markers. The expression of all markers was significantly associated with thyroid malignancy. The sensitivity for the diagnosis of thyroid carcinoma was 97.0% for galectin-3, 90.6% for HBME-1, 82.8% for p27, 63.5% for cyclin D1, 90.6% for CK19. The specificity of these markers was 93.2%, 71.2%, 76.3%, 86.4% and 79.7%, respectively. Among the four benign nodules which showed focal galectin-3 positivity, none showed staining to HBME-1, cyclin D1 and CK19. In comparison of PC and FC, the expression of galectin-3, cyclin D1, CK19 and HMWCK was significantly higher in PC than in FC. When comparing FVPC with FC, the expression of CK19 and HMWCK was significantly higher in FVPC. However, CK19 showed focal positivity in 32% and diffuse positivity in 9% of FC. Although the expression of HMWCK was confined to PC, the sensitivity for the diagnosis of PC was only 58.6%. In comparison of CPC and FVPC, the expression of galectin-3, HMWCK, cyclin D1 was significantly higher in CPC than in FVPC. We concluded that although galectin-3 has limitation due to focal staining in benign lesion, it is a useful marker in the distinction of benign and malignant thyroid tumors, especially when used in combination with HBME-1, CK19 and cyclin D1. CK19 and HMWCK can be aided in the diagnosis of PC. However, the low sensitivity of HMWCK and low specificity of CK19 may limit its utility.

#### P 601

##### IMPACT OF ACTIVATING BRAF MUTATION ON AGGRESSIVENESS IN PAPILLARY THYROID CANCER

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Activating mutations in the BRAF kinase gene have recently been reported in human papillary thyroid cancers. The aim of the present study was to determine the frequency of BRAF mutations in thyroid cancer and their correlation with clinicopathological parameters and various immunohistochemical markers including VEGF. We analyzed exon 15 of BRAF gene in 190 frozen or paraffin-embedded thyroid tumor tissues. A missense BRAF mutation was found in exon 15 in 105 of 166 papillary carcinomas (63.3%), 0 of 18 benign disease, 0 of 5 follicular carcinoma and 0 of 1 medullary carcinoma.

Mutation of BRAF and concomitant ERK activation may also influence the progression of papillary carcinomas. Examination of 166 patients with papillary thyroid cancer showed that BRAF mutation did not have correlation with clinical stage and distant metastasis, but BRAF mutation was more common in palpable thyroid cancer (p-value<0.05). Angiogenesis is very important process during the development and progression of solid tumors. Vascular endothelial growth factor (VEGF) is a major regulator of angiogenesis and could be produced by papillary thyroid cancer. To investigate the relationship between BRAF mutation and VEGF expression in the tumorigenesis of human thyroid, immunohistochemical study was performed. VEGF expression did not correlate with various clinicopathological parameters but, clearly correlated with tumor size. Papillary thyroid cancer with BRAF mutation exhibited more intensive VEGF expression (p-value<0.05).

Our results suggest that activating mutation of BRAF gene closely related to VEGF expression and more common in palpable thyroid cancer.

#### P 602

##### ROLE OF CYTOKINES AND DENDRITIC CELLS ON FIBROSIS IN THYROID PAPILLARY CARCINOMA AND HASHIMOTO'S DISEASE

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Introduction: Significant fibrosis is observed in both Hashimoto's disease (HD) and thyroid papillary carcinoma (TPC). TPC is a particularly suitable model to study the role of tumor-associated macrophages (M $\phi$ ) and/or dendritic cells (DC), because it is frequently associated with signs of immune reaction, including lymphocytic infiltration. On the other hand, interstitial cells such as fibroblast, DC and M $\phi$  are known to secrete various cytokines which have effects on connective tissue cells and endothelial cells in fibrotic lesions. Purpose: We tried to clarify whether the released cytokines from interstitial cells were related to development of fibrosis in the diseased thyroid. Materials and Methods: The role of cytokines such as TGF $\beta$ 1, CTGF, GM-CSF and TNF $\alpha$  in the development of fibrosis was examined with immunohistochemical staining (IHC) and in situ hybridization (ISH) using paraffin sections of the TPC and HD. The phenotype of the interstitial cells was examined by IHC. In addition, in vitro assays were performed using cultured fibroblasts (WI-38), thyroid carcinoma cells (HTC/C3) and differentiated U-937 cells including immature DC, and M $\phi$ , to determine how the released cytokines act on fibroblasts. The levels of cytokines, both in the cytoplasm and the culture medium, were examined using IHC, ELISA and RT-PCR. Results: In TPC, there was significant expression of TGF $\beta$ 1, CTGF, GM-CSF and TNF $\alpha$ , not only in fibroblasts and/or M $\phi$  in the stroma of the tumors, but also in the tumor cells and infiltrating DCs around the tumor cells. In the HD, mature DCs were seen mainly in lymph follicles, and immature DCs were focally observed around the degenerative or destructive thyroid follicles. Using in vitro assay, differentiated U-937 cells (mainly M $\phi$  and DC) were shown to significantly release GM-CSF and TNF $\alpha$ . When co-cultured with differentiated U-937 and/or HTC/C3 cells, respectively, WI-38 cells showed elongation of the cytoplasm and expressed cytokines. Conclusion: The results from both the in vivo and in vitro assays suggest that cytokines such as GM-CSF and TNF $\alpha$  released by fibroblasts and M $\phi$  as well as CD1a<sup>+</sup> cells, involve in the proliferation and differentiation of fibroblasts. This may contribute to the fibrosis development in TPC. However, in HD, the relationships between fibroblasts, DC, M $\phi$  and their released cytokines and fibrosis remain unclear.

#### P 603

##### GALECTIN 3, ONCOFETAL FIBRONECTIN, EMMPRIN, CYTOKERATIN 19, CITED1, AND TPO EXPRESSION IN THYROID TUMORS

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**Background:** Fine needle aspiration (FNA) has become the keystone diagnostic tool in the management of thyroid nodules. However this method has some limitations, such as its lack of sensitivity for follicular neoplasm and may not be informative in 5 to 20% of FNA samples. The use of molecular markers of malignancies could improve the diagnostic performance of these inconclusive or non-informative FNA. The choice for the most relevant markers needs to be performed on tissue samples.

The aim of this study was to determine the expression of 6 genes that may be effective in diagnosing cancer: galectin 3, oncofetal fibronectin, emmprin, cytokeratin 19, CITED 1, and TPO by analysing surgical thyroid tissue samples.

**Design:** Eighty six tissue samples were analysed and divided into 5 types: 11 normal thyroid tissue, 21 adenoma, 40 papillary carcinoma, 8 follicular carcinoma and 6 anaplastic carcinoma. For each sample, the gene expression was assessed compared to an ubiquitous gene RPLP0 using quantitative real time RT-PCR. The ratio concentration of studied genes over RPLP0 represented the level of expression of the studied gene in each sample. For the 6 studied genes, means and medians of these ratios were calculated for each type of tissue sample. These ratios were compared using an ANOVA and Scheffe tests (proc GLM Sas Institute).

**Results:** The emmprin and cytokeratin 19 levels of expression were comparable in all 5 types of tissue studied ( $p=0.6$  and  $p=0.4$ ). Galectin 3, fibronectin and CITED 1 levels of expression were significantly increased only in papillary carcinoma compared to normal tissue. The mean value of galectin was 1.19 (IC 95%:0.94-1.44) vs 0.13 (IC 95% : 0.08-0.18), mean value of fibronectin was 4.48 (IC 95%:2.5-6.06) vs 0.04 (IC 95% : 0.02-0.07) and mean value of CITED 1 was 6.94 (IC 95%:4.80-9.07) vs 0.20 (IC 95%:0.15-0.26). TPO was significantly underexpressed only in papillary carcinoma compared to normal tissue. The mean value of TPO was 0.6 (IC 95%:0.12-0.69) vs. 1.68 (IC 95%:1.15-2.22).

**Conclusion:** The emmprin and cytokeratin 19 gene did not seem effective in distinguishing different tissue types. The significantly increased levels of fibronectin, galectin 3 and CITED 1 and decreased levels of TPO found in papillary carcinoma compared to normal tissue may confirm the interest in these 4 genes as molecular markers of papillary cancer. An evaluation of these genes in cytology samples is now being conducted.

#### P 604

##### **DIFFERENTIATED THYROID CARCINOMAS : IMMUNOHISTOCHEMISTRY IS USEFUL TO PREDICT THE OCCURRENCE AND THE CHRONOLOGY OF LYMPH NODES METASTASES**

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**Background :** The poor outcome of differentiated thyroid carcinomas is difficult to predict, specially in pT1 ( $\leq 2$ cm) carcinomas. The diagnostic interest of various immunohistochemical markers has often been demonstrated but their prognostic value still remains unclear.

**Objective :** Are immunohistochemical findings in differentiated thyroid carcinomas correlated to the occurrence and the chronology of their lymph nodes metastases?

**Material and methods :** HBME-1, Galectin-3, Thyroperoxydase(TPO), Cytokeratin-19(CK19) and c-erbB-2 expression was studied in 41 thyroid carcinomas : 10 (N0) limited to the thyroid gland during a 7 to 9 years follow-up and 31 (N+) with lymph nodes metastases (12 with

synchronous, 10 with metachronous and 9 with synchronous and metachronous lymph nodes metastases).

**Results :** The tumors were 35 papillary carcinomas (PTC) and 6 follicular carcinomas (FTC), including one oncocyctic variant. 31PTC/35 coexpressed HBME-1, Galectin-3 and CK19, 2/35 expressed c-erbB-2 and 32/35 had a loss of TPO-expression (TPO staining in less than 60% of tumor cells). 6/6 FTC were stained with CK19, 5/6 with Galectin-3, 4/6 with HBME-1, 0/6 with c-erbB-2 and 6/6 had a loss of TPO-expression. 10/10 carcinomas with exclusive metachronous lymph nodes metastases were totally negative for TPO versus 8/21 others N+-carcinomas ( $p=0.001$ ).

7/8 N0-PTC had an intense (in more than 70% of the tumor cells) HBME-1 staining versus 5/27 N+-PTC ( $p<0.001$ ). None N+-carcinomas was negative for Galectin-3 contrary to 4/10 N0-carcinomas ( $p=0.002$ ). 9/9 N+-pT1-PTC associated an HBME-1/Galectin-3 coexpression and a loss of TPO-expression versus 1/5 N0-pT1-PTC ( $p=0.005$ ).

Staining in tumors and their lymph nodes metastases was globally the same for each antibody except for galectin-3.

**Conclusion :** HBME-1, Galectin-3 and CK19 (alone or associated to each others) and the loss of TPO-expression are highly sensitive for the diagnosis of PTC. A strong HBME-1 staining is often found in N0-PTC whereas galectin-3 had a 100% sensitivity for N+-carcinomas. A galectin-3/HBME-1 coexpression with a loss of TPO-expression is correlated to the occurrence of lymph nodes metastasis in pT1-PTC. A completely negative TPO-staining seems to be related to the occurrence of metachronous lymph nodes metastases. These results should prompt to modulate the actual consensual surveillance of patients with thyroid carcinomas, according to immunohistochemical pattern.

#### P 605

##### **BRAF T1799A MUTATION IN HUMAN THYROID PAPILLARY MICROCARCINOMA**

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**Background.** A hotspot mutation in exon 15 of BRAF gene, the T1799A transversion, has been shown to be a prevalent (28.8% - 68.6% in various series from different countries) and highly specific genetic alteration in human papillary thyroid cancer (PTC) in adult patients. BRAF mutation associates with certain histological variants of the tumor being found in a large proportion of PTC with classic papillary morphology and, vice versa, underrepresented in the tumors with follicular architecture. At the same time, little is known about the involvement of BRAF mutation in early stages of thyroid carcinogenesis such as papillary thyroid microcarcinoma (PMC) and its role in tumor progression.

**Purpose of the study.** Our investigation was set out i) to determine the prevalence of BRAF mutation in PMCs in patients from two geographically distinct groups, Japan and Russia; and ii) to evaluate a correlation between the presence of mutated BRAF and PMC morphology and biological behavior of the tumor.

**Materials and methods.** Tumor tissue was microdissected from serial sections of 15 PMCs from Japanese patients and 31 PMCs from Russian patients. DNA was extracted using Proteinase K/phenol-chloroform protocol. Fragment of BRAF exon 15 was PCR amplified and analyzed by direct sequencing.

Results. Mutated BRAF was detected in 13/46 (28.2%) of the tumors: 9/31 (29.0%) and 4/15 (26.6%) in Russian and Japanese individuals, respectively, displaying no signs of discrepancy in the mutational rates in patients with differing genetic background seen in PTCs. Occurrence of the BRAF mutation did not significantly correlate with patients' gender, age at presentation, metastatic indices as well as with papillary, mixed papillary and follicular, and solid/trabecular PMC histotype. On the contrary, tumors with follicular morphology significantly associated with the mutation-free genotype ( $P=0.018$ ). In the mixed-type tumors characterized by co-occurrence of well-differentiated and less differentiated components, the BRAF mutational frequency was significantly elevated ( $P=0.020$ ).

Conclusion. Results indicate that PMCs may have a spectrum of genetic events partly overlapping with that of PTCs. The BRAF T1796A mutation occurs in about one-third of PMCs irrespectively of the genetic background of patients and positively or negatively associates with particular tumor architecture whereas no correlation could be found with patients' demography and biological behavior of the tumor.

#### P 606

##### **BRAF T1799A MUTATION IN VARIOUS THYROID DISEASES**

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**BACKGROUND:** High prevalence of activating T1799A transversion in B Raf kinase-encoding gene (BRAF V600E) has been reported specifically in papillary thyroid cancer (PTC) varying 29-69% in series from different countries, but not in follicular thyroid cancer (FTC) and benign follicular thyroid adenomas (FA). With regard to other types of thyroid diseases information remains rather limited.

**PURPOSE OF THE STUDY:** In this investigation we determined BRAF mutational rate in medullary thyroid cancer (MTC), FTC, FA, nodular goiter (NG) and lymphocytic thyroiditis (LT) tissues along with that in PTCs in an attempt to elucidate the difference in BRAF alteration frequency among these entities.

**MATERIALS AND METHODS:** For the study we collected 2 MTCs, 5 FAs, 5 NGs, 2 LTs, 4 FTCs (1 case with lymph node metastasis) and 11 PTCs including 5 cases of follicular variant of PTC, 2 multi-focal PTCs with 2 lymph node metastases from adult patients (2 males and 27 females) aged 30-69 years at operation, residents of central region of Russia. We collected normal thyroid tissues for analyzing in all that cases also. The pathological diagnoses of all the tumors were made in accordance with WHO classification criteria (1988). Genomic DNA was isolated from fresh frozen surgical tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA) and analyzed for the BRAF mutation by mutant allele specific polymerase chain reaction. DNA extracted from PTC tissue previously determined to harbor mutant BRAF was used as a positive control.

**RESULTS:** In this series, we found no BRAF T1799A mutation in all eleven PTC cases as well as in any other type of tissue analyzed.

**CONCLUSIONS:** Our data demonstrate the absence of BRAF mutation in thyroid diseases such as medullary and follicular cancer, follicular adenoma, nodular goiter and lymphocytic thyroiditis. On the other hand, the fact that we were unable to detect BRAF T1799A transversion in 11 cases of PTC indicates that BRAF mutational rate is not too high in adult Russian patients suggestive that frequency of BRAF gene alteration in PTC may be substantially different in the

individuals with distinct genetic background (i.e. it is markedly lower in Russian patients in comparison with e.g. Asian individuals). Greater comprehension is necessary to correctly evaluate BRAF mutation prevalence in Russian patients with PTC.

#### P 607

##### **A NEW BRAF GENE MUTATION DETECTED IN A CASE OF SOLID VARIANT OF PAPILLARY THYROID CARCINOMA**

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BRAF gene mutations have frequently been detected in papillary thyroid carcinoma (PTC).

Moreover, there is a close association between the type of mutation and the PTC histotype: BRAFV600E is associated with conventional PTC and with histological variants of PTC displaying a prominent papillary growth pattern, whereas BRAFK601E is associated to the follicular variant of PTC.

We report the detection of a novel BRAF triplet deletion in a case of solid variant of PTC. The deletion leads to the replacement of a valine and a lysine by a glutamate in the BRAF activation segment (BRAFK600-1E) thus mimicking partially the two BRAF mutations previously described. Our study reinforces the existence of a close relationship between the occurrence of some types of BRAF mutation and some PTC histotypes. The genetic study of more cases of the solid variant of PTC is necessary to find whether or not there exists a significant association between the occurrence of BRAFK600-1E and such PTC histotype.

#### P 608

##### **PAX8-PPAR $\gamma$ TRANSLOCATION: ANOTHER COMMON FEATURE TO FOLLICULAR THYROID CARCINOMA AND FOLLICULAR VARIANT OF PAPILLARY CARCINOMA**

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Thyroid tumours are among the few epithelial tumours harbouring chromosomal translocations. Papillary thyroid carcinomas (PTC) may display RET/PTC and TRK rearrangements and follicular tumours often have the PAX8-PPAR $\gamma$  translocation.

The follicular variant of papillary thyroid carcinoma (FVPTC) is characterized by the cytologic nuclear features of PTC with a follicular pattern of growth. At variance with conventional PTC these tumours display a low frequency of RET/PTC rearrangements, low frequency of B-raf mutations (and of a distinct type from those found in conventional PTC) and a higher frequency of ras mutations.

In an attempt to further characterize the FVPTC we investigated the presence of PAX8-PPAR $\gamma$  translocation in FVPTC using interphase FISH, RT-PCR and immunohistochemistry.

By FISH analysis we found that 41% (9/22) of FVPTC cases display the PAX8-PPAR $\gamma$  translocation. All the cases which harboured the PAX8-PPAR $\gamma$  translocation showed a strong immunoreactivity for PPAR $\gamma$ , and the translocation was confirmed by RT-PCR in two of the cases.

The presence of PAX8-PPAR $\gamma$  translocation was associated with the presence of invasion and/or metastasization ( $p=0.0034$ ) and with the presence of multifocality ( $p=0.04$ ). We conclude that follicular carcinomas and FVPTC share the presence of PAX8-PPAR $\gamma$  translocation at a very similar frequency. We also found three cases of FVPTC displaying both PAX8-PPAR $\gamma$  translocation and Ras mutations. Finally, our data, together with the data already published, shows that FVPTC may be more closely related with FTC than with classical PTC.

#### P 609

##### THE EXPRESSION PROFILE OF DIFFERENTIATED THYROID CANCER: MULTI-GENE CLASSIFICATION APPROACH

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The diagnosis of thyroid cancer is still a field of many controversies, even among experienced pathologists. There is a paucity of efficient markers, both to differentiate benign and malignant cases and to recognize cancer subtypes. Thus, we apply the oligonucleotide microarray technology to study the expression profile of differentiated thyroid cancer and to select transcripts which differentiate between subclasses. We use state-of-the-art bioinformatic techniques, based on Support Vector Machines algorithms, to select not only the single "solitary" markers but rather the sets of genes which are taken into account cooperatively to enhance the diagnosis accuracy.

We start our analysis from the simplest model, the comparison between papillary thyroid cancer and normal thyroid tissue, mainly specimens from thyroid lobe contralateral to tumor.

In total, 82 thyroid samples were investigated by high density oligonucleotide microarrays (HG-U133A, Affymetrix): 48 PTC samples, 25 samples of macroscopically unchanged thyroid tissue and 9 benign thyroid lesions. We recently proposed (Jarzab et al., Cancer Res. 2005) a 20-gene classifier, trained on the initial group of 16 PTC and 16 normal samples, which took into account the interactions between genes and in our group outperformed any single-gene approach. It included DPP4, GJB3, ST14, SERPINA1, LRP4, MET, EVA1, SPUVE, LGALS3, HBB, MKRN2, MRC2, IGSF1, KIAA0830, RXRG, P4HA2, CDH3, IL13RA1, MTMR4. In the study we validate this classifier on the separate group of 50 samples and obtain 93% accuracy. The few misclassified tissues usually exhibit a low percentage of tumor cells in collected sample.

We further extend the analysis to the set of follicular neoplasms (10 follicular cancers and 10 follicular adenomas), where the specification of differentiating genes is much more difficult and it results in significantly lower accuracy.

Conclusion: The high diagnostic accuracy of the recently proposed own multigene PTC classifier has been confirmed.

#### P 610

##### IMMUNOHISTOCHEMISTRY IN THYROID TUMOUR PATHOLOGY

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##### Background and Aims:

Traditionally invasive growth is the only criterion of malignancy in follicular tumours of the thyroid, while nuclear

characteristics are the major criterion for papillary tumours, both papillary and follicular variant.

Our aim was to investigate the immunohistochemical phenotype of follicular epithelium and to characterize in more refined way follicular lesions of various origin.

##### Material and Methods:

Thyroid lesions and nodules encountered in daily practice over the last eighteen months were stained for various antibodies. Antibodies were used to demonstrate follicular cells (thyroglobulin, TTF1), oxyphilic cells (thyroglobulin, TTF1 and CK14) and C-cells (calcitonin, CEA and chromogranin A).

Thyroid malignancy associated antibodies used were: CK19, HMW keratin, HBME1, Galectin 3, RET-PTC and PAX8-PPAR $\gamma$ .

##### Preliminary Results :

HBME1 showed to be a most interesting valuable marker for follicular tumours, helping to distinguish follicular carcinoma from adenoma. Other useful stains in case of follicular carcinoma are Galectin3 (Gal3) and PAX-PPAR $\gamma$ .

In our experience Cytokeratin 19 is a very good marker for papillary carcinoma; papillary as well as follicular variants. Most papillary cancers stain for HBME1 and Gal3 as well.

##### Conclusions:

1. Immunohistochemical staining of thyroid nodules in general is mostly a valuable adjunct to confirm equivocal histopathological diagnoses.

2. In encapsulated, non-invasive tumours with follicular growth pattern but with focal papillary carcinoma nuclear features, in our hands a definite correlation was noted with CK19 positivity.

3. Oxyphilic cells stain for CK14.

Gal3 and HBME1 markers, helpful in distinguishing benign from malignant follicular tumours, may be positive in both benign as well as in malignant oxyphilic tumours.

4. In cellular areas in thyroid nodules and goitres, the positivity of some malignancy-associated markers is at present not well understood, but might be due to clonal heterogeneity.

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#### P 611

##### HER2/NEU STATUS IN THYROID TUMORS: A STUDY OF 92 THYROID CASES BY IMMUNOHISTOCHEMISTRY AND FLUORESCENCE IN SITU HYBRIDIZATION

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Introduction: HER2/neu overexpression and amplification have been shown in a variety of carcinomas particularly breast carcinomas. However, a limited information is available concerning thyroid neoplasms in this regard.

Aims: The aim of this study was to determine HER2/neu status by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) in benign and malignant thyroid neoplasms using low density manual tissue microarray.

Materials and Methods: For immunohistochemical study and FISH analysis, tissue cylinders of 4 mm diameter taken from

paraffin blocks of 92 thyroid tumors were arrayed in 8 recipient paraffin blocks. We analyzed HER2/neu status of 9 follicular adenomas, 50 papillary carcinomas, 19 medullary carcinomas, 10 anaplastic carcinomas and 4 follicular carcinomas by IHC (DAKO), using streptavidin biotin immunodetection system and by FISH (Q-biogene).

Results: A cytoplasmic staining with an intensity score changing between +1 and +3 was detected in various ratios in all groups except anaplastic carcinoma group immunohistochemically. However, none of the tumors showed diffuse strong membranous staining. Cytoplasmic and/or membranous staining was observed in 88%, 68.4%, 66.6% and 100% of papillary carcinomas, medullary carcinomas, follicular adenomas and follicular carcinomas, respectively. There was no statistically significant difference between papillary carcinoma group and other tumor groups with respect to staining. No statistically significant difference regarding staining was detected between papillary microcarcinoma and papillary carcinoma group, either.

Although HER2/neu amplification was detected in single tumor cells in some cases, HER2/CEP17 ratio was found to be lower than 2 in all tumors.

Conclusion: Cytoplasmic and/or membranous HER2/neu expression by IHC can be seen in thyroid tumors but the staining is not as strong or widespread as observed in breast carcinomas. Immunohistochemical expression does not correspond to a high level of amplification by FISH. The mechanisms and clinical impact of immunohistochemical HER2/neu expression remains unknown.

#### P 612

##### **DIFFERENTIAL EXPRESSION OF DYSADHERIN IN PAPILLARY THYROID CARCINOMA AND MICROCARCINOMA: CORRELATION WITH E-CADHERIN EXPRESSION**

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Introduction: Papillary carcinoma is the most common type of thyroid cancers. Microcarcinomas are small tumors (<1cm) of the same histologic type, usually clinically silent and frequently detecting as incidental findings. E-cadherin is a cell adhesion molecule involved in the progression of several malignant neoplasms. Dysadherin is a recently described cell membrane glycoprotein, which has an anti-cell-cell adhesion function and downregulates E-cadherin.

Purpose: The purpose of the study was to examine the expression of dysadherin and E-cadherin in papillary thyroid carcinomas and microcarcinomas and investigate whether there are differences in their expression.

Methods: The expression of E-cadherin and dysadherin was detected immunohistochemically in 20 papillary carcinomas and 20 microcarcinomas of the thyroid gland. The method was applied on formalin-fixed, paraffin-embedded tissue sections using the EnVision System (DAKO) and the monoclonal antibodies: NCC-M53 against dysadherin and E-cadherin (CM170B, Biocare Medical). The intensity, the expression pattern and the percentage of neoplastic cell staining was recorded and correlated with the histologic type.

Results: Dysadherin was not expressed in normal thyroid follicular cells. In papillary carcinomas low membranous expression was detected in 40%(8/20) of the cases, intermediate expression in 20%(4/20) and high expression in 15%(3/20). In microcarcinomas low expression was detected in 40%(8/20) of the cases, intermediate expression in 10%(2/20) and high expression in 0%(0/20).

Immunoreactivity for E-cadherin was detected in non-neoplastic follicular cells. In papillary carcinomas low expression was detected in 30%(6/20) of the cases, intermediate expression in 45%(9/20) and high expression in 20%(4/20). In microcarcinomas low expression was detected in 10%(2/20) of the cases, intermediate expression in 50%(10/20) and high expression in 40%(8/20). Increased dysadherin expression was correlated with aberrant (cytoplasmic) E-cadherin expression in most tumors.

Conclusion: Since dysadherin is not normally expressed in non-neoplastic thyroid follicular cells, it is conceivable that it plays a pivotal role in the neoplastic transformation of thyroid gland. Moreover its expression correlates with tumor size. In papillary thyroid tumors, dysadherin appears to downregulate E-cadherin expression, at least in part.

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##### **FIBRILLIN 1 EXPRESSION IN THYROID CARCINOMAS .**

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##### Introduction:

Fibrillin is an extracellular matrix glycoprotein ,a main component of microfibrills , with sites recognizable by integrin receptors, suggested to support cell attachment and to impact cell differentiation and migration. The aim of this study was to investigate and to compare the fibrillin expression in thyroid carcinomas at mRNA and protein level in cell lines, commercial available tissue microarrays and archival specimens,

since ECM proteins are suggested to be of great importance for the metastatic potential of carcinomas.

##### Material and Methods:

RNA was extracted from 13 thyroid cell lines (9 papillary Ca , 2 anaplastic Ca , 1 follicular Ca,and 1 medullary Ca) .RT-PCR analysis for Fibrillin- 1 mRNA was followed, using gene-specific primers.

Furthermore we investigated the fibrillin expression by immunohistochemistry in a commercial available tissue microarray (IMGEX IMH 319) including 42 papillary Ca, 7 follicular Ca, 1 PDC and 9 benign thyroid tissues, as well as in archival tissue from the files of the Pathology Department of the Athens University, including 33 thyroid carcinomas( 15 papillary, 4 follicular, 5 Huerthle cell-, 1 anaplastic and 8 medullary Ca).For immunohistochemistry an antibody against fibrillin 1 (Santa Cruz H-109) was used at dilution 1:100 (overnight) .The stain was evaluated semiquantatively (0 - +++)

##### Results:

RT-PCR analysis revealed Fbn-1 mRNA expression in all thyroid carcinoma cell lines, with highest expression in the follicular Ca cell line WRO, the papillary SW579 and the medullary TT, whereas lowest expression was found in the two anaplastic cell lines (ARO, FRO) and 1 papillary (BHP 18)

Fibrillin has been demonstrated immunohistochemically in cytoplasmic location in the tissue microarray as well as in the archival sections in almost all papillary (39-14), medullary(5), Huerthle cell-4) ,anaplastic(1) and poorly differentiated Ca (1), whereas it was absent in all follicular carcinomas.The most intense stain has been observed in papillary carcinomas, with a slight increased intensity in advanced stages.

##### Conclusions:

Our data indicate that, fibrillin 1 is strongly expressed by the neoplastic cells of thyroid carcinomas in different degree in the various histologic types and might be implicated in cell-stroma interaction in terms of signaling, attachment and migration.

#### P 614

### **DIAGNOSIS OF METASTATIC CARCINOMA OF THE THYROID GLAND BY FINE NEEDLE ASPIRATION CYTOLOGY. A REPORT OF CLINICAL AND MORPHOLOGICAL FEATURES OF A CASE WITH THYROID METASTASES FROM COLON ADENOCARCINOMA.**

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Clinically evident, diagnosed and surgically resected thyroid metastases are uncommon. In a series of 42 cases with nonthyroid carcinoma and thyroid nodules, thyroid metastases were found in 4 cases (2 – lung cancer, 1 – breast cancer, 1 – colon cancer) by ultrasound-guided fine needle aspiration cytology of the thyroid gland. We report the clinical and morphological features of a case of colon adenocarcinoma metastatic to the thyroid gland. The patient was a 56-year-old woman with progressively enlarging goiter and compression symptoms. A history of left hemicolectomy for colon adenocarcinoma with extracellular mucinous production - T3N0M0 (6 years ago), right lower lobectomy for lung metastasis (4 years ago) and chemotherapy was present. Thyroid ultrasound found a big tumor mass in the right lobe and a small one in the left lobe, and numerous enlarged neck lymph nodes. Fine-needle aspiration cytology of the thyroid showed atypical tall cells with an acinar pattern. Total thyroidectomy and lymph nodes' extirpation was done. Histological examination confirmed the presence of metastatic colon adenocarcinoma with moderate differentiation. Immunohistochemical staining of the thyroid tumor was negative for thyroglobulin but positive for CEA and cytokeratin 19. In conclusion ultrasound scan and FNA biopsy of the thyroid gland with cytology appear to be useful methods for the differential diagnosis of thyroid nodules in cancer patients, including colorectal carcinoma. The recognition of thyroid metastases is essential for the appropriate staging and therapy of primary carcinoma.

#### P 615

### **UTILITY OF HBME-1, CYTOKERATIN 19 AND GALECTIN-3 IMMUNOSTAINING IN THE DIAGNOSIS OF THYROID MALIGNANCY.**

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The aim of our study was to investigate the usefulness of immunohistochemical expression and immunolocalization of a panel of markers including HBME-1, CK-19 and galectin-3 in thyroid neoplasias and non-neoplastic lesions.

We evaluated 170 thyroid lesions including 148 neoplasias (84 papillary carcinomas, 38 follicular carcinomas, 18 follicular adenomas, 1 hyalinizing trabecular tumor, 5 medullary carcinomas, 2 anaplastic carcinomas) and 22 non-neoplastic lesions (12 adenomatous nodules and 10 Hashimoto's thyroiditis). The percentage of positive cells as well as the pattern of staining were analysed. The three markers were usually negative in normal thyroid tissue. This was helpful to differentiate lesions with follicular pattern, when there was doubt regarding the nuclear features of papillary

carcinoma. HBME-1, galectin-3 and CK-19 were expressed in 94%, 72.6%, 72.6% of the papillary carcinomas and in 63%, 21% and 21% of the follicular carcinomas. Furthermore, the staining pattern of the three markers (diffuse, focal segmental or focal cellular) was useful to differentiate neoplastic from non-neoplastic nodules. Lesions could be differentiated by their staining pattern. Diffuse reactivity predominated among papillary carcinomas, specially in the follicular variant of papillary carcinoma, in contrast to the focal segmental and focal cellular pattern found in follicular adenomas or carcinomas and adenomatous nodules. Counting the percentage of galectin-3 and CK-19 positive cells was useful for the differentiation between cases of follicular variant of papillary carcinoma and follicular carcinoma or adenoma.

Therefore we conclude that immunohistochemical staining features may be useful for the differential diagnosis of thyroid lesions. Diffuse positivity is indicative of malignancy. We also suggest that HBME-1 is a very sensitive marker for thyroid malignancy, but the three markers together may be helpful in special cases. This panel of markers discriminates the lesions with a follicular pattern, with special reference to the follicular variant of papillary carcinoma supported by FAEP, CNPq, FAPESP.

#### P 616

### **IMMUNOHISTOCHEMICAL EXPRESSION OF GALECTIN-3 IN BENIGN, MALIGNANT AND THYROID TUMORS OF UNCERTAIN MALIGNANT POTENTIAL**

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Background. Well differentiated encapsulated thyroid tumors with a follicular architecture are sometimes quite difficult for pathologists to make differential diagnosis between a definite malignant or definite benign category. Pathologists are compelled to classify the tumors having questionable nuclear changes and questionable or absent capsular invasion as well differentiated tumors of uncertain malignant potential (WDT-UMP). In a series of recent reports it has been found that galectin-3 immunostaining may be useful to distinguish benign and malignant thyroid tumors. Immunohistochemical expression of galectin-3, a human lectin, has been shown to be highly associated with a malignant behaviour of thyroid tumors.

Purpose of the study. We studied galectin-3 expression in a spectrum of obvious benign, tumors of border-line or uncertain malignancy and obvious malignant thyroid tumors. Materials and methods. Sixteen papillary carcinomas, two follicular carcinomas, five well differentiated thyroid carcinomas not otherwise specified (NOS), seventeen follicular adenomas and twelve thyroid tumors of uncertain malignant potential were examined. Monoclonal antibodies specific to galectin-3 (Novocastra Laboratories) and indirect streptavidin-biotin complex immuno-reaction were used for immunohistochemistry.

Results. Positive cytoplasmic immunostaining of tumor cells was seen in 14 papillary, 2 follicular carcinomas, 4 well differentiated carcinomas NOS, 2 follicular adenomas and 5 well differentiated thyroid tumors of uncertain malignant potential.

Conclusion. Galectin-3 is a reliable marker of well differentiated thyroid carcinoma. It can sometimes be expressed in benign tumors. Group of well differentiated thyroid tumors of uncertain malignant potential is seems to be a heterogeneous. The significance of positive immunostaining of tumor cells in obvious benign tumors and in some of the border-line group of tumors as well as negative immunostaining in some of the obvious malignant tumors needs to be further investigated.

#### P 617

### IMMUNOHISTOCHEMICAL EXPRESSION OF FASCIN IN GASTROENTEROPANCREATIC ENDOCRINE TUMORS

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**Introduction.** Fascin is a globular protein that organizes F-actin into well-ordered, tightly packed parallel bundles in vitro and in cells. Fascin is normally expressed by basal layer cells of the epidermis, neurons, glial cells, dendritic cells, endothelial cells and macrophages. Abnormal expression of fascin was reported in almost all cases of Hodgkin disease (in Reed-Sternberg cells), in T-cell lymphomas and in various types of carcinomas. In studies of non-small cell lung carcinoma and gastric adenocarcinoma high fascin expression is correlated with poor survival. Its immunoreactivity is variable in endocrine tumors (ETs). According to our knowledge, studies concerning the expression of fascin-1 in gastroenteropancreatic (GEP) ETs have not been reported. The aim of the present study is to investigate the expression of fascin in GEP-ETs.

**Material and Methods.** A total 30 cases of GEP-ETs were included in this study. A panel of immunohistochemical markers was used for diagnosis. GEP-ETs were classified according to the latest WHO criteria in 13 cases of well differentiated ETs "carcinoids" and 17 cases of endocrine carcinomas (well or low differentiated). The primary tumors were located in the foregut (13-43,3%), midgut (14-46,7%), or hindgut (3-10%) respectively. All the cases were immunohistochemically tested using the monoclonal antibody that recognizes the C-terminal region of the fascin molecule. Twenty pancreatic and gastrointestinal tissue specimens with non-specific reactive changes were also tested for fascin expression.

**Results.** Diffuse cytoplasmic staining of fascin was found in neoplastic cells of 4/17 GEP endocrine carcinomas. These tumors had a high MIB-1 labeling index (over >50% of neoplastic cells). All the well differentiated ETs were non-reactive for fascin. The endocrine cells of Langerhans islands of pancreas and those of the GI tract were also negative.

**Conclusion.** High expression of fascin is reported in large-cell neuroendocrine carcinomas of the lung and in all cases of small-cell lung carcinoma. In typical or atypical carcinoids of the lung fascin is detected in a small number of cases. In our study only 4/17 cases of GEP endocrine carcinomas were positive for fascin. On the contrary all the well differentiated GEP-ETs were non-reactive. These results show that overexpression of fascin-1 in GEP-ET is not a common finding. Further extensive studies are essential to define the role of fascin expression in these neoplasms.

### P 618 RELATIONSHIP BETWEEN IMMUNOHISTOCHEMICAL EXPRESSION AND PLASMA CONCENTRATION OF TOTAL GHRELIN IN PATIENTS WITH GASTRIC NEUROENDOCRINE TUMOURS.

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**Background:** Ghrelin is a 28-amino acid growth hormone secretagogue occurring predominantly in the gastric mucosa. Ghrelin-immunoreactive (IR) cells in normal stomach also express vesicular monoamine transporter-2 (VMAT-2).

Ghrelin has been reported in ECLomas type I and III and in regions with ECL cell hyperplasia.

**Aim:** To characterise neuroendocrine (NE) tumours of the human gastric corpus with respect to the occurrence of ghrelin-IR cells and relate the findings to plasma (P) total ghrelin concentration.

**Material and Methods:** The study included ECLomas type I (n=4), type III (n=5), one gastric NE carcinoma and one malignant gastric ghrelinoma. Antibodies against chromogranin A, synaptophysin, synaptic vesicle protein 2, VMAT-1, VMAT-2 and ghrelin were used for immunohistochemistry. P total ghrelin was measured using a commercial RIA kit (Linco Research, Mi, USA).

**Results:** Ghrelin immunoreactivity was found in varying frequency in neoplastic cells of ECLomas type I (VMAT-2-IR) and in virtually all cells of the ghrelinoma (VMAT-2 negative), but were not observed in type III ECLomas nor in the NE carcinoma. Immunoreactivity for ghrelin was detected among the ECL hyperplastic cells in the mucosa of ECLomas type I. In ECLomas type III and in the NE carcinoma slight diffuse hyperplasia of ghrelin-IR cells was also seen in the peritumoural mucosa. P total ghrelin levels were within the reference range (<5 ig/L) in all ECLomas and in the NE carcinoma, despite the presence of neoplastic and/or hyperplastic ghrelin-IR cells. Increased P concentration of total ghrelin was only observed in the patient with the malignant gastric ghrelinoma, who had more than 400-fold increase (2100 ig/L).

**Conclusion:** In spite of neoplastic and hyperplastic ghrelin-IR cells in ECLomas type I and ghrelin cell hyperplasia in the surrounding mucosa of the neoplastic tissue in type III and in the NE carcinoma no elevation of P total ghrelin concentration was detected. However, in the patient with the ghrelinoma the P total ghrelin was markedly elevated. Thus, expression of ghrelin in neoplastic (excluding the ghrelinoma case) and hyperplastic cells in the gastric mucosa does not seem to give rise to elevated circulating levels of ghrelin.

### P 619 PANCREATIC MICROADENOMATOSIS IN PATIENTS WITH AND WITHOUT THE MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 SYNDROME SCHLENGER Regina\*, ANLAUF Martin\*, PERREN Aurel\*\*, SCHMID Sonja\*\*, BAUERSFELD Juliane\*, WIEBEN Daniel\*, KOCH Christian A.\*\*\*, KOMMINOTH Paul\*\*, DRALLE Henning\*\*\*\*, WEIHE Eberhard\*\*\*\*\*, HEITZ Philipp U.\*\*, KLÖPPEL Günter\*

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**Introduction:** Microadenomatosis of the endocrine pancreas has been suggested to represent a hallmark of multiple endocrine neoplasia type 1 (MEN 1). Purpose of the study:

On the basis of several observations we hypothesize that microadenomatosis may also occur independently of MEN 1 and that it may show features distinct from those in MEN 1.

**Methods:** Pancreatic tissue specimens from 40 patients were analyzed, using immunohistochemistry, confocal laser scanning microscopy and morphometric methods. The MEN 1 and Von-Hippel-Lindau (VHL) status were assessed clinically and by PCR based mutational analysis. Results:

Twenty seven of the 31 MEN 1 patients were found to have microadenomatosis in addition to single macrotumors. Most microadenomas and macrotumors expressed glucagon, with pancreatic polypeptide and insulin following in frequency. In

nine of the 40 patients with pancreatic microadenomatosis neither MEN 1 nor VHL germline mutations could be found. Five of these nine patients suffered from hyperinsulinism caused by multiple microinsulinomas, while the four others had multiple clinically silent microglucagonomas. The nontumorous pancreatic tissue from both MEN 1 and nonMEN 1 patients contained proliferative endocrine cell lesions in the ducts and the islets. Conclusions: Microadenomatosis in MEN 1 is the basis for the development of single macrotumors, which may cause a hormonal syndrome. Microadenomatosis is not specific for MEN 1 or VHL, but may occur independently and cause a functional syndrome. In both conditions microadenomatosis is preceded by various preneoplastic proliferative changes of endocrine cells.

#### P 620

#### ARE PANCREATIC ENDOCRINE TUMORS A MANIFESTATION OF THE NF1 PHENOTYPE? MOLECULAR ANALYSIS OF A PATIENT WITH VON RECKLINGHAUSEN SYNDROME

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Introduction: Patients with a von Recklinghausen syndrome (NF1) carrying NF1 germline mutations are predisposed to endocrine tumors including pheochromocytomas and duodenal somatostatinomas. It is unclear whether the rarely reported occurrence of pancreatic insulinomas in NF-1 patients represents a coincident finding or whether insulinomas are a rare manifestation of the NF1 syndrome.

Aims: To determine the relationship between NF1 and insulinomas, we analyzed blood and tumor tissue of an insulinoma for NF1 mutation, allelic loss of the NF1 gene and its expression in a patient with von Recklinghausen syndrome. Patient: The NF-1 patient presented with recurrent episodes with weakness, sweating, and occasional loss of consciousness. A MRI showed a 2.5 cm tumor in the body of the pancreas. A tumor enucleation was performed and histology revealed a well differentiated pancreatic endocrine carcinoma (WHO 2004) with a micrometastasis in a peripancreatic lymph node. Immunohistochemistry showed expression of insulin and proinsulin.

Methods: Mutation analysis was performed by direct sequencing of the peripheral blood and tumor tissue. Allelic loss (LOH) analysis of the NF1 gene was done using the intragenic microsatellite markers D17S1166, NF1.PCR3 and D17S1849 on 17q11.2. For expression analysis, a RT-PCR was designed spanning intron 3 of the NF1 gene including the mutated site in exon 4.

Results: Mutation analysis of peripheral blood leukocytes confirmed a 4 base pair deletion in exon 4 starting at codon 167 (499 del TGTT). LOH analysis of tumor tissue revealed retention of both NF1 alleles. While RT-PCR of peripheral blood showed biallelic expression of both the wild-type NF1 and the mutated form, RT-PCR of tumor extracts showed a sole expression of the mutated NF1 allele.

Conclusion: These results suggests that the transcription of the wild type NF1 allele is suppressed, probably by epigenetic mechanisms. This is a strong indication for a causal relationship between the insulinoma and the von Recklinghausen disease. It can be hypothesized that insulinomas are a rare manifestation of the NF1 phenotype. Furthermore, the NF1 gene must be considered as a candidate tumor suppressor gene for insulinomas and other PET, since

the second allele can be inactivated by other mechanisms than 17q LOH.

#### P 621

#### NESIDIOBLASTOSIS: A RARE CAUSE OF HYPERINSULINEMIC HYPOGLYCEMIA IN ADULTS.

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INTRODUCTION: Nesidioblastosis is an uncommon cause of hyperinsulinemic hypoglycemia in adults. In this case report we will present an adulthood nesidioblastosis.

CASE REPORT: 73 years old man was admitted to the emergency service of Kocaeli University Medical Faculty with the complaint of senkop. During first examinations, the blood glucose levels were between 10-29 mg/dl. Although there was not any preoperative radiological findings of pancreatic mass, decision of surgical examination for the diagnosis and treatment of insulinoma was made. During surgery, 85% distal pancreatectomy was performed because of the lack of pancreatic mass during operative exploration.

The resection material was in 12X4X1,5 cm dimensions. All the pancreas was embedded in 24 blocks. Serial sections were applied to each block for three times and tumoral mass was not observed. In histopathological examination abnormal shaped Langerhans islands, ductulo-insular complexes, endocrin buddings from ductal structures, small clustures and single endocrine cells were observed in the pancreas parachyma. The insulin immunostaining was observed more than 80% in Langerhans islands, small clustures and single cells in the paranchyma. The typical peripheric alpha cell ring was not observed in some islands in glucagon immunostaining. The mean Langerhans island diameter was 270µ.

After the operation the patient's general condition deteriorated because of his respiratory problems and sent to intensive care unit. The blood glucose levels were fluctuated between 90-110 mg/dl. The patient died because of the respiratory problems in postoperative 48. day.

CONCLUSION: The diagnosis of adulthood nesidioblastosis was made according to clinical and histopathological features. Although insulinoma is the most common reason of deep hyperinsulinemic hypoglycemia the entity of nesidioblastosis must be in mind for differential diagnosis of this situation.

#### P 622

#### SUBTOTAL PANCREATECTOMY FOR PERSISTENT HYPERINSULINAEMIC HYPOGLYCAEMIA – CASE REPORT

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The syndrome of persistent neonatal and infantile hyperinsulinaemic hypoglycaemia (PHHI) has long recognized, its pathogenesis remain to be elucidated. Two different forms, focal and diffuse, have been reported. The diffuse form corresponds to the lesion previously described as "nesidioblastosis". Persistent hyperinsulinemic hypoglycemia (PHHI) rarely appears after infancy. We present a normally developed 4.5 year old boy with severe hypoglycemia resistant to medications. Treatment with glucose infusions did not keep his glycemia normal. He was hypoglycemic (initial glycemia 1.9 mmol/l) and no ketosis was detected. He

required 140 mg/min iv glucose for maintenance of glycemia within normal range. The insulinemia was 44  $\mu$ U/ml at glycemia of 2.2 mmol/l (ratio 20:1). Hyperinsulinemia was persistent. Imaging of the pancreas by ultrasound and CT was normal. Diasoxide in a dose of up to 6 mg/kg showed a transitional effect. After two months the child became severely hypoglycemic again requiring permanent high doses of iv glucose. Glucagon and somatostatin did not correct the hypoglycemia as confirmed by continuous glucose monitoring. Subtotal pancreatectomy was performed. Methods and results: Intra-operative frozen section were performed on small pancreatic specimens taken from the different parts of the gland allows one to determine the type of lesion ( focal or diffuse). The paraffin block obtained from subtotal pancreatectomies were sliced and stained with routine staining HE, reticulin, PAS and immunohistochemical stains: insulin, proinsulin, somatostatin, glukagon, pancreatic polipeptid, P57 and SUR-1. Hystology of the pancreas showed numerous normally configured islets that are quite numerous with variably sized. They are irregularly distributed in the acinar tissue and interlobular stroma. The structure was normal with insulin cells in the center and glucagon, somatostatin and pancreatic polypeptide cells at the periphery. The islets were not at rest. Labeling for P57 and SUR-1 was normal. There was no excessive release of insulin. The child is in good health 14 months after surgery and does not require any treatment. Continuous glucose monitoring showed normal values of glycemia. Last check up showed normal HbA1c (5.0%) and insulin level (10.4  $\mu$ IU/ml). Histology and the clinical course are not typical for a diffuse form or a PHH1.

#### P 623

##### ANEUPLOIDY AND LOW P21/WAF1 EXPRESSION IN MALIGNANT PARANGLIOMAS

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**Background:** Clinical and histopathological features do not reliably distinguish between benign and malignant paragangliomas. Additional markers that might be useful prognostic indicators in the pathological assessment of these tumors are sought. **Aim:** to determine if p21/WAF1, p27Kip1, p53 and Ki-67 expression, and DNA content correlates with clinical behaviour of the paragangliomas. **Design:** Patients submitted to surgery for sporadic paraganglioma from sites anywhere with follow-up, were included in this retrospective case-control study. Gender, age, tumor size and metastases were obtained from medical records. Malignancy was defined as the presence of metastasis and/or extensive local invasion. **IHQ** detection of p21/WAF1, p27 Kip1, p53 and MIB-1 against Ki-67 antigen, was blindly performed. The reactivity was evaluated as percentage of positive nuclear immunoreactivity. For measurement of DNA content one block of each case was selected. Student t test was used. **Results:** The study group included 31 patients, 23 women and 8 men. Mean follow-up was 41 months (range, 0-123). Twenty-seven were benign and 4 malignant tumors. Three of the malignant tumors were present in men. Mean age of patients with benign and malignant tumors was  $48 \pm 11.3$  years and  $33 \pm 12.3$  years, respectively ( $p=0.031$ ). The mean size for benign and malignant tumors was  $4.5 \text{ cm} \pm 1.9$  and  $7.5 \text{ cm} \pm 8.3$ , respectively ( $p=0.093$ ). Recurrences were observed in 2 patients (7%). p21/WAF1 expression was positive in 50% malignant versus 89% benign tumors. Only one benign case was positive for p53. p27Kip1 and Ki-67 expression no showed significant differences between groups.

All malignant tumors were aneuploid versus only 23% of benign tumors. **Conclusions:** The present study showed that malignant paragangliomas were related to less age, higher tumor size, aneuploidy and less p21/WAF1 expression. This results suggest that p21/WAF1 can be inactivated in malignant paragangliomas inducing proliferation of neoplastic cells.

#### P 624

##### ADRENAL LIPOMAS: REPORT OF TWO CASES AS INCIDENTAL AUTOSY FINDING.

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#### INTRODUCTION

Adrenal lipomas are extremely rare, benign, non-functioning neoplasms, with very few cases recorded in the medical literature. Most cases have been incidental findings, usually during autopsy investigation of deaths from an unrelated cause. The size can be variable, ranging from one up to twelve centimetres. The follow up is limited to exceptional cases diagnosed during life and the outcome of these patients have been excellent.

#### CASE REPORTS

**Case 1:** A 70-year-old man is found dead in a beach. Medico-legal investigation and autopsy confirm that the death was due to drowning. A small tumour is noted in the right adrenal gland. It is a soft, rounded, yellow mass, with 1 cm in its largest dimension. Histopathological examination reveals a typical lipoma.

**Case 2:** A previously healthy 45-year-old man collapsed and dies while taken to the hospital. Autopsy examination shows that both adrenal glands are enlarged. The left weights 92 gr and a yellow-tan mass occupies most of the cut surface. Histopathologically corresponds to a pheocromocytoma, which was considered responsible for the death.

The right adrenal gland weights 18 gr. and has a 2 cm, soft, yellow mass. Histopathological examination reveals a lipoma.

#### COMMENTS

We report two cases of adrenal lipomas, one of them associated to pheocromocytoma in the contraateral adrenal gland. To the best of our knowledge, this association has not been previously reported. Since lipomas are very unusual in this location, in both cases the whole mass was submitted for histopathological examination to rule out other possibilities such as myelolipoma or liposarcoma.

Histopathologically, adrenal lipomas are similar to conventional lipomas arising elsewhere in the body, a feature non contributive to asses its pathogenesis, which remains unknown. The relation between myelolipoma and lipoma has been discussed and a continuum spectrum between both neoplasms has been proposed, but never proved.

Our cases are typical, since they were asymptomatic and only discovered at autopsy. Although they present little difficulty in the histopathological diagnosis, their knowledge is merit because the incidental discovery of these neoplasms has increased during the last years.

#### P 625

##### HISTOPATHOLOGICAL STUDY OF PRENATAL STRESS ON ADRENAL GLAND TOTAL VOLUME AND ABSOLUTE VOLUME OF GLOMERULOSA, FASCICULATE, RETICULARIS LAYERS AND MEDULLA IN 21 DAYS NEONATE RATS: A STEREOLOGICAL STUDY

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Pregnant mothers may expose to various stresses and it may impact on their embryos. It affects the structural development of brain, gland and body weight. The detail of this histopathological changes have been little researched. The base of this study was the prenatal stress effects on adrenal gland stereological.

Sprague-Dawley rats were kept in standard condition for two weeks. The two female with one male were caged overnight. Vaginal pluge and sperm detection in vaginal smear confirmed the pregnancy. Then 30 pregnant rats were divided into two groups as control and experimental. The experimental group was exposed to stress from the first to the end of the pregnancy. The stress was induced three times a day, each time lasting 45 minutes. 21 days after birth one animal from each dam was selected and its adrenal gland was removed for tissue processing, and sterological study. 12 sections were selected by random systemic sampling and the Cavalier's method was used to calculate the gland volume. The statistical analysis revealed significant differences between the total and absolute volume of glomerulosa, fasciculate, reticularis layers ( $P < 0.001$ ) and medulla ( $P < 0.05$ ) in control and experimental animals.

It can be concluded that prenatal stress causes total adrenal gland and cortical layers hypertrophy and reduces the volume of adrenal medulla.

#### P 626

##### **DGGE MUTATION ANALYSIS OF RET, VHL, SDHB AND SDHD IN A SERIES OF EXTRA-ADRENAL PHEOCHROMOCYTOMAS**

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Pheochromocytomas (PCC) are rare tumours that generally arise from the chromaffin tissue of the adrenal medulla, but approximately 10% may also develop from extra-adrenal tissues. Twenty-five to 30% of patients with adrenal PCC show germline mutations in either rearranged during transfection protooncogene (RET, associated with multiple endocrine neoplasia type 2), VHL (associated with von Hippel-Lindau disease), succinate dehydrogenase subunit B (SDHB) or succinate dehydrogenase subunit D (SDHD). So far, little is known about the occurrence of mutations in these susceptibility genes in extra-adrenal PCC, as only few studies have investigated these tumours in limited numbers of patients. Therefore we have investigated a large group of extra-adrenal PCC for the presence of mutations in RET, VHL, SDHB and SDHD. We have performed denaturing gradient gel electrophoresis (DGGE) mutation analysis on a series of 32 extra-adrenal PCC. Mutation analysis of SDHD showed two patients with previously described mutations D92Y and L95P in exon 3, whereas there were three patients with previously undiscrised SDHB mutations: S100F and S123P in exon 4, and C243S in exon 7. No mutations were found in RET and VHL. We conclude that SDHB and SDHD might be involved in the development of a small proportion (15%) of the extra-adrenal PCC, whereas it seems that VHL and RET play no role.

#### P 627

##### **EVALUATION OF HISTIDINE DECARBOXYLASE EXPRESSION IN NEUROENDOCRINE TUMORS OF DIFFERENT SITES BY IMMUNOHISTOCHEMISTRY AND REAL-TIME RT-PCR.**

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Immunohistochemical (IHC) expression of HDC has been proposed as a marker for normal ECL-cell of the stomach and related neuroendocrine tumors (NETs). HDC immunoreactivity (IR) has been recently detected in NETs other than gastric ECL-cell tumors, such as intestinal, bronchial and pancreatic well differentiated NETs (WDNETs) and in poorly differentiated neuroendocrine carcinomas (PDNECs) of the lung. To date, the only available anti-HDC antibody is a polyclonal directed against the whole recombinant protein. This raises the possibility of a cross reaction with DDC, which shares a large piridoxal-5'-phosphate-dependent domain with HDC.

Aim of this work was to study the HDC-IR in a series of 107 NETs and to validate the IHC results in a subset of cases by the analysis of HDC and DDC mRNA expression using quantitative real-time PCR technique.

We studied 51 WDNETs (8 gastric and 43 extragastric) and 56 PDNECs (11 of the lung and 45 extrapulmonary). Anti HDC antibody (Eurodiagnostica, Malmo, SW) was employed with standard immunoperoxidase technique on sections from formalin fixed and paraffin embedded tumor samples. Fluorescence-based real-time RT-PCR assay was performed on 37 cases (26 WDNETs- 7 gastric, 5 ileal, 7 pancreatic, 7 bronchial- and 11 PDNECs of the lung), using specific probes and primers for HDC and DDC.

HDC-IR was observed in 43/51 WDNETs (84%) with no significant differences among various sites. 32/56 PDNETs (57%) were HDC-positive, including all lung carcinomas and a fraction ranging from 33% to 55% from other sites. Quantitative real-time PCR showed an increased expression of HDC in HDC-IR gastric, ileal and pancreatic WDNETs, while DDC expression was absent or lower than in normal controls. By contrast, HDC mRNA levels were very low in HDC-IR bronchial carcinoids, while DDC was highly expressed in all cases. In addition, all lung PDNECs showed HDC mRNA levels below the normal control, whereas high levels of DDC mRNA were found in all cases.

This study shows that anti-HDC antibody against the whole recombinant protein cross-reacts with DDC. Therefore, HDC-IR in NETs should be critically evaluated and validated with a specific molecular approach. Our data demonstrate that HDC is expressed in ileal and pancreatic WDNETs, in addition to gastric ECL-cell tumors. On the other hand, HDC-IR in bronchial WDNETs and in lung PDNECs should be considered as a cross reaction with DDC.

#### P 628

##### **ANGIOGENESIS IN PITUITARY ADENOMAS BY VASCULAR ENDOTHELIAL MARKERS**

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##### **Introduction**

Angiogenesis is a dynamic process, essential for embryogenesis, morphogenesis, tumorigenesis and other biological processes, being the most important factor for growth and proliferation of tumors.

##### **Aim**

We proposed to evaluate angiogenic phenotype by quantified the vascular density for estimation the biological behavior and invasiveness to pituitary adenomas.

#### Materials and methods

For quantified the vascular density, we used histochemistry (Gomori stain) and immunohistochemistry analysis (for vascular endothelial markers CD31/CD34, FVIII and the most potent angiogenic molecule - VEGF).

#### Results

We have investigated 30 paraffin-embedded pituitary adenomas: 21 functioning and 9 non-functioning; 22 with local invasiveness, 16 invasive in cavernous sinus; 4 were recidives.

Immunohistochemical analyses of CD31/CD34, FVIII, demonstrate variable degrees of vascular density in different types of pituitary tumors. There were differences in the fields of the same tumor, and also in the different types of tumor, angiogenesis being more pronounced in the front of invasive than in the middle of tumor.

CD34 was expressed in 100% of cases, follow by FVIII in 83,3% of cases.

There is a good correlation between CD34 and FVIII expression.

We observed a positive correlation between vascular density and invasiveness, angiogenesis is more pronounced in invasive pituitary tumors.

VEGF expression is more prominent in certain adenoma subtypes; stronger in invasive pituitary adenomas.

#### Conclusions.

Analyses of vascular density may provide useful information regarding angiogenesis and their role in determining overall angiogenic phenotype and their tumor behavior.

#### P 629

### ROLE OF NITRIC OXIDE IN THE MODULATORY EFFECTS OF OVARIAN HORMONES UPON BEHAVIORAL INDICES OF ANXIETY IN FEMALE RATS

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Nitric oxide, as neurotransmitter and neuromodulator is considered to be involved in several physiologic processes within the central nervous system. The objective of the present study is to determine the influence of this molecule on the indices of anxiety regulated by ovarian hormones.

The study was conducted on 150 sterilized female rats. Estradiol Benzoate (10 ig/kg/SC), progesterone (25 mg/kg/SC), alone or both, were administered to the animals in different groups. Some groups received an additional dose of either L-name (60 mg/kg/IP) or L-arginine (100 mg/kg/IP) before the behavioral test. Then the anxiety indices based on open and closed arms exploration, were estimated in an elevated-plus maze test.

Finally, blood samples were obtained to measure serum nitrite-nitrate concentration (the metabolite of nitric oxide) using Griess reaction.

Our study showed an anxiolytic effect of progesterone and progesterone+L-name, along with a decrease in the nitrite-nitrate concentration. While the administration of estradiol alone or together with L-arginine showed an angiogenic effect and a reduction in nitrite-nitrate concentration.

The findings suggest the possible involvement of nitric oxide in the process of anxiety induced by ovarian steroid hormones.

#### P 630

### CHONDROMYXOID FIBROMA OF THE TIBIA. A CASE REPORT

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The chondromyxoid fibroma (CMF) is a rare benign osseous tumour. It represents less than 1% of the primitive osseous tumours and 2% of the benign osseous tumours. Its radiological aspect is strongly evocative. Its histological aspect is misleading sometimes of a chondrosarcoma or a chondroblastoma. The diagnosis confirmation rests on anatomo-radiological confrontation.

A 28 years old man has consulted for metaphyso-epiphyseal tibial osseous pains . X-Rays demonstrated a metaphyso-epiphyseal lytic excentric limited tibial image without periosteal reaction nor invasion of the soft tissue. The anatomopathological exam for surgical curettage confirmed the diagnosis of CMF.

The CMF is a rare benign osseous tumour. It poses problems of differential diagnosis with a chondrosarcoma. The anatomo-clinical and radiological confrontation is needed for diagnosis confirmation. The prognosis of this entity is good.

#### P 631

### ACTIVATION OF THE JAK1 – STAT3 SIGNAL TRANSDUCTION PATHWAY IS ASSOCIATED WITH PATHOGENESIS AND PROGRESSION OF HUMAN CHONDROSARCOMAS

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Introduction: Chondrosarcomas represent the second most frequent primary bone malignancies. However, comprehension of the molecular mechanisms underlying their pathogenesis is far from thorough. The Signal Transducer and Activator of Transcription (STAT) family proteins are transcription factors pivotal in mediating cytokine signaling. Among them, STAT3 is frequently activated in human malignancies and transformed cell lines and is involved in tumorigenesis. Activation of STAT3 is regulated by JAK1, a member of the Janus Kinases family proteins. Recent studies have demonstrated that STAT3 interacts with the promoter of c-fos proto-oncogene, enhancing its transcription. In vitro and in vivo data suggest that the onco-protein c-Fos is overexpressed in human chondrosarcomas.

Purpose: To test the hypothesis that functional upregulation of the JAK1 – STAT3 pathway and induction of c-Fos, are implicated in the pathogenesis and progression of human chondrosarcomas.

Materials and method: We employed immunohistochemistry to assess the protein levels of JAK1, its substrate STAT3, the phosphorylated/activated form of STAT3, p-STAT3 and c-Fos, in 21 benign chondroblastic lesions (14 osteochondromas, 7 enchondromas), 36 chondrosarcomas (13 Grade 1, 15 Grade 2, 8 Grade 3) and in normal cartilage.

Results: Positive immunostaining for JAK1, STAT3, p-STAT3 and c-Fos was observed in 94.4%, 100%, 97.2% and 94.4% of the chondrosarcomas, and in 42.9%, 61.9%, 23.8% and 42.9% of the benign tumors, respectively. Normal cartilage was devoid of these immunoreactivities. The cellular levels of all proteins were significantly correlated to each other (Spearman's Rho 0.699-0.892, p<0.001 for each correlation). Significantly higher expression levels of all proteins were detected in grade 3 compared to grade 2/1 chondrosarcomas, and to benign chondroblastic lesions (Mann-Whitney U test, p<0.001 for each tumor grade pair, Kruscal Wallis p<0.001).

Conclusions: Our findings provide novel evidence that the JAK1/STAT3 signal transduction pathway and the onco-protein c-Fos are functionally operative in the neoplastic change of chondroblastic cells and the development and progression of human chondrosarcomas. Evaluation of the

expression levels of c-Fos and JAK1 - STAT3 signalling cascade components, may facilitate an improved prediction of the tumor's clinical behaviour and potentially be exploited in designing patient-tailored treatment regimens.

#### P 632

### IMPLICATION OF JNK/ERK-AP-1/ -RUNX2 "CROSS-TALK" IN CARTILAGE LOAD-TRIGGERED CHONDROGENESIS

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**Introduction:** Mechanical loads influence chondro-osteogenesis and thus skeletal morphology. However, the exact mechanisms by which mechanical stimuli are transduced in chondrocytes remain obscure. Recent data have documented the involvement of the JNK and ERK MAPK signaling pathways and the transcription complex AP-1 in chondroblastic differentiation, proliferation and maturation. Furthermore, in vitro studies have demonstrated the paramount importance of the transcriptional regulator Runx2 in chondroblast biology.

**Purpose:** To explore and characterize the mechanotransduction events occurring in chondroblasts under altered functional loading. To this end, the roles of JNK/ERK-AP-1 signaling cascades and Runx2 were investigated.

**Materials and Method:** Fourteen-day-old female Wistar rats were divided into 2 groups: the first group was fed whole pellets (simulating physiologic mastication), while the second group was fed soft diet (lowered temporomandibular joint (TMJ) loading). Animals were sacrificed at 6, 12, and 48 h post-experiment initiation and biopsy material was obtained from their TMJ condyles at each time point. Immunohistochemistry was performed to evaluate the expression levels of pc-Jun (c-Jun's phosphorylated/active form), c-Fos (principal AP-1 partner of c-Jun), JNK2 (major c-Jun kinase), p-JNK (phosphorylated/active species of JNK2), p-ERK (ERK's phosphorylated/active counterpart) and Runx2, on each TMJ specimen.

**Results:** The protein levels of all the examined signaling mediators/effectors were markedly increased in animals fed with hard diet, throughout the experimental process (ANOVA,  $p < 0.001$  for all). Tukey post-hoc analysis revealed that protein expression levels were significantly higher in cartilage from the hard, compared to cartilage from the soft food group, at 48 and 12 h. The cellular levels of the proteins were positively and significantly correlated to each other (Kendall's tau = 0.601-0.812,  $p < 0.001$ ).

**Conclusions:** 1) Functional cartilage loading induces the MAPK cascades; 2) Coordinate targeting of AP-1 and Runx2 transcription factors by mechano-triggered JNK and ERK appears to "fine tune" chondroblastic differentiation/maturation; 3) Deciphering the molecular events underlying the mechanotransduction phenomena is important, in order to effectively combine mechanical therapy with pharmacological intervention to control cartilage growth, augment cartilage repair and prevent cartilage degeneration.

#### P 633

### MESENCHYMAL CHONDROSARCOMA – MULTICENTRIC OR METASTATIC LESION ATANCKOVIC Mirjana, SOPTA Jelena, NAGULIC Mirjana, TULIC Goran

We present a case of mesenchymal chondrosarcoma in a 25 year old man who had painless tumour of m.vastus lateris sinistri invented after trauma. Radiographic examination showed soft tissue mass with irregular calcification of femoral region.

Diagnostic evaluation disclosed a hematoma in obs., but MRI will show an aggressive soft tissue mass in the reg.femoris with areas of calcification.

One month later patient noticed a weal of eyelid inferior and swelling with protrusio bulbi oculi dextri. CT scans showed retrobulbar infiltrative mass soft tissue density. Tumor mass present at the right frontal sinus was 18-20 H.U.density.

Surgical resection was done at the same time of retrobulbar and reg.femoris tumors mass. Microscopically, tumours exhibited biphasic pattern, cellular and cartilage. The majority of the tumours were composed of sheets or aggregates of small round and spindl cells with hyperchromatic nuclei that resemble those of Ewing sarcoma. Somewhere cells were arranged in a manner suggestive of hemangiopericytoma. Poorly circumscribed cartilaginous areas are blend with the undifferentiated tumour cells. Rarely, the cartilaginous foci were well defined. The histological and imunohisthemical diagnosis was – mesenchimal chondrosarcoma.

Two months after operation it was marked on the skin small lesions of abdominal wall and reg. bucae. Pathohystological verification was done and diagnosis was – mesenchymal chondrosarcoma. At the same time on verthebral body of Th 2-4. Radiographic examination showed small osteolytic lesions, but those lesions didn't examine pathohystologicali. Chemotherapy (ADM) was apply 3,5 months after resection, but because of recurrens of retrobulbar tumour and its invasion at the cranium with simptoms of intracranial high pressure – patient died 8 months after diagnosis was made.

A critical step of presented case is to ascertain whether it is a multicentric soft tissue and bone mesenchymal chondrosarcoma or the first discovered localisation is primary tumour and the others - metastasis.

#### P 634

### CONDROBLASTOMA, A RETROSPECTIVE STUDY OF 13 CASES OVER A PERIOD OF 6 YEARS.

POP Doina, POP Florinel

Chondroblastoma is a rare benign tumor of the epiphysis encountered commonly in the 2nd decade benefiting exclusively from surgery.

Many histopathological pitfalls associated with this tumor make it a great mime of osseous tumors, especially of osteosarcoma, which is by far more frequent in this age interval and of giant cell tumor, sharing with it the same location. The extreme heterogeneity of this tumor coupled with its local aggressiveness, in long standing cases, awakes suspicion about malignancy. Because of extra compartmental involvement in 8 out of 13 cases, fine needle aspiration was the fist diagnostic procedure, which is a simple maneuver, sometimes in the range of the pathologist's competence and very often rewarding, if not with extreme precision of diagnosis at least as malignant/benign supposition.

Frequently our guess was giant cell tumor (5 out of 8 cases), and only in 1 case the chondroblastoma diagnosis was carried out in this stage of diagnostic procedures. In two cases the aspirate was very cellular, mitoses were noticed as well as chondro and osteoid matrices, facts that arose suspicion of malignancy and urged for biopsy. As for the biopsy the pathologist task was not easy. histochemistry ( Acid periodio-Schiff, Gordon-Sweet and Alcian Blau and immunohistochemistry( S-100 protein, neuron specific enolase, osteopontin, Ki-67 and PCNA) were performed and sometime contributory to the final right decision.

Those cases staged G1(Enneking), were submitted to surgical excision with thorough curetting after benign intraoperative frozen sections .

The definitive paraffin diagnosis was carried out after scrupulous sampling and integration of all histopathological aspects some of which could be very intriguing if taken apart. Our presentation intends to show most of them which are related to the presence of condro-osteoid matrix, metaplastic areas and mitotic activity.

Immunohistochemistry was of little help in differential diagnosis so that one's experience and radiological correlation are essential.

Our study tries to catch the many faces of this tumor and establish an algorithm of diagnosis emphasizing on radiological-histopathological correlation.

#### **P 635**

#### **PROTECTIVE EFFECTS OF VITAMIN D3 ON METHYLPREDNISOLONE ACETATE INDUCED LOSS OF BONE METABOLISM MARKERS AND BONE MINERAL DENSITY IN THE LUMBAR SPINE OF RAT.**

Aligholi Sobhani Ph.D., Fatemeh Moradi, MSc., Parichehr Pasbakhsh Ph.D., Iraj Ragerdi Kashani, MSc.

**Introduction:** Although some vitamins have been shown to prevent glucocorticoids induce osteoporosis in short time, the magnitude of this effect remains to be clarified. The aim of this prospective study is the evaluation of protective effect of vitamin D3 on methylprednisolone acetate induced osteoporosis in rats.

**Methods and Materials:** Total duration of the experiment was four weeks. Twenty-four male spargue Dawly rats (8 week old and 180 g weight) were randomly divided into four groups: Group A (n=6), was a base line control or normal animals. Group B (n=6), was treated only normal saline (0.9%), group C (n=6), was treated methylprednisolone acetate (0.2 mg/kg) subcutaneously for 4 weeks (3 times per a week) and finally group D (n=6) were administered methylprednisolone acetate resemble to group C and treated by Vitamin D3 (0.1 µg/kg dissolved in ethanol daily). For evaluation of biochemical agents changes in the serum, level of calcium, osteocalcine and acid phosphatase were measured before and after treatment. Also, bone mineral density (BMD) of lumber vertebrae was measured by dual energy x-ray absorptiometry (DEXA).

**Results:** The results showed that, the serum calcium level unaffected ( $P>0.05$ ) by methylprednisolone acetate in all groups before and after treatment, but, the serum osteocalcine level and bone mineral density of lumbar vertebrae were significantly ( $p=0.05$ ) decreased in group C compared with groups A and B. In group D serum osteocalcine level increased again significantly ( $p<0.05$ ) but increasing of bone mineral density and bone mineral content were not significant ( $P>0.05$ ). Also, the serum acid phosphatase level increasing in group C was treated by vitamin D3 (in Group D), but was not significantly ( $p>0.05$ ).

**Conclusions:** The findings of present investigation indicate that by using of vitamin D3 in methylprednisolone acetate treated rats could increase bone formation and decrease bone resorption.

**Key words:** Methylprednisolone acetate, Vitamin D3, Osteoporosis, Bone Markers Metabolism, BMD and Rat.

#### **P 636**

#### **FIBRONECTIN IN HUMAN BONE TISSUE REMODELLING**

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The extracellular matrix component fibronectin (fn) has fundamental functions in cell attachment, differentiation, proliferation and in cell migration. Embryonic forms of cellular fn, named EDA-fn and EDB-fn, are generated by alternative splicing. They are detected in proliferating tissue, wound healing and tumor invasion.

This study aimed to quantify fn and its splice variants in the most frequent highly malignant human bone tumor (osteosarcoma) in comparison to bone fracture healing tissue as a model for a physiological bone repair process.

**Material and Methods:** A real-time quantitative RT-PCR assay (LightCycler System, Roche) was developed to quantify levels of fn, EDA-fn and EDB-fn mRNA. Relative expression was correlated to cell proliferation and differentiation (detected immunohistochemically by Ki67 and CD31 marker) and to the level of osteoid formation.

**Results:** Both, osteosarcoma and bone fracture healing tissue, did express higher levels of the embryonic fn variants (EDA-fn, EDB-fn) compared to unspliced fn. No significant differences were found in the EDA-fn and EDB-fn mRNA expression comparing tissue remodelling in malignant or physiological repair processes. EDA-fn and EDB-fn expression in osteosarcoma did correlate to the osteoid formation.

**Conclusions:** Embryonic fn splice variants are a strong marker for active granulation and tissue remodelling processes in human bones comparable with wound healing processes. The stimulating effect of fn splice variants on reparative and proliferative processes can be found in physiological bone remodelling as well as in malignant transformation. EDA-fn and EDB-fn mRNA expression levels are a marker for the malignant differentiation in osteosarcoma.

#### **P 637**

#### **TGF BETA 1 INFLUENCE ON OSTEOBLASTS IN PATIENTS WITH NONSYNDROMIC CRANIOSYNOSTOSIS**

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Nonsyndromatic craniosynostosis (NC) is characterized by premature closure of cranial sutures in children. The aim of our investigation was to evaluate osteoblastic cell events that lead to NC. 10 children (from 8 months to 5 years old) incision biopsies were studied. All children had an isolated NC. Stenosed cranial sutures were removed during the cranioplastic operation, fixed, decalcified with the trilon B, paraffinized. Light microscopy, immunohistochemistry was performed with labeled, specific anti-TGF beta 1 and R1, Ki 67 antibodies.

We found mature osteons, fragments of the osteons, intermediate lamellas, osteon-like structures in the cranial biopsies. Osteoblastic cells lining the bone fronts were located on the inner side of sutures. We did not find in the biopsies the serrated margins of sutures in opposite to the control. We discovered the focuses of ossification as well as the areas of bone atrophy. Within affected sutures we revealed premature ossification, increased extent of subperiosteal bone formation and complete suture obliteration instead of connective tissue zone and osteogenic cells as seen in normal cranial sutures. The same criteria of the increased osteogenesis were seen about 3 cm from a closed suture. In conjunction with increased osteogenesis the morphological changes of the osteolysis were observed: the close situated serration resorption line, the dedifferentiation of the osteones,

the osteoclastic resorption, "oncosis." There were opened haversian canals and spaces in the inner plate of the bone.

The immunohistochemistry showed the expression of TGF beta 1 and R1 on the osteoblasts and overexpression of the same factor on the osteoblasts within the inner plate of the cranial bone. We found increased osteoblastic proliferation with deposition of Ki 67 in these areas. The TGF beta 1 is one of the factors regulating cell-to-cell and cell-to-matrix interactions, proliferation, differentiation and maturation of the osteoblasts. Also this factor stimulates osteogenic activity of osteoblasts and accelerates osteogenesis. The activated osteoblastic cells grew at a significantly increased rate and produced significantly more serum osteocalcin than the control (119,3 ng/ml vs. 31,5 ng/ml,  $p < 0,05$ ).

In general the morphological changes of the cranial bones were typical for cynostosis in all children, but the quantitative and qualitative features were different. Maximal TGF beta 1 expression in the cranial biopsies proposes a key role of this factor for NC due to stimulation of the osteoblast proliferation, differentiation and maturation. Our results indicate that an increased maturation of osteoblastic cells in the suture margins leads to the premature ossification in NC.

#### P 638

### CLINICAL AND PATHOLOGICAL ASPECTS IN DISTAL FEMORAL PATHOLOGICAL FRACTURES DUE TO METASTASIS

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The present paper analyzed the clinical, pathological and therapeutic aspects in a group of 15 patients with surgically removed metastasis at the distal femur level. The mean age was of 52,5 years. 9 patients showed a history of malignancy (lung carcinoma- 5 cases, larynx carcinoma- 1 case, breast cancer- 2 cases and prostate cancer- 3 cases). All patients were operated for the pathological fractures by tumor exeresis, cavity sealing with acrylic cement and internal fixation, with immediate mobilization. Histopathological examination showed similar differentiation degrees in metastasis and the primary tumor in 7 cases. 3 primary tumors were found while 3 metastasis remained with an unknown primary tumor. Even if the osteosynthesis was performed on pathological bone it insured the patient the required mobility that was balanced with the survival period.

#### P 639

### THE FRA-1, FRA2 AND JUNB, JUND TRANSCRIPTION FACTORS ARE INVOLVED IN THE SIGNALING CASCADE TRANSDUCING MECHANICAL LOAD IN CONDYLAR CHONDROCYTES

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Introduction: The chondrocytes of articular condylar cartilage proliferate, hypertrophy and finally undergo apoptosis, being replaced by osteoblasts. Mechanical loading influences the growth of the condylar cartilage, and the mechanical

environment of the temporomandibular joint (TMJ) regulates the biological and histological features of the cartilage tissue via differentiation of proliferative cells into mature chondroblasts. Animal studies have shown that changes in the amount of chewing force and reduced masticatory function result in alteration of the overall growth of the mandible, hence decreased condylar cartilage growth activity. The Jun (c-Jun, JunB, JunD) and Fos (c-Fos, Fra-1, Fra-2) family of proteins are components of the dimeric transcription factor AP-1. They are activated by extracellular stress and play critical roles in bone remodeling and programmed cell death/apoptosis.

Purpose: The aim of this study was to examine the implication of the AP-1 constituents Fra-1, Fra-2, JunB and JunD in the signaling pathway of mechanical loading in the condylar cartilage. Furthermore, the association of mechanical load to cell differentiation and apoptosis through the involvement of the above factors was investigated.

Materials and Method: Fourteen-day-old female Wistar rats were divided into 2 groups: the first group was fed hard food (simulating physiologic mastication), while the second group was fed soft diet (resulting in lowered TMJ loading). Animals from both groups were sacrificed at 6, 12 and 48 h after the experiment initiation and histologic sections were obtained from their TMJ condyles at each time point. The condylar cartilage of both groups was immunostained against Fra-1, Fra-2, JunB and JunD.

Results: Our data revealed up-regulation of all the examined members of the AP-1 transcription factor family in all stages of differentiation of chondrocytes derived from mandibular condylar cartilage.

Conclusions: Mechanical loading of condylar chondrocytes results in early apoptotic phenomena of these cells and subsequent replacement by osteoblasts, most likely affecting the overall growth of the condylar head. Elucidating the signaling pathways of mechanical load in condylar cartilage cells may unravel new routes for innovative treatment approaches of patients suffering from dentofacial deformities.

#### P 640

### CALCANEUS AS A LOCALIZATION OF SOLITARY UNICAMERAL BONE CYST

SOPTA Jelena, ATANACKOVIC Mirjana

The calcaneus is a skeletal site no so frequently affected by solitary unicameral bone cysts (SBC). In the period of 30 years at the Bone Biopsy Register of the Institute of Pathology, School of Medicine, Belgrade in calcaneus have been registered: 3 chondroblastomas, 5 osteochondromas, 2 morbus exostosis, 4 chondrosarcomas, 1 osteoid osteoma, 1 osteosarcoma, 1 Ewing sarcoma, 1 giant cell tumor, 3 aneurysmal bone cysts and 17 solitary bone cysts. Calcaneal SBC accounts 3,25% of SBC of all localizations and 44,7% of all diagnosed tumors in calcaneus.

The diagnosis of calcaneal SBC based on radiographic features was possible in almost all cases. On CT scans and MRI scans the cysts were filled with fluid. A CT scan showed a central hypodense area of -120 Hounsfield units representing fat in the fluid-fluid cyst (differential diagnosis intraosseous lipoma).

After curettage cystic membrane was pathological observed. Histological features of SBC in calcaneus, are slight different from SBC of long tubular bones. The cyst membrane is usually thinner (in 2 cases absent), the zone of bleeding are more marked (9 cases), with cholesterol crystals (7 cases). Histologic proof of cementum-like substance (8 cases) showed that calcaneal cysts are true SBC and excluded intraosseous lipoma. Cementum-like substance was diagnosed more frequent in calcaneal (43%) than in long bone (10%) SBC. In 2 cases were presented histological characteristic of superposed aneurysmal bone cyst.

In 80% of cases cysts were diagnosed on a characteristic and typical location- the trochlear processus, Ravelli's calcaneal triangle. The nature and clinical importance of calcaneal cysts is controversial and not clear. The risk of pathologic fracture is unpredictable and surgical treatment is not always necessary. SBC in calcaneus is "self-limited" disease, and in significant percent of cases were described spontaneous filling of the defect.

#### P 641

##### **HISTOPATHOLOGICAL CLASSIFICATION OF PERIPROSTHETIC LOOSENING MEMBRANES**

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**Aims:** To find a consensus on defined histopathological criteria for a standardized evaluation of the periprosthetic membrane from loose hip and knee endoprostheses.

**Methods:** Based on histomorphological criteria, four types of the periprosthetic membrane are defined: Wear particle type (detection of foreign body particles; macrophages and multinucleated giant cells occupy at least 20% of the area; type I); infectious type (granulation tissue with neutrophilic granulocytes, plasma cells and few if any wear particles; type II); combined type (aspects of type I and type II occur simultaneously; type III); indifferent type (neither criteria for type I nor type II are fulfilled; type IV).

Periprosthetic membranes from 370 patients (f=217, m=153, mean age 67.6 years, mean time to revision surgery 7.4 years) were analyzed according to the abovementioned criteria.

**Results:** Incidences of the histopathological membrane types: type I 54.3%, type II 19.7%, type III 5.4%, type IV 15.4%, not assessable 5.1%. The mean time between primary arthroplasty and revision surgery was: type I 10.1 years, type II 3.2 years, type III 4.5 years and type IV 5.4 years. The inter-observer reproducibility (three pathologists) was sufficient (87.1%, Cohen's kappa=0.805). The correlation between histopathological and microbiological diagnosis was high (89.7%).

**Conclusion:** Together with numerous co-workers, we propose a feasible classification system, enabling a standardized diagnostic evaluation of periprosthetic membrane samples. Furthermore, the importance of non-infectious, non-particle-induced prosthesis loosening (membrane type IV), which was observed in 15.4%, is highlighted.

#### P 642

##### **ONCOGENIC OSTEOMALACIA (OO): REPORT OF TWO CASES**

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Oncogenic osteomalacia (OO) is rare and characterized by vitamin D inefficiency, hypophosphatasia and loss of phosphate in urine. OO is mostly linked to benign mesenchymatous tumours. We report two cases.

The first concerned a woman of 67 who benefited from treatment for postmenopausal osteoporosis. The second one concerned a 52-year-old man who had an initial diagnosis of male osteoporosis. Both were treated by alendronate and vitamin D. Despite this treatment, these patients showed bone fractures and progressively increasing asthenia. New biological exams assessed dramatic hypophosphatemia and an

abnormal phosphate tubular absorption. Calcic metabolism was normal. A bone biopsy was performed on the woman showing osteoporosis and rickets. For each case, biological results allowed to exclude a parental etiology. Tumours were located using CT-scan. For the woman, the tumour measured 15 mm beneath abdominal subcutaneous fat. For the man the tumour was located under the sole of the foot and measured at 30 mm. Both tumours showed the same histological aspect. They were composed of fusocellular cells. These cells were not atypical. They were surrounded by an often mineralized myxoid stroma. Vascularization showed a hemangioperitic pattern. Bone pain, clinical signs and hypophosphatemia normalized a few months after removing of the tumour. Oncogenic osteomalacia are most often linked to benign mesenchymatous tumours mostly located in bone or soft tissue. They have several histological aspects. They secrete 'Fibroblast Growth Factor' FGF23 which leads to loss of phosphate in urine by inhibiting phosphate tubular reabsorption.

#### P 643

##### **QUANTITATIVE ANALYSIS OF PERIOSTEAL CIRCULATION IN LONG BONES**

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The important role that periosteum plays in healing bone injury and its use as a potential source of osteoprogenitor cells is of high clinical interest. Periosteum is important for bone vascularization and osteogenesis. Periosteal tissue is divided into an outer fibrosis and an inner - active and proliferative layer with intrinsic periosteal blood system. So far few data are available about the quality of periosteal circulation especially regarding different bones and different bone segments. Aim of this study was to quantify microcirculation of inner layer of intact periosteum. We examined periosteum of radius and tibia, taken from 4 sheep 1 to 2 years old. Material for microscopic examination was fixed in 10% neutral buffered formalin, decalcinated in a mixture of formic and sulfuric acid, embedded in paraffin and stained immunohistochemically for CD34 and von Willenbrands factor according standard protocol (DAKO, Denmark). Two samples from each periosteum, 1 cm length respectively, were processed. From each sample 5 fields were analyzed microscopically using a x25 objective and computer-based image analysis system (ISSA, VAMSTECH, Zagreb, Croatia). Periosteal thickness and capillary count were accessed. Capillaries were counted in inner periosteal layer in a determined field of 10 000µm<sup>2</sup>. Analysis of intact periosteal microcirculation yielded a mean capillary number of 5, and a mean periosteal thickness of 100µm. Our quantification of the periosteal capillaries indicates no significant differences between periosteum taken from radius and those taken from tibia. From our data we concluded that they represent a basis for studying periosteal capillary circulation during the process of healing or its damage caused by osteosynthesis.

#### P 644

##### **REPAIR OF BONE DEFECTS BY ANIONIC COLLAGEN MATRICES**

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The aim of this study was to evaluate the repair of critical-size bone defects by three anionic collagen matrices in rats. Samples of the collagen matrices with varying electric charge density were implanted in 4-mm cranial bone defects with empty defects as controls. Cranial bone was retrieved after 3,

7, 15, 30, 60 days and 1 year and submitted to radiological, histological, immunohistochemical and ultrastructural analysis. Texture analysis was done on digitalized microscopic images. Two of the matrices allowed a quick recovery of the bone structure. After two months, full recovery of the bone defects was seen. The collagen matrix with the highest electric charge density still had remnants at the periphery of the newly formed bone at the same period. The anionic collagen matrices were actively incorporated into the mineralized bone matrix, without signs of resorption prior to the mineralization process. At the beginning of the regeneration process insulin-like growth factor-I and bone morphogenetic proteins 2 and 4 were found in direct contact with anionic collagen matrices, revealing a possible mechanism of osteogenesis stimulation by the anionic collagen matrices. One year after implantation, all defects were fully recovered. Texture analysis showed decreasing values of entropy and the fractal dimension during the experiment. The changes of the fractal dimensions followed a power law for each matrix. Morphologically, the bone formed by the matrix with the highest charge density had a more mature aspect compared with the less electronegative matrices, which could also be demonstrated by comparison of the fractal dimensions. These results show that the anionic collagen matrices are effective osteoconductive biomaterials, showing a great potential for clinical application.

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#### P 645

##### **THE ROLE OF THE EXTRACELLULAR MATRIX IN THE BONE LAMELLA ARCHITECTURE**

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**Objective:** The bone lamella participates to the formation of two components of the bone, compacta and the spongy part of the bone. The bone lamella, a constitutive part of the bone extracellular matrix was studied using a microanatomic study of the endochondral ortomorphogenesis; a study of the bone lamellar elements in trabecular bones, and a study of the bone lamellar system in bones with mixed osteogenesis.

**Materials and methods:** 53 bones from the axial skeleton (vertebra, sternum), 54 bones from the lower limb skeleton (femur, tibia, calcaneus). Stains: HE, Van Gieson, Gömöri.

**Results:** The research on the extracellular matrix from the cortex of the tibia diaphysis showed us a continuity of the interosteonal collagen lamella fascicles with those from the osteon's periphery. This doesn't concord with the classic images. The collagen lamella fascicles' stereotopography can be studied using mathematical methods – the parametric equation of a Moebius band. The collagen fibers from the bone lamella in the osteons from compacta of tibia diaphysis are birefringent and parallel with each other. Some fascicles are tangent to the oval spaces.

**Conclusions:** The stereodistribution of the collagen lamella fascicles is variable with the osteogenesis and the mechanic forces hereupon the bones are or will be submitted. The application of the mathematic methods of algebra topology and the finite element opens new ways in the modeling and the creation of abstractisation of the bone lamella system, on one way and in the evaluation of the hamato-pathological lesions on the bone biopsy on the other way.

#### P 646

##### **EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION IN CHORDOMA**

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Epidermal Growth Factor Receptor (EGFR) overexpression has been found in various tumors with poor outcome.

Chordoma is a neoplasm arising from the embryonal rest of the notochord. The most common locations are both ends of the spine, sacral bone and head and neck locations. Surgery and radiation therapy are the most commonly used treatment modalities. Purpose of the study was to evaluate an EGFR expression status in 21 cases of chordoma diagnosed at the MCS Cancer Center Institute between 2002 and 2004. Immunohistochemistry with anti EGFR antibody as well as Fluorescence In Situ Hybridisation (FISH) were utilised on the slides obtained from the paraffin blocks. Ten cases showed strong expression of EGFR receptor, eleven cases were negative. At the time of the evaluation, six cases with EGFR expression presented with metastatic disease. Four cases negative for EGFR expression showed metastatic disease. In many cases of chordoma surgical and radiotherapy treatment is not effective. New treatment modalities are needed. Anti EGFR (cetuximab) treatment approach might be a supplementary method.

#### P 647

##### **GIANT CELL TUMORS OF THE BONE: A RETROSPECTIVE STUDY OF 33 CASES.**

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**Introduction:** Giant cell tumors (G.C.T) of the bone are rare. They represent 5 to 10% of all primary bone tumors and 20% of benign lesions. They are often locally aggressive neoplasm characterized by large numbers of uniformly distributed, osteoclast-like giant cells and a more diagnostically pertinent background population of plump, epithelioid to spindle mononuclear cells.

**Aims:** To describe the epidemiological, clinical, radiological features and test the relationship between histological grading of Jaffe et al., established in 1940, and the prognosis.

**Materials and methods:** Thirty three cases of G.C.T of the bone were examined during 28-year period from 1970 to 1997. After studying the epidemiological, clinical and radiological features, the histological criteria of Jaffe et al. (1940) were performed for each case.

**Results:** Thirty three cases of G.C.T were retrieved with a range of age between 16 and 66 years with an average of 32.8 years. Female outnumbered male (23 vs. 10). Ninety-four (94%) were located in the long bones, the most frequent sites being the lower end of the femur (10 cases), the upper end of the tibia (6 cases) and the lower end of the radius (6 cases). Twenty cases (64%) occurred around the knee. Radiologically, 24 cases (77.4%) occupied the epiphysial and metaphysial regions. The tumor was centrally placed in 23 cases (69.7%) and eccentrically placed in 10 cases (30.3%). The tumor showed an expanded lytic lesion with fine to moderately coarse trabeculations in 26 cases (78.8%) and a pure lytic appearance in 7 cases (21.2%). Histological grading of Jaffe et al. resulted in 12 cases of grade I, 20 cases of grade II with 3 cases of grade II+ and 1 case of grade III. Statistical analysis revealed no significant correlation between all morphological parameters and prognosis.

**Conclusion:** Our epidemiological, clinical and radiological findings are in agreement with published data. Furthermore, there is no relationship between the histological grading and the prognosis.

#### P 648

##### **A RETROSPECTIVE MULTIVARIATE ANALYSIS OF MYXOFIBROSARCOMAS: WITH A SPECIAL**

### EMPHASIS ON MARGIN STATUS AS A CRITICAL PROGNOSTICATOR

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**Introduction:** Myxofibrosarcomas (MFS) are characterized by tumor progression with increased metastases after relentless local recurrences (LR). It remains challenging to predict outcomes without a prudent survival analysis to assess parameters of primary lesions. Previous series suggested that LR could not be predicted by any factor, whereas few have uniformly examined prognostic factors by appropriate multivariate analyses. **Purpose:** The aim of this study was to identify critical clinicopathological variables related to outcome by Cox regression model. **Methods:** 70 primary MFS were retrospectively identified after review and graded by the FNCLCC scheme. Survival was compared by log-rank test with regard to recurrence-free survival (RFS), metastasis-free survival (MeFS), disease-specific survival (DSS), and overall survival (OS). **Results:** Follow-up (FU) was obtained in 61 cases with a median duration of 32 months (range: 2-201). Among them, there were 32 men and 29 women with a median age of 61 years. The size of primary tumors ranged from 1.5 to 24 cm (median, 5.5). The surgical margins were considered positive in 21 cases (34%). At last FU, 27 patients developed at least 1 LR, 14 patients experienced distant metastasis, 13 patients died of MFS, and 4 patients died of other causes. The cumulative 3-year rates of RFS, MeFS, DSS, and OS were 51%, 72%, 80%, and 74%, respectively. By univariate analysis, positive margins ( $p=0.0003$ ) were the only significant factor for worse RFS. Mitoses;  $\geq 20/10\text{HPF}$ , high grade (grade 2,3), positive margins, tumor necrosis ( $\geq 10\%$ ), prominent nuclear pleomorphism, and AJCC stage 3 were significantly correlated with MeFS, while factors associated with reduced DSS and OS included the former 3, together with tumor necrosis and nuclear pleomorphism found respectively significant for DSS and OS. By multivariate analysis, only high mitoses ( $p=0.0009$  for DSS;  $p=0.0034$  for OS) and positive margins ( $p=0.0203$  for DSS;  $p=0.0034$  for OS) remained independent for poorer patient survival. However, tumor necrosis ( $p=0.0053$ ) was independently associated with MeFS, apart from high mitoses ( $p=0.0086$ ) and positive margins ( $p=0.0142$ ). **Conclusions:** Margin status is a critical prognostic determinant in MFS, not only proved as the single significant factor for reduced RFS but also independently predictive of metastatic propensity and patient survival as is high mitotic activity. However, tumor necrosis;  $\geq 10\%$  independently predicts worse MeFS alone.

#### P 649

### CALCIFYING APONEUROTIC FIBROMA. THE FIRST CASE REPORT COMPRISING COMPLETE HISTOLOGICAL, IMMUNOHISTOCHEMICAL, ULTRASTRUCTURAL AND CYTOGENETIC CHARACTERISTICS OF TUMOUR AND A REVIEW OF THE LITERATURE

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**Introduction:** Calcifying aponeurotic fibroma (CAF) is a rare soft tissue tumour that primarily occurs in children and adolescents and has a strong predilection for hands and feet. It has been described as locally invasive, recurring tumour without the ability to metastasize. Since initial report by

Keasby, more than 100 cases have been documented in the literature. However, only a few reports presented histological, immunohistochemical and ultrastructural characteristics of the tumour.

**Aims:** The aim of the study is a presentation of CAF with morphological, and cytogenetic analysis of tumour. This is the first description of ultrastructural features of CAF in childhood, as well as the first note concerning cytogenetic study of the tumour.

**Material and methods:** A 2 year-old girl was operated on because of a nodule, 2.5 cm in diameter, localized in soft tissue of hypothenar of the left hand. Surgical exploration of the lesion revealed a rather dull-separated tumour attached to ulnar nerve and vessels. The mass was excised non-radically. Small fragments of tumour tissue were taken for cytogenetic and ultrastructural studies. Finally the material was fixed in 10% buffered formalin's solution, and embedded in paraffin blocks. Paraffin-embedded material was examined histologically and with immunohistochemistry, using the LSAB2+ kit.

**Results:** The lesion was characterized by a fibromatosis-like proliferation of fibroblastic cells, admixed with cartilage-like islands with areas of calcification. Spindled fibroblasts infiltrated striated muscles as well as nerves and vessels, and they were usually organized in well-orientated fascicle. Chondroid-like foci were built of swollen cells lodged in lacunae. Despite the focal cellularity of the lesion, mitotic figures were scarce, and did not exceed 2 per 50 high-power fields. MiB-1 index reached 9.6. Both spindle and chondroid tumour cells were stained with antibodies against vimentin, H-caldesmon, CD99, and S100 protein (diffuse reaction), as well as smooth- and muscle- specific actin (focal reaction). On the contrary, they lacked keratins, EMA, desmin, BCL-2, CD34, CD57, ER, PR and P53 expression. On ultrastructural examination a biphasic texture consisting of fibroblasts and cartilage cells could also be distinguished. The karyotype of tumour cells was normal.

**Conclusion:** Our findings favor the origin of CAF from fibroblastic cells which differentiate into fibrocartilage and/or hyaline cartilage.

#### P 650

### SOLITARY FIBROUS TUMOUR OF THIGH AND INGUINAL REGION. THE HISTOLOGICAL, IMMUNOHISTOCHEMICAL, ULTRASTRUCTURAL AND CYTOGENETIC CHARACTERISTICS OF TWO CASES AND A REVIEW OF THE LITERATURE

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**Introduction:** Extrapleural solitary fibrous tumours (SFTs) are uncommon mesenchymal neoplasm. Slightly more than 100 cases were reported. Most of them were localized in the upper respiratory system and the soft tissues of head and neck region. SFTs of the extremities are exceptionally rare. We found only eight such cases in the available literature. They pose problems in the differential diagnosis, especially from synovial sarcoma.

**Aims:** The object of the study is the clinico-morphological characteristics of 11 (including our two cases) described SFTs of the thigh and inguinal region and the estimation of prognostic significance of selected morphological and cytometric parameters of the tumour.

**Material and methods:** A 61-year-old woman and a 39-year-old man were admitted to the Centre of Oncology with the circumscribed, palpable masses localized in soft tissue of the thigh and inguinal region. The tumours were totally excised and both patients live with no evidence of tumour recurrence

49 and 12 months, respectively. Tumour tissues for light microscopy were fixed in 10% buffered formalin and embedded in paraffin. Representative paraffin blocks were chosen for immunohistochemical studies. Small fragments of fresh tumour tissue were taken for ultrastructural, cytometric and cytogenetic studies.

Results: Histological texture of tumours was characterized by a combination of hypocellular and hypercellular areas separated from each other by thick bands of hyalinized collagen and hemangiopericytoma-like vessels. The tumour cells of both cases were positively stained with antibodies against CD34, BCL2, CD99, and progesterone receptor. The first tumour showed additionally the small foci of necrosis. The proliferative activity of tumours was variable. The cellular areas of the first tumour presented up to 13 mitoses per 10HPF and 11.0% MIB-1 index. FCM study of these cells revealed 7.2% S-phase fraction (SPF) and aneuploidy (DI=2.08). Conversely, the second tumour was diploid and characterized by low mitotic rate (6/10 HPF), MiB-1 index (7.2%) and SPF (0.3%). Cytogenetic analysis of this tumour showed 46,XX normal karyotype.

Conclusion: 1)High proliferative activity, aneuploidy, and focal necrosis do not have to indicate the malignant behaviour of SFT. 2)Lack of the (X,18) translocation facilitates the differentiation between SFT and synovial sarcoma.

#### P 651

##### ACRAL MYXOINFLAMMATORY FIBROBLASTIC SARCOMA

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INTRODUCTION:Acral myxoinflammatory fibroblastic sarcoma (AMIFS) was described as different entity in the larger group of myxomatous soft tissue tumors in 1997 and 1998 by three different authors. These tumors have quite characteristic clinical presentation and morphology. The lesion has morphologically myxoid or myxohyalen presentation and multinodular growth pattern. In this present study we report on three cases of AMIFS seen in our department to discuss their clinicopathological features and emphasize the rare occurrence of it's invasion to neighboring bone tissue.

MATERIALS and METHOD:We found 3 cases of AMIFS in our register in 1999 to 2004. We applied routine H&E sections and also immunohistochemical stains as vimentin, CD68, CD34, S100 protein, SMA and cytokeratin. Clinical features were obtained from archive records.

RESULTS and CONCLUSION: All of the cases were adult men of ages 29, 36, and 56. Lesions occurred in the 4.th and 5.th metacarpophalangeal joints of the right hand (first case), in the right elbow wrist (second case) and in the right toe thumb distal interphalangeal joint (third case). Tumors were composed of epithelioid to spindle cells containing myxoid zones and various inflammatory cells. In some foci, Reed-Stenberg like giant cells were seen. Immunohistochemically, tumors were diffusely positive for vimentin. Various numbers of cells were also positive for CD 68. Only one case was poorly positive for CD34 and SMA. All of the cases were negative for S-100. In contrast to the previous literature, we observed neighboring bone invasion in two cases. In follow up period, one case had regional lymph node metastasis. This newly described, both morphologically and clinically distinct tumor must be kept in mind when dealing with a tumor locating in distal extremities and must be in differential diagnosis with epithelioid sarcoma and myxoid or inflammatory type malignant fibrous histiocytoma.

#### P 652

##### A SINGLE-TEAM EXPERIENCE OF LIMB SPARING APPROACH TO ADULTS WITH HIGH-GRADE MALIGNANT FIBROUS HISTIOCYTOMA

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Introduction: Malignant fibrous histiocytoma (MFH) is the most common subtype of soft-tissue sarcoma (STS). When located in a limb, MFH, as well as other STS, are currently treated with limb sparing surgery (LSS) followed by radiation therapy (RT).

Patients: From October 1994 through October 2002, 42 adult patients with high-grade limb MFH were approached by LSS followed by RT.

Results: Our results reflect a single-team experience with multi-disciplinary limb sparing approach towards adult patients with limb high-grade MFH, and point to several important conclusions. High grade MFH can be treated by surgery and postoperative radiation therapy, and controlled to yield a 10-year relapse free survival of 62% and a 10-year overall survival rate of more than 80%. Recurrences of MFH tend to occur during the first 2 years of follow-up, and become less frequent thereafter. Relapse free survival was affected mainly by location in the lower limb versus the upper limb, irrespective of the tumor size. Patients who had their diagnostic biopsies in another medical center had a greater tendency to local and systemic relapse probably due to compartmental and systemic contamination with tumor cells. This observation supports the need for early referral of patients with limb masses suspicious of sarcomas, to a specialized center for careful diagnosis and multidisciplinary therapy.

Conclusions: Our results are satisfactory and are within the accepted ranges. It seems that the most important key words for disease-free survival is the team experience, starting with biopsy, surgery, and post-operative radiation therapy. All other factors are tumor-biology dependent, and thus far are beyond our control.

#### P 653

##### PRIMARY HEMANGIOPERICYTOMA OF THE TIBIA A CASE REPORT.

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Primary hemangiopericytoma of the bone is a very rare vascular tumor. It can occur any where but the most common sites are pelvis, proximal femur, humerus, rachis, sacrum and mandible.

We report a case occurred in a patient aged 53 years who consulted in June 2004 for pain of the left knee evolving since 3 years and swelling.

Standard X-ray showed a lytic metaphyso- epiphysary tumor of the upper of the left tibia with cortical permeation and extension into surrounding soft tissues and fibula.

Giant cells tumor and chondrosarcoma were evoked. Biopsy concluded to a grade 2 hemangiopericytoma.

Immunohistochemical staining showed no expression of FVIII, actin and CK by tumoral cells.

The tumor was treated by en bloc resection followed by a chemotherapy.

The result was good. The patient presented neither recurrence nor metastasis 6 months after the treatment.

#### P 654

##### PECOMA OF ABDOMEN: A UNIQUE CASE

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**BACKGROUND:** The World Health Organization recently recognized a family of neoplasms showing at least partial morphological or immunohistochemical evidence of a putative perivascular epithelioid cell (PEC) differentiation. These tumors include angiomyolipoma (AML), clear cell 'sugar' tumors of the lung (CCST), lymphangiomyomatosis (LAM), clear cell myxoid melanocytic tumors of the falciiform ligament and distinctive clear cell tumors at various other sites. Pcomas are rare neoplasms of pericytes, mainly affect young women. Pcoms have been identified in uterus, lung,, vulva, breast, prostate, rectum, jejunum, liver, cardiac septum, falciiform ligament soft tissue and in the abdomen with tuberous sclerosis.

**CASE PRESENTATION:** A 29 years old woman without previous history was admitted with a painless palpable mass in her abdomen. Laboratory tests were normal apart of ECHO revealing a mass up to 8cm in her abdomen. The mass were easily identified and removed by laparoscopy. There were no sign of Tuberous Sclerosis.

The tumor was well circumscribed, encapsulated, measured 8x5x3cm. Cut surface was red, nodular, with visible blood vessels. Microscopy revealed a well developed vascular network in the vicinity of which a pleomorphic cellular component was recognised. Neoplastic cells at the immediate vascular location are epithelioid and spindle shaped whereas in areas away from vessels, resemble smooth muscle cells.

Epithelioid cells show variable morphology, some having clear cytoplasm and others eosinophilic with "ground glass" configuration. Most display small, normochromatic round nuclei and other have large oval nuclei with prominent nucleoli. Immunostaining shows diffuse positivity in HMB-45 and SMA focal for S-100, and negativity for cytokeratins and desmin.

Although most of these tumors have behaved in a benign fashion, they should be considered tumors of uncertain malignant potential given previous reports of recurrence and metastases. Our patient remains free of disease 36 months later.

#### P 655

##### **LIPOMATOUS HEMANGIOPERICYTOMA (ADIPOCYTIC VARIANT OF SOLITARY FIBROUS TUMOR) OF THE ORBIT**

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Hemangiopericytoma (HPC) of the orbit is a rare tumor presenting with slowly progressive proptosis, ocular motility impairment and visual loss. In 1995, a newly variant of HPC termed lipomatous hemangiopericytoma (LHPC), was described. Only two cases arising in the orbit have been previously reported.

The authors describe a new case of orbital LHPC arising in a 64-year old woman who presented a 1-year history of pain in the right orbital region and a progressive right proptosis. Computed tomography scans revealed a well-delineated extraconal mass of 3x3x1.5cm affecting the superomedial area of the orbit. No signs of bony erosion or globe indentation were visible. Histological examination revealed an entirely encapsulated lesion composed of a haphazard proliferation of

spindle, fibroblast-like cells alternating with hypocellular foci. The stroma was collagenous, with vascular spaces displaying a prominent hemangiopericytoma pattern (stag horn configuration) and lipomatous areas made of mature adipocytes was found. Mitotic figures were absent, and no cellular atypia, necrosis, or vascular invasion was observed. Immunohistochemical staining revealed an intense and diffuse positive reactivity to CD99, CD34 and bcl-2. The patient was free of recurrence when examined 9 months after surgery.

The aim of this study is to discuss the clinicopathologic features, including the immunohistochemical staining profile and ultrastructural appearance of this distinctive tumor, and briefly discuss the relationship between HPC and solitary fibrous tumor (SFT) of soft tissue, a neoplasm with many clinical and pathologic similarities.

#### P 656

##### **ANGIOSARCOMA OF BONE**

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The purpose of this work is to describe a rare case of an angiosarcoma of bone and to discuss about the low frequency and the variant behavior and histological appearance of the malignant vascular bone tumors.

**Method and results:** A male 47 years-old with a 4-year history of lower back pain was operated for a lumbar disk herniation, but during his transportation from the operation theater a pathological intertrochanteric fracture of the right hip was reordered.

The magnetic resonance imaging (MRI) showed a large soft tissue mass extending anteriorly with infiltration of the bone marrow to the cartilaginous level of the femur head. The histological examination revealed variable shapes of vascular anastomotic channels lined with large atypical endothelial cells with solid sheaths formations, pseudopapillary infolding arrangements and foci of necrosis. On immunohistochemical investigation many neoplastic cells were positive in factor-VIII and UEA-I endothelial markers. The treatment included a radiotherapy (300 rad/per day for 13 days) followed by an amputation and total hip replacement.

**Conclusion:** The malignant vascular bone tumors are very rare accounting less than 1% of the bone malignancies characterized by unknown etiology and variable biologic behavior and histological appearance.

#### P 657

##### **MORPHOLOGICAL FEATURES OF 23 MALIGNANT VASCULAR TUMORS**

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**Introduction:** Due to its rarity, information and experience about malignant vascular tumors are limited. Although many immunohistochemical techniques are available, determination of grade still requires a detailed morphological investigation. **Methods:** We present histological features of 23 malignant vascular tumors, 8 of which had a prior diagnosis of angiosarcoma. Tumor size, mitotic index, pleomorphism, cellularity, presence of necrosis and grade are determined for

each case. Chi square test and linear regression analysis are used and  $p < 0.05$  is accepted as significant.

Results: Age at diagnosis was 6-77 years with a median of 35 years and male to female ratio was 1.5. Extremities (8) were most common primary site following head and neck (5); sacrum (3); parenchymal organs including colon (2), liver (1) and spleen (1); abdominal wall (1); inguinal (1) and breast (1). Tumor size, varied 1 cm up to 20 cm in diameter (median 5.5 cm), was  $> 3$  cm in 14 of cases, 9 of which were high grade lesions. Infiltrative pattern (15/23) and epithelioid cellular characteristic (15/23) were dominant. Majority of lesions were hemorrhagic (83%) and necrotic (70%). Mainly, pleomorphism and cellularity have contributed to grade of tumor. Decision giving for high grade tumor was directly associated with high pleomorphism ( $p < 0.00$ ) and tumor diameter ( $> 3$  cm,  $p < 0.02$ ). In cases of intermediate pleomorphism, cellularity has assisted on grading. After a total reexamination of all 23 vascular tumors 11 of them were categorised as high grade lesions. Follow up information were available for 7 of 23 patients: Five of them were alive for 5 years and 2 of them, both having high grade tumor, were dead of disease in 2 years.

Conclusion: Following an algorithm as consideration of pleomorphism, size and cellularity of malignant vascular tumors has a key role in determination of grade, thus the diagnosis of angiosarcoma. Still the need for more objective findings such as molecular studies is obvious on the way to specific diagnosis of malignant vascular tumors.

#### P 658

##### **METASTATIC ANGIOSARCOMA ARISING IN AN EPITHELIOID HEMANGIOENDOTHELIOMA OF AN LIGATED ARTERIOVENOUS FISTULA IN A IMMUNOSUPPRESSED PATIENT AFTER KIDNEY TRANSPLANTATION**

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#### Introduction:

About one third of angiosarcomas develop in association with certain pre-existing conditions such as benign hemangiomas, synthetic vascular grafts or other foreign material. Immunosuppression in the setting of organ-transplantation is associated with the development of malignant tumors, most commonly carcinomas or lymphomas. Sarcomas are relatively infrequent. We report a male patient with chronic renal failure, who underwent kidney transplantation. The patient presented with an epithelioid hemangioendothelioma at the site of a ligated arteriovenous fistula constructed for haemodialysis at the left cubital region. Within 2 years a transformation into an angiosarcoma occurred. The patient died of multiple metastases.

#### Purpose of the study:

This is rare case of an epithelial hemangioendothelioma in an immunosuppressed patient at the site of an AV-fistula with malignant transformation into an angiosarcoma. It is important to keep in mind, that immunosuppressed patients can develop sarcomas in such circumstances.

#### Material and methods:

Formalin-fixed and paraffin-embedded archival material of biopsy and surgical specimen, as well as autopsy material of primary tumor and metastases were examined on HE-stained section and immunochemistry was evaluated using antibodies to CD31, CD34 and factor VII.

#### Results:

The initial biopsy and the surgical specimen showed a solid proliferation of a vascular tumor composed of short strands, cords and solid nests of rounded to slightly spindled eosinophilic endothelial cells, some of them with intracytoplasmic lumina containing erythrocytes. The autopsy specimens of the recurrent tumor at the primary site as well as

the metastases (lungs, bone, axillary lymph nodes) showed classical features of an angiosarcoma with positive staining with antibodies against CD31, CD24 and factor VII.

#### Conclusion:

It is important to keep in mind, that immunosuppressed patients in addition to carcinomas or lymphomas may also develop sarcomas. Immunosuppressed patients with ligated AV-fistula do have a higher risk to develop a malignant tumor in the shunt. Especially we would like to draw attention, that angiosarcoma may arise in benign or intermediate vascular tumors under such circumstances.

#### P 659

##### **EPITHELIOID HEMANGIOENDOTHELIOMA OF THE SOFT TISSUES IN A WOMAN'S ARM: A CASE REPORT AND REVIEW OF THE LITERATURE**

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Introduction: Epithelioid hemangioendothelioma (EHE) is a rare vascular neoplasm arising in soft tissue, bone, lung and liver. This tumor is often considered to have an intermediately aggressive nature and a long clinical course depending on the sites involved.

Aims: To present the clinicopathologic and immunohistochemical features of a rare vascular tumor and to review differential diagnosis.

Material and methods: A 44-year-old woman presented with an expansive soft tissue mass of her left arm. Tumor resection was performed with light microscopic and immunohistochemical analysis.

Results: Macroscopic examination revealed a fusiform perivascular mass measuring 22 / 13 mm and infiltrating the surrounding adipocytic and skeletal muscle tissues. Histological examination revealed short strands, cords or solid nests of rounded to slightly spindled or epithelioid eosinophilic cells. The stroma was hyalinized and focally calcified. Some tumor cells displayed endothelial differentiation as evidenced by intra-cytoplasmic lumina (vacuoles) containing erythrocytes. Mitotic activity was estimated as 1 mitosis / 10 HPF. Immunohistochemical study revealed tumor cells immunoreactivity for CD 31, Ulex europeus and vimentin.

To date, neither recurrence nor metastases have been observed 10 months after initial diagnosis.

Conclusion: EHE of soft tissue is a rare borderline vascular tumor with unpredictable prognosis. This uncommon tumor should be considered in the differential diagnosis of other tumors such as carcinomas and malignant epithelioid schwannoma. The role of immunohistochemistry is crucial.

#### P 660

##### **VASCULAR MALFORMATIONS:**

##### **CLINICOPATHOLOGIC CLASSIFICATION**

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#### Objectives:

The classification of vascular anomalies (vascular malformations and vascular tumours) has been a matter of debate. A first consensus was obtained at the International Society for the Study of Vascular Anomalies (ISSVA, Hamburg 1988) on separating infantile hemangiomas and other vascular tumours from vascular malformations. A common scheme of classification, based on the main vascular constituent was then adopted (Rome 1996). A more detailed classification of vascular malformations is still to be

achieved. We present the classification we have used for several years in our interdisciplinary group, based on clinical and pathological data.

Classification:

**A - Truncular vascular malformations**

- Affecting arteries, veins, or lymphatics
- Anomalies of length, diameter, course, or number
- Direct arteriovenous fistulas

**B - Tissular (non truncular) vascular malformations**

Capillary Malformations (Port Wine Stains and other telangiectasias)

Venous Malformations (common, nodular, with glomus cells)

Lymphatic Malformations (capillary type, venous type, cystic type)

Arteriovenous Malformations

Soft Tissue Angiomatosis

**C - Combined vascular malformations (associate 2 or more defined entities from A and/or B)**

Hemo-lymphatic malformation

Arteriovenous-lymphatic malformation

...

**D - Complex malformations**

One or more from (A), (B) or (C) may be associated with non vascular anomalies of bone, soft tissues or viscera in eponymous syndromes.

Sturge-Weber syndrome, Klippel-Trenaunay syndrome, Parkes Weber syndrome, Bean syndrome, Proteus syndrome, etc.

Conclusion: This classification has proved to be convenient for our clinical, imaging, and pathological practice. The clinical and pathological features of the main types of vascular malformations will be illustrated.

**P 661**

**CRANIAL FASCIITIS OF CHILDHOOD WITH UNDERLYING**

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A ten-year old girl presented with a spontaneous, rapidly growing but painless subcutaneous mass over the left temporal region of the scalp. An initial biopsy of the mass showed features of cranial fasciitis, a variant of nodular fasciitis first described by Lauer and Enzinger in 1980. Cranial fasciitis is considered a benign fibroblastic lesion of likely myofibroblastic origin but uncertain etiopathogenesis, though some authors have found an association with previous local trauma. Radiographically, there was a lytic skull lesion with destruction of both the inner and outer tables of left temporal bone. Computed tomography (CT) scan done to visualize extent of tumour showed intracranial extension and partial destruction of the greater wing of sphenoid but no midline shift. The tumour had destroyed the calvarial bone as well as part of the zygoma. Dura was pushed but not infiltrated. Peroperatively, there was a large, firm tumour of high vascularity. Cut section had a gritty feel and a 'honey-combed' appearance. Histopathological examination showed cellular septae separating angiectoid cavernous spaces without endothelial lining. Multinucleated osteoclast-like giant cells and reactive bone formation were present. A diagnosis of solid variant of aneurysmal bone cyst was offered. Features of cranial fasciitis were restricted to the superficial part of the lesion which also showed intercellular haemorrhages. The authors hypothesize that the cranial fasciitis in this child was a reactive response to intralesional haemorrhages from the aneurysmal bone cyst. Thus cases of cranial fasciitis must be thoroughly worked up to rule out other underlying pathology. The child is well eight months post follow-up. This is the first such report in world literature.

**P 662**

**ALTERATIONS OF THE  $\beta$ -CATENIN PATHWAY IN SARCOMAS OF THE PULMONARY ARTERY(SPA)**

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**Aims:** The Wnt signaling pathway plays an important role in cell fate determination mediated by stabilization of the  $\beta$ -catenin complex through APC. Thus,  $\beta$ -catenin activation is an important step in the tumorigenesis through induction of transcription and proliferation via c-myc, cyclin A and cyclin D1. Since activation mutation of  $\beta$ -catenin/APC has been shown to occur in a variety of tumors we investigated the expression and mutation of the involved molecules.

**Methods:** Expression of  $\beta$ -catenin, cyclin A, cyclin D1 and mismatch repair proteins hMLH1, hMSH2, hMSH6 were examined by immunohistochemistry in 18 SPA and microsatellite instability (MSI) using the recommended reference panel for colorectal cancer. In addition, sequencing of exon 3 of the  $\beta$ -catenin gene was performed. Subsequently, survival analysis was performed for  $\beta$ -catenin, cyclin A and cyclin D1.

**Results:** In 8 cases a membrane staining and in 3 cases additional cytoplasmic staining of  $\beta$ -catenin was observed. 1 case showed some cells with nuclear staining and 3 cases were negative. Mutation analysis of  $\beta$ -catenin revealed wildtype sequence of Exon 3 in all examined cases. Cyclin D1 was expressed in most cases while cyclin A was present in few tumors. MSI could only be detected in 2 cases of the SPA. Interestingly, this case also showed nuclear staining for  $\beta$ -catenin. In addition, 4 cases revealed a LOH at the APC gene locus. Nuclear expression of hMLH1, hMSH2 and hMSH6 was detected in 17 cases. Survival analysis revealed a significant better survival for cyclin D1 positive tumors.

**Discussion:** LOH of APC was the prominent observation in our series supporting the view that misfunction of this tumor suppressor gene may play a role in the tumorigenesis of some SPA. However,  $\beta$ -catenin showed only nuclear translocation in 1 case, which was the only patient who had also MSI. Thus, alteration of the  $\beta$ -catenin pathway and MSI seem to play a minor role in the proliferative activity of SPA in our series.

**P 663**

**PREVALENCE AND SEVERITY OF AA AMYLOIDOSIS IN RHEUMATOID ARTHRITIS - A RETROSPECTIVE CLINICOPATHOLOGIC STUDY OF 161 AUTOPSY PATIENTS**

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**Statement**

AA amyloidosis (AAa) may exist in different stages of its development at the time of death.

AAa is a progressive cumulative process in each individual patient. The different stages of AAa found at death in a population of RA patients represent the progression of the pathological process of amyloid A protein deposition, i.e. the chronological development of AAa can be followed by studying its characteristics in individuals who died in various stages of the disease.

The aim of this study was to determine

(1) the prevalence of systemic AAa in RA, (2) the mortality due to AAa, (3) the clinically missed diagnosis of AAa, (4) the severity (extent) of amyloid A deposits in RA patients.

#### Patients and Methods

A randomized autopsy population of 161 in-patients with rheumatoid arthritis (RA) was studied.

AAa was diagnosed histologically.

The mortality and the clinically missed diagnosis of AAa were analyzed retrospectively by reviewing the clinical records and histological slides.

The degree of AAa was evaluated semi-quantitatively: the numerical values of amyloid A deposits were summarized in different tissue structures of the heart, kidneys, liver, and lungs of RA patients with AAa.

#### Results

(1) Systemic AA amyloidosis was observed in 34 (21.1%) of 161 cases. (2) Seventeen (50 rel%) of 34 patients died of uremia. (3) In 9 (26 rel%) of 17 amyloidosis was recognized clinically. (4) The severity of AAa distributed according to the curve of Gauss. Arranged according to increasing values the cases of 'severity' showed an exponential growth curve.

#### Discussion

Only about one quarter of existing amyloidosis was clinically recognized.

AAa was recognized clinically in end stage of the disease, characterized by severe amyloid deposits, and with clinical evidence of renal insufficiency and uremia.

Clinical signs of renal involvement (nephrotic syndrome) refer to an advanced stage of amyloidosis, with a worse prognosis.

Early clinical stages characterized by minimal, or small amounts of amyloid deposits remained clinically undetected.

In the autopsy population of this study minimal amyloid A deposits indicate the early stage of the pathological process; these patients died of other diseases before they developed massive deposits of AAa. In other words, by determining the extent and location of amyloid A protein deposition in different structures of various organs, one may estimate the stage of AAa in a given case. Staging has a prognostic value.

#### P 664

##### A VERY CURIOUS LESION OF THE SCALP

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The presence of ectopic meningeothelial tissue on the scalp can occur under different forms: typical or rudimentary meningocele, meningeothelial hamartoma, primitive meningioma of the scalp, or extension of an internal or external skull meningioma on the scalp. Skin or subcutaneous hamartomas are extremely rare. A sixteen-month-old child presented with a hamartoma on the scalp with ectopic meningeothelial elements. Since birth, this child had an occipital middle cyst, evoking a meningoencephalocele with associated lipoma. Clinically, this was a non evolutive unique lesion, measuring 1,4 cm, well-demarcated, rounded and soft, with no modification of the skin. The RMI scan showed a middle nodular tumor with liquid aspect looking like either a meningocele or an epidermal cyst. The lesion was removed, and the post-surgical course was uneventful. Histologically, well-circumscribed mixture of vascular structures, conjunctive bundles and adipose cells was observed in the deep dermis, the entirety without specific architecture. The characteristic element was the presence of numerous epithelioid cells, set up in irregular bundles or clusters, closely mixed with dermis. Some seemed to merge with the vessels, and sometimes seemed to form anastomotic structures which can mime a malignant vascular tumor. Immunostains witnessed the meningeothelial origin of the epithelioid cells by the strong positivity of EMA and vimentin. Moreover, the negativity of

CD34, CD31 and cytokeratin dismissed a vascular origin. The purpose of this case is to remind of the histological criteria of this rare entity. It also helps us to distinguish it from an atypical vascular proliferation and even from an angiosarcoma. Mix of meningeothelial cells with conjunctive tissue and vessels, also allows to differentiate it from a cutaneous meningioma. On the histogenetic view, the presence of meningeothelial component could be explained by ectopic meningeothelial remnants on the scalp, and therefore must be considered as the part of an hamartomatous process.

#### P 665

##### RARE ASSOCIATION : OSTEOSARCOMA AND ANEURYSMAL BONE CYST

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The diagnosis of the lesions bony depends of the clinic, the radiology and the histology. However, the association of a malignant bony tumor and a pseudo tumor made this difficult diagnosis. The aneurysmal bone cyst is the result of a specific pathophysiologic change, which is probably the result of trauma or a tumor-induced anomalous vascular process. Osteosarcoma is rare preexisting lesion of aneurysmal bone cyst. A 38-year-old male presented with a painful progressive swelling in his right knee, without further complaints, which had been present for 5 weeks. Radiological examination was found to have a well-defined 5cm lytic lesion in the right tibial head and neck. The cortex was thinned. Surgical excision and histological exam showed a double tumorous proliferation:vascular and cellular. The diagnosis of aneurysmal bone cyst is the likeliest. However, an fibroblastic osteosarcoma cannot be eliminated. This rare association makes difficult to take therapeutic decision: curettage or amputation and chemotherapy. Bone tumors represent a group of tumors of various dignity. In spite of this, single tumor entities may display strong morphological resemblance to each other which can in turn result in deep difficulties in different diagnosis.

#### P 666

##### IMMUNOEXPRESION OF TNF-ALPHA AND P38 TRANSDUCTION PATHWAY MEMBERS IN NORMAL AND PATHOLOGICAL (BENIGN HYPERPLASTIC AND CANCER) HUMAN PROSTATE.

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#### P 667

##### AKT INHIBITION ENHANCES APOPTOSIS AFTER TNF-ALPHA ADMINISTRATION IN PROSTATE CELL LINE LNCAP BUT NOT IN PC3.

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INTRODUCTION: Akt/PI3K play an important role in TNF-alpha resistance because has been reported like a survival factor in addition to NF-kappaB. Akt interferes with the apoptotic machinery by phosphorylating thus sequestering the

pro-apoptotic Bcl-2 family protein, caspase-9 and promoting NF-kappaB-dependent cytoprotection via transcriptional activation of a plethora of downstream target genes. In neutrophils, PI3-K/Akt inhibition prevents p38 and ERK1/2 activation.

**PURPOSE:** The aim of this study was investigate Akt/PI3K role (pro- or anti-apoptotic) after TNF-alpha administration in prostate cancer cell lines (LNCaP and PC3).

**MATERIAL AND METHODS:** Two establish human prostate cancer cell lines LnCap (CRL-1740) and PC3 (CRL-1435) were used. TNF-alpha treatments were carried out with TNF-alpha at several concentrations and selectively with specific pharmacological Akt/PI3K (LY294002). Apoptosis determination was evaluated by DAPI and flow cytometry. MAPKs activation was measured by Western blot.

**RESULTS:** TNF-alpha induces apoptosis in LNCaP when we increased TNF-alpha concentration (10, 20, 50, 75, 100 ng/ml) while this treatment has no significant effect on PC3 cells viability. Akt inhibition by LY294002 showed a significative apoptosis increment in LNCaP but not in PC3.

**CONCLUSION:** Constitutively activate PI3-K/Akt promotes cellular survival and resistance to chemotherapy and radiation in prostate cancer cells. Furthermore, showed that Akt is involved in the activation of NF-kappaB by tumour necrosis factor alpha (TNF-alpha). In our study PI3-K/Akt seems to be involved in TNF-alpha apoptosis regulation in LNCaP and may be through different survival factors as NF-kappaB.

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#### **P 668**

#### **CYCLOOXYGENASE-2 EXPRESSION CORRELATES WITH LOCAL CHRONIC INFLAMMATION AND MICROVESSEL DENSITY IN BENIGN PROSTATE HYPERPLASIA AND PROSTATE CANCER**

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**BACKGROUND.** Chronic inflammation has been suggested to be linked to the development and progression of prostate cancer. Up-regulated cyclooxygenase-2 (COX-2), which can be induced by elevated levels of proinflammatory cytokines, may play a role in influencing cell proliferation, differentiation, apoptosis, or angiogenesis. This study aimed to derive data from human BPH and prostate cancer tissues to investigate whether chronic inflammation and angiogenesis were correlated with the expression of COX-2. **METHODS.** Detection of COX-2 expression was performed on a total of 88 patients, including 45 BPH and 43 prostate cancers. Double-IHC technique were used with the combination of: COX-2/CD3, /CD68, /CD20, to investigate the COX-2 expression and the relationship with chronic inflammation; COX-2/Ki-67 and /Bcl-2, to determine the proliferation or apoptosis; COX-2/ CK34/ÅE12 and /CK8, to detect the lay of epithelial cells; and COX-2/CD31, to show the microvessel density (MVD). **RESULTS.** A. In BPH, COX-2 expression was detected in prostate luminal epithelial cells in all 45 samples. The overall percentage of COX-2 positive glands was 7.5%, distributed as: 0.2% in normal prostate tissue, 25.7% in PAH, and 11.9% in simple atrophy. It was evidently that COX-2 expression was mainly found in atrophic glands, which commonly infiltrated with inflammatory cells. T-lymphocytes and macrophages were the predominant inflammatory cells related to the COX-2 expression. COX-2 expression was also associated with Bcl-2 immunostaining and the COX-2 positive epithelial cells had higher proliferation index. B. COX-2 positive staining was detected in 40/43 cancer samples with the very heterogeneous expression. Elevated COX-2 expression was associated with high Gleason score. COX-2-positive areas were noted with high T-lymphocyte and macrophage densities than COX-2 negative tumor areas. MVD were also found higher in COX-

2-positive areas than in COX-2-negative tumor areas.

**CONCLUSIONS.** This study demonstrates a novel relationship between COX-2 expression and the local chronic inflammation in situ. The data demonstrate that T lymphocytes and macrophages, by releasing pro-inflammatory cytokines, appeared to play an important role in the induction of COX-2 expression in prostate epithelium, both benign and malignant cells. It is likely that COX-2 may be an effective therapeutic target in prostate cancer treatment.

#### **P 669**

#### **THE KI-67 EXPRESSION COULD BE USEFUL IN THE DIFFERENTIAL DIAGNOSIS OF PROSTATIC INTRAEPITHELMIAL NEOPLASIA AND DUCTAL ADENOCARCINOMA**

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**Background :** Cribriform and /or papillary prostatic lesions observed on limited tissue, such as needle biopsy, can pose diagnostic dilemmas. One such area of difficulty is the distinction between papillary and/or cribriform prostatic high-grade intraepithelial neoplasia (HG-PIN) and ductal adenocarcinoma.

**Design :** Over 48 months, we identified 17 cases of ductal adenocarcinoma and 17 cases of HG-PIN from radical retropubic prostatectomy specimens. The HG-PIN lesions were in all cases associated with an acinar prostatic adenocarcinoma component. For each case, we evaluated the proliferative activity, assessed by Ki-67 immunohistochemistry. **Results :** The majority of ductal adenocarcinomas (82%) were composed of mixed papillary and cribriform patterns, with the remaining demonstrating pure papillary or cribriform patterns. The HG-PIN lesions showed a papillary, cribriform, or mixed papillary/cribriform architecture. The proliferative activity, defined as Ki-67 labeling index, was statistically higher in ductal adenocarcinoma (mean: 33%; range: 21%-66%) as compared to HG-PIN (mean 6%; range: 2%-15%), with no overlap in the Ki-67 indices (p,0.0001).

**Conclusion :** A combination of histologic features and measurements of cellular proliferation may be helpful to distinguish HG-PIN from ductal adenocarcinoma in limited prostatic tissue samples.

#### **P 670**

#### **DOES AMACR/P63 ANTIBODY COCKTAIL DETECT NON SIGNIFICANT PROSTATIC CARCINOMA? A CORRELATIVE STUDY BETWEEN BIOPSIES AND RADICAL PROSTATECTOMIES.**

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**Introduction :** AMACR/p63 antibody cocktail used in the context of atypical small acinar proliferations suspicious for malignancy provides an increased sensitivity in cancer diagnosis on prostate biopsies. However the risk to detect by this way non significant cancer is not estimated.

**Aim of the study :** to evaluate in radical prostatectomy the significance of prostate cancers that were diagnosed preoperatively using AMACR/p63 cocktail.

Methods : among the 525 prostate biopsies performed in year 2004, 37 cases were selected with cancer diagnosis made only after AMACR/p63 immunohistochemistry, 17 among these 37 cases were treated by radical prostatectomy and reviewed. Non significant cancer in radical prostatectomy was defined as an intraprostatic tumour with a Gleason score < 7 and a volume <0.5 cc.

Results : median number of biopsies was 21 (15-21) with tumour detected only in 1 biopsy (n=14), or 2 to 4 biopsies (n=3); Gleason score was 5 (n=1), 6 (n=15) or 7 (n=1), median total tumour length was 0.85 mm (0.4-4.6) with a median percentage involved of 0.3% (0.2-2.1). In radical prostatectomy, cancer was significant in 10 cases (Gleason score was 7 (n=9) or 8 (n=1), with a tumour volume above 0.5 cc in 5 cases (1.23-2.28), stage pT2 (n=8), pT3a (n=1) or pT4 (n=1) and positive margins in 3 cases). Cancer was not significant in other 7 cases but no stage pT0 was observed. Median PSA at diagnosis was 7.1 ng/mL in significant cancers (3.9-40) and 6.8 ng/mL in non significant cancers (5.2-14) without statistical difference.

Conclusion : the high rate of significant prostate cancer among those diagnosed preoperatively using AMACR/p63 immunohistochemistry confirms the strong interest of this ancillary technique in clinical practice.

#### P 671

##### **ROLE OF TOUCH IMPRINT CYTOLOGY IN THE DIAGNOSIS OF PROSTATE CORE NEEDLE BIOPSIES**

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Introduction: Patients at increased risk for prostate cancer with previously negative biopsies pose a diagnostic challenge.

Purpose: The aim of this study was to investigate the diagnostic accuracy of touch imprint cytology of core needle biopsies of the prostate.

Material and Methods: All patients were suspect for having prostate carcinoma. A total of 452 transrectal prostate needle biopsies were performed from 56 patients. The age ranged from 45 to 87 years, the median age was 68 years.

From each fresh biopsy cylinder two touch imprints were prepared which were stained with Diff-Quik and Papanicolaou methods. After formalin-fixation of the core biopsies a first histological diagnosis was made. The results of the touch imprints and the diagnosis of the first histological examinations were compared. Later on, up to 60 fine step serial sections from all biopsy cylinders, which were inconclusive or negative for carcinoma were prepared. The cytological and histological findings were evaluated by two pathologists.

Results: Needle biopsies: The routinely histologically examined slides of 352 biopsies and 259 touch imprints revealed no carcinoma. A carcinoma was diagnosed in 100 first histologically examined slides and in 193 touch imprints. In 93 touch imprints, a carcinoma was detected, which could not be identify in the first histological examination. While in the following serial sectioning in 336 slides no carcinoma was detected, 116 slides showed a carcinoma at deeper level. In 16 biopsy cylinders a carcinoma was diagnosed which was not identified in the first histological examination. In 77 imprints which were positive for carcinoma no carcinoma was detected even after serial sectioning.

Patients: While the routine histological examination of 27 patients revealed carcinoma, the touch imprint cytology showed carcinoma in 38 patients. In the following fine step serial sectioning of 29 histologically cancer-free patients, 26 patients remained to be negative. The fine step serial sectioning, however, displayed additional carcinomas in 3

patients who were initially negative in the first histological examination.

Conclusion: The additional use of touch imprint cytology of prostate core needle biopsies can improve the diagnostic accuracy. Touch imprint cytology of the prostate can particularly be useful as an adjunct in patients with repeated negative histology or in cases with previous inconclusive histological results.

#### P 672

##### **EXPRESSION OF E-CADHERIN AND PROTEINS FROM THE CYCLINE-DEPENDENT KINASE INHIBITORS (CDKI) FAMILY IN PROSTATE ADENOCARCINOMA (PAC)**

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INTRODUCTION: In the androgen-independent, metastatic PAC there is currently no successful therapy. Therefore we need to improve our understanding of markers which can be helpful in determining the metastasizing potential. It has been proposed that progression may be related to the presence of neuroendocrine differentiation, proteins from the CDKI family (p21, p27) and adhesion molecule E-cadherin. Decreased expression of this proteins may be associated with PAC invasion potential. The aim of our study was to evaluate if E-cadherin, p21 and p27 are valuable to identify individuals at risk for biologically active PAC.

MATERIAL AND METHODS: Archival tissues from 75 patients with PAC after total prostatectomy and 75 controls with BPH (and a 10-year PAC-free follow-up) were labeled for E-cadherin, p21 and p27. Analyzed neoplasms were subdivided into two groups: GL(5-6) (Gleason score 5-6) 48 cases (64%) and GL(7-9) 27 cases (36%). Immunoreactivity for E-cadherin was quantified using morphometric computer analysis. LM patterns were saved (mean 30 for case) and the percentage of cells with positive membranous reaction per 1000 cells was calculated. For p21 and p27 the percentage of positively stained nuclei was counted (per 1000). The results obtained were compared with the histological grade, Gleason score, staging system.

RESULTS: In BPH strong membranous reaction for E-cadherin was evident in all cases (in more than 80% of cells). Regions of PAC were less intensively stained. In the GL(5-6) the percentage of positive cells was above 80% in 34 cases (70.89%) when in the GL(7-9) group it was lower than 80% in 13 cases (48.74%) (p<0.036). Correlations between E-cadherin immunostaining pattern and histological malignancy were found. Decreased expression (less than 10% positively stained nuclei) for p27 was observed in 18 cases (66.71%) and 39 cases (81.25%) respectively in above mentioned groups (p<0.032). The percentage of positively stained nuclei was above 50% in all control cases. Loss of immunoreactivity was correlated with histological grading and staging. There were found no significant differences for p21.

CONCLUSIONS: The results obtained confirm the importance of E-cadherin and p27 as strong biomarkers for PAC. Decreased expression of this proteins is associated with aggressive phenotype in PAC and may provide additional information to decide which patients need more intensive monitoring or treatment (chemoprevention).

#### P 673

##### **EXPRESSION OF MOLECULES OF INTERCELLULAR INTERACTION IN PROSTATE CANCER**

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**INTRODUCTION:** It is known, that losses of an intercellular adhesion resulting in to disconnection of tumour cells, and also the changes of extracellular matrix can promote development of metastatic disease. It is doubtless, that the tumour cells at interaction, and also at construction of a stroma the same molecules use, as normal tissues, though the character expression of those or other factors in various phases of a cancerogenesis can be various. We investigated features expression of some adhesive molecules in prostate cancer.

**MATERIALS & METHODS:** Tissue samples were taken from 211 patients with prostate cancer, their age ranging between 51 and 82 years. Tissues sections were subjected to immunohistochemical study with a monoclonal antibody in to human ICAM-1 (clone My13), VCAM-1 (clone 1.4C3), CD31 (clone JC/70A), N-Cadherin (clone 3B9), P-Cadherin (clone NCC-CAD-299), E-Cadherin (clone HEC11), collagen I $\alpha$ 1 (clone C1Y 94), Laminin (clone 4C7), Tenascin (clone TN2) (Dako, Zymed). An EnVisionTM; +System (AES) complex staining kit (Dako) was used.

**RESULTS:** The obtained data allow revealing some differences of normal and tumour cells, and also nor-mal stroma and stroma shaped tumour. In particular, in samples of a normal tissue predominates expression of laminin,  $\beta$ - and  $\alpha$ -Cadherins in comparison with other molecules. We reveal ICAM-1 and tenascin rarely and absolutely misses V $\beta$ 1. At highly differentiated tumours reaction with laminin and collagen I $\alpha$ 1 increase reliable. At moderate differentiated tumours reliable decreases expression of  $\beta$ - and  $\alpha$ -Cadherins in comparison with a normal tissue and the expressiveness of reaction with CD31 and collagen I $\alpha$ 1 of increases. In poorly differentiated tumours cells demonstrate tenascin expression and absence or weak degree of reaction of remaining investigated molecules. It was founded that tumours demonstrating decreasing expression of cadherin ( $p=0,3$ ) and laminin ( $p=0,3$ ), and increasing expression of tenascin ( $p=0,05$ ) are accompanied by the more frequent development of the biochemical relapse during three years after radical prostatectomy.

**CONCLUSION.** Thus, for the prostate cancer characterisation the most important are CD31, cadherin, laminin and tenascin. The changing of their expression is accompanied by the increasing of the frequency of the development of the biochemical relapse.

#### P 674

##### **DIAGNOSIS OF LIMITED PROSTATE CANCER IN BIOPSY TISSUE: A BAYESIAN ANALYSIS.**

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**Background:** Currently the threshold in the histologic diagnosis (dx) of prostate cancer (ca) from atypia/suspicious (A/S) is not fixed. Emphasis is now given to use as many methods as possible to increase sensitivity in the dx of ca. The question becomes whether or not continuing this approach has merit with respect to improving patient outcome (PO). A Bayesian approach was used to analyze the question.

**Design:** The existing relevant literature was searched for the numbers needed for input into the problem. Unlike prior considerations of this question, the initial point to begin calculating probabilities was where a single pathologist identifies a focus of A/S vs. ca. or even misses an A/S focus. From this point the logical progression of types of studies to review include: pathologist reproducibility (repro.) in the dx of prostate ca, use of immunohistochemistry (ihc) in dx, prostatectomy findings (pf) given a dx of limited ca, findings on repeat bx after dx of A/S or benign dx with persistent increase in PSA, disease progression (dp) based on pf, dp

after biochemical failure, mortality/morbidity statistics, and watchful waiting (ww) as a tx.

**Results:** Studies of pathologist repro. in the dx of limited prostate ca are sparse. Repro. of the ihc methods currently being advocated to help in the decision of limited ca vs. A/S are virtually nonexistent. Possible coin flips therefore abound at the earliest phases of the problem. Then patients with a dx of A/S often show obvious ca after repeat bx., and conversely the dx of limited ca at bx often leads to insignificant ca found after prostatectomy. Ww in many clinical settings has not been proven to unfavorably compare with aggressive treatment. Pathologists rarely consider morbidity in their evaluation of patient outcome, and leave the input of this number as a blank.

**Conclusions:** At this juncture a defined threshold or set of specific criteria should be set before a dx of prostate ca should be given. Based on this study it appears that any questionable focus less than 0.5mm in a single bx core without Gleason grade 4 features could be regarded as A/S with ww recommended as tx. Diagnosis of ca in foci smaller than 0.5mm (GS  $\leq$ 6), despite many talented individuals feeling comfortable in doing so, should await better validation of technique.

#### P 675

##### **THE ROLE OF LAMININ-5, VLA-6 AND TENASCIN IN PROSTATE ADENOCARCINOMA SPREADING**

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**Introduction.** The process of tumor invasion is accompanied with the significant alterations in expression of different matrix components responsive for cell adhesion and cell migration and contribute to the carcinoma spreading. In prostate adenocarcinoma we investigated the expression of some matrix proteins and integrins have been reported to be highly expressed in many other cancers. The results were compared with respect to histological and clinical peculiarities.

**Materials and methods.** Cryostat sections and the primary cultures of prostate tissue from 20 patients were immunohistochemically examined using mAb against human tenascin, two forms of Fn, TSP-1, lam, lam-2, lam-5, VLA-2, VLA-6.

**Results.** Excepting laminin-5, VLA-6 and tenascin we didn't find any correlation with tumor characteristics and revealed variable patterns of expression of the other investigated antigens. All samples showed a complete loss of laminin-5 in focuses of invasion and in sharp contrast a strong positive staining of this antigen in adjacent hyperplastic benign areas. The loss or production of laminin-5 was exceptionally stable feature as it was confirmed in all primary cultures from histologically distinct zones. The immunostaining for VLA-6 was obviously positive in basal layer of all hyperplastic glands and generally negative or decreased in association with malignancy but it was newly observed and even further enhanced in only some cases at the invasive edge of tumor. Tenascin as well as VLA-6 showed positive staining in stroma of hyperplastic areas and it was highly prominent in a few samples, where it arose in epithelial carcinoma cells and partially increased in periglandular stroma of carcinoma foci. Analysis of the relationship of the VLA-6 and tenascin patterns with various clinicopathological characteristics and the patient state revealed that the tenascin presence in carcinoma cell cytoplasm and increased emergence of VLA-6 associated with lymph node metastasis and advanced clinical stage.

**Conclusion.** The complete loss of laminin-5 in prostate adenocarcinoma is a principal property of invasion and would suggest a less stable epithelial-stromal junction. Partial loss and later new reinforcing of VLA-6 and tenascin at the invasive margins of tumor could reflect a different stages of tumor growth or invasion and also indicate a contributive role of these antigens in migration of malignant cells and disruption of normal integrin signaling.

#### P 676

##### **EZRIN EXPRESSION IN PROSTATE CANCER AND BENIGN PROSTATIC TISSUE**

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**INTRODUCTION.** The membrane-linking protein ezrin is highly expressed in several types of human cancers and correlations between its immunoreactivity and histopathological data as well as patient outcome have previously been shown. However, such studies have not yet been done on human prostate cancer.

**PURPOSE.** This study assesses ezrin protein expression in a series of clinical specimens.

**METHODS.** Immunohistochemical analysis was used to characterize patterns of ezrin expression in prostatic carcinoma and benign epithelium in 103 radical prostatectomy specimens. Ezrin immunoreactivity (IR) was scored 0 to 3 (absent, weak, moderate or strong staining). Agreement between three independent observers was calculated.

**RESULTS.** Ezrin IR in prostate cancers was moderate or strong in 70% of specimens while negative or only weakly positive in benign epithelium. Interobserver agreement of IR score was substantial (mean weighted kappa 0.70, range 0.67 - 0.73). Ezrin expression correlated with Gleason score ( $p = 0.016$ ) and seminal vesicle invasion ( $p = 0.006$ ) but not with extraprostatic extension or margin status. No correlation with biochemical recurrence after prostatectomy was found ( $p = 0.19$ ). Urothelial and squamous metaplasia invariably showed moderate or strong ezrin expression. Epithelium of seminal vesicles and ejaculatory ducts was always intensely positive.

**CONCLUSION.** Ezrin was expressed in the majority of prostate cancers and correlated with adverse prognostic factors. Interestingly, high levels of ezrin IR were observed in benign metaplastic epithelium and in seminal vesicles.

#### P 677

##### **CO-OPERATION BETWEEN THE ANDROGEN RECEPTOR AND THE ERBB FAMILY PROTEINS IN ANDROGEN-INDEPENDENT PROSTATE CANCER**

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**Introduction:** The androgen receptor (AR) plays a crucial role in the development of androgen-independent prostate cancer (AIPC). There is also considerable evidence for significant activation of AR by growth factor receptors like the erbB

family. Preliminary results have shown that the modulation of the AR function is mediated by the erbB-2/erbB-3 pathway and not by erbB-1 (EGFR).

**Aim:** To analyse the relationship between AR and erbB-1-4 expression in relation to AIPC development.

**Material and methods:** We used indirect immunohistochemistry for determination of the above mentioned expressions in prostate cancer samples obtained from 147 symptomatic AIPC patients where prostatic biopsies were available both before the start of androgen deprivation and after AIPC development. Mild, moderate or strong membrane-specific staining was recorded semiquantitatively using H-score (intensity of staining 1-3 x % of positive cells). Relevant statistical methods were used to analyse the results.

**Results:** We found a statistically higher expression of erbB-1 and erbB-2 in cases with AIPC and a significantly shorter survival of patients with high expression of erbB-2 after AIPC development. The levels erbB-3 and erbB-4 proteins were unchanged. Expression of AR positively correlated with expression of erbB-2 in advanced cases with metastases.

**Conclusions:** These data suggest that the erbB family proteins pathway is a critical target in hormone-refractory prostate cancer and that pharmacological inhibition of these receptors may provide a powerful approach to treatment of advanced prostate cancers.

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#### P 678

##### **IMMUNOHISTOCHEMICAL EXPRESSION OF P21WAF1, P27KIP1, CYCLIN D1, P53 AND KI-67(MIB-1) IN PROSTATE INTRAEPITHELIAL NEOPLASIA AND ADENOCARCINOMA, WITH EMPHASIS ON PIN WITH MICROINVASION (PINMIC)**

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**INTRODUCTION:** Prostate intraepithelial neoplasia (PIN) is the most likely precursor of adenocarcinoma (PCA) in the prostate, but the morphologic process in which PIN become PCA is poorly understood. Therefore, PIN with microinvasion (PINmic) could be the intermediate step in this morphologic continuum.

**PURPOSE:** The aim of this study is to present the differential expression of some G1-S modulators of the cell cycle in PINmic as compared with normal prostate (N), PIN, and PCA.

Thirty-seven formalin-fixed, paraffin-embedded samples from radical prostatectomies which contained foci of N (n=37), PIN (n=32), PINmic (n=22) and PCA (n=18) were used. Immunohistochemical expression of p21, p27, cyclin D1, p53 and MIB-1 was quantitated by the grid counting method and the corresponding labelling indices (LI) were determined. Statistical analysis included one-way ANOVA and Scheffé's test for post-hoc multiple comparisons.

**RESULTS:** We found an increase in the mean LI for all markers from N to PIN, PINmic and PCA. Significant differences between N and PIN, PINmic and PCA were characterized by down-regulation of p27 and up-regulation of the other markers investigated (ANOVA). Multiple group comparisons showed differences between all groups for cyclin D1 LI (N: 1.45±0.76, PIN: 2.49±0.99, PINmic: 3.68±1.02, PCA: 4.76±0.82) ( $p < 0.001$ ) and p53 LI (N: 1.55±1.09, PIN: 4.90±2.03, PINmic: 5.36±2.06, PCA: 9.02±4.02) ( $p < 0.001$ ), with PINmic showing values between PIN and PCA.

**CONCLUSION:** G1-S phase modulators of the cell cycle are frequently altered in a continuum from N to PIN, PINmic and PCA. PINmic should be considered an intermediate lesion from PIN to PCA and therefore it could be included as a morphologic variant of PIN.

**P 679****FUSION OF MRI AND HISTOLOGY IMAGES IN PROSTATE CANCER: A HELPFUL TOOL FOR PROSTATE CANCER TREATMENT**

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Prostate cancer (PC) is the first cancer in terms of incidence and the second leading cause of death among men in France with 40309 new cases and 10004 deaths in 2000. Needle core biopsy is used for the diagnosis of prostate cancer by urologists worldwide. MRI has shown to be sensitive and specific for prostate imaging and is a useful diagnostic tool. It is critical to acquire that the best possible protocols for biopsy to improve PC treatment. The aim of this study was (i) : to compare retrospectively preoperative MRI images with histological data in order to improve pre-therapeutic disease staging, (ii): to evaluate feasibility and precision of a system of fusion of IRM and histopathological data and (iii) to collect data in order to constitute a 3D computerized prostate surface model with the major sites of prostate cancer, thus to improve biopsy, but also in order to perform medical interventions through the use of passive, semi-active, or active guiding systems.

This study has included 4 cadaver prostates and 8 specimen of radical prostatectomy. MRI imaging was available for all specimens. Cadaver prostates were examined by MRI after resection, patients who underwent prostatectomy had MRI examination before surgery.

Before examination of the prostates after the recommendations of the Stanford protocol, size, weight and volume were evaluated. To be able to reconstruct histology, parallel needles were inserted into the prostate from the apex to the base. These biopsy tunnels were labelled by ink. After staining of the histological sections, all slices were reconstructed and scanned. Contours of organ and tumor were defined. Using deformable modeling techniques, 3D computerized prostate surface models were reconstructed from prostatectomy specimens with localized prostate cancer. A 3D computer simulation system was developed to accurately depict the anatomy of the prostate and all individual tumor foci.

Registration accuracy determined on composite images was visually estimated to about 2-3mm. The composite image quality may be rather limited for MRI retrospective analysis. Composite images of histology/MRI have been defined, to improve the cancer detection. Histological/MRI fusion is still very promising to improve the pre-therapeutic staging of PC, to optimize the biopsy strategy by application of a computer aided system of reference, to increase positive biopsy rate. This may

**P 680****NEW DATA ABOUT ETHNIC PECULIARITIES OF LATENT CANCER OF PROSTATE OF THE INHABITANTS AROUND FORMER NUCLEAR TEST SITE (SEMIPALATINSK, EAST KAZAKHSTAN)**

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Introduction: The study of prevalence pathogenesis and morphogenesis of prostate cancer (PC) is relatively restricted in countries of Asia. Ethnic variations in cancer incidence in the region are unknown.

Purpose of the study: Study of ethnic peculiarities of latent cancer of prostate of the inhabitants of Semipalatinsk.

Materials and Methods: Morphological analysis of prostates was performed on sequential gross autopsy material of histotopographic sections among 778 inhabitants of Semipalatinsk region in eastern Kazakhstan. The population in this area consists mainly of Europeans (68.1%, mean age at death: 55.3 yrs) and Asians (31.9%, mean age at death: 45.4 yrs).

Results: Cancer of prostate was discovered in 223 cases, PC occurred 2.5 times more often among the persons of Europeans origin, than among the Asians (35.5% vs. 14.1%,  $P < 0.05$ ). Of interest also is that in Europeans PC incidence increased with age, while among the Asians it decreased up to the 7th decades, and later ( $P < 0.05$ ). HGPIN was also more frequent in the Europeans population than Asians (35.5% vs. 16.1%,  $P < 0.01$ ). However Asians had more HGPIN in the 4th and 5th decades and Europeans in the older population. Low Gleason scores (GS) were more frequent in younger Asians. Also Europeans tend to have more foci of cancer than Asians.

Conclusions: Ethnic peculiarities were discovered among the inhabitants of Semipalatinsk region, Kazakhstan with latent cancer of prostate. Asian patients have a lower incidence of PC that happens earlier in life and is less aggressive biologically. This is further evidence of ethnic predisposition to the cancer of this origin.

**P 681****NEUROENDOCRINE DIFFERENTIATION AND ANDROGEN EXPRESSION IN BENIGN AND NEOPLASTIC PROSTATE EPITHELIUM**

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Introduction. A substantial proportion of primary prostatic adenocarcinomas contain a subpopulation of malignant cells expressing a neuroendocrine phenotype. Neuroendocrine cells (NEC) are considered important for regulating prostatic cell growth and differentiation. Androgen, acting via the androgen receptor (AR), is associated with the development and progression of prostate cancer. We determined the incidence and pattern of distribution of NEC and AR in prostate hyperplastic and cancer tissue and correlated their expression with tumor differentiation and invasiveness.

Material and Method. Antibodies to chromogranin A (CgA) and neuron specific enolase (NSE) were used for the immunohistochemical detection of NEC in formalin fixed, paraffin embedded prostate tissue. We immunohistochemically evaluated 70 prostate cancer (PCa)-30 well, 20 moderately and 20 poorly differentiated-, 55 prostate intraepithelial neoplasia (PIN)- 30 PIN1, 10 PIN2, 15 PIN3- and 60 prostate adenosis (PA) specimens for CgA, NSE and AR expressing tumor cells. Neuropeptide immunoreactivity was scored as (-) no staining, (+)  $< 2\%$ , (++) 2-25% and (+++)  $> 25\%$  of isolated cells staining positive. AR expression was categorized according to the scoring of the estrogen receptor expression in breast cancer.

Results. Neuroendocrine differentiation (NED) (1% of tumor cells) was noted in 16% of well, 25% of moderately and 50% of poorly differentiated PCa specimens; in 16% of PIN1 and 66% of PIN3; and in 17% of PA cases. AR expression was

positive (weak-moderate) in 50% of well and 100% of moderately differentiated PCa, and moderate-strong in 75% of poorly differentiated PCa specimens.

Conclusions. NED is upregulated in prostatic carcinogenesis, with higher levels of expression in PIN and carcinoma compared with normal cells and hyperplastic tissue. In prostate cancer, NED was increased with PCa dedifferentiation; the same percentage was demonstrated in PIN, which in turn increased in high PIN grades. AR immunoreactivity was more pronounced in benign than malignant prostatic epithelium and became stronger as the tissue became increasingly neoplastic. No negative association between NED status and AR expression was noted in prostate carcinoma. The NEC may play a key role in the regulation of prostatic growth and differentiation.

#### P 682

##### **BASAL CELL CARCINOMA OF THE PROSTATE: REPORT OF ONE CASE.**

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#### INTRODUCTION

Basal cell carcinoma (BCC) is a very rare neoplasm, with only a few cases published in the literature.

#### CASE REPORT

A 51-year-old male presented with urinary obstruction and hematuria. Serum PSA was normal. The cystoscopic examination showed a nodule located in prostatic urethra that was biopsied and diagnosed as basal cell carcinoma of the prostate.

A radical prostatectomy was performed.

#### MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded tissue was available from the biopsy and the radical prostatectomy specimen.

Immunohistochemical analysis was performed using a universal second antibody kit that used a peroxidase-conjugated labeled-dextran polymer (EnVision, Peroxidase/DAB; Dako-Cytomation Glostrup, Denmark).

#### RESULTS

Histologically, there was a proliferation of solid nests of uniform basophilic cells with scant cytoplasm and large nuclei with prominent nucleoli. Mitotic figures were inconspicuous.

The immunohistochemical study showed strong and diffuse positivity for BCL-2 and a high proliferation index (MIB-1).

Cytokeratin -34betaE12 was focally positive. Cytokeratin -7 and p53 were also positive.

Prostate-Specific Antigen (PSA), Prostatic Acid Phosphatase (PAP), Leukocyte Common Antigen (LCA), chromogranin, synaptophysin, Neuron-Specific Enolase (NSE), S-100 and p63 were all negative.

Microscopic examination of the radical prostatectomy specimen showed a predominance of a basal cell type solid pattern, but focally there were some cribriform areas. The neoplasm involved more than one-half of the right prostatic lobe (pT2b) and contacted with the inked surface, but periprostatic adipose tissue and striated muscle were free of tumor. Perineural invasion was present. No vascular invasion was identified.

#### DISCUSSION

Basal cell proliferative lesions of the prostate include lesions ranging from the common basal cell hyperplasia to the uncommon basal cell carcinoma.

Histological features that help to distinguish benign from malignant lesions are the presence of atypical cells with large nuclei and prominent nucleoli showing an infiltrative pattern, perineural invasion, extraprostatic extension or invasion of the bladder neck, necrosis and stromal desmoplasia.

In the case described herein necrosis was only focally seen and the stroma showed areas of myxoid change.

However, the strong and diffuse positivity for BCL-2 and the presence of a high proliferation index are extremely helpful in the diagnosis.

#### P 683

##### **KI 67 AND P53 IMMUNOHISTOCHEMICAL EXPRESSION IN PROSTATE CANCER**

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Prostate carcinoma is the most common male cancer and represents a serious health problem.

The aim of this study is to correlate P53 tumor suppressor protein, with tumor grade and proliferative activity. Clinical data were obtained from case notes.

Materials and methods:

Paraffin-embedded samples from 23 patients with prostate carcinoma were assessed for pattern, Ki 67 and P53 expression. Biotinylated secondary antibodies and the streptavidin-biotin peroxidase complex were applied according to the manufacturer's instructions (LSAB2 kit, DAKO, Denmark).

Immunohistochemical analysis was performed for the expression of Ki 67 and p53. Target retrieval solution was used.

Results and Conclusions: All the carcinomas expressed Ki67, 12 having > 25% nuclear marking. Four patients who were strongly positive for p53 and had a high Ki67 score survived only one year after diagnosis. Survival ranged from 1 to 13 years after diagnosis.

This study confirms that the expression of P53 correlates with Ki67.

#### P 684

##### **PROLIFERATING CELL NUCLEAR ANTIGEN ANALYSIS IN PROSTATIC NEEDLE BIOPSY – CORRELATION WITH PSA**

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Effective management of prostate cancer is dependent on adequate pathological reporting of the surgical specimen.

Immunohistochemistry has become an important tool in surgical pathology and the evaluation of PCNA and PSA, specific immunohistochemical marker for tumors of prostatic origin, became now routine practice.

Aims: To evaluate the expression of PCNA and PSA in prostate needle biopsy specimens and transurethral resection of the prostate and correlation with tistular PSA and other prognostic indicators including age, Gleason score and surviving period for some patients.

Materials and methods:

Biological markers (PSA, PCNA) were performed on 61 patients with prostate cancer. Biotinylated secondary antibodies and the streptavidin-biotin peroxidase complex were applied according to the manufacturer's instructions (LSAB2 kit, DAKO, Denmark).

Diaminobenzidine was used as a chromogen for visualization. The slides were counterstained with light hematoxylin.

Results and Conclusions:

PSA immunoreactivity decreased in adenocarcinomas with higher Gleason score.

PCNA increases progressively from well differentiated through poorly differentiated cancer; correlation with cancer stage is strong. In the less differentiated cases, the percent of positive nuclei is higher than the average. PCNA correlates with clinical stadialization and degree of tumor metastasis.

#### P 685

##### **VIRTUAL SLIDES QUALITY ASSESSMENT TEST FOR EARLY PROSTATE CANCER ON A NATIONWIDE SCALE IN FRANCE**

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**Background:** PSA screening increases the number of cancer cells detected at a low stage (pT1c), with only small foci found on biopsy specimens. In order to reduce the number of potential over- and under-diagnoses, a study was carried out through a quality assessment test on a nationwide scale in France on virtual microscopic slides.

**Materials and methods:** Twelve cases of prostatic specimens (biopsies, TRUP) displaying carcinoma and their mimics were selected: prostatic carcinoma (n=6), mimickers of cancer (n=5), high grade PIN (n=1). Among carcinoma there were Gleason score  $\leq$  6 (n=2), Gleason score  $>$  6 (n=4), with small focus (n=5). Mimickers were atrophy (n=2), cribriform clear cell hyperplasia (n=1), basal cell hyperplasia (n=1), and sclerosing adenosis (n=1).

For each case an H&E slide was digitalized on a CD-ROM with the NAVIQAP® virtual slide system from Samba Technologies.

The CD-ROM was sent to 191 junior or senior pathologists scattered all over the country. The answer form included main items like benign lesion, prostatic carcinoma, need for new biopsy, and secondary items for the sub-classification of the lesions and the Gleason score in case of malignancy. After the deadline for the responses, each participant was given access to the results and comments on the CD-ROM.

**Results:** 130 answers were analyzed. Some mimic lesions were misinterpreted as carcinoma: sclerosing adenosis (37%), atrophy (2 -10%), basal cell hyperplasia and cribriform clear cell hyperplasia (2% of the participants). HGPIN was misinterpreted as a benign lesion by 13%, and over-diagnosed as carcinoma by 5% of the pathologists. Small foci of carcinoma were misinterpreted as benign lesions in respectively 6, 7 and 18% in Gleason score  $>$  6, and in 40 and 48% in Gleason score  $\leq$  6. Ductal carcinoma was misinterpreted as a benign lesion by 5% of the participants.

**Conclusion:** This study demonstrated the ability to test on a large scale the pathologist accuracy on usual prostate lesions. Its results can be used to focus teaching efforts on obvious potential mistakes in a "the real life" setting.

#### P 686

##### **HER-2/NEU ONCOGENE EXPRESSION IN PROSTATE CARCINOMA: EVALUATION OF GENE AMPLIFICATION BY FISH**

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**Introduction:** The variability in the clinical and biological behaviour of prostate cancer and the inadequacy of markers used for assessing prognosis leads to investigate for new prognostic parameters. There has been an increasing interest on Her-2/neu oncogene and studies which investigate its role in prostate carcinomas have given variable results.

**Aim:** The aim of the present study is to investigate whether Her-2/neu oncogene has a role in the initiation and progression of prostate carcinoma.

**Methods:** Radical prostatectomy specimens from 74 patients who have prostate carcinoma were included in the study. Tissue array blocks were constructed by taking 2-6 cores 4mm in diameter for each tumour specimen using punch biopsy needle. Overexpression of Her-2/neu protein was assessed in areas of carcinoma, low grade intraepithelial neoplasia (LGPIN), high grade intraepithelial neoplasia (HGPIN), and benign prostate hyperplasia (BPH) by immunohistochemistry (IHC). Fluorescence in-situ hybridization (FISH) method was used to investigate Her-2/neu gene amplification in areas of carcinoma in 56 cases. The results of both methods were compared in correlation with gleason grade, stage of tumour and preoperative serum prostate specific antigen levels.

**Results:** Her-2/neu protein was expressed in a cytoplasmic/membranous manner in the study cases. The expression of Her-2/neu oncoprotein significantly increased from areas of BPH to PIN and reached the highest degree in areas of carcinoma (p=0). Her-2/neu overexpression by IHC was observed in 66,21 % of prostate carcinomas while Her-2/neu gene amplification by FISH was present in 4,05 % of prostate carcinomas. There was no relationship between the ratio of Her-2/neu protein expression or gene amplification and gleason grade, stage of tumour and preoperative serum prostate specific antigen levels.

**Conclusion:** The degree of expression of Her-2/neu oncoprotein increased from areas of BPH to PIN and reached the highest degree in areas of carcinoma by IHC though no correlation with gleason grade, stage of tumour and preoperative serum prostate specific antigen was found. Her-2/neu gene amplification was observed in very few of the carcinoma cases, thereby making it difficult to comment on the significance of Her-2/neu protein expression in prostate carcinoma. Therefore, studies on larger series of prostate carcinoma representing different stages are necessary to clarify its significance.

#### P 687

##### **THE SIGNIFICANCE OF THE IMMUNOEXPRESSION OF KI 67 AND P21 IN PROSTATE BIOPSY TISSUE**

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The diverse clinical outcome of patients with prostate cancer necessitates additional examination of biopsy tissue to achieve individual approach of treatment. Some clue can be provided by immunohistochemical establish abnormal expression of p21 or Ki 67. The aim of this study was to investigate the expression of Ki67 and p21 and to analyze their relationship in BPH and prostate cancer. **METHODS:** The expression of the Ki67 and p21 was examined by immunohistochemistry on paraffin embedded tissues sections from patients with BPH, PIN and prostatic carcinoma.

**RESULTS:** A total of 52 patients, from 52 to 81 years old, were diagnosed: 9 benign prostatic hyperplasia, 11 prostatic intraepithelial neoplasia and 32 adenocarcinomas. The specimens were examined by immunohistochemistry for Ki67 and p21. We found that BPH and PIN showed no or weak immunostaining for p21 and Ki 67, ranging from 0 to 3%, with one exception. Ki 67 was found positive mainly in prostates with PIN, revealing the premalignant nature of the lesion. In Gleason score 2-6 group Ki 67 showed equal percentage of cells in proliferation, and no p21 expression. The adenocarcinomas with high Gleason score revealed high proliferative index. The incidence of expression and the

percentage of Ki 67 positive cells increased with Gleason score. In the examined biopsies, different zones revealed different expression of Ki67, with tendency to increase in less differentiated areas. Overexpression of p21 was found only in four cases mainly in the areas with high Ki67 index.

**CONCLUSION:** Although the immunoeexpression of Ki67 and p21 cannot help to differentiate between BPH, PIN and cancer, it provides additional information to find patients needing close clinical follow up. Their expression serves as independent prognostic factor after PSA, Gleason score and stage, and shows the effect of treatment and AIPC turn down. If the data are known from biopsy before the operation they can help to choose the most efficient treatment. The high proliferative index in biopsies of patients without cancer screens those needing close clinical and biochemical control.

#### P 688

##### **COMPARISON OF NUCLEAR VOLUME IN PROSTATIC INTRAEPITHELIAL NEOPLASIA AND PROSTATE CARCINOMA**

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**Introduction.** Prostatic intraepithelial neoplasia (PIN) is the most precancerous lesion of prostatic carcinoma, one of the most common malignant diagnosed tumor and there are no accurate technique for predicting their evolution. Gleason system has been shown to accurately predict prognosis for low and high grades PINs, but for prostate carcinoma is of limited prognostic use. We have been interested in using a computer-based quantitative measurement to determine a more accurate assessment of nuclear dimension.

**Materials and methods.** There were analyzed histologic sections from 82 biopsies and 40 transrectal resections of prostate carcinoma. The biopsy tissue block and tumor fragments were paraffin included and quantitative analysis was performed on hematoxylin and eosin-stained histological sections with a computer-based program. All distinguishable nuclei in a microscopic measurement field were systematically selected for the standard measurement and for stereologic estimation of volume-weighted mean nuclear volume by the „point-sampled intercept” method. The lesion measured in each samples of primitive tumor was always the most severe and 40 nuclei were measured in each case.

**Results.** The stereologic results indicate a significant increase in nuclear volume from low grade PIN ( $301.0532 \pm 68.1322$ ) to high grade PIN ( $387.11563 \pm 74.5381$ ) and prostatic carcinoma ( $400.2371 \pm 88.5272$ ). It is a significant difference between the two grades of PINs, but the differences between high grade PIN and prostatic carcinoma was not significant.

**Conclusion.** Our study provides a more-objective differentiation of the two grades of PINs and demonstrates that stereologic nuclear volumetry adds significantly to the correct and objective classification and prediction of these lesions and is of capital importance in patient management.

**KEY WORDS:** NUCLEAR VOLUME MEASUREMENT, PROSTATE CARCINOMA, PROSTATIC INTRAEPITHELIAL NEOPLASIA.

#### P 689

##### **EXPRESSION OF CLEAVED CASPASES 3 AND 8 IN HUMAN PROSTATE CARCINOMAS**

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Prostate carcinoma (Pca), one of the most common cancer in men, is a major health problem, due to its morbidity and mortality in the growing elderly man population. New diagnostic and prognostic markers are needed, and the carcinogenesis of Pca remains to be clarified.

The disruption of the balance between cell proliferation and apoptosis in favor of apoptosis is responsible for the abnormal growth of the prostatic gland during neoplastic development. The chief effectors of the apoptotic pathway are the caspase family that induce cell death through activation in cascade of different caspase members. Upstream, caspase 8, an initiator caspase, cleaves all other known caspases. Downstream, caspase 3, as executioner, is at the center of apoptotic process by cleaving cellular substrates and inducing DNA fragmentation.

The aim of this study was to evaluate the cleaved caspase 3 (Casp3) and 8 (Casp8) proteins expression in prostate cancers to better understand the role of the apoptotic pathway in these tumors.

Immunohistochemical study was performed on prostatic tumors (with tumoral and normal tissues in each case) from 30 patients. The cases were separated in low grade tumors (Gleason score  $\leq 6$ , n=12) and high grade tumors (Gleason score  $>6$ , n=18). For each case, paraffin sections was incubated with rabbit polyclonal antibodies raised against Casp3 or Casp8.

Casp 3 expression was significantly decreased ( $p= 0.0006$ ) in cancers compared with normal tissues. Moreover, this expression was also decreased in tumoral tissues from low grade ( $p= 0.0025$ ) and from high grade ( $p=0.0005$ ) tumors, compared with normal tissues. By contrast, no significant changes ( $p>0.05$ ) in Casp8 expression was detected in tumoral tissues from all the 30 cases or inside the subgroups (low or high grade) compared with normal tissues.

The loss of Casp3, the key protein in the execution of apoptosis, in prostate cancers compared with normal tissues suggests an inhibition of the apoptotic process in these tumors. Furthermore, the decrease in Casp 3 expression was observed even in low grade tumors, making of this activated protein a potential early marker of tumoral transformation. Nevertheless, how caspase 3 is inactivated in Pca remains unclear. It couldn't be accounted on the loss of activation of caspase 8 since its expression was not modified in Pca. A potential posttranscriptional deregulation of caspase 3 activation in Pca could be hypothesized.

#### P 690

##### **IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LOCALIZED PROSTATE CANCER.**

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**Introduction:** classical predictive factors for prostate cancer seem to be sometimes insufficient. Advances in molecular biology and immunohistochemistry allow us to study molecules related with the cell cycle in prostatectomies with prostate cancer.

**Aims:** To define predictive value for prognosis of different molecules implicated in the biology of prostate cancer; using immunohistochemistry for p-21, bcl-2 and ki-67 in radical prostatectomies of localized prostate cancer in order to determine association with biochemical relapse.

**Material and methods:** We used immunohistochemistry to analyze the expression of ki-67, p21 (waf1/cip1) and bcl2 protein in 96 radical prostatectomies with localized prostate cancer. The results were related with biochemical relapse (seric PSA more than 5ng/ml) using statistical analysis with Kaplan-Meier curves and test log-rank. Gleason score and tumour stage were related.

**RESULTS:** Biochemical relapse was found in 22 cases (22%). Patients with overexpression of p21 and Bcl-2 were more prone to have biochemical relapse ( 36 months follow-up), but without statistical association. Patients with overexpression of Ki-67 had 3,15 more possibilities to have biochemical relapse, with statistical signification ( $p < 0,001$ ).

**CONCLUSIONS:** Ki-67 overexpression seems to be a good prognostic predictive factor.

#### P 691

##### **SINGLE-MINDED 2 HOMOLOG GENE (SIM2) IS OVEREXPRESSED IN PROSTATE CANCER**

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The single-minded homolog 2 gene (SIM2) is present within the Down's syndrome critical region on chromosome 21 and encodes a transcription factor involved in development. A short (SIM2-s) and long (SIM2-l) isoform of the SIM2 transcript have been identified. In a recent study, we identified SIM2 among highly upregulated genes in 29 malignant prostate tumours (T), compared with 33 benign (B) prostate samples, using a 40k human cDNA microarray. SIM2 was identified as the second most consistently upregulated named gene in T versus B samples, only surpassed by AMACR. The purpose of our study was to validate the expression of the SIM2 gene and SIM2 isoforms, and examine the expression and prognostic impact of SIM2-s protein expression in prostate cancer. The upregulation of SIM2 was confirmed in a subset of samples using oligonucleotide microarrays. SIM2-s and SIM2-l specific and SIM2 total TaqMan Assays-by-design were employed for quantitative real time PCR (qRT-PCR) on an expanded material of 47 prostate T and 38 B total RNAs, including 31 T/B pairs. SIM2-s, -l and -total were significantly elevated in malignant vs. benign prostate tissues by 3.3, 6.3 and 3.8 fold (median), respectively. However, SIM2-s upregulation was paralleled by SIM2-l upregulation in tumours, contrasting previous studies. Low level expression of both SIM2 isoforms was also detected in benign prostate tissue. In order to examine a larger patient series, a tissue microarray consisting of paraffin embedded prostate cancers from 104 patients, treated by radical prostatectomy, was subjected to immunohistochemistry using an anti-SIM2-s specific antibody. A moderate/strong staining was noted in 44 of 103 carcinomas as opposed to a weak to negative staining in the remaining cases. Moderate/strong expression of SIM2-s was associated with adverse prognostic variables like high histological grade, extra-prostatic extension and elevated serum-PSA. The 13 year survival was 98.1 % vs. 72.8 % in patients with weak/negative vs. moderate/strong SIM2-s expression, respectively ( $P=0.008$ , log rank test). In conclusion, the SIM2 gene was upregulated in prostate cancer as validated by oligonucleotide microarrays and qRT-PCR, and SIM2-s protein expression was found to be associated with adverse prognostic variables and reduced patient survival. These findings may prove relevant as SIM2 has been proposed as a possible drug therapy target.

#### P 692

##### **COMPARISON OF 34BE12 AND P63 IN VARIOUS LESIONS OF THE PROSTATE; ATYPICAL SMALL ACINAR PROLIFERATION (ASAP), LOW AND HIGH GRADE ADENOCARCINOMA AND HIGH GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA (PIN)**

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**Objective:** The basal cell-specific cytokeratin antibody (34BE12) is widely used to aid diagnosis of prostate cancer. Recently p63, a nuclear protein, was identified as useful basal cell-specific marker.

In this study, specimens from prostate needle biopsies, transurethral resections of prostate (TURP), open enucleations and radical prostatectomies were examined. We compared staining patterns of 34BE12 and p63 on the lesions of atypical small acinar proliferation (ASAP), low and high grade adenocarcinoma and high grade prostatic intraepithelial neoplasia (PIN).

**Results:** It was a statistical significance in basal cell staining with 34BE12 and p63 in the foci of ASAP. In some cases, 34BE12 was stained basal cell layer as complete and strong, whereas p63 was stained fragmented and strong. In three cases, both stains did not stain basal cell layer.

In other lesions, there was not a statistical significance for staining of basal cell layer with 34BE12 and p63. However, some different features of both stains were observed.

**Conclusion:** ASAP is not a new tumor or precursor entity. Whereas the high predictive value of p63 for subsequent adenocarcinoma warrants close follow-up and repeat biopsy.

The most commonly used immunohistochemical marker in the diagnosis of prostate cancer is 34BE12. However, p63 is almost as sensitive and specific as 34BE12 in diagnosing prostate cancer in needle core biopsies. Both stains are usually equally effective in demonstrating basal cells in benign glands. p63 is a basal cell-specific marker that could be used as a complementary test for the traditionally used 34BE12 in difficult cases. An important difference from 34BE12 is its consistent strong nuclear signal, which is easier to interpret.

#### P 693

##### **CORRELATIONS OF SMALL (3MM OR LESS) FOCUS OF PROSTATE CARCINOMA ON NEEDLE BIOPSY WITH RADICAL PROSTATECTOMY SPECIMEN.**

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**Purpose of the study :** In order to analyse the radical prostatectomy follow up of small foci of prostate cancer diagnosed on biopsies, we performed a multicentric retrospective study from 1999 to 2004.

**Methods :** We studied 107 consecutive patients, with a small focus of cancer (less or equal to  $\leq$  3 mm length) on only one biopsy core, a Gleason score  $\leq$  7, and evaluated the pT stage and the tumour volume on subsequent radical prostatectomy specimen.

**Results :** Mean age of patients was 64,6 years (46-83 years). Mean PSA concentration was 9,12 (0,6-33) (no data available for 5 patients).

The average number of prostate cores per biopsy was 7,94 (5 – 21). Average total length biopsies was 83,69 mm (13-170). 45 patients had a focus  $\leq$  1 mm, 30 between 1 and 2 mm and 32 between 2 and 3 mm. 15 patients had Gleason score  $\leq$  5, 73 of 6, and 19 of 7.

The whole prostate was embedded in paraffin ; cancer staging was reevaluated according to TNM 2002 classification. Volume of cancer was measured according to an elliptical model : length x width x height x 0.4. Average tumour volume at radical prostatectomy was 1,1cc (0,001 – 11,8).

pT Stages pT2a, pT2b, pT2c, pT3 ( $\leq$  1mm : 13, 3, 26, 3; 1-2 mm : 3,6, 13, 8; 2-3mm : 5, 4, 16, 7)

volumes  $\leq 0,2$  cc;  $0,2-0,5$ cc;  $> 0,5$ cc ( $\leq 1$  mm : 16, 6, 23;  $1-2$  mm : 7, 6, 17;  $2-3$  mm : 11, 6, 15)

A small focus  $\leq 1$  mm was statistically associated with an organ confined cancer ( $p=0,02$ ), and associated with pT2a stage ( $p=0,04$ ). Paradoxically, it was not associated with a small cancer volume ( $\leq 0,5$ cc  $p=0,47$  and  $\leq 0,2$ cc  $p=0,77$ ). PSA average value was correlated with cancer volume ( $p=0,02$ ) and a volume  $\leq 0,5$ cc was highly correlated with PSA  $<10$ ng/ml ( $p=0,001$ ).

There was not a significant association between Gleason score and cancer volume ( $p= 0,47$ )

On multivariate analysis no association was found between cancer volume and length of cancer on biopsy, adjusted on PSA and gleason score ( $p>0,5$ ).

Conclusion : This study demonstrates a strong association between small foci of tumour ( $\leq 1$  mm) on one prostate biopsy core and pT2a stage (but not with tumour volume) on prostatectomy. Those paradoxical results justify a prospective study on a series of patients with larger number of biopsies (i.e. 12 to 18) to determine whether length of tumour on biopsy can or not identify a subset of patients with good prognosis.

#### P 694

##### PATHOLOGICAL SECRETIONS IN PROSTATIC ADENOCARCINOMA

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**Introduction** The presence of pathological intraluminal secretions in prostatic adenocarcinoma, i.e. pink amorphous secretions, crystalloids, blue – tanged mucinous secretions, is always suspicious, but not diagnostic for prostate cancer. Between 10% and 23% of acinar – forming prostatic adenocarcinoma contain intraluminal crystal – like structures referred to as crystalloids, and are often associated with amorphous, intraglandular eosinophilic secretions. The aim of our study was to analysis the expression of these morphological signs in prostatic adenocarcinoma.

**Methods** Research was performed in 80 patients with prostatic adenocarcinoma, at the Institute of Pathology Clinical Centre Nis. The specimens were fixed in 10% formaldehyde and embedded in paraffin. The sections were stained with HE, PAS and HID-AB pH 2,5 methods.

**Results** The most common changes present in mucous secreting carcinomas were neoplastic glands lumen distension (43/80 – 53,75%), basophilic secretion (21/80 – 26,25%), presence of extracellular mucins (7/80 – 8,75%) and presence of crystalloids (22/80 – 27,5%). Presence of all morphological signs was recorded in carcinomas of Gleason grade 1-4A. Crystalloids were more often present in well differentiated and moderately differentiated neoplastic glands. Lumen distension was typical for Gleason grade 3 and 4A. The association of those morphological features with mucin secretion was confirmed with special methods for mucin detection (PAS and HID-AB).

**Conclusions** This study clearly showed that pathological intraluminal or extraluminal secretions are very often in prostatic adenocarcinoma and it can use as supportive features of prostatic cancer.

#### P 695

##### TUMOR MARKER DETECTION BY MEANS OF RT-PCR: ANALYSIS OF PARAFFIN-EMBEDDED SENTINEL LYMPH NODES OF PROSTATE CANCER PATIENTS

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**Introduction:** For the improvement of pathological diagnosis RT-PCR based expression analysis of tumor markers seems to

be a highly promising method. However, the value of this technique for routine diagnosis has to be proven by application on paraffin-embedded tissue and direct correlation with immunohistochemical data.

**Material and Methods:** From 242 routinely paraffin-embedded SLNs obtained from 85 prostate cancer patients two immediately adjacent 8  $\mu$ m sections were cut, one of which used for IHC and the other for RNA-extraction.

The obtained RNA was reverse transcribed and PCR for the detection of various tumor markers was performed. The specificity and sensitivity of these RT-PCRs was compared with the immunohistochemical results, as well as with the other pathological and clinical data of the respective patients. Moreover, for the RT-PCR positive specimen the tumor marker expression was quantified by real-time PCR and a correlation with the micromorphometric data was performed.

**Results:** For the tumor marker panel tested, the sensitivity of the RT-PCR as well as the negative predictive value was 99 % for detection of immunohistochemically identified tumor cells in the SLNs. All non-prostate cancer SLNs used as negative controls were RT-PCR negative. Of the 175 immunohistochemically negative prostate cancer SLNs 25 were RT-PCR positive. Of these 8 were from pN1(i+)(sn) patients and 14 from pN0(i-)(sn) patients with biochemical relapse. The real-time RT-PCR data corresponded well with the histopathological distinction between isolated tumor cells, micro- and macrometastases.

**Conclusions:** RT-PCR based techniques are feasible on single sections of paraffin material and thus can be used as a supportive methods in pathological diagnosis of prostate SLNs. This opens the possibility for extremely sensitive qualitative and even quantitative analysis of tumor marker expression. For prostate cancer patients RT-PCR analysis of appropriate tumor markers may be useful as an additional prognostic factor for the identification of patients with increased risk of biochemical relapse.

#### P 696

##### ANATOMO-PATHOLOGICAL FEATURES IN MUCINOUS PROSTATE CARCINOMA

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Signet-ring adenocarcinoma of the prostate is an unusual variant of prostatic carcinoma, with a high grade of malignancy. But with increasing use of needle biopsies as the primary mode of diagnosis, pathologists should be familiar with the full range of aspects of this tumor and its mimics. We report the cases of two male patients, with ages of 65 and 72 years, presented with urinary retention. Seric Prostatic specific antigen (PSA) level was increased in both cases. Fine needle biopsies were performed and fragments were fixed in formalin and embedded in paraffin. Histochemical reactions (mucicarmin, Alcian blue) and immunohistochemical methods (PSA) were applied. Over 90% of prostate samples were infiltrated by a poorly differentiated carcinoma with mucinous and signet ring patterns. The signet-ring cells presented abundant clear or vacuolated cytoplasm and ovalar, vesicular, eccentrically placed nuclei, with small nucleoli. The overall grade was assessed as Gleason grade 5 and immunohistochemical staining for PSA was positive in both cases. This aggressive tumor is an uncommon but distinct variant of primary prostatic carcinoma which should be distinguished from metastatic disease. The expression of prostate associated antigens such as PSA represents the diagnostic of prostatic origin.

**P 697****DUCTAL CARCINOMAS OF THE PROSTATE: A CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY**

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The aim of our study was to confirm the expression of prostate specific antigen (PSA) in ductal carcinoma of the prostate, and to analyse p53, and Ki-67 expression in these tumors. Paraffin-embedded samples from 12 patients with ductal carcinoma of the prostate were assessed for pattern, mitotic count and the presence of a microacinar carcinoma component. There were 6 pure ductal and 6 mixed microacinar and ductal carcinomas. Sections were stained immunohistochemically for the expression of PSA, p53 and Ki-67. Six of the ductal tumors had a papillary pattern whilst the others had a cribriform appearance. The mitotic rates in the ductal areas were high in the tumors from 8 of the 12 patients. PSA immunohistochemistry was positive in all cases. Two of the tumors were strongly positive for p53 protein. All the ductal carcinomas expressed Ki-67. This study confirms the expression of PSA in ductal carcinomas of the prostate. The percentage of tumors expressing p53 was similar to that published for high-grade microacinar carcinomas. The results for Ki-67 suggest that ductal tumors have higher scores than microacinar tumors, but further studies are necessary to ascertain this aspect. As half of the patients with ductal tumors had co-existent microacinar tumors, we advise transrectal prostatic biopsies in patients diagnosed with pure ductal carcinomas on transurethral resection specimens, to exclude high-grade microacinar carcinomas.

**P 698****EXPRESSION OF CYCLIN A, CYCLIN B1, AND HIGH MOLECULAR WEIGHT CYTOKERATIN (34-BETA-E12) IN PROSTATIC CANCER**

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Cytokeratin 34-beta-E12 staining is a useful tool in confirming and establishing of prostatic cancer diagnosis. Our aim was to investigate, whether cyclin A and B1, as a proliferative markers, have additional importance.

Forty three cases of patients with primary prostatic adenocarcinoma obtained from needle biopsy in Department of Urology, Clinical Centre of Serbia, Belgrade.

Cyclin A, cyclin B1 and cytokeratin 34-beta-E12 proteins expression were immunohistochemically assessed by semiquantitative evaluation using anti-human cyclin B1 monoclonal antibody (M 3530, DAKO) and anti-human cytokeratin 34-beta-E12 monoclonal antibody (N1553, DAKO). We examined relationship of this immunohistochemical markers with values of Gleason score, PSA and F/T PSA in serum.

Cytokeratin expression and Gleason score were in inversion correlation ( $p < 0.05$ ). Decreased cytokeratin expression was followed by increased expression of cyclin A ( $p < 0.05$ ), but without statistically significant increase of cyclin B1 expression ( $p > 0.05$ ). However, strong correlation between cyclin A and cyclin B1 expression were observed ( $p < 0.01$ ).

PSA value and Gleason score were in very strong direct correlation ( $p < 0.001$ ). We could not find any significant relationship between F/T PSA and other analysed parameters. Increase of Gleason score which has prognostical significance, clinically has been followed by higher serum PSA levels and immunohistochemically by decreased expression of cytokeratin. Direct significant connection between Gleason score and cyclin A and B1 has not found.

We conclude that only 34-beta-E12 staining is still reliable immunohistochemical marker which improves prostatic carcinoma diagnosis on needle biopsies.

Key words: prostatic cancer, Gleason score, cyclin A, cyclin B1, cytokeratin 34-beta-E12, PSA, F/T PSA, needle biopsy

**P 699****INTRAOPERATIVE FROZEN SECTIONS ANALYSIS OF THE POSTEROLATERAL MARGINS DURING NERVE SPARING LAPAROSCOPIC RADICAL PROSTATECTOMY: TECHNICAL ASPECTS**

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Introduction : nerve sparing procedure during laparoscopic radical prostatectomy need to perform a dissection closer to the posterolateral margins than without preservation of the neurovascular structures. Using intraoperative frozen sections (IFS) analysis on the posterolateral margins was unfrequently reported before during open or laparoscopic radical prostatectomy and could be an improvement of the technique allowing more patients to benefit from nerve sparing for preservation of potency in case of negative margins.

Purpose of the study: describe the technical aspects of the technique that we currently use in patients candidates to a nerve sparing procedure.

Methods: A total of 242 (uni or bilateral) IFS analysis were realised during nerve sparing laparoscopic prostatectomy between April 2002 and March 2005 for potent patients with clinically localized prostate cancer (T1&T2). After completion of the nerve sparing procedure and interfascial dissection the prostate is immediately remove. A specimen (2.5/4cm x 1cm) is cut by the surgeon from base to apex on one or both side of the prostate posterolaterally on the area of contact of the neurovascular bundles. The specimen including the capsule and 4 mm of the underlying prostatic parenchyma is immediately sent to the pathologist. After inking of capsular side 12im frozen sections are performed followed by toluidine blue staining and analyzed. Positive surgical margins are defined as tumor at the inked surface. In case of positive margins the ipsilateral neurovascular bundle is removed at the end of the procedure.

Conclusions: The usefulness of IFS during laparoscopic radical prostatectomy remains to be proven but seems to allow the surgeon to offer a nerve sparing procedure more frequently without compromising cancer control.

**P 700****A MORPHOLOGICAL STUDY OF STARTING SITES OF INVASION FROM PROSTATIC INTRAEPITHELIAL NEOPLASIA (PIN) TO PROSTATIC ADENOCARCINOMA**

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Introduction: High-grade prostatic intraepithelial neoplasia (PIN) has been considered as the most likely preinvasive stage of prostatic adenocarcinoma. However starting mechanisms of invasion from PIN has not been completely investigated not only from the view of genetics but also

morphology admittedly. Thus we examined a large number of complete serial sections so as to find out starting sites of invasion, and also we reconstructed 3D- images from the sections in order to visualize the sites and to examine them morphologically.

**Materials and methods:** Used were two prostatectomy specimens having extensive PIN with multiple invasive foci. After formalin fixed and paraffin embedded, five blocks were selected and sectioned serially in 3µm thickness completely. After HE stained and immunohistostained for high molecular weight cytokeratin (34bE12) in aid of searching for micro-invasion, invasive lesions connected with PINs were searched microscopically. Then, the lesions were reconstructed into 3D-images with computer-assisted 3D-reconstruction system.

**Results:** Total amount of the serial sections reached 824 sheets containing many invasive lesions, while only six sites were considered to be invasive lesions apparently connected with PIN. Furthermore, serial sections and 3-D images revealed the early invasive sites were distinctly diminutive compared from the surrounding invasive lesions.

**Conclusion:** The study confirmed morphologically that PIN was preinvasive lesion of prostatic adenocarcinoma. While starting sites of invasion were hardly discovered and the area were obviously smaller than the surrounded invasive lesions.

#### P 701

##### **EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION IN BASAL CELLS OF NORMAL, ATROPHIC, HYPERPLASTIC PROSTATIC GLANDS, AND PIN. A TISSUE MICROARRAY STUDY**

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**Background:** Epidermal growth factor receptor (EGFR) is involved in the autocrine regulation of basal and luminal cell growth in the prostate. Basal cells (BC) of the prostate act as reserve-regenerative compartment, and are important in the maintenance of normal glands and in the development of prostatic diseases. Expression of EGFR in BC has been documented, but modification of this expression under pathologic conditions has received relatively little attention. The present tissue microarray (TMA) study has focused on EGFR expression in BC of normal (N), atrophic (A), hyperplastic (H), and dysplastic (PIN) glands from patients with prostatic adenocarcinoma (PCa).

**Design:** 227 radical prostatectomies were reviewed and 4 TMAs were

constructed, with a total of 849 cores (3.7 cores/case). In addition to representative foci of PCa, the cores included 451 areas with benign or dysplastic lesions (N:57; A,168; H,146; PIN, 80). The slides were immunostained with a monoclonal EGFR antibody (H11, DakoCytomation), using the Envision system (DakoCytomation, Glostrup, Denmark). The results were scored in a four-tiered scale (0, 1+, 2+, 3+). The percentages of cores with EGFR-positive BC in N, A, H or PIN glands in each intensity category were compared using a Chi-square test.

**Results:** The distribution and relative percentages of positive cores for each category are summarized in the table. None of the observed differences was statistically significant.

**Conclusion:** 1. EGFR expression in BC is predominantly of low (1+) intensity. 2. The EGFR expression profile of BC in hyperplasia is similar to that of atrophic, and only slightly different from that of normal glands. 3. Strong positivity (3+) is observed more often in BC of glandular hyperplasia, but moderate and strong positivity are overall more extensively observed in BC of PIN. 4. EGFR expression in BC may be relevant in the pathogenesis both of hyperplasia and PIN.

#### P 702

##### **BASAL CELL HYPERPLASIA OF THE PROSTATE**

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**Introduction:** Prostatic basal cell proliferations is a benign condition in a background of nodular hyperplasia typically found in the transition zone and usually identified in transurethral resection or prostatectomy specimens. Some studies report an incidence of BCH in prostate between 8.9% and 10.2%. BCH show a wide spectrum of morphologic features, ranging from focal basal cell hyperplasia to florid adenoid basal cell hyperplasia. Unusual histological patterns are rare and the distinction between benign and malignant lesions is difficult to establish. Morphologic evidence of malignancy include infiltrative growth, extraprostatic extension, perineural invasion, local recurrence or metastases, necrosis and stromal desmoplasia. When the lesion is well delineated, the term adenoma has been used (BCA). Hyperplastic lesions and adenoid cystic carcinomas have cytoplasmatic immunoreactivity to high molecular weight keratins, but the basaloid carcinoma does not have it. **Purpose of study:** The aim of this study is to remark this entity because basal cell proliferations are uncommon lesions and sometimes can mimic prostatic intraepithelial neoplasia and carcinoma. **Materials and methods:** A 65-year-old white male underwent a retropubic simple prostatectomy. The serum prostate-specific antigen (PSA) was 2,8 ng/ml. We collected 18 cases with the diagnosis of BCH and BCA that were reported in this Department over a 35 year period (1970-2005). **Results:** Gross abnormalities were multinodular masses of variable size. Sectioning showed multiples nodules tan-white and uniformly firm. The largest one measuring 2.5 cm. **Microscopic findings:** in a background of benign nodular hyperplasia the largest nodule was well circumscribed and containing small nests of basal cells of two or more cell layers thick surrounded by a few concentric layers of compressed stroma. The basal cells were plump, with large and uniform nuclei, scant cytoplasm and inconspicuous nucleoli. Our diagnostic was basal cell hyperplasia with nodular pattern. **Conclusion:** BCH are uncommon lesions in the prostatic gland and can exhibit a morphologic ranging. Accounted for 18 cases reported in our files emphasizing the unusual of these diagnosis and the architectural and cytology features of ordinary and unusual patterns of BCH that even more closely mimic prostatic intraepithelial neoplasia and carcinoma.

#### P 703

##### **RETRACTION CLEFTING AND PROSTATIC INTRAEPITHELIAL NEOPLASIA IN NEEDLE CORE BIOPSIES**

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**Introduction:** In comparison to normal glands retraction clefting is more common and prominent around neoplastic acini and therefore represents an additional criterion for the diagnosis of prostatic adenocarcinoma. Prostatic intraepithelial neoplasia (PIN) composed of abnormal proliferation within the prostate ducts, ductules, and large acini, premalignant foci of cellular dysplasia and carcinoma in situ without stromal invasion is the most likely precursor of prostatic adenocarcinoma. However, in the literature, we were not able to find data on the relationship between clefts and prostatic intraepithelial neoplasia.

**Purpose of the study:** To determine the presence and extent of retraction clefting in glands with prostatic intraepithelial neoplasia (PIN) in needle core biopsies.

**Methods:** We analyzed needle core biopsies from 35 consecutive biopsies of patients with PIN diagnosed at the Department of Pathology, Sestre milosrdnice University Hospital. Specimens were fixed in 10% buffered formaldehyde, embedded in paraffin, cut at 4 µm and

routinely stained with hematoxylin and eosin. Glands with PIN were analyzed on high power field (400x) and classified in three groups: group I (no clefting), group II (clefting in up to 50% of gland circumference) and group III (clefts in more than 50% of gland circumference). Normal glands within the same biopsy served as a control.

Results: The age of the patients ranged from 53 to 84 years (median 67.8 years), with the PSA value from 4.2 to 121 ng/ml. In glands with PIN, clefts in more than 50% of gland circumference were not found. There were 8 PIN (22.8%) cases with clefting in less than 50% of circumference (group II) and 27 (78.2%) cases with no clefting.

Conclusion: Our results showing the lack of retraction clefting in PIN cases suggest the influence of stromal reaction in the development of clefts around neoplastic acini of prostatic adenocarcinoma and therefore clefting should not be considered as an aid in the differential diagnosis of PIN.

#### P 704

##### METABOLIC AND T2-RELAXATION TIME

##### MAPPING OF PROSTATE USING 1H MRS

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PURPOSE: We propose quantitative description of metabolic and T2-relaxation time alteration of prostate tissue for differential diagnosis between prostatic carcinoma (PC) and benign changes such as hyperplasia (BPH) and inflammation.

MATERIALS AND METHODS: Two groups of patients are examined by 1.5 T Magnetom Vision System (SIEMENS). The 1-st group (PG) includes 10 patients (age range 34-76), the 2-nd group (VG) consists of 4 healthy asymptomatic volunteers (age range 32-48). For building of the metabolic map the 1H MR-spectra are recorded with 2D CSI sequence: TR/TE = 1500/60 ms. In T2-relaxation time measurement the 1H MR-spectra are recorded in two regions of the prostate (in the central zone (CZ) and in the peripheral zone (PZ)) with SVS STEAM sequence: TR/TE = 1500/60,80,100, 140,180 ms. All spectra are recorded with and without of water suppression. The water signal of prostate tissue as an internal standard for calculating of content of main metabolites is used.

RESULTS: Lesions identified on T2-weighted images were analyzed using the following criteria: low-signal nodule (well circumscribed, rounded low-signal area), low-signal zone (irregular, less circumscribed low-signal area), heterogeneous zone (mixed, high- and low-signal area), central gland asymmetry. From 1H MR spectra the peak areas of main metabolite signals: citrate (Cit, 2.6 ppm), creatine (Cr, 3.0 ppm) and choline (Cho, 3.2 ppm) and the mean peak height ratios of  $R = \text{Cit}/(\text{Cr}+\text{Cho})$  are obtained. The values  $R > 1.12$  are indicative for PC,  $R > 2.30$  – for BHP,  $R > 4.14$  – for normal regions of prostate tissue. In the VG the mean values of T2 for water (Wat), and for Cit, Cr, and Cho are the following: T2 CZ (Wat)=(71.3+0.56) ms, T2 CZ (Cit)=(170.2+0.23) ms, T2 CZ (Cr)=(134.5+0.3) ms, and T2 CZ (Cho)=(254.13+0.81) ms; T2 PZ (Wat)=(123.5+0.74) ms, T2 PZ (Cit)=(216.8+0.79) ms, T2 PZ (Cr)=(241.2+0.15) ms, T2 PZ (Cho)=(323.7+0.2) ms. In the PG the values of T2 (from 56.4 to 93.2 ms) are indicative for water signal in PC, and (from 98.7 to 117.4 ms) - for BHP.

CONCLUSION: Metabolic and T2-relaxation time mapping can be helpful for diagnosis and characterization of clinically localized PC and also for the estimation of efficiency of therapy.

#### P 705

##### IS P63 A RELIABLE MARKER FOR PROSTATE BASAL CELL EPITHELIUM? IMPLICATIONS FOR PROSTATE CANCER DIAGNOSIS

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Introduction. The diagnosis of prostate malignancy is often based on the absence of basal cells. Diagnostically reliable identification of prostatic basal cells has depended on staining for high molecular weight cytokeratin. The p63 gene, a member of the p53 gene family, has also been shown to be expressed in the basal epithelial cells of normal human prostatic epithelium. Additionally, irregularities in p63 expression in multiple organs have been associated with epithelial carcinogenesis; p63 may protect prostatic epithelial cells against neoplastic transformation, perhaps through a tumor suppressor function. We examined the differential expression of p63 protein and cytokeratin 903 in prostatic adenocarcinoma (PCa), intraepithelial neoplasia (PIN), atypical adenomatous hyperplasia (AAH) and benign prostatic hyperplasia (BPH).

Material and Method. We performed immunohistochemistry with monoclonal antibodies for p63 and cytokeratin 903 on specimens of PCa (70 cases: 30 well, 20 moderately and 20 poorly differentiated), PIN (55 cases: 30 PIN1, 10 PIN2, 15 PIN3) and AAH (60 cases).

Results. 100% of the PCa specimens were negative for p63 and cytokeratin 903 immunoreactivity. In PIN1-3, p63 expression was weak-moderate and focal in 100%, and cytokeratin 903 staining was weak and focal in 89% of the cases. Weak-moderate and focal expression of p63, as well as weak and focal expression of cytokeratin 903 were also observed in 85% and 70% of AAH cases, respectively. In BPH, p63 glandular expression was more extensive than cytokeratin 903.

Conclusions. In contrast to normal and preneoplastic prostatic tissue, prostate adenocarcinomas do not express p63. The latter seems to be a reliable marker for prostate basal epithelium, such as cytokeratin 903, and can potentially increase the specificity for discerning cancer from high grade PIN or atypical prostate lesions, thereby reducing diagnostic uncertainty. In prostate cancer (PCa), p63 can be considered a potential reliable method for facilitating its pathologic diagnosis.

#### P 706

##### ATYPICAL PROSTATIC BASAL CELL

##### PROLIFERATIONS: MORPHOLOGIC AND

##### IMMUNOHISTOCHEMICAL STUDY OF 4 CASES

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Background: Besides usual basal cell hyperplasia commonly observed in prostatic adenoma, atypical basal cell proliferations are observed in less than 0,1% of samples provided from transurethral resection (TUR) or adenectomy. Florid and/ or adenoid hyperplasia can be observed on repeated resections during years without aggravation, but a small subset of lesions displays a malignant evolution with extra prostatic invasion and /or distant metastases.

**Design:** Because of the rare occurrence of these lesions and the limited follow-up information available in elderly population, the histologic criteria of malignancy are not clearly established. We report 4 cases of florid basal cell hyperplasia and/ or carcinoma in attempt to precise these criteria.

**Results:** Patients were respectively 58, 73, 90 and 92 years old without previous prostatic surgery. An unusual basal cell proliferation was observed in almost the whole prostatic tissue obtained by TUR (1 patient) or adenectomy (3 others). The lesions varied from small to large lobular sheets of basaloid cells with frequent cystic patterns to basaloid and/or comedo-like patterns with central necrosis. In 2 cases, adenoid cystic (AC) like pattern surrounded by dense hyaline stroma was predominant. In the 2 other cases there were large sheets of basal cells associated with infiltrative patterns suspect of malignancy. P63 was expressed by 50 to 100% of basal cells at the periphery of the lobules in AC like patterns and was negative in basaloid ones. Mib-1 expression was respectively 40% and 60% in the 2 cases suspect of malignancy and 10 and 30% in the 2 others. Bcl-2 was intensely expressed in 100% of cells of basaloid and/or comedo-like patterns, weakly and heterogeneously elsewhere. Without additional treatment the 4 patients are free of disease 2 years after surgery.

**Conclusion:** Morphological differences exist among these lesions in correlation with immunohistochemical profile. Basaloid proliferations displaying large cohesive pseudoinfiltrative and comedo-like patterns suspicious of malignancy are associated with a Mib 1 index > 30%, a strong bcl-2 expression and a lack of p63 expression. Considering the difficulty to assess extra prostatic or perineural extension in limited samples and the accurate infiltration except on total prostatectomy specimen, these morphologic and immunohistochemical features could be used to induce a more aggressive therapeutic approach in patients with long life expectancy.

#### **P 707**

##### **PROSTATIC MIXED EPITHELIAL-STROMAL (PHYLLODE) TUMORS: A REPORT OF 4 NEW CASES**

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We report 4 additional cases of mixed epithelial-stromal tumor (phyllode tumor of the prostate) arising in men aged 27 to 64 years. The presenting clinical symptoms included urinary retention (2 cases), hematuria (1 case), and hematospermia (2 cases). The tumors formed prostatic masses measuring from 6 to 12 cm in diameter.

The diagnosis was suggested in 2 cases based on needle biopsy. In all cases the whole tumor was resected and patients were well from 4 months to 5 years after surgery.

Histologically, tumors showed a non-neoplastic epithelial component with cystic glands, an elongated cleft-like space and stromal proliferation with occasional high cellularity and cellular atypia. There was no increased mitotic activity. The epithelial component was positive for cytokeratin and PSA. The stromal cells were positive for vimentin in all cases, smooth muscle actin in 2 cases, desmin in 2 cases, and occasionally CD34.

These tumors are of uncertain malignant potential and need to be distinguished from benign lesions (leiomyomas and stromal hyperplasia with bizarre nuclei), and from stromal sarcomas and leiomyosarcomas.

These four cases as well as most cases reported in the literature behave in an indolent fashion but recurrences and progression to sarcoma can occur. Thus, when the diagnosis is suggested based on needle biopsy, total resection of the lesion is required.

#### **P 708**

##### **RECIPIENT DERIVED COLLAGEN TYPE III IN HUMAN TRANSPLANTED HEARTS**

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**Introduction:** Transplantation of donor hearts is often associated with progressive development of interstitial myocardial fibrosis and alterations in composition and organisation of the extracellular matrix. Changes in cardiac interstitial collagen network are thought to contribute to abnormal stiffness and loss of function of the myocardium. Fibroblasts are the main producers of type I and type III collagens, the major interstitial collagens found in the heart. In transplanted hearts, intragraft fibroblasts consist of two cell populations. Donor-derived fibroblasts pre-exist in donor organs, whereas host-derived fibroblasts are progressively immigrating as mesenchymal progenitors from the circulation to the allograft.

**Purpose of the study:** To determine the contribution of these two distinct fibroblast populations to progression of myocardial fibrosis, we studied endomyocardial biopsies from eight male patients who had received hearts from female donors.

**Methods:** In these sex-mismatched patients two frequent genetic polymorphisms at the collagen III locus were determined by polymerase chain reaction-based restriction enzyme digestion, both in donor allografts and in the corresponding explanted recipient heart. Based on differences the of collagen-genotype in donor and recipient tissue, we selected 40 endomyocardial biopsies by hematoxylin eosin staining and determined population-specific collagen expression using single nucleotide polymorphisms.

**Results:** Total number of donor derived cells in biopsies determined by short tandem repeat analysis was up to 70%. Chromogene in situ hybridization with probes specific for Y-chromosomes and immunohistochemistry staining for CD68 demonstrates the majority of macrophages to be of donor origin. The amounts of interstitial collagen type I and type III increased in a time-dependent manner within cardiac allografts. Years after transplantation a substantial number of Y-chromosome-positive recipient-derived cells was non-inflammatory, non-endothelial spindle-shaped fibroblast-like cell types. A considerable quantity of collagen III mRNA can be attributed to recipient-derived fibroblasts in transplanted hearts.

**Conclusion:** Our data confirm the existence of a substantial number of fibrosis-mediating immigrated recipient-derived fibroblasts in cardiac allografts. Furthermore, this suggests an active, essential role for recipient-derived fibroblasts in the process of fibrosis in transplanted human hearts.

#### **P 709**

##### **PULMONARY LEUKOCYTOCLASTIC VASCULITIS AFTER HEART-LUNG TRANSPLANTATION: A NEW CASE SUGGESTING ACUTE HUMORAL REJECTION.**

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Acute pulmonary rejection is primarily cell-mediated and is characterized by perivascular mononuclear infiltrates. Acute vascular rejection with humoral component has only been suspected in few previously reported cases. Diagnostic criteria are not well established. We present a case of pulmonary vasculitis possibly related to such a rejection.

A 33-year-old female patient had an initially uneventful double lung transplantation for primary pulmonary hypertension. There were no panel-reactive anti-HLA antibodies. From day 19, she developed fever, progressive bilateral lung opacities with hilar lymphadenopathy and irregularities of the bronchial mucosa. Pulmonary function did not deteriorate. No was no sign of angiitis in other organs. No infection could be identified, beside the donor being HHV8+. Antineutrophil cytoplasmic antibodies were absent. Treatment with high doses of corticoids was started at day 58 and lead to rapid abatement of fever and progressive disappearance of lung opacities. The patient is well 6 months after her transplantation.

Bronchioloalveolar lavage at day 20 showed 90% of macrophages often containing nuclear debris and with important hemosiderosis. Transbronchial lung biopsy at day 20 was normal but bronchial and transbronchial biopsies at day 45 showed leukocytoclastic capillaritis. An open lung biopsy, performed at day 58 confirmed a widespread leukocytoclastic vasculitis involving capillaries, veins and arteries with foci of necrosis. A peribronchial lymph node was necrotic. There was no usual criterion for acute rejection. Special stains showed no micro-organism and there was no viral inclusion. Immunofluorescence on cryostat sections for C4d deposition was not conclusive. A second open lung biopsy, performed at day 109 during the treatment of a persisting pneumothorax, showed normal pulmonary parenchyma.

Acute humoral rejection is difficult to ascertain. However in rare cases similar to this one, clinical and pathologic data may suggest this diagnosis.

#### P 710

##### **HUMAN HEART EXPLANT CONTAIN CELL POPULATION DIFFERENTIATING IN VITRO INTO MYOFIBROBLASTS**

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**Introduction.** In human and experimental animal there is now incontrovertible evidence that new myocytes may be generated throughout life in response to physiological and pathological stimuli. Adult heart stromal system has been proposed to consists of mesodermal stem cell that are capable of self-renewal into de novo cardiomyocytes. The rate of cell renewal is slow and low. **Purpose.** Sections from heart explants of patient enderwent heart transplanation were cultured in vitro. This procedure was perform to obtain a population for future supporting failed heart with cultured 'stem cell' transplantation. **Material.** Multiple fragment from human heart explants were cultured in vitro. **Method.** Number of staining procedures were performed to confirm phenotypes of growing cells (FGF, Titin, actin, myosin, vimentin, desmin, HLA class I and II, CD31, CD34, CD68 including electron microscopy. **Results.** The prolonged culture of heart fragments was the source of de novo growing cell population. **Conclusion.** It has been demonstrated that failed heart in contrast to normal has had precursor cells exhibits growth potentials to generate population of cells with immunocytochemical and ultrastructurel characters of myofibroblasts.

#### P 711

##### **EXPRESSION OF PCNA GENE, NUCLEOLAR ORGANIZER REGION ACTIVITY AND NUCLEAR PLOIDY IN INTERVENTRICULAR SEPTUM**

##### **CARDIOMYOCYTES FROM CHILDREN AND ADULTS WITH OHCMF**

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**Aim:** to estimate PCNA expression, nucleolar organizer region activity and nuclear ploidy in interventricular septum cardiomyocytes from patients with obstructive hypertrophic cardiomyopathy (OHCMF).

**Materials and methods:** material from 61 patients with OHCMF was included into the study. Among those, 19 were below 21 y.o. and named as 'children'. The rest 41 patients were over 21 y.o. and named as 'adults'. The material was obtained upon the surgical interventions as well as upon express autopsies from the right side of the interventricular septum. The samples were stained with hematoxylin-eosin technique and by the method of nucleolar organizer regions silver staining. Expression of PCNA in cardiomyocyte nuclear was studied by immunohistochemistry. DNA content was estimated by Felgen method on dissociated cardiomyocyte nuclei and assessed by cytophotometry. One hundred tissue lymphocytes were used as a control for diploidal DNA content.

Clinical and echocardiographic data were obtained from case histories.

**Results:** the mean age in the children group was 14±1,1, ranging from 5 to 21 y.o. The mean age in the adult group was 46,4±1,9, ranging from 27 to 82 and being statistically different from the children group (p<0,000). The mean age of OHCMF diagnosis was 4,5±1,1 in children group, ranging from several days to 14 years. In the adult group this parameter was 38±2 and ranged from 2 to 69, being statistically different from children (p<0,000). There was no statistical difference between children and adults in LVMI (276,1±28,6± and 252,1±16g/m2) and IVS thickness in the outflow tract (2,89±0,14 and 2,54±0,08cm). In the children group compared to adult group there was a significant increase in AgNORs parameter ( 25,9±0,58 and 20,9±0,6, p<0,000), PCNA-positive cardiomyocytes (6,9% ±0,9 and 3,8%±0,4 p<0,001) and DNA content (15,2±2,2 and 9,9 ±1,4 p<0,017). The mean number of maximal cardiomyocyte DNA content was higher in children group, then in adult group as well (53,5±7 and 30,7±2,2 p<0,002).

**Conclusion:** the children group with OHCMF is characterized by highest degree of myocardial hypertrophy, accompanied by increase in PCNA expression, increase in cardiomyocyte ploidy and increase in ribosomal biogenesis, estimated by AgNOR content in cardiomyocytes form right side of asymmetrically hypertrophied interventricular septum.

#### P 712

##### **MORPHOLOGICAL CHANGES IN HIBERNATING MYOCARDIUM**

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**Introduction:**

Hibernating myocardium represent a prolonged but potentially reversible myocardium contractile dysfunction caused by chronic myocardial ischemia. The purpose of this study was to describe the morphological changes in diskinetical areas and their correlation with the functional investigations.

**Material and methods:**

In 48 patients with chronic ischemic heart diseases, areas of left ventricular hibernating myocardium were identified by angiography, thallium scintigraphy and dobutamine echocardiography. On biopsies removed during by interventions, ultrastructural and various histological and immunohistochemical investigation were effectuated.

**Results:**

The morphological changes are suggestive for embryofetal dedifferentiations and summarized as following: loss of the contractile structures, fragment glycogen granules, small mitochondria, absence of sarcoplasmic reticulum and T tubules, redistribution of chromatin, the presence of rough endoplasmic reticulum and  $\alpha$ -smooth muscle actin, the decrease of cardiotonin, the expression of titin in puncted pattern. In the extracellular matrix were increased early matrix such for fibronectin, tenascin, collagen type I and III and fibroblasts. Also were observed stunned changes, cellular degeneration, apoptosis and reparative fibrosis.

**Conclusions:**

Hibernating myocardium is a transient incomplete adaptation to reduced coronary blood flow, an embryofetal dedifferentiation, that impose early revascularization.

**P 713**

**EXPRESSION OF THE NUCLEOLAR ORGANIZER REGION'S ARGENTOPHYLLIC PROTEINS, TUMOR SUPPRESSOR GENE P53 AND CASPASE-3 IN CARDIAC SAMPLES FROM PATIENTS WITH IDIOPATHIC RESTRICTIVE CARDIOMYOPATHY.**

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**Aim**

Our aim was to study the expression of argentophyllic proteins in the nucleolar organizer regions, tumor suppressor protein p53 and caspase-3 in cardiac samples from patients with idiopathic restrictive cardiomyopathy (IRCM).

**Material and methods**

The express-autopsy cardiac samples from five patients with IRCMP were studied by hematoxylin-eosin, Van Gieson and Congo Red staining and well as AgNOR staining. Immunostaining of p53 and caspase 3 were performed with DAKO monoclonal antibody. Cardiac samples from eight healthy people, died due an incidental cranial trauma were used as controls.

**Results**

In all study samples we found oxyphylic amorphous protein depositions, which were positive for Congo Red staining only in two cases. Along with morphologically normal nucleoli in atrial and ventricular cardiomyocytes we identified micronucleolar, or atypical extranucleolar localization of argentophyllic proteins. The latter were more often seen in atria than in ventricles. The stromal cellularity was markedly increased, in some stromal cells 1-8 small, dot-like inactive nucleoli were observed. Positive staining for caspase-3 was present in cardiomyocyte cytoplasm and cardiomyocyte nuclei were positive for p53 staining. In the control samples, atypical extranucleolar localization of argentophyllic proteins were not present, as well as positive caspase-3 and p53 staining. Most of the stromal cells in the control cardiac tissue contain one dot-like nucleolus.

**Conclusion**

Cardiomyocyte nuclei from patients with IRCMP, complicated by terminal congestive heart failure, are characterized by increased p53 expression along with increased cytoplasmatic caspase-3 expression, which might illustrate p53-dependent apoptosis process. Atypical localization of argentophyllic proteins in cardiomyocyte and

stromal cell nuclei as well as presence of micronucleoli might reflect the impairment of the protein synthesis. It is also important to note, that besides signs of structural and functional abnormality of the nucleoli in all study cases oxyphylic amorphous protein depositions were found.

**P 714**

**DISEASE GENETICAL SYNDROMES AND CONGENITAL HEART**

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**INTRODUCTION:** Congenital heart diseases (CHD) mainly develop as a consequence of multifactor influence, i.e. some develops as partially genetically induced anomalies. Therefore some CHD are often associated with different genetic syndromes.

**PURPOSE:** The aim of the study was to evaluate the incidence of CHD associated with genetic syndromes, as well as to analyze the types of CHD found in particular entity.

**MATERIAL AND METHOD:** At the Institute of Pathology, Medical School University of Belgrade, during the period from 1926-2004, a total number of 1399 cases of CHD were diagnosed. This investigation was made by analysis of data from standard autopsy protocols, and especially the designed forms for CHD cases. All autopsies were performed using standard or modified Rokitansky technique.

**RESULTS:** Our results showed that the most common association of CHD was with Down syndrome, found in 42 cases (3% of all CHD). Out of them, in 26 cases the atrio-ventricular septal defects (AVSD) was found; in 7 cases ventricular septal defect (VSD), in 6 cases atrial septal defect (ASD), and in 1 case there were trilogia of Fallot, aortic atresia and persistent ductus arteriosus. Besides Down syndrome, we found association of CHD with four cases of Edwards's syndrome, and one case of Turner syndrome. In three cases of Edwards's syndrome, isolated VSD was found, and in one case bicuspid pulmonary valve with fibroelastosis of both ventricles was present. In only case of Turner syndrome, coarctation of aorta and patent ductus arteriosus was found.

**CONCLUSION:** Our findings, made on very large autopsy series, indicate a relatively low percentage of CHD associated with different genetic syndromes. However these findings are in agreement with data from the literature, indicating that predominant influence in the development of CHD should be looked outside of genetic disorders.

**P 715**

**ENDOMYOCARDIAL BIOPSY FINDINGS IN PATIENTS WITH MYOTONIC DYSTROPHY**

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**Introduction:** Endomyocardial biopsies (EMB) are powerful diagnostic tool in specific cardiomyopathies, including patients with different neuromuscular diseases and heart problems. Although among indications for EMB, usual non-pathognomonic findings in these entities are the reason for limited series in the literature. In patients with myotonic dystrophy (MD) it has been shown changes such as myofibrillar degeneration, mitochondrial abnormalities, focal myocarditis, fibrosis and fatty infiltration of the myocardium, and especially changes in the conduction system.

**Methods:** This study presents the results of EMB findings in 10 patients with MD. The biopsies were carried out using King's biptome, followed by routine histological procedure.

Results: In two patients with severe MD, biopsy specimens showed changes compatible with border-line myocarditis. In five patients with moderate to severe forms of MD, we found fibrosis and fatty infiltration of the myocardium, in addition to degenerative changes and hypertrophy of muscle fibers. Three patients with mild MD had non-specific degenerative and hypertrophic myocardial changes.

The histological changes described above were present in patients without cardiological symptoms and in those with normal ECG and echocardiographic findings. Only two of the 10 patients in whom EMB was performed complained of fatigue and occasional palpitations, while the rest of the patients were asymptomatic. One patient with focal myocarditis had ECG signs of left bundle branch block and echocardiographic evidence of reduced left ventricular contractility. Five patients with signs of endomyocardial fibrosis only had an abnormal Q-wave on ECG recordings. The remaining five patients with border-line myocarditis and/or with degenerative and hypertrophic myocardial changes had normal ECG and echocardiographic findings. Conclusions: These results stress the significance of endomyocardial biopsy in detecting myocardial pathologic changes in patients with MD.

**P 716**  
**MYOCARDIAL FLUORESCENCE MEASUREMENTS AS MORPHOPATHOLOGICAL CRITERIA FOR DETERMINING THE DURATION OF MYOCARDIAL INFARCTION**

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The study aimed towards finding new criteria for the determination of the duration in time of myocardial infarction (MI). 52 hearts from persons deceased from MI, in which MI beginning was documented clinically and by ECG, were selected. 30 hearts of persons deceased almost instantaneously from trauma served as controls. In each case we did 1000 measurements of myocardial primary fluorescence (MPF) at the wavelength of 360 nm, both in the necrotic (ischaemic) zone (NZ) and in extrainfarct zones (EZ). During 8-12 hrs from MI beginning, the MPF decreased concordantly in both zones, being  $89.6 \pm 2.3\%$  of controls. At 24 hrs after MI onset MPF in NZ was  $78.8 \pm 4.3\%$ , in EZ  $84.4 \pm 3.1\%$  of controls. Minimal NZ MPF was at 4-5th and 9-10th days ( $66.3 \pm 2.5\%$  and  $62.6 \pm 2.1\%$  of controls,  $p < 0.001$ ). EZ MPF at 4-5th days was  $77.4 \pm 3.5\%$ , at 9-10th days  $82.3 \pm 2.2\%$  and at 14-15th days  $85.7 \pm 2.8\%$ , remaining even up to 30-35th days of MI  $7.3 \pm 0.8\%$  below controls. MPF variations in time were approximated by splines. By solving a system of equations, which included formulae for EZ and NZ MPFs, it was possible to determine the time elapsed from the MI onset, with an accuracy of 9.6 hours.

**P 717**  
**APOPTOSIS IN ISCHEMIC CARDIOMYOCYTE INJURY**

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It is believed that necrosis, apoptosis and reversible injury differentially contribute to cardiac functional loss in ischemia. Although apoptosis has been studied extensively as a pathogenic factor in cardiovascular pathology, its role in myocardial infarction remains not fully understood. THE AIM of the study was to evaluate apoptosis in ischemic myocardium and assess its pattern of distribution.

36 specimens of the myocardium were obtained during necropsy (performed within 6 h from death) from patients who died of acute cardiac failure within 24 h from the onset of the ischemic attack. M/F ratio was 1:1, median age – 62.

Coronary arteries were 50-80% stenotic, with no signs of thrombosis. Myocardium from patients who died from brain hemorrhage was included for control purposes. Basing on clinical (ECG, enzyme assays) and morphologic data (basic fuchsin stain and polarized light microscopy). 5 groups were built with duration of ischemia from 0-1h, to over 24 h. Standard protocol was created for taking samples from the myocardium considering position towards ischemic region and heart anatomy. Bcl-2, caspase 3 and p53 were studied immunohistochemically (reagents from Dako Cytomation, Denmark).

Bcl-2 and caspase 3 were localized in peri-infarction zone, with increasing intensity from 6 to over 24 h of ischemia. Intranuclear positivity for p53 was demonstrated in isolated cardiomyocytes (CMs) and only in sudden cardiac death. TUNEL method was used to prove apoptotic DNA fragmentation. The distribution of TUNEL-positive CMs showed mosaic pattern in sudden cardiac death and was multifocal in peri-necrotic region in developed infarction. Marked chromatin clumping was seen in apoptotic cells. Strong statistical correlation between the apoptotic rate and contracture damaged cells in the myocardium was shown. By polarized light microscopy apoptotic CMs were localized in areas with marked contracture changes.

WE CONCLUDE that apoptosis contributes to the development of CM injury in ischemia. Apoptotic death of CMs may lead to electric instability of the myocardium in early ischemia and cause sudden cardiac death. Apoptosis of the peri-infarction CMs may precede extension of the necrotic area. Further studies of apoptotic death of CMs in ischemic heart disease may help to elaborate novel therapeutic strategies, aimed at regulation of apoptosis.

**P 718**  
**MORPHOLOGY OF CHOLINERGIC INNERVATION IN ISCHEMIC HEART DISEASE**

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The sequence of changes in the vessels, cardiomyocytes and neurocytes in ischemic heart disease remains unsolved, but they are strategically important question for choice of pharmacological measures direction at the same time. The purpose of the present work was to study the morphological state of the parasympathetic innervation of the heart in ischemic heart disease.

The hearts of patients died with acute and chronic form of ischemic heart disease and hearts of persons who died due to cranial trauma were subjected to morphological study. The histological, histochemical, morphometric methods were used.

The results of the study have demonstrated that the parasympathetic plexuses had consistent topography with highest density near the heart hilum. The density of the ganglia was reduced in cardiosclerosis. Degenerative and sclerotic changes of all cholinergic elements were established. The number of the ganglia and density of the filaments did not correlate with level of coronary stenosis. The change of the form of perivascular neurocytes has been revealed with signs of perinuclear chromatolysis, edema, and augmentation of cell dimensions. Thus the vacuolization begins from periphery of the cells with the subsequent growth of vacuoles and movement of the nuclei to periphery. Study of cholinergic innervation revealed pronounced depression of acetyl cholinesterase activity.

The nervous fibers settle mainly along of vessels of microcirculatory bed. Both myelenic, and amyelenic nervous fibers are turgent, with signs of pronounced argyrophilia. Thus the fibers are impregnated irregularly with alternating of light and dark fields. The varicose thickenings, leakage of neuroplasm, microlocuses of destruction and fragmentation are marked; cytoplasm with signs of vacuolization.

So, deparasympathetization of myocardium could be one of the factors in pathogenesis of myocardial ischemia.

#### P 719

### CARDIOMYOCYTE REMODELING, A NEW ASPECT OF ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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**Background.** Progressive deterioration of mainly right ventricular function due to fibro-fatty tissue infiltration and ongoing loss of myocytes is a characteristic feature of the arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). Histopathological investigations of endomyocardial biopsy samples have diagnostic value but till now were not dedicated to elucidate the cellular mechanisms underlying myocyte loss except apoptosis, and disease progression.

The study was aimed to show if structural cardiomyocytes remodeling might underlie the ARVC/D development and progression.

**Methods.** Endomyocardial biopsy specimens of the right ventricle wall were obtained from 7 paediatric pts. (4F, 3M, aged from 6 to 17 years) with ARVC/D established according to McKenna et al. (1994) criteria. Samples obtained from every heart were fixed in 4% buffered formaline and embedded in paraffin blocks, and in 2.5% glutaraldehyde in cacodylate buffer and embedded in Epon blocks. Serial paraffin sections stained hemotoxylin-eosin, AZAN, PAS and PAS with diastase, and immunohistochemically with antibodies anti-desmin, smooth muscle alpha-actin, vimentin were analysed. Ultrathin sections prepared from trimmed area bordering fibro-fatty tissue were routinely stained for EM examination.

**Results.** Some myocytes adjacent to fatty tissue showed vacuolisation, myolysis and accumulation of glycogen. Immunohistochemistry revealed that some of these cells exhibited abnormal pattern of desmin labelling at the level of Z-lines and positive staining for smooth muscle alpha-actin simultaneously. Electron microscopical studies showed in some cardiomyocytes lysis of contractile apparatus, accumulation of glycogen, loss of sarcoplasmic reticulum and T-tubules, polymorphic mitochondria and dispersion of nuclear chromatin. Both immunohistochemistry and electron microscopy presented related cell alterations probably.

**Conclusions.** Changes in ARVC/D in distribution and expression of proteins involved in contraction and excitation are probably phenotypic resemblance to fetal cardiomyocytes, what points the dedifferentiation of these cells. Dedifferentiation of cardiomyocytes might actually be considered survival states aimed to endure the altered conditions. Whether this state could be reversible needs further studies.

**Acknowledgement.** This work was supported by project QLG1-CT-2000-0191 financed by the UE.

#### P 720

### QUANTITATIVE BASES OF COMPROMISED CARDIAC CONTRACTILITY DURING THE LATE STAGE OF SEPSIS

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**Introduction.** Previously it is reported an association between sepsis and myocardial edema and histologic changes, but the anatomic base of sepsis-induced myocardial depression is incompletely understood. The objective of the present study was to determine whether are prerequisite morphometric

myocardial parenchymal or microcirculatory changes for the development of contractile dysfunction in sepsis.

**Material and methods.** We have studied fourth-eight patients, who died of sepsis caused by abdominal infections and were been postmortem examination. The fragments from all cavities of the heart have been processed by paraffin technique and have been examined for qualitative diagnosis. The quantitative studies have been done with a video digital interactive program that utilized standard measurement of cardiac muscle fibers and stereology of percentual volumes of myocardial components.

**Results.** Our studies provide that histological changes are also a feature of myocardial injury in human sepsis. We noted alterations in the myocardium that consisted of interstitial miocarditis, interstitial edema, and muscle-fibers necrosis. We remarked differences between heart cavities, ventricles being more affected by myocytolysis and destruction of capillary wall. Morphometry relieved the increased of percentual volumes of interstitial space (22.1% vs. 13.5% in witness hearts) and myocardial capillaries occupied by leukocytes (16.8% vs. 4.1% in witness hearts).

**Conclusion.** We conclude that that objective myocardial structural changes and microcirculatory injuries may contribute to decreased ventricular contractility during sepsis.  
**KEY WORDS:** CARDIAC CONTRACTILITY, MYOCARDIUM, QUANTITATIVE ANALYSIS, SEPSIS

#### P 721

### HEMORRHAGIC MYOCARDIAL INFARCTION

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**Objective:** Hemorrhagic myocardial infarction is one of the rarest kinds of myocardial necrosis. It is result from thrombolysis, hemorrhagic disturbances or mechanic trauma.

**Aim:** The aim of the work was the characterization of morphology of myocardial hemorrhagic infarction and its differences with ischemic myocardial necrosis (myocardial infarction).

**Material and methods:** The material consisted of 30 cases of macroscopically hemorrhagic myocardial infarction. Comparative group consisted of 20 cases of ischemic, pale myocardial infarction. The clinical history of disease was 48 hours. The studies were based upon histology and confocal laser scanning microscopy.

**Results:** Hemorrhagic myocardial infarction was characterized by the bulk of interstitial erythrorrhages, paucity of inflammatory infiltration, preserved continuity of cardiocytes hyperemia of coronary veins, compression of coronary arterioles and nerve trunks. In confocal laser microscopy the myocardial fibers shows no increased autofluorescence and fractures, but dominant feature was its thinning by interstitial hemorrhages.

**Conclusion:** Hemorrhagic myocardial infarction is a diverse morphological entity, closely resembled with hemorrhagic complications.

#### P 722

### MYOFIBROBLASTS IN THE EVOLUTION OF HUMAN MYOCARDIAL INFARCTION

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Recent studies have shown that cardiac fibroblasts and myofibroblasts play an important role in healing and remodeling processes after myocardial infarction (MI). Despite extensive research, the exact mechanisms are not

completely understood yet. The aim of our study was to analyze the distribution and immunophenotype of fibroblasts and myofibroblasts in the normal human heart and MI.

**Methods.** Autopsy samples of infarcted heart tissue from 15 patients with MI of different duration, and samples of heart tissue from 5 healthy persons who died in accidents, were included. Immunohistochemistry was performed by a sensitive peroxidase-streptavidin method on formalin fixed, paraffin-embedded tissue, using monoclonal antibodies against  $\alpha$ -smooth muscle actin (SMA), CD34, CD31, vimentin, desmin, activated caspase-3, Ki67, TGF $\beta$ -1 and TGF $\beta$ -1 receptors.

**Results.** In normal hearts, there were scattered vimentin and CD34-positive fibroblasts in the subendocardial space and around intramyocardial blood vessels. TGF $\beta$ -1 was focally expressed on capillary endothelial cells.

Myofibroblasts were found in MI which were at least 5 days old; they were vimentin and SMA positive and desmin negative ('VA' phenotype). Some were labeled with Ki67, and some with anti caspase-3. Myofibroblasts as well as most of inflammatory cells and endothelial cells also expressed TGF $\beta$ -1 and TGF $\beta$ -1 receptors.

In old scar, some myofibroblasts retained SMA and TGF $\beta$ -1 receptor expression.

**Conclusions.** 1. We found CD34-positive fibroblasts in the interstitium of the normal myocardium. These cells might be one of the origins of myofibroblasts, similarly to other tissues.

2. Myofibroblasts in MI were of the 'VA' phenotype. 3. Myofibroblasts, inflammatory cells and endothelial cells in MI also expressed TGF $\beta$ -1 and TGF $\beta$ -1 receptors suggesting that TGF $\beta$ -1 might be important in the healing process after MI as suggested by experimental studies 4. Some myofibroblasts were activated caspase-3 positive showing that apoptosis is an important mechanism in transformation of granulation tissue into a scar. 5. Some myofibroblasts in old scars after MI retained SMA expression, in contrast to dermal wound healing, where expression of SMA is transient. It has been suggested that this is due to the continuous mechanical stress caused by contraction and relaxation of the surrounding viable myocardium.

#### P 723

#### CLINICAL SPECTRUM OF CARDIAC LYMPHOMAS

Mary N Sheppard

Cardiac lymphomas are rare and there is often a delay in diagnosis. We present six cases where the clinical presentation varied from sudden death to infarction to pericardial effusion.

Two cases presented with large pericardial effusions in children. Both were T cell lymphomas. One child died suddenly after draining the effusion shortly after admission to hospital.

One T cell lymphoma presented as multiple areas of infarction due to vascular involvement with diagnosis only being made 6 months after presentation when a 29 year old male developed cutaneous lesions.

One large B cell lymphoma presented as a suspected bacterial endocarditis on an aortic homograft in a 57 year old male.

One case in an 81 year old presented with sudden death due to widespread infiltration by chronic lymphocytic leukaemia. The final case presented as a sudden death in a 57 year old man following cardiac transplantation six years previously. He had widespread microscopic involvement by a B cell lymphoma.

All these reflect the wide variation in cardiac involvement by lymphoma.

#### P 724

#### A NOVAL METHOD OF QUANTITATIVE FUNCTIONAL MORPHOLOGY ASSESSING CONTRACTILE RESERVES OF MYOCARDIUM USING HISTOLOGICAL SECTIONS

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#### Introduction

The important information for cardiology and cardiosurgery obtained from morphological examination of heart muscle biopsies is the volume of the myocardial contractile potential. Currently this information can only be obtained by methods of quantitative functional morphology generally requiring electron microscopic examination (EME).

#### Aim

The aim of the study was to develop a method to determine the contractile reserve of the cardiac muscle using histological sections examined by light microscope (LM).

#### Materials and Methods

Cardiac muscle samples were collected and investigated as following: a) postmortem heart muscle from patients died of acute myocardial infarction and healthy people died in accidents, b) myocardium specimens from normal and physically exercised rats as well as animals with acute pancreatitis, c) the cardiac biopsy tissue from patients with valvular heart disease. We applied conventional histology, electron microscopy, stereology, micromechanography, statistics, mathematical modeling and quantitative functional morphology.

#### Results

A novel mathematical model was developed, which makes it possible to determine the contractile ability of cardiac muscle (fmyo) using quantitative morphological parameters obtained by LM. The parameters ( $V_v$  = volume density;  $S_v$  = surface density) take into account the most essential quantitative and qualitative changes of muscle fibres (mf), capillaries (cap) and interstitium (int). The following equation with a modular construction and an open architecture was derived:

$$f_{myo} = K \cdot V_{vmf} \cdot \left\{ \frac{1}{V_{vcap}} + \left[ \frac{1}{(0,0064 \cdot S_{vcap})} + (V_{vint}/0,0073) + \frac{1}{(0,0016 \cdot S_{vmf})} + (V_{vmf}/0,053) \right] \cdot (1/v_1) \right\}^{-1}$$

The index (K) shows the relative level of myocytolysis. The ratio (1/v<sub>1</sub>) is considered the standard value for this model. The (-1) is the power for equation.

The statistically strongly correlated concordance of myocardial contractility data from the same hearts determined by using micromechanography from one side and by using EME, LM examination from the other side, supports the validity of the new mathematical model.

#### Conclusion

Our results demonstrated that the quantitative functional morphology using histological sections is a quick, reliable and easily applied method in assessing myocardial contractility. We suggest this novel method for routine evaluation of vital capacities of myocardium.

#### P 725

#### CARDIAC ANGIOSARCOMA

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#### Introduction:

Primary tumours of the heart are rare. 80-90% are benign. The most common malignant tumour is angiosarcoma, representing 40% of cardiac primary sarcomas. Patients are usually adult, more frequently men. We present a man with angiosarcoma of the right atrium.

**Clinical history:**

Otherwise healthy 22-year-old man was admitted to the hospital due to severe chest pain a day after a mild blunt chest trauma. ECG and chest x-ray were normal. Transthoracic echocardiogram demonstrated a moderate pericardial effusion and a mass measuring 3x3 cm, protruding into the right atrium above the tricuspid annulus, without obstruction of the orifice. The angiography showed abnormal vascularization originating from the right coronary artery. The tumour was excised but excision was not radical due to extension of the tumour. The patient refused urgent cardiac transplantation, which was performed six months later.

Macroscopically, the excised tumour was multilobular, dark, growing around right coronary artery. In explanted heart residual tumour measuring 6x4,5x3,5 cm infiltrating pericardium and a satellite tumour were found.

Microscopically, there were irregular vascular channels, papillary fronds lined by atypical endothelial cells, compressed capillary channels without recognizable lumina, sheets of anaplastic and spindle cells with extravascular erythrocytes. There were mitoses, some necrosis, invasion of blood vessels and nerves. Tumour extended to the resection margin in both samples.

**Discussion:**

Histologically, angiosarcoma of the heart has three major differential diagnostic possibilities, haemangioma with papillary endothelial hyperplasia, spindle cell and Kaposi sarcoma, which usually forms small nodules on pericardial surface with minimal infiltration of the heart. Most cases of cardiac angiosarcoma are diagnosed at the time of autopsy. Metastases, most often in lungs, are present at the time of presentation in 66-89% of patients. Prognosis is extremely poor, mean survival only 3 months, but survival up to 53 months has been reported. Complete resection is usually not possible, because of extensive local spread. Heart transplantation is the best option. Radiation therapy and chemotherapy may offer some temporary relief. Our patient is currently on radiation therapy, without any signs of disease, 11 months after presentation of the tumour.

**P 726****METASTATIC SQUAMOUS CELL CARCINOMA MIMICKING CARDIAC MYXOMA**

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**Introduction:**

We report a case of squamous cell carcinoma of the uterine cervix, presenting with cardiac metastasis in a 42-year-old woman who had a radical hysterectomy for carcinoma of the cervix. The tumor was a poorly differentiated squamous cell carcinoma with extensive vascular invasion, involving the cervix, lower uterine segment, myometrium and parametrial soft tissue. The patient received radiotherapy and chemotherapy. Two years later the patient was admitted for symptomatic right atrial mass (clinical impression: Atrial myxoma).

**Methods:**

The original tumor and cardiac metastasis was analyzed for expression of low and high molecular weight cytokeratins, Ki-67, and p53.

**Results:**

The first intraoperative frozen sections exhibited prominent myxomatous stroma leading to an initial impression of myxoma. Subsequent intraoperative sections showed obvious squamous cell carcinoma.

Immunohistochemical study of the tumor showed expression of low and high molecular weight cytokeratins and high Ki-67 positivity. The original tumor and cardiac metastatic squamous cell carcinoma did not demonstrate any degree of

p53 expression. Interestingly, the myxomatous stroma exhibited marked Ki-67 positivity.

**Conclusion:**

Patients with squamous cell carcinoma of the uterine cervix with extensive vascular invasion are at the risk of vascular invasion and hematogenous dissemination.

Metastatic squamous cell carcinoma to the right atrium can mimic atrial myxoma by CT and echocardiogram. Furthermore, a myxoid stromal reaction with high proliferative index can lead to an initial misdiagnosis of atrial myxoma.

**P 727****PATHOLOGICAL FINDINGS IN SUDDEN CARDIAC DEATH**

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Sudden cardiac death results from an acute ischaemic event or a ventricular arrhythmia arising in an inflamed, scarred, hypertrophic or genetically abnormal heart. To determine the relative contributions of these causes we made a prospective study of all cases of sudden cardiac death in adults of any age over a one year period.

Three hundred and twenty one cases of out-of-hospital sudden cardiac death occurring within one hour of the onset of symptoms were studied. Each case was graded according to the certainty of the cause of death. Grade 1 cases were the certain result of ischaemia and had clear evidence of coronary artery thrombosis or myocardial infarction. Grade 2 cases had evidence of healed myocardial infarction and at least one coronary artery < 1mm in diameter. Grade 3 cases had coronary artery narrowing alone while Grade 4 had cardiac hypertrophy only. Grade 5 was unexplained cardiac death (SADS). In 107 cases (33%) there was coronary thrombosis or acute infarction. In 163 further cases there was coronary artery narrowing. 107(33%) had an associated myocardial scar (Grade 2) whilst 56 (17%) did not (Grade 3). In 45 cases (14%) non ischaemic heart disease was present (Grade 4) and in 5 there was no obvious cardiac pathology (SADS).

The 33% incidence of acute ischaemia in sudden death is lower than in two studies from the 1990s (47% and 52%). Whether this is the result in changes in autopsy practice or a real change in the pattern of disease is uncertain. Our incidence of SADS is lower than in a recent UK study, but one that set an upper age of 65 years. Sudden death due to unexplained cardiac hypertrophy is important and has been underinvestigated.

**P 728****SPONTANEOUS AORTIC DISSECTION. AN AUTOPSY STUDY OF 28 CASES.**

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Background: Spontaneous aortic dissection is caused either by a tear in the intima or by an intramural haematoma preceded by rupture of the vasa vasorum. The dissection is caused by degeneration of the media, which is seen in different conditions like hypertension, heart valve diseases, pregnancy, cocaine abuse and certain syndromes.

Material and methods: In a 12-year period (1992 - 2003), 28 cases of sudden unexpected death were autopsied at the Institute of Forensic Medicine, University of Aarhus, and diagnosed as aortic dissection. The cases represent different clinical manifestations, diagnostic difficulties and different localizations of the dissection.

Results: Of the 28 cases 11 were females aged between 20-82 years (mean 47,2) and 17 were men aged between 31-80 years (mean 49,5).

In terms of De Bakey classification, 60 % were classified as Type I, 40 % as De Bakey Type II and none were classified as De Bakey Type III. Varying symptoms including, chest pain, nausea, dyspnoea, headache and sweating were reported in 68%, whereas 32% had been asymptomatic. 60% had had medical contact and of these 21% had been hospitalized of which 50% had been misdiagnosed as coronary thrombosis, and two as gallstone and gastric ulcer. Aortic dissection was only diagnosed in one case by CAT-scan. The examinations done at admission were, electrocardiography (100 %), blood test (83 %), echocardiography (33 %), chest X-ray (16 %) and CAT-scan in the only pre-mortem correctly diagnosed case.

Conclusion: The diagnosis is difficult because the symptoms often mimic other conditions like gallstone, peptic ulcer and coronary thrombosis. With the symptoms in mind one should consider aortic dissection as a possibility, if the electrocardiography and blood tests are inconclusive for coronary thrombosis. The common used diagnostic procedures are CAT-scan, MR-scan, transoesophageal echocardiography and angiography, which have different accessibility, advantages and disadvantages.

The only chance of saving lives is to diagnose aortic dissection as early as possible, even though the prognosis is poor.

The only known treatment is surgery.

#### P 729

##### **AUTOPSY FINDINGS IN CORRECTED TRANSPOSITION OF THE GREAT ARTERIES**

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Introduction: congenitally corrected transposition of the great arteries (C-TGA) represents the combination of atrio-ventricular and ventriculo-arterial discordance, with completely normal circulation, but with many associated cardiac anomalies.

Purpose: The aim of this study was to analyze the cases of C-TGA (sex, age and causes of death, atrial situs), and presence of associated cardiac and/or extra-cardiac anomalies.

Material and Methods: during the period from 1926-2004, a total number of 1.399 cases of congenital heart diseases (CHD) were diagnosed. All autopsies were performed using standard or modified Rokitsansky technique. C-TGA was found in 24 cases (1.7 %).

Results: There were 15 males (62.5%) and 9 females (37.5%); in 20 cases the heart was in situs solitus (83.3%) and in 4 in situs inversus (16.7%). The viscera was always in situs solitus. Majority of the patients had 2 or more associated cardiac anomalies (20 ASD, 9 VSD, 3 AVSD, 2 PDA), and only 1 case had no communications between heart chambers; in 7 cases we found 1 communication, in 15 cases 2, and in 1 case 3. In addition, pulmonary atresia was present in 7 cases (29.2 %), and pulmonary stenosis in 4 (16.7%). Anomalous connection of pulmonary veins was found in 5 cases (20.8%), and anomalies of the coronary arteries too (hypoplasia in 3 and single coronary artery in 2). Majority of the patients died within the first month of life (13 or 54.2%), 6 within the first year (25%), 2 in the second year (8.3%), 1 in the third year (4.2%), and 2 lived over 20 years (8.3%). Extra-cardiac causes of death were predominant within the first year of life: bronchopneumonia (12 or 50%), atelectasis (4 or 16.7%), cerebral hemorrhage (3 or 12.5%), acute heart failure (2 or 8.3%), and myocarditis/endocarditis (1 or 4.2%).

Conclusion: Our results showed male predominance, situs solitus predominance, and the most often cause of death were

extra-cardiac complications. Majority of patients died within the first year of life, and the most often associated cardiac anomalies were: ASD, VSD, pulmonary atresia or stenosis, and AVSD.

#### P 730

##### **PECULIARITIES OF VULNERABLE PLAQUE FORMATION**

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Vulnerable plaque formation exposed to rupture is the main cause of the acute coronary syndrome. The factors that promote the progression of atherosclerotic lesions instability are not clearly understood. In this study we showed the difference in extracellular matrix components and cellular distribution in vulnerable and stable plaques.

Methods and Results. Human carotid and coronary endarterectomy specimens (n=27) were obtained from patients with stenosis of >65%, as demonstrated by duplex ultrasonography and angiography. Plaques were histologically classified as either fibrous (stable), vulnerable (unstable) or plaques with features of instability – large lipid core, numerous inflammatory infiltrates and neovascularization but having thick fibrous cap. Immunohistochemical staining was used to analyze the expression of CD68, aSMA, collagen type III, fibronectin, laminin and thrombospondin-1. Macrophage (CD68 positive cells) infiltrates were greater in vulnerable plaques and plaques with features of instability. Laminin and thrombospondin-1 immunostaining was not detected in normal arteries (n=5) and was larger in vulnerable plaques and plaques with features of instability than in stable plaques. The matrix components collagen type III and fibronectin showed variable distributions in all groups. There were no significant differences in aSMA immunostaining between different lesions also.

Conclusions. Our results suggest that laminin and thrombospondin-1 as well as CD-68 positive macrophages may be involved in the formation of vulnerable plaques and plaques with features of instability.

#### P 731

##### **AORTIC DISSECTION, A LIFE THREATING DISEASE, TREATED BY USING „BIO-GLUE” SURGICAL ADHESIVE IN THE SURGICAL WALL REPAIR**

Doina Butcovan, Cleopatra Borza - Department of Pathology Grigore Tinică-Cardiology Center Iasi

Using Bio-Glue the cardiovascular surgery becomes a minimal invasive technique. Biologic glued anastomoses are obtained by applying a conventional technique of anastomosis indicated for severely altered aortic walls. We present a 55 years old man, without significant known history, having at presentation an anterior thoracic sudden violent pain, with duration of 1 hour and half. The patient was admitted at Cardiology Center Iasi with the suspicion of aortic dissection, which was confirmed after a complete cardiovascular evaluation. The histological examination of the surgical biopsic aortic specimens showed the morphological substrate of the disease represented by medial degenerative lesions that were evidenced by using routine morphological techniques. The study revealed the importance of a complete cardiovascular evaluation and a rapid diagnostic for indicating an effective treatment and, in this way, for preventing an unpredictable complication. Concluding, we can appreciate that this biologic glue allows a good anastomosis, and a truly minimally invasive cardiovascular surgery may be possible.

#### P 732

### **CD44 IS LOCATED IN PLEXIFORM LESIONS OF IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION**

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**Introduction:** The plexiform lesion in idiopathic pulmonary arterial hypertension (IPAH) is characterized by thin-walled microvessels with abnormal endothelial cells (ECs) proliferation. The pathogenesis of plexiform lesions has not been fully clarified. Recently, CD44 has been reported to regulate collateral artery formation via leukocyte trafficking and several growth factors. We hypothesized that CD44 is involved in formation of plexiform lesions of IPAH.

**Methods:** Expression of CD44 was examined on lung specimens including 46 plexiform lesions from 7 IPAH patients and normal lung tissues from 3 autopsy cases as control. To define localization of CD44, the following antibodies were used: CD31 for ECs, CD68 for macrophages, CD45Ro for T lymphocytes and alpha -smooth muscle actin for smooth muscle cells.

**Results:** ECs in 44 out of 46 plexiform lesions (96%) expressed CD44, while in patent muscular arteries from IPAH and muscular arteries from control lung tissue were negative for CD44. Intense expression of infiltrating leukocytes in plexiform lesions or perivascular accumulating leukocytes (mainly T lymphocytes) in lung tissue from IPAH was also observed.

**Conclusion:** CD44 expression was increased in ECs and leukocytes of plexiform lesions. The result indicates that CD44 might play an important role in the formation of plexiform lesions in IPAH.

### **P 733**

#### **CHAGAS' DISEASE AND SUBSETS T CELL**

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The present work reviewed endomyocardial biopsy specimens taken in the Instituto de Investigaciones Cardiovasculares de la Universidad de los Andes Mérida, Venezuela and Instituto do CoraVão, São Paulo, Brasil, from 1986 to 1998 and, looking for different CD4+/CD8+ T cell ratios in patients with chronic heart failure. For immunohistochemistry, T.cruzi antigens and CD4+ and CD8+ T cells in paraffin histological sections were quantified. The sections came from 4 groups of chagasic patients: A.- Acute phase (12 pts); B.- Indeterminate form (21 pts); C.- Cardiac form with arrhythmia (10 pts); and D.- Chronic heart failure (11 pts).

Groups A, B, C and D presented respectively: 100%, 10%, 40% and 45% of myocarditis; 58%, 0%, 10% and 0% with T.cruzi (+) antigens; with a mean CD4+/CD8+ T cell ratio of 0,75; 0,90; 1,17 y 0,79. The Pearson Correlation Test between mean numbers of CD4+ T and CD8+ T cells was high in groups A and B but not in groups C and D. Acute phase is characterized by myocardial inflammatory infiltrate with a balanced CD4+/CD8+ T cell immune response, associated with presence of parasite antigens. Indeterminate phase shows a lesser degree of myocarditis and balanced T cell immune response. Chronic cardiac forms are associated with myocarditis, but the quantity of T.cruzi antigens is much smaller and not detected by biopsy; an unbalanced immune response, with low CD4+/CD8+ T cell ratio, may be contributing to the persistence of active chronic myocarditis and injury of non-infected myocardial fibers.

### **P 734**

#### **PATHOLHISTOLOGICAL CHANGES IN AORTOCORONARY VENOUS GRAFTS**

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The aim of this paper is to present autopsy, histomorphological and immunohistochemical findings in aortocoronary saphenous vein bypass. Aortocoronary vein grafts removed at autopsy from 15 patients who died within one month up to 2 years after the bypass operation were examined. Histomorphological analysis was performed on paraffin sections stained with hemalaun eosin, Van Gieson elastica and Trichrome Masson. Immunohistochemical analysis for smooth cell actin, desmin, vimentin, CD 68, MAC 387, LCA, CD 20, and CD 43 (DACO products) was performed. A technique of LSAB+ immunoperoxidase (DAKO) was used with prior microwave antigen retrieval in citric acid buffer (pH 6,0). The main immunohistological finding were presence of intraluminal thrombotic masses, and endothelial damage with slight hyperplasia of tunica intima. Immunohistochemically we found a few smooth cell actin and vimentin positive cells in the intima. There were not found inflammatory cells, except at the anastomotic sites (CD68+, MAC 387+ and a few LCA+ cells). Positivity for the muscle cell actin in the intima suggests presence of adhesive molecules and cytokines that influence migration and hyperplasia of these cells, as well as in formation of atherosclerotic changes that occurred in the wall of the vein grafts.

### **P 735**

#### **ULTRASTRUCTURAL ALTERATIONS OF THE PLACENTAL BARRIER UNDER HYPOXIA CONDITIONS**

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The electron microscopic investigation of placenta under hypoxia condition caused by maternal iron-deficiency anaemia and EPH-gestosis was carried.

Electronograms from 30 placentas, including 10 cases of EPH-gestosis, 10-maternal anaemia and 10-uncomplicated pregnancy (controls) were studied stereometrically by point count method at magnification X 5600.

Pathological ultrastructure's alterations of a placental barrier, including a damage of microvilli, thickening of basal membranes both of epithelium and endothelium, a stromal fibrosis were found. Compensatory proliferations of cytotrophoblast in cases of EPH-gestosis and in anaemia group; as well as hyperaemia of foetal vessels in EPH-gestosis group were seen too. The increase of the syncytiotrophoblast volume fraction (VF) in anaemia group (35,71±0,66 vs. 32,19± 0,26 % in controls), as well as cytotrophoblast, epithelium and endothelium basal membranes VF's both in anaemia (A) and gestosis(G) groups had registered

( correspondingly: A- 2,76± 0,04; 3,52±0,02; 0,57±0,06; G- 1,05±0,03; 4,76±0,03; 0,74±0,04 vs. 0,76±0,04; 3,04±0,01; 0,38±0,12% in controls, P<0,05). The capillary lumen increased to 14,70±0,26% in gestosis group (13,5±0,21% VF in controls, P<0,05).

Pathological changes of a placental barrier could be a structural basis of a placental insufficiency. It was suggested that the process of ultrastructural remodelling of the placental membrane may compensate for different pathologies at the terminal parts of the villous chorion, which is essential base of the morphological correction of a placental insufficiency.

### **P 736**

**ULTRASTRUCTURAL STUDY OF THE EFFECT OF EXPERIMENTAL STAINING OF THE ANTERIOR LENS CAPSULE IN ALBINO RABBITS ON THE CORNEA AND ANGLE**

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**Purpose:** To study the value and safety of staining the anterior lens capsule in albino rabbits.

**Methods:** The experiment was divided into 3 stages. First, the capacity of different concentrations of crystal violet solution to stain the anterior lens capsule of postmortem albino rabbit eyes was tested. The toxicity of different concentrations of the dye (2% to 0.25%) was then tested to determine the highest concentration that was nontoxic to the cornea and trabecular meshwork. The third step was to detect possible toxicity of lower concentrations (0.1% to 0.05%). Different concentrations were injected into the anterior chamber of the rabbit eyes. The eyes were examined after 1 and 3 days, 1 week, and 1 month using light microscopy and scanning and transmission electron microscopy.

**Results:** Different concentrations of crystal violet stained the anterior lens capsule, allowing for easy capsulorhexis. The use of the 2% and 1% concentrations was accompanied with irreversible damage to all corneal layers. The use of the 0.5% concentration caused damage to stromal keratocytes and endothelium. The use of the 0.25% concentration did not damage any corneal layer of the trabecular meshwork. Lower concentrations of 0.1% and 0.05% also stained the capsule, providing good visibility for successful capsulorhexis, and were less toxic to the corneal endothelium.

**Conclusion:** Staining the anterior lens capsule with 0.25% to 0.05% concentrations of crystal violet solution caused no injury to the cornea or trabecular meshwork in albino rabbit eyes.

**P 737**

**LIVER INJURY IN HEAT STROKE: ELECTRON MICROSCOPY STUDY.**

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It is common that thermal injury results in widespread injury of liver. The purpose of the study is to investigate the ultrastructural changes of liver in the patients with heat stroke. **Methods:** A total 12 young soldiers with heat stroke, liver biopsy specimens were obtained within one week after the onset of heat stroke. Specimens were examined in electron microscope.

**Results:** Ultrastructural abnormalities of hepatic tissues were found in 11 cases. The changes of liver are as following: fat droplets in hepatocytes, increased autophagic vacuoles in cytoplasm, increased collagen fibers in space of Disse, dilated endoplasmic reticulum, cell debris and glycogen in sinusoid, and outer membrane disruption of hepatocytes. In one of the cases, the liver showed no remarkable change.

**Conclusions:** In this study, various degrees of liver injury were found in most of the patients with heat stroke. The histological changes of liver evidence that direct thermal injury and tissue hypoperfusion.

**P 738**

**LET'S DO THE TWIST - A MODEL FOR THE ASSEMBLY OF CYTOPLASMIC AND NUCLEAR INTERMEDIATE FILAMENT PROTEINS**

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Recently, intermediate filament (IF) proteins and especially the nuclear lamins have attracted wide attention as mutations in the lamin A-gene have been linked to various diseases such as lipodystrophy, dilative cardiomyopathy, muscular dystrophy, neurodegenerative disorders and progeria. Still the function of IF proteins is poorly understood. The ability of IF proteins to self-assemble into higher-order structures is conserved among species and thus seems to be key for protein function. Nuclear lamins and cytoplasmic intermediate filament proteins share the primary steps of assembly but ultimately form different structures in vivo.

Here we propose a model for the self assembly of nuclear lamins and cytoplasmic IF proteins which, on the basis of known interactions, explains the differences in assembly properties: IF proteins share a common tripartite architecture consisting of an alpha-helical central rod domain flanked by non-helical domains. The alpha-helices wind around each other resulting in dimeric coiled-coils. The dimers in a second step can interact basically in four different ways, one parallel and three anti-parallel, resulting in higher order structures such as unit-length filaments and ultimately filaments in case of the cytoplasmic IF proteins and head-to-tail polymers and paracrystals in case of the nuclear lamins.

Nuclear lamins and cytoplasmic IF proteins are distinct in that nuclear lamins have a 42 amino acid insert in their rod domain. Our model explains, why this insert leads to a different behavior of assembly. We propose that IF filaments are twisted structures emerging from surface lattices formed by dimetric IF proteins assembled via the known four basic dimer-dimer interactions and we extend this model onto the nuclear lamins, the geometry of which finally leads to other higher order structures.

**P 739**

**THE ENDOMETRIAL PINOPODES INVESTIGATION IN WOMEN WITH UNEXPLAINED INFERTILITY.**

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**Introduction** The aim of the study was to evaluate the peculiarity of pinopodes formation in luminal uterine epithelial cells in women with unexplained infertility.

**Materials and methods** We have investigated the luminal epithelial cells morphology of endometrium in 16 patients with unexplained infertility and 10 healthy women from couples with male infertility. Double endometrium biopsy was taken on day LH+6/7 and LH+8/9. Specimens were examined with scanning electron microscopy for detection of pinopodes.

**Results** The SEM pictures of pinopodes were obtained in all the patients. Scanty pinopodes were present in 6 (37,5%) cases; the same stage of development in both biopsies or arrested pinopodes in 2 (12,5%) cases; high variability of the pinopodes shape and size in 8 (50%) cases; ciliated cells were absent in 4 (25%) cases.

In all the women from couples with male infertility were abundant or moderate pinopodes.

**Conclusion** We can consider, that the disturbances in pinopodes formation, maturation and ciliated cells quantity is one of the causes of implantation failure.

**P 740**

**REVERSIBLE AND IRREVERSIBLE LUNG CHANGES AFTER EXOGENOUS SURFACTANT TREATMENT WITH IMMUNO-ELECTRON MICROSCOPY STUDIES**

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Exogenous surfactants are widely used in prevention and treatment of respiratory distress syndrome in newborns. There are also several attempts to use the same mode of therapy in

treatment of acute respiratory distress syndrome in adults but the results are still unsatisfactory.

The aim of the present study was evaluation of influence of exogenous surfactant on cells of air-blood barrier and the outcomes on its morphology in prolonged observations. The studies were conducted on animal model. The single dose of commercially available surfactant (Curosurf plus synthetic analogue of SP-B-HA) was introduced under anesthesia and intubation. Lung tissue samples were taken for morphologic evaluation after 1, 6, 24 hours and 5, 10, 21 and 42 days. Light and electron microscopic techniques as well as morphometric studies were used.

In the first periods of observation after administration of exogenous surfactant atelectasis, congestion, alveolar edema and erythrorrhagies were observed. After 5 days those changes started to decline and since the 10th day of observations first signs of alveolar fibrosis were recognized. Using the immuno-electron microscopic studies evidence of incorporation of exogenous surfactant into membrane systems of air-blood barrier cells was found.

In conclusion it should be stressed that exogenous surfactant internalize with cell membranes and cause lung injury. The injury of the lung parenchyma leads to alveolar fibrosis.

#### P 741

##### **ULTRASTRUCTURE IN FAMILIAR HYPERTROPHIC CARDIOMYOPATHY WITH ARG723GLY MUTATION**

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The Familiar Hypertrophic Cardiomyopathy is considered as the most prevalent (1/500) hereditary cardiomyopathy. At least ten genes are related to its appearance. In our hospital we have assessed a new mutation in three families over 30 carriers, that has demonstrated a worse prognosis. This is a Arg723Gly mutation in the gene that codifies for the heavy chain of beta-myosine. The carriers of this mutation show a non-obstructive form of the disease with evolution to clinical symptoms of cardiac failure that starts at the age of forty. The evolution of the patients is progressive heart failure leading to death at a mean age of 50 years, so they have to be treated with an implantable cardioverter-defibrillator or finally with heart transplant. Two of these patients needed a cardiac transplant, so we had the opportunity of studying these hearts histopathologically. Grossly they were within normal measurements, but a marked fibrosis was seen in the microscopical study. We performed electronic microscopy of both, that showed peculiar cytoplasmic granules, measuring 8-10 microns. These granules were dilated endoplasmic reticulum with granular material in dense areas.

As a conclusion this is the first time that a genetic based cardiomyopathy has been related to an abnormal morphological change. This contribution can be of help in order to make the diagnosis of these patients.

#### P 742

##### **SOME EFFECTS OF ARTIFICIAL EXTRACELLULAR MATRIX ON FUNCTION AND MORPHOLOGY OF HUMAN ENDOMETRIAL EPITHELIAL CELLS IN VITRO.**

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The importance of extracellular matrix (ECM) in development and function of different cells has been reported but little is known about its role in human endometrial epithelial cells.

We have examined effects of artificial ECM (Matrigel) on the function and morphology of human endometrial epithelial cells using molecular techniques and electron microscopy. Endometrial samples were removed, with informed patient consent and Ethics Committee approval, from 17 previously fertile women undergoing total abdominal hysterectomy. The tissue was dissociated and centrifuged to provide an epithelial-rich suspension which was cultured either on plastic or seeded into Matrigel to produce polarized cells. Cells on plastic were undifferentiated, flattened and squamous but cells in polarized cultured were well-developed, tall, columnar and similar to secretory phase cells in vivo. The amount of total RNA in cells grown on Matrigel (23±1.5 pg/cell) was more than double that in cells grown on plastic (9.1±1.4 pg/cell). These results suggest that ECM plays an important role in function and morphology of human endometrial epithelial cells in vitro.

#### P 743

##### **MULTIHORMONAL ADENOMAS OF PITUITARY. APPLICATION OF ELECTRON- AND IMMUNOELECTRON MICROSCOPY IN THE DIFFERENTIAL DIAGNOSIS**

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Pituitary adenomas are classified on the basis of hormones produced by the neoplastic cells detected by immunohistochemical stains. The pivotal part in pituitary tumors diagnosis is based on the electron microscopy investigation. Immunoelectron microscopy using single and double staining enables to identify cell types not detectable by routine methods. It is important to recognize some rare subtypes of pituitary tumors with specific biology and prognosis.

Aim. Evaluation of practical implementation of electron microscopic techniques in diagnosis of pituitary adenomas.

Material consists of 470 consecutive cases of surgically resected pituitary tumors. In pituitary adenomas were diagnosed (424 cases), clinically there were 147 patients with symptoms of acromegaly, 71 patients with hyperprolactinemia, 59 cases of Cushing's syndrome, 6 cases of Nelson syndrome, 3 cases of TSH hypersecretion and 138 clinically nonfunctioning adenomas. We selected 48 cases for current study.

Methods. All cases were diagnosed by routine histological examination and immunohistochemical identification of pituitary hormones (growth hormone, prolactin, ACTH, TSH, FSH, LH and alpha-subunit) and then examined with transmission electron microscope. Immunoelectron microscopy was done using a postembedding immunogold technique.

Results. Immunohistochemical analysis demonstrated the presence more than one pituitary hormone in the about 40% of pituitary adenomas. Immunopositivity was encountered in 27.5% of nonfunctioning tumors. Ultrastructural analysis provided proper diagnosis of selected cases (e.g. subtypes of silent corticotroph adenomas or mixed GH/TSH adenomas). Double immunogold labeling greatly facilitated the distinction among mixed growth hormone/prolactin, mammosomatotroph and acidophil stem cell adenomas.

Conclusions. Electron microscopy analysis was valuable to provide a useful informations regarding the diagnosis of rare multihormonal pituitary adenomas. At present, in the routine pathological diagnosis of pituitary tumors, immunoelectron microscopy (as well as molecular biologic methods) still are regarded as research tools. These methods has to be limited for cases in which they are necessary.

#### P 744

### **CYTOLOGICAL HISTORY OF 104 WOMEN WITH INVASIVE CERVICAL CANCER.**

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**Objective:** The aim of this study was to analyse the cervical cytological history of women with invasive cervical cancer.

**Methods:** Cases of invasive cervical cancer diagnosed between 2000-2003 were identified from six Pathology departments covering a Health Region in Catalonia (Spain). For each case we retrieve from the laboratories data base: age, histology, cervical smear histories in minimum 5 years preceding the diagnosis, time from last negative and time from last abnormal screen.

**Results:** During this period, we identified 104 patients with invasive cervical cancer. Seventy-five per cent of cases were squamous carcinoma and 26% adenocarcinomas. 81.8% (85/104) had not a cervical smear reported as negative one year before of the diagnosis of the cervical cancer. The average age of these patients was 57 years. Eleven patients (11%) had had an occasional normal cervical smear an average of four years before of the diagnosis. After review, we estimated that 15% were reported as false negative

**Conclusion:** A majority of cases of invasive cervical cancer occur in women without evidence of cervical screening history. In a country with very low incidence rates of cervical cancer and opportunistic screening, specific actions to reinforce coverage among menopausal women may be justified.

### **P 745**

#### **NEW INSIGHT INTO THE EFFECT OF HIV ON HPV INFECTED CELLS**

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The role of HPV in the carcinogenesis of intraepithelial and invasive anogenital lesions is currently well established. E6 and E7 oncoproteins of HPV 18/16 high risk genotype are known to inactivate p53 and pRb pathways. The role of HIV in cervical carcinomas associated with HIV infection is, however, less well known. Several studies have described an increased prevalence of both cervical HPV infection and invasive cervical cancer among HIV-positive women compared to HIV-negative ones. A high recurrence rate of invasive cervical cancer after standard treatment has been noted in HIV-positive women and the severity of these lesions seems to be inversely correlated to immune function. This combination of events typically results in a more aggressive phenotype. A primary means by which HIV infection may influence the pathogenesis of HPV-associated cervical pathology is by molecular interaction between HPV and HIV genes. Although not yet well defined, an upregulation of HPV E6 and E7 gene expression by HIV proteins such as Tat has been postulated by some authors. With this in mind, we investigated the role of HIV in cervical carcinomas both by transfecting HeLa cells, a cervical carcinoma cell line, with Tat and by analyzing HIV positive or negative cases.

Testing HIV negative cases by immunohistochemistry, we found a recurring pattern of pRb2/p130 expression. In precancerous lesions we found two distinct layers: a proliferating one, composed of Ki-67+/pRb2- cells, and a superficial one composed of Ki-67-/pRb2+ cells. In cancerous lesions all the cells showed staining for both markers, suggesting that the growth control exerted by pRb2/p130 may be lost due to the binding with HPV E6 and E7. We then tested the effect of the expression of the HIV-1 Tat protein in HeLa cells. After Tat over-expression in HeLa cells we observed a significant reduction of cell cycle inhibitor transcription and an increase in the levels of proliferating markers, such as Cyclin A, at the mRNA level.

These preliminary results seem to suggest that HIV may contribute to carcinogenesis by promoting cancer progression, because in HIV-positive women the disease may be aggressive and less responsive to treatment.

### **P 746**

#### **EXPRESSION OF CYCLIN-DEPENDENT KINASE INHIBITORS P16 INK4A IN PREMALIGNANT UTERINE CERVICAL LESIONS; ITS ASSOCIATION WITH DEGREE OF DYPLASIA, HUMAN PAPILLOMAVIRUS (HPV) STATUS AND COMPARISON WITH PROLIFERATION MARKERS LIKE KI67 AND AGNORS.**

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**BACKGROUND:** HPV is recognized as a casual agent for cervical carcinomas. Assimilation of HPV oncogenes E6 and E7 into the host DNA promotes upregulation of cyclin dependent kinase inhibitor (CDKI) p16 detectable by monoclonal antibody in the developing cervical cancer cells.

**STUDY DESIGN:** The aim of this study is to examine the potential of p16 (INK4A) as a biomarker and to determine its utility as an HPV marker on a spectrum of cervical reactive and neoplastic lesions.

**METHODS:** A series of 172 cervical tissue samples (20 reactive, 88 CIN1, 54 CIN2, 10 CIN3) were analyzed by immunohistochemistry for expression of p16 (E6H4, CINtec™, DAKO) and Ki67 (Mib-1, DAKO) as well as by in situ hybridization for high risk HPV testing (INFORM® VENTANA) and by histochemical staining (Ploton) for AgNORs assessment. The distribution of p16 staining was graded as patchy, diffuse basal and diffuse full thickness. Pearson's  $\chi^2$  test analyzed the relationship between p16, CIN, HPV, Ki67 and AgNORs expression.

**RESULTS:** In CIN1 p16 was positive in 48% ( $p < 0.001$ ) with 74% showing patchy, 21% diffuse basal and 5% diffuse full thickness; in CIN2 p16 was positive in 70% ( $p < 0.001$ ) with 3% showing patchy, 34% diffuse basal and 63% diffuse full thickness; in CIN3 p16 was positive in 100% ( $p < 0.001$ ) with 30% showing diffuse basal and 70% diffuse full thickness. The difference in the proportions of biopsies showing patchy p16 staining in CIN1 (LSIL) and diffuse full thickness staining in CIN2/3 (HSIL) was significant ( $p < 0.001$ ). High risk HPV increased from 52% in CIN1 to 94% in CIN2 and to 100% in CIN3 ( $p < 0.001$ ); in CIN1 39% of high-risk HPV positive biopsies were p16 positive ( $p < 0.001$ ) showing 61% patchy, 28% diffuse basal and 11% diffuse full thickness distribution; in CIN2 69% were p16 positive (3% patchy, 29% diffuse basal and 68% diffuse full thickness) while all high-risk HPV positive CIN3 biopsies were p16 positive (30% basal and 70% diffuse full thickness).

Ki67 and AgNORs pattern of distribution were similar: limited to basal layer in LSIL extending to middle and superficial layers in HSIL, correlated with degree of neoplasia, also if they were expressed in more reactive conditions than p16.

**CONCLUSIONS:** Diffuse full thickness p16 expression in lesion with high and intermediate-risk HPV appears a good marker of persistent high risk HPV infection and might be useful to discriminate in equivocal cases HSIL from LSIL and to select LSIL with higher risk of neoplastic progression.

**P 747**

**KI-67 EXPRESSION IN THE INVASION FRONT AS AN ADDITIONAL INDEPENDENT SIGNIFICANT PROGNOSTIC FACTOR INFLUENCING RECCURENCE IN EARLY STAGE CERVICAL CARCINOMAS**

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**INTRODUCTION:** The attempts to determine the prognostic significance of biological markers and their relation to human papillomavirus (HPV) infection in cervical cancer have yielded controversial results.

**AIMS:** The aims of this retrospective study were to correlate alterations of cell proliferation, growth, differentiation and apoptosis regulatory proteins in early stage cervical carcinomas with HPV infection, histopathological and clinical parameters, and to estimate their prognostic significance.

**METHODS:** Expression of Ki-67, p53, mdm-2, bcl-2, c-erbB-2, EGFR protein, as well as estrogen and progesterone receptors was evaluated by immunohistochemistry in operative specimens of 83 patients with early stage cervical carcinoma. The results were assessed semiquantitatively in the surface area, center and invasion front of each tumor as a percentage of the immunostained cells and/or intensity of immunostaining for each protein. The presence of HPV was assessed by conventional in situ hybridization (ISH) technique and catalyzed reporter deposition signal amplification ISH using mixed biotinylated probes to identify types 6/11, 16/18 and 31/33 or 31/33/51.

**RESULTS:** In our case series 73 patients had a tumor limited to the uterine cervix less than 4 cm in diameter (pT1b1), while 10 patients had larger neoplasms belonging to pT1b2 category. Pelvic lymph node involvement was found in 20 patients. During the follow-up period (range, 65 -181, mean, 121 months) recurrences were observed in 9 patients. The 5, 10 and 15-year disease-free survival rate was 92.7%, 90.8% and 86.6%, respectively. Among the 18 variables pelvic lymph node involvement (P=0.0008), tumor diameter (P=0.035), depth of stromal invasion (P=0.029), histotype (P=0.0009), grade (P=0.056), HPV DNA presence (P=0.056), HPV type (P=0.043), as well as bcl-2 (P=0.035), mdm-2 (P=0.051), EGFR (P<0.0001), and Ki-67 (P=0.031) expression in the tumor's invasion front were identified as important predictive indicators of recurrence in the univariate analysis. Independent significant prognostic factors for disease-free survival in multivariate analysis were the histotype, HPV DNA presence and Ki-67 expression.

**CONCLUSIONS:** The invasive front of carcinomas proved to be the most important area for tumor prognosis. In addition to the detection of HPV presence and morphological parameters, Ki-67 evaluation could be used in selecting appropriate therapeutical approaches in patients with early stage cervical cancer.

**P 748**

**HUMAN PAPILLOMA VIRUSES DO NOT PLAY AN AETIOLOGICAL ROLE IN ADENOSARCOMAS OF THE UTERINE CERVIX**

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Primary adenosarcomas of the uterine cervix are exceedingly uncommon neoplasms. Although high-risk human papilloma virus (HPV) types have been linked to cervical carcinomas, with demonstration of integrated virus in the nuclei of the malignant epithelial and sarcomatoid components, their role in the histogenesis of adenosarcomas is unclear. The aim of this study was to determine if HPV's play an aetiological role in cervical adenosarcomas. The HPV status of 7 adenosarcomas of the cervix was investigated by non-isotopic in situ hybridisation (NISH) and polymerase chain reaction (PCR). NISH was carried out using digoxigenin labelled probes to HPV types 6, 11, 16, 18, 31 and 33. PCR employed GP5+/GP6+ primers to the HPV L1 gene. Neither the benign epithelial components nor the malignant stromal components of the 7 neoplasms harboured nuclear NISH signals for the HPV types investigated. Amplimers of the HPV L1 gene could also not be detected by PCR in any of the tumours studied. HPV's do not appear to play an aetiological role in the pathogenesis of uterine cervical adenosarcomas. This suggests that a different histogenetic pathway for this rare tumour type must exist.

**P 749**

**THE USEFULNESS OF HYBRID CAPTURE II (HC2) AS SCREENING OPTION IN LOW-RESOURCE SETTINGS OF LATIN AMERICA.**

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**Objective:** Hybrid Capture II (HC2) to detect high-risk HPV (hr-HPV) in our ongoing multi-centre trial was performed and compared to other optional testing screening tools [cytology (conventional and liquid-based {LBC}), screening colposcopy, Visual Inspection with acetic acid (VIA), Visual Inspection with Lugol's Iodine (VILI): conventional and self-sampling], for cervical cancer in Brazil and Argentina, in order to test the potential use of HC2 as a primary option for cervical screening. Purpose: A cohort of 12.107 women attending four clinics (Campinas, Sao Paulo, Porto Alegre, and Buenos Aires) are randomised into the 8 diagnostic arms. Women testing positive with any of the tests are referred for colposcopy, and cervical biopsies are used as the gold standard to assess performance characteristics (SE, SP, PPV, NPV, ROC) of the HC2 test. Methods: All cases with cytological abnormalities were referred to colposcopy and biopsies were taken according clinical evaluation. The HC2 protocol was performed according to the instructions of the manufacturer (Digene Co., Gaithersburg, MD, USA). In estimation of the viral load, samples with relative light units

(RLU) > 20 were considered to harbour a high viral load and, those with 5-19.9 were intermediate, and those with 1-4.99 were low. Only high risk HPV was tested. Chi-square test was used to analyze correlations between categorical data, with Pearson's correlation and Fisher's exact test, and calculating OR and their 95%CI where appropriate. In all statistical analysis, the  $p < 0,05$  were regarded as significant. Results: The sensitivity and specificity for CIN3 were 96.5 (95.1-97.9) and 66.1 (62.6-69.6), respectively. For CIN 2 the results were quite similar: 90.4 (88.2-92.6) and 69.0 (65.6-72.4). When we adjusted the results for Roc Curve (Receiver Operating Characteristics) the Sensitivity and Specificity were very robust: 82.5% and 82.3% for CIN3, and 83% and 76.3% for CIN2. Conclusion: In our settings, positive hr-HPV-DNA showed an important agreement with high-grade lesions of the cervix. The Public Health authorities should seriously evaluate the potential use of HC2 as primary option in order to optimize the sensitivity of cervical cancer screening. Moreover, HC2 performance might be improved by the association of cytology (preferentially, LBC) performed either after HC2 or concomitantly.

#### P 750

### **CERVICAL INTRAEPITHELIAL NEOPLASIAS POSSESS ANGIOGENIC ACTIVITY. IMMUNOHISTOCHEMICAL AND MORFOMETRIC STUDY**

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**Introduction.** Angiogenesis is the fundamental process through which new blood vessels are made. Angiogenesis is necessary for growth of solid tumors. On the other hand, cervical intraepithelial neoplasias (CIN) represents the proliferational state of the epithelium with biological characteristics of carcinoma.

**Purpose.** This research was performed to answer whether cervical intraepithelial neoplasias are able to provoke neovascularisation - angiogenesis.

**Methodes.** The blood vessel count along the basement membrane of the epithelium and the total number of blood vessels of given microscopic view field were compared between 30 specimens of CIN 1, 30 specimens of CIN 2, 30 specimens of CIN 3 and two control groups each of them containing 30 specimens of cervical tissue without pathohistological abnormality and differing from each other in the fact that specimens of control group K 1 were taken from patients who underwent hysterectomy for reasons unrelated to CIN and group K 2 of specimens taken from patients with CIN 3 lesions but from parts with no pathohistological abnormality. Samples were treated with immunohistochemical methods using anti-F-VIII and anti-CD 31 markers. Quantitative parameters were processed using quantitative morphometric analysis in program 'ISSA for windows', produced by VAMS, Zagreb. The results were statistically analysed by method testing analysis of variance (ANOVA) based on Fischer's (F-test) as the criterion of significance.

**Results.** Statistically significant difference was found in the blood vessel count directly below or along the basement membrane between CIN 1, CIN 2, CIN 3 and the control group K1 using both immunohistochemical methods ( $P < 0,05$ ) and between CIN 3 and the control group K 2 ( $P < 0,05$ ). Statistically significant difference was found in the total number of blood vessels between control groups K 1, K 2 and CIN 3 only using method F-VIII ( $P < 0,05$ ). The blood vessel count close to the basement membrane of epithelium between CIN 1 and CIN 3, and CIN 2 and CIN 3 in both immunohistochemical methods was significantly different ( $P < 0,05$ )

**Conclusion.** Since there is a significant difference in the blood vessel count along the basement membrane between normal and dysplastic epithelium, and that number increases with the

rise of CIN grade, it was concluded that CIN lesions are angiogenic.

#### P 751

### **P16 PROTEIN EXPRESSION IN HISTOLOGICAL MATERIAL OF CERVIX AND ITS CORRELATION WITH HPV INFECTION**

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Cervical carcinoma is a multistage process. It arises from defined precursor as low-grade intraepithelial lesions (LSIL), referred to as high-grade intraepithelial lesions (HSIL). An important factor in the pathogenesis is the high-risk HPV types (HR-HPV) infection. The HSIL may be initiated by deregulated expression of HR-HPV oncogenes E6, E7 in replicating epithelial stem cells. An indicator of active expression of viral oncogenes is the overexpression of the cyclin-dependent kinase inhibitor p16.

A total of 339 routinely processed cytological specimen were analyzed. The material was taken consecutively for Pap test and for HPV-DNA hybrid capture assay (HC<sup>TM</sup>, Digene). The cytological reports were negative for intraepithelial lesion or malignancy, LSIL to HSIL and cells of undetermined significance (ASC-US). HR-HPV positive cases were used for further investigation. Amplification of  $\beta$ -Globin was followed by subtyping of HPV 16-DNA with conventional PCR. A total of 73 cases of cervical carcinoma and its precursor lesion were diagnosed out of these 339 cases of routinely cytological cervical specimen. The histological report showed condylomatous atypia, cervicitis, mild dysplasia (CIN I), moderate dysplasia (CIN II), severe dysplasia (CIN III), carcinoma in situ (CIS) and invasive cancer (Ca). Formalin-fixed, paraffin-embedded tissue blocks were used for the immunocytochemical expression of p16 protein with CINtec<sup>TM</sup> p16INK4a histology kit (DakoCytomation, DN).

23 specimens with histological reports of condylomata acuminata, cervicitis and CIN I showed positivity for HR-HPV (HC<sup>TM</sup>) in 79%, HPV 16-DNA (PCR) in 37% and p16INK4a in 26%.

63% of the Pap-test cases were diagnosed as LSIL or negative for intraepithelial lesion and malignancy.

50 specimens with histological results of CIN II to CIN III, CIS and Ca showed HR-HPV (HC<sup>TM</sup>) positivity in 97% and for HPV 16-DNA (PCR) in 76% and in 87% for p16INK4a. 49% of the Pap-test cases were diagnosed as HSIL, CIS, Ca and 16% of the Pap-cases were reported as ASC-US.

Our results indicate that the cyclin-dependent kinase inhibitor p16 is strongly overexpressed in correlation with the transforming activity of the viral oncogenes of all HR-HPV types, but particular the HPV 16. The p16INK4a expression increases with the progression of dysplasia.

#### P 752

### **EXPRESSION OF MMP-1, -2 AND TIMP-1, -2 IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE CERVICAL CANCER**

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Destruction of extracellular matrix is one of the most important step for the metastasis and invasion of cancer cells. Matrix metalloproteinase-2(MMP-2) specifically cleaves collagen type IV and MMP-1 digests interstitial collagen type I and III. These proteolytic activities are regulated by stoichiometric binding of specific, locally produced tissue inhibitor of metalloproteinases(TIMPs). The aim of this study was to investigate the expression of MMP-1, MMP-2, TIMP-1 and TIMP-2 in cervical intraepithelial neoplasias(CINs) and

invasive squamous cell carcinomas. Paraffin-embedded tissue sections from 36 CINs (10 CIN I and 26 CIN II & III) and 60 invasive squamous cell carcinomas. We investigated the expression of MMP-1, MMP-2, TIMP-1 and TIMP-2 by using immunoperoxidase staining. MMP-1 expression rate in invasive carcinoma (66.7%) was higher than that of CIN I (10%) and CIN II & III (11.5%) ( $p < 0.0001$ ). Expression rates of MMP-2 in CIN II & III and invasive carcinoma were 42.3% and 48.3%, but no MMP-2 expression was found in CIN I ( $p = 0.0162$ ). TIMP-1 expression rate in CIN I (60.0%) was higher than that of CIN II & III (34.6%) and invasive carcinoma (16.7%) ( $p = 0.0078$ ). Expression rates of TIMP-2 in stromal cells of CIN II & III and invasive carcinoma were 30.8% and 46.7%, respectively. But no TIMP-2 expression was found in CIN I ( $p = 0.0114$ ). No significant correlations between MMP-1 and TIMP-1, MMP-2 and TIMP-2 were found. The presence of MMP-1 in tumor cells may enhance the invasive ability, but MMP-2 expression is an early event during malignant transformation in cervical neoplasia. TIMP-1 have inhibitory activity in progression in cervical neoplasia, but TIMP-2 may enhance the progression of cervical neoplasia through activation of MMP-2.

#### P 753

##### **EFFICIENCY OF IMMUNOHISTOCHEMICAL P16 EXPRESSION AND HPV TYPING IN CERVICAL SQUAMOUS INTRAEPITHELIAL LESION GRADING**

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Diagnosing and grading cervical cancer precursors is challenging at some point. In search of a solution, this study detects presence of HPV and investigates the staining pattern of p16, a cyclin-dependent kinase inhibitor whose overexpression is reported to be typical for dysplastic and neoplastic epithelium of cervix, on the basis of distribution and intensity, immunohistochemically.

H&E stained slides from cervical loop excision materials formerly diagnosed as LSIL (15 cases) and HSIL (20 cases), were reviewed. An immunohistochemical panel consisting of p16, all HPV types (Viroactiv® HPV Screening Kit) and HR (high risk)-HPV types (16, 18, 33, 35, 39, 45, 56, 58) (Viroactiv® HPV High Risk Kit) was applied. Nuclear staining was interpreted as positive for HPV evaluation. Both nuclear and cytoplasmic staining were accepted as positive for p16. Staining of p16 was evaluated as rare, basal and full thickness according to the distribution and the intensity was classified as weak, variable and strong. Chi-Square Test and Fisher's Exact Test were used for statistical analysis.

All HSIL cases (20/20) and % 80 of LSIL cases (12/15) were positive with p16. Of p16 (+) HSIL cases, epithelial distribution was % 50 full thickness, % 45 basal and % 5 rare. The staining intensity of the same cases were % 70 strong, % 20 variable and % 10 weak. Of p16 (+) LSIL cases, epithelial distribution was % 58.3 basal and % 41.7 rare. No cases of LSIL showed full thickness p16 positivity. The staining intensity of the same cases were % 25 strong, % 16.7 variable and % 58.3 weak. % 48.6 of all cases (17/35) was positive with screening kit (all HPV types). % 31.4 of all cases (11/35) was positive with HR-HPV. The distribution of this positivity was as % 35 of HSIL and % 26.6 of LSIL cases. All HPV type positivity rate was % 48.6, the distribution being % 50 of HSIL and % 46.6 of LSIL cases.

P16 is a highly sensitive marker of cervical epithelial dysplasia. Strong and full thickness staining of p16 in the cervix epithelium is highly supportive of HSIL diagnosis, while weak and basal/rare staining is in the favor of LSIL. All HPV positive cases were also p16 positive but no statistically significant relationship between HPV positivity and the intensity and the distribution of p16 was found. Although

HPV is very sensitive for the detection of the infection, it is not helpful in the grading of SIL since an unignorable rate of HR-HPV positivity (% 26.6) was detected in LSIL group.

#### P 754

##### **THE ROLE OF CELL CYCLE REGULATORS IN THE MULTISTEP PROCESS OF HPV-ASSOCIATED CERVICAL CARCINOMA**

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High-risk HPV (HR-HPV) types have long been implicated in the pathogenesis of cervical carcinoma with a reported interaction between HR-HPV oncogenic proteins and cell cycle regulators. We investigated the involvement of aberrant expression and coexpression of some cell cycle related genes in the multistep process of HPV16/18-associated cervical carcinogenesis.

Forty-three invasive squamous cell carcinoma (ISCC), 38 carcinoma in situ (CIS), 11 high grade dysplasia (HGD), 18 low grade dysplasia (LGD) and 20 normal cervical tissues were evaluated for aberrations in p53, mdm2, Rb, cyclin D1, cyclin E, CDK4, p21waf, p27 KIP1, p16INK4A and SPF using immunohistochemistry, flow cytometry and molecular techniques.

There was a significant increase in the expression level of cyclin E, CDK4, Rb, SPF and a significant decrease in the expression of p27 KIP1 in the sequence from normal mucosa to ISCC. Aberration involving cyclin D1, p21waf, p53, mdm2 and p16INK4A were detected in CIS and ISCC cases only.

In conclusion, infection of normal cervical mucosa by HR-HPV types leads to dysregulation of the cell cycle control via altering the expression of some cell cycle related genes. Aberrations involving p27 KIP1, cyclin E, Rb and CDK4 are early events, whereas aberrations involving the p53 pathway, cyclin D1 and p16INK4A are late events in the genetic cascade of cervical carcinogenesis. Together, these alterations lead to acceleration of the cell cycle with an increased proliferation rate and acquisition of more genetic damage.

#### P 755

##### **DETECTION OF HPV IN TISSUE BIOPSIES: A COMPARATIVE STUDY OF TWO VERSIONS OF INNO-LIPA SYSTEM AND MY09/11 PRIMERS**

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**INTRODUCTION:** we have detected the different HPV genital genotypes by PCR in paraffin-embedded biopsies since 1993 using the primers MY09/11, that amplify a 452-bp DNA fragment. Since 2003 we have used the INNO-LIPA system version 1 (IL-1). IL-1 amplifies a short 65-bp DNA fragment followed by reverse hybridization with type-specific probes of the 25 most relevant genotypes. Since 2004 we have used the second version of this system (IL-2).

**METHODS:** during 2003-4 we performed both MY and IL-1 tests in 56 biopsies. During 2004-5 we performed both MY and IL-2 tests in another 56 biopsies. All the biopsies showed pathologic findings of HPV related lesions. The presence of HPV DNA, the total number of viral genotypes and the presence of mixed infections were compared between MY and IL-1 (group 1) and MY and IL-2 (group 2).

**RESULTS. GROUP 1:** MY detected HPV DNA in 40 biopsies (71%) and IL-1 in 53 biopsies (94%). The total number of viral genotypes detected by MY was 42 and by IL-1 was 71. Mixed infections were detected by MY in 2

biopsies (5%) and IL-1 in 11 biopsies (20%). The viral genotypes detected by MY and IL-1 were the same in 25 biopsies. IL-1 detected more viral genotypes than MY in 20 biopsies and MY detected more than IL-1 in 2 biopsies. GROUP 2: MY detected HPV DNA in 46 biopsies (82%) and IL-2 in 49 biopsies (87%). The total number of viral genotypes detected by MY was 50 and by IL-2 was 58. Mixed infections were detected by MY in 4 biopsies (8%) and IL-2 in 7 biopsies (14%). The viral genotypes detected by MY and IL-2 were the same in 31 biopsies. IL-2 detected more viral genotypes than MY in 11 biopsies and MY detected more than IL-2 in 5 biopsies. IL-1 and 2 typified 4 cases of MY-unknown viral genotypes in both groups.

CONCLUSIONS: IL-1 was more sensible than MY in the detection of HPV DNA, several viral genotypes and mixed infections. IL-2 has reduced these differences in sensitivity. The material used in this study does not allow us to determine whether IL-1 is over-detecting HPV DNA (false positive results) or IL-2 is under-detecting HPV DNA (false negative results). However, IL-2 reveals no discordances compared with MY.

#### P 756

##### RETROSPECTIVE STUDY OF 68 PATIENTS WHO HAD COLPOSCOPY-GUIDED BIOPSY: COMPARISON OF THE CYTOLOGY RESULTS WITH HISTOLOGY

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Reducing morbidity and mortality is the main aim of the health system in the world. The incidence of cervical cancer has been declining in the last decades due to the Papanicolau (PAP) smear which is the preferred screening test for cervical squamous cell carcinoma and its precursors. A zero error standard does not exist in cytopathology. The false negativity or positivity were determined by many factors. We aimed to examine the final histological findings in patients who had a cervical intraepithelial lesion (CIN/dysplasia), atypical squamous cells of undetermined significance (ASCUS) on PAP smears and underwent colposcopy-guided biopsy.

This study is a retrospective review of 68 smears obtained from patients who had colposcopy-guided biopsy between 2002 to 2004 in a single center where 10000 PAP smears have been screening annually. All cases were examined by experienced gynaecopathologists.

Of 17 patients who had CIN 1 on the PAP smear, 15 had CIN 1 or higher lesion and 2 had benign changes (inflammation, reparation) on the final histology. All of 11 patients who had CIN 2 on the PAP smear, had at least CIN 1 on the biopsy. Only in one of the 21 patients diagnosed as CIN 3 on the PAP smear, dysplasia can not be found on the final histology. We later found out that this patient has recently received chemotherapy for leukemia. Of 14 patients diagnosed as ASCUS, six had CIN 1, two had CIN 2, four had CIN 3 and two had benign changes such as reparation and inflammation. Five patients who had benign cytologic changes, had undergone colposcopic examination, which revealed CIN 1 in three and CIN 2 in two patients.

Based on the results of our study, we determined a 7.7% of false negativity rate and a 4.8% of false positivity rate for the PAP smear examination. The efficiency of the PAP smear was 88.2%.

After the reassessment of patients having benign cytologic features, we found that sampling errors, which were possibly related to small lesions or those exfoliating few cells, were

practically the main reason of the false negativity in our study. It is well known that chemotherapy induces cellular atypia mimicking those of dysplasia or carcinoma, which was present in one of our cases. Even with optimal conditions, about 5-10 % of false negativity exist in cervical cytology.

#### P 757

##### STANDARDS FOR THE HISTOPATHOLOGICAL REPORTING OF UTERINE CERVICAL NEOPLASIA

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Current practices in cancer pathologies has resulted in pathologists globally joining together in the elaboration of a standard histopathological report of samples for uterine cervical neoplasia. The accomplishment of this form was possible with the support of the INCa (Institut National du Cancer), the DGS (Direction Générale de la Santé), the DHOS (Direction de l'Hospitalisation et de l'Organisation des Soins) and the InVS (Institut National de Veille Sanitaire). This required the following stages. Firstly, the constitution of a multicentric work group. Secondly, the feasibility tests to use this form. Finally, its validity. A regular update is planned in accordance with the standard types of literature. This standardised report form and its handbook have been developed with the assistance of bibliographical references, actual standardised reports, publications, ANAES recommendations, WHO proposed classifications, UICC, and a consensus of experts. This document should replace the original and be part of the computerised medical records. Its intention is to be used as a shared document between the pathologists and the practitioners. It will supply the practitioner with appropriate data for patient care. It takes into account or not organised screening tests. It allows a homogeneous procedure in the diagnosis of uterine cervical neoplasia. It supplies cancer registers with the necessary data for the epidemiological surveillance, and finally it facilitates the elaboration of computerised data for the relevant pathologies. The proposed report will contain precise data on the practitioner, the pathologist, the patient and the samples. It will give the most complete macroscopic and microscopic diagnosis as possible. Thus providing the key elements to allow for an objective prognosis and a therapeutic follow up of the patient. It will be succinct, clear, homogenous and standardised. Therefore, facilitating a better understanding by all practitioners and pathologists.

#### P 758

##### DETECTION OF HPV GENOTYPES IN CERVICAL LESIONS BY THE HPV DNA CHIP AND SEQUENCING

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Introduction: Cervical cancer is the second most common cancer in terms of both incidence and mortality worldwide. Recently, there is some agreement that the availability of high sensitive human papillomavirus (HPV) tests offers the potential for replacement of conventional cytologic screening

programs. In addition, the importance of HPV testing has been increasing because genetic typing is required to predict clinical progression. Digene hybrid-capture (HC-2) technology was widely used for it is highly sensitive, and if appropriately used, highly specific as well. A newly introduced HPV detection technique in cervical lesion, the HPV DNA chip test, contains 24 HPV probes and has the advantage of being able to detect 24 HPV types at once.

**Objective:** In this study, we evaluated the accuracy of HPV DNA Chip test for detection and typing of HPV in cervical lesions by comparing with result of HPV DNA sequencing of same samples. In addition, we desired to find out the types which were not detected in HPV DNA Chip test by HPV DNA sequencing of HPV-other types.

**Methods:** The HPV DNA sequencing was performed in samples of 282 patients, where specific HPV type had been shown in HPV DNA Chip test. The sixteen cases where multiple HPV types had been found in HPV DNA Chip test were included in 282 cases. The sequencing was also performed in HPV-other type samples of 95 patients, where positive in HPV-PCR, but specific HPV type had not been found.

**Results:** In 257 cases (91.1%) of 282 cases, the HPV types of the HPV DNA sequencing test was in agreement with types of the HPV DNA Chip. In 16 cases (5.7%), the sequencing types were different from the types of HPV DNA Chip. But, in nine of 16 cases, types in HPV DNA sequencing were absent types in HPV DNA Chip test. The interpretation of HPV DNA sequencing was impossible in nine cases (3.2%). The HPV DNA sequencing test of 95 cases of HPV-other type showed that the sequencing types from 94 cases (98.9%) were absent types in HPV DNA chip test. In sequencing test of HPV-other type, HPV-81 (20.0%), HPV-62 (14.7%), HPV-84 (13.7%), and HPV-61 (13.7%) were frequently detected.

**Conclusion:** HPV DNA chip is an accurate method for detecting the 24 HPV genotypes.

#### P 759

##### **DNA VERSUS RNA BASED METHODS FOR HUMAN PAPILLOMAVIRUS DETECTION IN CERVICAL NEOPLASIA**

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**Background.** Given the results summarized in the 2005 IARC monograph, HPV testing is likely to be implemented as the primary screening test within few years. HPV tests detecting either HPV DNA or HPV E6/E7 mRNA are now commercially available, and needs to be validated before inclusion in clinical practice. The aims of this study were to compare DNA-based and mRNA-based methods for detection of high-grade cervical neoplasia.

**Methods.** HPV prevalence was analyzed in 383 women with positive index cytology, selected from gynecology clinics. All patients were investigated by a new PAP smear, histology and two commercially available HPV tests: Hybrid Capture II (Digene Corporation) and the Pre Tect HPV-Proofer (NorChip AS). Cases with positive DNA test and negative mRNA test, and cases with high-grade histology and negative HPV tests were retested with PCR and sequencing. We regarded the infection as latent or transient if sequencing revealed a HPV type included in both assays.

**Results.** High-risk HPV was detected in 99.7% of the histological confirmed high-grade lesions (CIN2+) (290/291). The DNA test was positive in 95% (275/291), and the mRNA test was positive in 77% (225/291) of the histological confirmed high-grade lesions. All invasive carcinomas were mRNA positive. The DNA test was significantly more often positive in benign and low-grade lesions, some of which were found to be false positive due to cross-contamination with unrelated types. High-grade histology was detected in 83% of women with normal cytology and positive mRNA test. Latent

or transient infections were detected in 11 low-grade and 12 high-grade preinvasive lesions. Sequencing revealed high-risk HPV types included only in the DNA test in 35 high-grade preinvasive lesions, HPV 52 and 58 were the most prevalent HPV types.

**Conclusions.** These HPV tests have potential to improve the detection rate of high-grade cervical neoplasia, with some limitations. The mRNA test seems to be more appropriate for risk-evaluation. Larger scale, population based studies are necessary to evaluate the predictive values of HPV testing in Norway. National monitoring of HPV testing should be obligate and incorporate cytology and histology results in order to give recommendations for follow-up.

#### P 760

##### **SQUAMOUS CELL CARCINOMAS OF THE VULVA: ASSOCIATED EPITHELIAL LESIONS AND VALUE OF P16 AND P53 IMMUNOSTAINING.**

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**Introduction and aim:** Two types of vulvar squamous cell carcinomas (SCC) have been delineated: human papillomavirus related (HPV+) and unrelated (HPV-) SCC. HPV+ tumors are basaloid or warty (B/W), are considered to arise in relatively young women, are frequently associated to undifferentiated vulvar intraepithelial neoplasia (VIN) and over express p16. HPV-tumors are keratinizing (KER), arise in elderly patients, are frequently associated with differentiated VIN, lichen sclerosus (LS) and squamous cell hyperplasia (SCH), and show a high proportion of p53 mutations that result in p53 immunohistochemical positivity. However, some overlap between HPV+ and HPV- tumors exist. The aim of our study was to evaluate the correlation between the two major types of SCC and concurrent epithelial lesions and determine whether p16 immunostaining could be a good discriminator between these forms.

**Methods:** All patients diagnosed of vulvar SCC treated in our institution with radical vulvectomy or partial resection from 1995 to March 2005 were included. 84 patients were identified. 4 to 20 slides of every case (mean 8), including tumor and non-neoplastic tissue, were reviewed focusing on histological type of tumor and concurrent epithelial changes. Immunohistochemical analysis of p16 and p53 was performed.

**Results:** 70 (83,3%) were KER SCC, whereas 14 (16,6%) were B/W type (9 and 5 respectively). LS and SCH were present respectively in 36 and 60% of KER and in 7 and 21% of B/W SCC (p<0.05). VIN was detected in 47% of KER and in 43% of B/W SCC. p53 was positive in 50 cases (67% of KER, 7% of B/W) and p16 in 18 cases (7% KER, 93% B/W). p16 and p53 positivity were almost always mutually exclusive. Interestingly, 3 out of 5 keratinizing SCC positive for p16 showed undifferentiated VIN, suggesting a relationship with HPV, and the only basaloid SCC negative for p16 was intensely positive for p53 and showed differentiated VIN and LS in the non-neoplastic skin, indicating an HPV-unrelated pathway.

**Conclusion:** p16 and p53 immunostaining, seem to discriminate adequately between HPV-associated and HPV-unrelated vulvar squamous cell carcinomas. A small percentage of keratinizing SCC are probably related to HPV infection, whereas a few tumors not associated to HPV may have a basaloid or warty histology.

#### P 761

##### **EXTRACELLULAR SIGNAL-REGULATED KINASE 2(ERK 2) EXPRESSION IN CERVICAL**

### **INTRAEPITHELIAL NEOPLASIA AND CARCINOMA OF THE CERVIX.**

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The HPV protein E5 seems to be weakly oncogenic and has been suggested to potentiate the transforming activity of E7. The molecular mechanisms of E5-induced cell proliferation are transmitted through the activation of potent transcription factors by the ERK/MAPK signaling pathway. The key event in E5 induced cell proliferation involves the activation of the mitogen-activated protein kinases (MAPK), which include a pair of protein kinases known as ERK1 and ERK2.

The aim of this study is to investigate the usefulness of ERK2 expression in predicting the grade of CIN lesions as well as to compare the expression with that of cervical carcinoma.

Immunohistochemistry was performed on paraffin embedded sections of 76 CIN lesions (20 CIN1, 20 CIN2 and 36 CIN3) and 30 cervical carcinomas (21 squamous, 9 adenocarcinomas) using standard procedures with the application of the ERK2 monoclonal antibody (Santa Cruz Biotechnology, USA 1/300 dilution). A semiquantitative scoring into 3 categories was used as follows: 1. slight staining equivalent to that of normal squamous epithelium 2. moderately increased staining cytoplasmic and/or nuclear 3. intense diffuse cytoplasmic and/or nuclear staining.

Results: The frequency of strong ERK2 expression increased from 0% for CIN1 lesions to 61% for CIN3 lesions. Specifically the majority of CIN1 lesions (60%) showed a weak cytoplasmic expression in the basal and parabasal layers equivalent to that of the normal squamous epithelium while 40% showed a moderately increased staining. In CIN2 lesions 40% of the cases displayed either a weak equivalent to normal (20%) or intense (20%) expression while the majority (60%) showed a moderately increased expression. Finally in CIN3 lesions only 11% showed weak ERK2 expression while 28% and 61% of the cases expressed ERK2 moderately and strongly respectively. In cervical squamous carcinomas moderate and strong expression was found in 91% of the cases in approximately equal percentages (48% moderate, 43% strong) while in cervical adenocarcinomas all the cases showed either strong (67%) or moderate (33%) expression.

Conclusions: According to our study a direct relationship between the increasing grade of the CIN lesion and the intensity of ERK2 staining was found. Moderate and strong ERK2 expression was related to high grade lesions (CIN3 or cancer). All cervical adenocarcinomas showed either moderate or intense ERK2 expression while in 10% of squamous cervical carcinomas only weak equivalent to normal staining was found.

### **P 762**

#### **HUMAN PAPILLOMAVIRUS TYPES IN SQUAMOUS CELL CARCINOMAS AND ADENOCARCINOMAS OF THE UTERINE CERVIX IN REGION OF RIJEKA, CROATIA**

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Introduction: The association between human papillomavirus (HPV) infection and cervical cancer have been well established. The different carcinoma-associated HPV types have been reported world wide. However in Croatian population, such data have not been reported to date.

Purpose of the study: To analyse the frequency and distribution in HPV types in two different histological forms of uterine cervical carcinoma among the patients in region of Rijeka, Croatia.

Material and Methods: A retrospective analysis was undertaken on formalin fixed paraffin embedded specimens of 54 squamous cell carcinoma (SCCs) and 40 adenocarcinomas (ACs), including 15 adenocarcinoma in situ, from patients treated in Clinical Hospital Rijeka. HPV genotyping was performed by consensus and type specific primers directed PCR. The results were correlated with clinicopathologic parameters i.e. age of the patients, FIGO stage, histological subtype and grade of the lesions.

Results: Of the 94 cases examined, 87 (92.5%) were HPV DNA positive; 50/54 (92.6%) of SCCs and 37/40 (92.5%) ACs. Eight different HPV genotypes were detected: 6/11, 16, 18, 31, 33, 52, 74 and undetermined type X. HPV types 6/11, detectable in 9.1% of HPV positive cases, were observed as a part of coinfection, except in one case of AC. HPV 16 was the predominant type in SCCs, detectable in 26/50 (52.0%) of cases, whereas HPV 18 was the most common type in ACs 25/37 (67.5%). HPV 31 and 33 accounted for 16.0% and 8.0% of HPV positive SCCs and 8.1% and 5.4% of HPV positive ACs, respectively. In addition, multiple HPV types were found in 21/87 (24.1%) of carcinomas, without significant correlation to the histologic type, with 19.9% in ACCs and 29.0% in ACs. No relationship was found between viral type and any of the clinico-pathologic parameters analysed.

Conclusion: Our results indicate that cervical carcinomas are largely associated with HPV types 16, 18, 31 and 33 in our study group in Rijeka region, with statistically significant predominance of HPV 16 in SCCs (52.0%) and HPV 18 in ACs (67.5%).

### **P 763**

#### **CYTOKERATIN 17 AND P63 IN METAPLASTIC AND NEOPLASTIC CERVICAL LESIONS**

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The transformation zone of the cervix is the place where most malignancies appear. The epithelial turn over seems to differ here from that of more stable structures in the vicinity. The occurrences of squamous metaplasia makes this zone more prone to malignancies but the reason why this happens remains a matter of debate. The extension of the stem cell theory to solid tumors has prompted new insight into tumor genesis. Classically it is admitted that a bipotent stem-like cell, the subcolumnar cell, is the origin of squamous metaplasia of the cervix. In this study we identified expression of p63, a homologue of the tumor suppressor gene p53 and cytokeratin 17 (CK17) in metaplastic and neoplastic cervical lesions. Cervical tissue from archival paraffin blocks was available from 30 patients which had variable lesions: mature squamous metaplasia, moderate and severe dysplasia and squamous invasive carcinoma. We noted p63 expression consistently in the nuclei of reserve cells, hyperplasia of the basal layer of the dysplastic and neoplastic epithelium of ectocervix, while CK17 was sporadically found in the basal layer of ectocervical epithelium and always present in the basal cells of squamous metaplastic epithelium. Also, CK17 has increased expression during progression of CIN, and diffuse stained in squamous carcinoma. Our results concluded that a considerable number of premalignant and malignant lesions of the uterine cervix express CK17 and p63 which is also found in the progenitor reserve cells.

### **P 764**

## **LARGE CELL NEUROENDOCRINE CARCINOMA OF THE UTERINE CERVIX: A CLINICOPATHOLOGIC STUDY OF FIVE CASES.**

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**Background:** Cervical carcinoma in not developed countries is the first cause of death for woman's cancer, in Mexico 9,913 new cases were reported on 2000 whit 4,620 deaths in the same year. The large cell neuroendocrine carcinoma (LCNC) of the uterine cervix is a rare entity and few cases have been reported in the international literature. The LCNC have been described as aggressive tumor with "organoid" growth patterns (trabecular, insular and solid). The neoplastic cells are large whit vesicular nuclei, prominent nucleoli, atypical mitosis (3-12 per high power field) and necrosis.

**Design:** All the cases identified as small cell neuroendocrine or undifferentiated cervical carcinoma from a period from 1991 to 2003 were revised separately by three pathologist using the criteria described in the background. Immunohistochemical staining with cromogranin and synaptophysin were done. Clinical information was obtained from patient's charts.

**Results:** Five patients were selected using the previous mentioned criteria from a pool of 103 cases; the tumors where no consensus in the diagnosis were obtained were excluded. Patients age ranged in 43-61 years. One patient was stage IIA and four patients were stage IIB. Maximal dimensions were 8 cm. Two patients were treated with surgery, chemotherapy and radiotherapy, two patients with chemotherapy and radiotherapy without surgery and one patient was lost, this patient was associated whit adenocarcinoma of the colon treated whit surgery and chemotherapy. Immunohistochemistry study was done, all the cases were positive for cromogranin and synaptophysin. Disease progression was documented in three patients; two with cerebral metastasis, one with node metastasis; one patient had persistent disease. Two patients are alive whit disease 17 and 18 months after diagnosis and two die with disease 6 and 8 months after diagnosis.

**Conclusions:** large cell neuroendocrine carcinoma is a rare neoplasia frequently confused with small cell neuroendocrine carcinoma or undifferentiated carcinoma. The patients had a high grade tumors whit advanced disease, and despite aggressive treatment, the patients have had progression and early recurrence.

### **P 765**

#### **SMALL AND LARGE CELL NEUROENDOCRINE CARCINOMAS OF THE UTERINE CERVIX. A REPORT OF 10 CASES**

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**BACKGROUND:** Small (SCC) and large cell (LCC) neuroendocrine carcinomas of the uterine cervix are rare and highly aggressive neoplasms. Their association with the integration of human papilloma virus (HPV) DNA of the types 16 or 18 has been documented in many studies.

**AIMS:** The purpose of this study is to present the clinical, histopathological, immunohistochemical characteristics and

the presence of HPV DNA in ten cases of SCCs and LCCs of the uterine cervix.

**METHODS:** Seven patients with primary SCCs and three patients with LCCs of the uterine cervix were diagnosed at our department between 1989 and 2004. Clinical data were retrieved from the patients' files and included age, recurrence and survival. Routinely processed operative and/or biopsy specimens were used for immunohistochemical stains and hybridization procedures. Primary antibodies against several epithelial, neuroendocrine, mesenchimal and proliferative markers were included. The presence of HPV DNA was assessed by conventional in situ hybridization (ISH) using probes for HPV 16/18 and 31/33/51 and polymerase chain reaction (PCR), using three primers (MY09/11, GP5+/GP6+, E6). Six of the patients were surgically treated. Postoperatively, four received chemo and/or radiotherapy, two rejected further treatment and one patient was lost to follow-up. The other four patients underwent conservative treatment due to advanced disease.

**RESULTS:** The patients' age ranged from 25 to 71 years. Histologically, the tumors showed trabecular, nesting or a sheet-like pattern, with areas of necrosis and frequent mitoses. Their neuroendocrine nature was confirmed by diffuse positive immunostaining for neuron-specific enolase and low molecular weight cytokeratins. Focal positivity for chromogranin, synaptophysin and S100, together with pancytokeratin and EMA, was evident in the majority of the tumors. Their aggressive potential was confirmed by high Ki-67 proliferative index (50-90%). HPV types 16/18 were found in 4 tumors using ISH, and HPV 16 in additional 3, using PCR (type 16). 3 of the patients developed distant metastases and died 7-48 months after receiving partial or full treatment, while 6 are alive and without evidence of disease after 4-38 months.

**CONCLUSIONS:** SCCs and LCCs are highly aggressive neoplasms. However, early diagnosis and combined therapy may improve survival in some patients. Although mainly a morphologic diagnosis, immunohistochemistry may help in the diagnosis of SCC and LCC.

### **P 766**

#### **EVALUATION OF TUMOUR NECROSIS FACTOR (TNF ALPHA ) IN EUTOPIC & ECTOPIC ENDOMETRIA OF WOMEN WITH ENDOMETRIOSIS & ENDOMETRIUM OF HEALTHY WOMEN**

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To achieve a successful implantation, in addition to adequate embryonic quality, an appropriate endometrium is mandatory. Some alterations have been found in the eutopic endometrium of women with endometriosis that could be responsible, at least in part, for the subfertility in these women.

The aim of this study is to compare eutopic & ectopic endometrium of women with endometriosis with that of healthy controls, regarding expression of TNF alpha using immunohistochemical staining. Also, to gain more information about the possible role of cytokine TNF alpha in autocrine & paracrine growth regulation of endometriosis.

The study included 30 patients having endometriosis & endometriotic cysts in the ovary (study group). Those patients were subjected to laparoscopy to confirm the diagnosis & obtain a biopsy of the endometriotic cyst, besides an endometrial biopsy. The control group included 30 healthy fertile women without endometriosis, they did laparoscopy for tubal ligation followed by endometrial biopsy. For both groups the biopsies obtained were immunohistochemically stained & examined for TNF alpha expression.

The results revealed higher expression of TNF $\alpha$  in eutopic endometrium of patients having endometriosis than endometrium of healthy fertile female controls, with a highly statistically significant difference. These results have been confirmed by using image analyzer computer system which measured TNF  $\alpha$  expressed in tissues quantitatively by measuring the optical density. Optical density was then expressed in the form of sum grey & mean grey. The sum grey of TNF  $\alpha$  expression in eutopic endometrium of endometriosis patients was higher than that of healthy women (control group).

This study shows that increase TNF  $\alpha$  expression in eutopic endometrium & ovarian endometriosis may explain why endometriosis develop in certain females, as TNF  $\alpha$  was implicated as one of the factors related to the attachment & growth of the ectopic endometrium through its angiogenic & paracrine activities. Besides high TNF  $\alpha$  expression in the endometrium of patients having endometriosis may be a cause of endometriosis-associated infertility through affecting implantation.

#### P 767

##### **THE EVALUATION OF MALIGNANCY POTENTIAL IN ENDOMETRIAL HYPERPLASIA BY USING PROLIFERATION MARKERS (PCNA AN KI-67)**

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**Introduction and objects :** Endometrial hyperplasia can be described as proliferation of endometrial glands by disordered number and shape with an increase in gland/stroma ratio. The aim of the study is, to evaluate the malignancy potential of endometrial hyperplasia by using proliferating cell nuclear antigen (PCNA) and Ki-67. Which are known as proliferation markers.

**Methods :** We evaluated 62 patients (20 of them control group), ages between 25-62, whom are operated or taken specimen by D&C in Zekai Tahir Burak Womens Health and Education Hospital, between years 2002-2003. After routine procedures of preparation, pathologic specimens are labeled with PCNA and Ki-67 by using immunohistochemical methods. For statistical analyses, Anova and Post Hoc Tukey tests were used. ( $p < 0,05$ )

**Results :** In complex atypic hyperplasia, both PCNA and Ki-67 expression was higher than in control group, with a statistical significance.

**Discussion :** We suggest that, these proliferation markers (PCNA and Ki-67) can be useful specially to determine the malignancy potential of complex atypic hyperplasia of endometrium.

#### P 768

##### **HISTOPATHOLOGIC CHANGES AFTER UTERINE ARTERIAL EMBOLIZATION WITH TRIS-ACRYL GELATIN MICROSPHERES**

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**Introduction:** Uterine artery embolization (UAE) as an alternative treatment of uterine fibroids and adenomyosis uteri becomes increasingly popular. Because there is considerable uncertainty with respect to the wanted and unwanted morphological changes induced by UAE we conducted a histopathologic study on post UAE tissue specimen. **Methods:** A total of 173 women were treated with UAE using tris-acryl gelatin microspheres (TGMS) for either symptomatic

adenomyosis or leiomyoma. Surgical specimen of 8 women who underwent subsequent myomectomy or hysterectomy were evaluated by conventional histology and immunohistochemistry.

**Results:** TGMS were readily apparent in both macroscopy and routine histology. In patients with fibroids, TGMS accumulated preferably in medium size vessels in the direct tumor vicinity. In patients with adenomyosis, a random distribution of TGMS was noted throughout the outer myometrium. A granulomatous foreign body reaction occurred in the vicinity of particles, eventually followed by formation of vascular pseudoaneurysms and complete vessel destruction. Leiomyoma treated with UAE showed either fibrinoid necrosis, coagulative necrosis or no change at all. Increased mitotic activity and tumor cell polymorphism was evident in some but not all treated smooth muscle tumors. Foci of adenomyosis remained unaltered.

**Conclusions:** In conclusion, after UAE with TGMS, particles were identified predominately but not exclusively at the periphery of fibroids. Pathologists must be aware of the morphologic changes induced by UAE in leiomyoma, to avoid misinterpretation of induced tissue alterations as signs of malignant tumor growth.

#### P 769

##### **PTEN IMMUNEXPRESSION IN RANDOMLY SELECTED ENDOMETRIAL POLYPS**

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Endometrial polyps are common in women over 40 and are thought to arise due to some type of hyperplastic process. Although most of them are benign, carcinoma developing in polyps is a rare but well-known condition especially in postmenopausal women. Recently, PTEN inactivation has been shown to be a common event in endometrial carcinogenesis. Also PTEN loss has been documented in some normal-appearing endometrial glands of 43% of premenopausal women suggesting that PTEN loss might be an early step in endometrial carcinogenesis. In order to search for the PTEN expression pattern in endometrial polyps we investigated the immunohistochemical expression of PTEN in 50 randomly selected endometrial polyps. The morphology of polyps were atrophic (n:21), functional (n:17), inactive (n:12) and hyperplastic (n: 1). PTEN was lost in more than 50% of the glands in 88% (n:17) of atrophic polyps. Also in the 2 of the 3 polyps with secretory epithelium there was diffuse PTEN loss. On the contrary there was diffuse PTEN expression in 75% of proliferative glands in functional polyps. None of the glands with PTEN loss showed atypical histological findings. In polyps containing both proliferative and inactive or atrophic glands, PTEN-null glands were restricted to atrophic or inactive glands. Expression of PTEN in stromal cells also was parallel to that of the epithelia. This data shows that in polyps PTEN is expressed strongly in proliferative epithelium but it is commonly decreased or lost in any kind of nonproliferative epithelium. Although it is strongly suggested that PTEN-inactivated glands could be 'latent precancers' care should be taken in evaluating the immunexpression in the absence of morphologic evidence of atypia. In conclusion, we think that finding PTEN inactivated glands in a polyp could be valuable in proliferative type glands but in normal-appearing nonproliferative epithelium it seems like a normal event.

#### P 770

##### **IMMUNOHISTOCHEMICAL EXPRESSION OF P63 IN ENDOMETRIAL POLYPS: AN EVIDENCE OF THE MAINTENANCE OF A BASAL CELL**

IMMUNOPHENOTYPE  
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**Introduction:** Endometrial polyps likely originate from basal layer but their biology underlying is not fully understood. Recently, p63, an important protein in regulating epithelial proliferation and differentiation, has been described as a marker of basal/ reserve cells in female genital tract.

**Objective:** Our objective was to verify if p63 is expressed differently in postmenopausal endometrial polyps and adjacent endometrium.

**Design:** In this study we evaluated the p63 expression in 36 specimens of postmenopausal endometrial polyps, obtained by hysteroscopic polypectomy realized in a tertiary care university hospital, using a monoclonal antibody clone 4A4 (1:200), which recognizes all p63 variants.

**Results:** The majority of endometrial polyps (94.4%) were p63-positive. Otherwise, only 5.6% of adjacent endometrium had presented rare nuclei immunostaining for p63 ( $P < 0.0001$ ). Endometrial polyps had presented homogeneous pattern of distribution of p63 immunostaining.

**Conclusions:** Our results are an evidence of the maintenance of a basal cell immunophenotype in postmenopausal endometrial polyps. Furthermore, they suggest that P63 has a role in the pathogenesis of endometrial polyps and emphasize the necessity of more studies about P63 role and its different isoforms in the biological and clinical behavior of endometrial polyps.

**Key-words:** endometrial polyps, menopause, basal cells, P63.

#### P 771

### HISTOPATHOLOGIC DIAGNOSES OF THE ENDOMETRIUM OBTAINED BY CONSECUTIVE BIOPSY PROCEDURES IN 1699 FRENCH AND 2887 ISRAELI WOMEN.

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**Introduction:** Histologic examination of the endometrium is a common diagnostic procedure in peri and menopausal women with abnormal bleeding and/or thickened endometrium by ultrasound (US).

**Purpose:** This study, performed in France and Israel, was undertaken to compare frequency of histologic diagnoses in both populations and correlate the results with clinical information.

**Methods:** Hematoxylin-eosin stained slides of endometrial formalin fixed, paraffin embedded tissue from 1699 French and 2887 Israeli women aged 45 and above were examined independently by pathologists in each country.

**Results:** Proliferative endometrium was present in 22.3% of the French and 28.3% of the Israeli cases. Secretory endometrium including menstrual was seen in 10.1% of French and in 12% of Israeli patients. Atrophic endometrium, mostly in menopausal patients was diagnosed in 48.4% of the French and 32.2% of the Israeli population. No tissue was available for histologic evaluation in 5.5% of the French and in 10% of Israelis, most of them menopausal. Chronic endometritis was diagnosed in 0.2% of Israelis but none in

French patients. Thus, in 86.3% of the French and in 82.5% of the Israeli women, the histology was within normal range of the age group studied. Endometrial polyps were found in 9.4% of the French and in 9.0% of Israelis. Endometrial adenocarcinoma was diagnosed in 2.1% of French and 2% of Israeli women. The only significant discrepancy between the French and Israeli series was simple hyperplasia without atypia diagnosed in 1.7% of the French and 6% of the Israelis. Complex hyperplasia was rare in both populations (0.1%). Atypical hyperplasia was also rare (0.4% in the French and none in the Israelis). Most likely these cases were classified as well differentiated endometrioid carcinoma.

**Conclusion:** Histopathologic diagnoses of endometrium in French and Israeli populations showed with few exceptions remarkable similarities. Thickened endometrium (measured by US) and abnormal bleeding were not restricted to women with polyps, hyperplasia or carcinoma, but were present in the entire histopathologic spectrum including in atrophy.

#### P 772

### EXPRESSION PATTERN OF OSTEOPOINTIN (OPN) IN ENDOMETRIAL CARCINOMA – CORRELATION WITH EXPRESSION OF THE ADHESION MOLECULE CEACAM1

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**Introduction:**

OPN and CEACAM1 have diverse biological functions in the uterus throughout the estrous cycle and have been shown to interact with integrin beta3. OPN is a glycoprotein of the extracellular matrix, which has been shown to mediate cellular migration and invasion and to contribute to tumorigenesis in several types of cancers. CEACAM1 is an adhesion molecule of the carcinoembryonic antigen family which we have recently found to be expressed in endometrial cancer and which has been shown to be down-regulated in colorectal and breast cancer.

**Purpose:**

Endometrial carcinoma is the most frequent invasive malignancy of the female genital tract in developed countries. The present study was designed to investigate the expression pattern of OPN in the normal human endometrium and in endometrial carcinomas and to correlate it with the expression of CEACAM1.

**Material and methods:**

In the present study, immunohistochemistry and immunofluorescence with specific antibodies were performed on a series of 20 normal endometrial samples and 40 endometrial carcinomas to investigate the expression pattern and cell-type specific localization of OPN and to correlate it with the expression of CEACAM1. In addition, western blot was performed on normal human endometrium and endometrial neoplasia.

**Results:**

Strong OPN expression with a consistent cytoplasmic localization in epithelial glandular cells was observed in the normal human endometrium in 80% of the samples of the proliferative and secretory phase (score 8-12). CEACAM1 showed similar results. Strong expression of OPN could be observed in 35 (87,5%) of the 40 analyzed endometrial carcinomas. Of the 40 analyzed tumors, 19 (47,5%) were in the high score (8-12) category with a strong OPN expression level, nine tumors of 40 (22,5%) showed a moderate score (4-7) category. With increasing malignancy grade, increasing areas with low OPN expression level or complete loss of OPN expression could be observed. CEACAM1 had shown similar results and was found to be co-expressed with OPN in normal human endometrium and in endometrial neoplasia.

**Conclusion:**

The expression pattern of osteopontin in endometrial tissue indicates that it might play a role in the pathogenesis of

endometrial cancer (possibly as a functional complex with CEACAM1) and to be useful as an additional diagnostic marker for such lesions. The correlation between pathological stage and OPN suggests a role for OPN in tumor prognosis.

**P 773**

**MALIGNANT LYMPHOMA OF THE UTERUS: A CLINICOPATHOLOGIC ANALYSIS OF 14 CASES**

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**Introduction:** Non-Hodgkin's lymphoma involving uterus is uncommon. Most tumors arise in the cervix while those of the uterine corpus are extremely rare. We evaluate the clinicopathologic features of 14 cases in Taiwan and reclassify them using new WHO classification.

**Methods:** Fourteen cases of Non-Hodgkin's lymphoma involving the uterus were retrieved from the pathology file of Chang Gung Memorial Hospital from 1987 to 2004 and reclassified by new WHO classification. Pathology slides, immunohistochemical study, and clinical information were reviewed.

**Results:** Patients ranged from 20 to 78 years (mean, 51.8 years). Of fourteen cases, eleven had primary tumor involving uterus at the presentation and three were secondary cases. The most common presenting symptoms were vaginal bleeding (43%). Histologically, nine tumors were diffuse large B-cell lymphoma (DLBCL) (5 centroblastic variant, 4 immunoblastic variant), three were follicular lymphoma and two were Burkitt's lymphoma. Six cases (43%) involved cervix (5 DLBCL, 1 Follicular) and were stage IE or IIE. Three cases (21%) involved corpus (all DLBCL) only and were all stage IVE. Five cases (36%) involved both cervix and corpus (2 Burkitt's, 2 Follicular, 1 DLBCL) and were stage IIIIE or IVE. Follow-up information was available for 10 cases and ranged up to 75 months (mean, 26.9 months). Two follicular lymphomas relapsed in uterus transformed to diffuse large B-cell lymphoma 2 and 26 months after the diagnosis and were both stage IV cases. All low-stage (IE or IIE) cases received chemotherapy and were all alive without recurrence for 1-75 months (mean, 29.2 months). Two stage IV cases died 0.5 and 2 months after the diagnosis.

**Conclusion:** Our study showed that malignant lymphomas involving cervix are all low stage irrespective of histological type. However, those involving uterine corpus (with or without cervical involvement) are of high-stage disease and more aggressive tumor type. Two cases of Follicular lymphoma showed transformation to diffuse large B-cell lymphoma.

**P 774**

**AN UTERINE PECOMA : A CASE REPORT**

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Epithelioid mesenchymal tumors of the uterus were previously considered to be either from smooth muscle or endometrial stromal lineage, but recently a new family of lesion have been described including HMB45 + epithelioid cells with clear to granular cytoplasm and perivascular distribution.

We described an uterine PECOMA in a 33 years old woman that tumor cells were positive for HMB45 but negative for epithelial and smooth muscle markers.

**P 775**

**LOW GRADE ENDOMETRIAL STROMAL SARCOMA OF THE UTERUS WITH EXTENSIVE ENDOMETRIOID GLANDULAR DIFFERENTIATION**

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**Introduction:** Low grade endometrial stromal sarcoma (LGESS) is a rare tumor of uterus. Occasional cases may cause problems in differential diagnosis because of variable patterns: epithelioid cells, sex-cord-like differentiation, benign or malignant endometrioid glands, smooth muscle metaplasia, and myxoid areas. We reported a rare case of LGESS with extensive glandular differentiation.

**Case report:** A 29-year-old woman presented with a painless palpable mass in the left lower abdomen for a month. She went to local hospital and a left ovarian mass with suspect of malignancy was noted under sonography and the CA-125 was elevated. She received left salpingo-oophorectomy, and hormone therapy after surgery for four months. Then she was transferred to our hospital for further management. Hysterectomy was performed. On gross examination, an ill-defined, tan to yellowish elastic multi-nodular mass measuring 8 cm in maximal diameter was in the myometrium. Histologically, the tumor diffusely penetrated the myometrium and lymphovascular space as irregular tongues or nests. It composed of oval or spindle tumor cells with rich network of delicate small arterioles and focal myxoid and fibrous stroma. The cells exhibited mild nuclear pleomorphism and a mitotic rate of 10/10 HPF. Most of the tumor also contained diffusely benign-appearing endometrioid glands. Immunohistochemical stains showed that the tumor cells were positive for ER, PR, smooth muscle actin, and CD10, and negative for desmin.

**Conclusion:** The differential diagnosis of LGESS with extensive glandular differentiation includes endometriosis, adenomyosis, endometrial stromal sarcomas arising in adenomyosis or endometriosis, and adenosarcomas. It is important that LGESS with extensive glandular differentiation in myometrium not to be mistaken for adenomyosis.

**P 776**

**DEPICTION OF ANGIOGENIC AND LYMPHANGIOGENIC FACTORS INCLUDING VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF-C), PROLACTIN (C-17), PLATELET DERIVED GROWTH FACTOR (PDGF-A), FIBROBLASTS GROWTH FACTOR -1 (FGF-1), AND THEIR EXPRESSION IN 90 CASES OF ENDOMETRIOID AD CATALANO Edison M.D., WEIDMANN James BSCT HTL (ASCP) Cooper University Hospital, Camden, NJ USA**

**Introduction:** PDGF-A (N-30) is a mitogen for mesenchymal cells, which plays an important role in the angiogenesis and progression of tumor. The Vascular Endothelial Growth Factor C is a member of the family of growth factors, which is an important endothelial cell mitogen and is associated with an increase in angiogenesis and lymphangiogenesis. The increase in expression of Prolactin (C-17) protein has been related to a more aggressive tumor behavior.

The fibroblastic growth factor-1 (FGF-1) is a growth factor that stimulates the proliferation of mesenchymal, epithelial and neural ectodermal cells.

**Purpose:** This study was to investigate the relationship of the overexpression of VEGF-C, Prolactin (C-17), PDGF-A (N-30), and FGF-1 in 90 cases of invasive endometrioid adenocarcinoma, the effects of these factors over the angio and lymphangiogenesis. Also, through the survival tables we evaluate the prognostic significance of overexpression of these factors, as well as the relationship with the staging of the tumor and degree of differentiation.

**Methods:** Formalin fixed and paraffin embedded sections of the endometrial tumors were obtained from 90 cases. Immunohistochemical staining was obtained for VEGF,

Prolactin (C-17), PDGF-A (N-30) and FGF-1, and the measurement of the action of these factors, over the vascular proliferation evaluated with CD-34 and the microvascular density (MVD).

Summary of Results: In the 90 cases studied there was a perfect correlation in 50% of the cases between the VEGF-C and the increase in angiogenesis. Also, Prolactin and PDGF-A (N-30) showed an excellent correlation between the antibody staining (overexpression), in relationship to the tumor differentiation, staging and survival statistics.

In general the staining of this antibody, FGF-1 (C-19), was lighter in comparison with the previous and shows a diffuse cytoplasmic staining with no nuclear staining. There were more negative cases and the only positive cases were only 2+ or 3+. The correlation; however, with the survival was excellent, as well as with the staging and degree of tumor differentiation.

Conclusion: The overexpression of the factors VEGF-C, Prolactin (C-17), PDGF-A (N-30) and FGF-1 (C-19) demonstrated a cytoplasmic overexpression of these antibodies that activates the angio and lymphangiogenesis and leads to a more rapid tumor growth, higher stage of the disease and more aggressive clinical behavior demonstrated with the statistic analysis.

#### P 777

#### ABERRANT CDX-2 EXPRESSION IN SQUAMOUS MORULES IN ENDOMETRIOID ADENOCARCINOMA AND BENIGN ENDOMETRIAL LESIONS. -IMMUNOHISTOCHEMICAL ANALYSIS OF HOMEBOX GENE TRANSCRIPTION FACTOR CDX2 AND PDX1-

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Introduction: Cdx2 protein is a intestine-specific homeobox gene transcription factor that plays an important role in the regulation of differentiation of intestinal epithelium. Only a few data exist with Cdx2 expression of endometrial lesions. Pdx1 is also a homeobox gene that plays an essential role in the development or exocrine and endocrine of pancreas. Pdx1 expression is observed not only in the pancreas but also in Brunner's glands and pyloric glands. Little is known whether Pdx1 is expressed in the endometrium.

Purpose of Study: To investigate the potential role of Cdx-2 and Pdx-1 in endometrial lesions, we performed immunostaining for Cdx-2 and Pdx-1.

Methods: We selected 17 cases of normal or benign endometrial lesions (NBEs), 20 cases of Atypical endometrial hyperplasia (AEH)/endometrioid adenocarcinoma (EAC) with Squamous metaplasia (SqM), 24 cases of AEH/EAC without SqM. We defined that SqM is consisted of morules (MOs) and/or mature squamous cells (SCs). Cdx2 and Pdx1 expression was evaluated in formalin-fixed, paraffin-embedded materials. All cases were immunostained with the Envision+ method (DAKO), using a monoclonal anti-Cdx2 antibody (CDX2-88, 1:200 BioGenex), and a polyclonal rabbit anti-Pdx1 antibody (1:50 TransGenic Inc.). We used the following scoring system for Cdx2 and Pdx1 immunostaining: 0= negative; 1=1% to 10%; 2=11% to 50%; 3=51% to 100%.

Results: Four of 13 NBE cases contained foci of MO, where intense immunoreactivity for Cdx2 was seen. All AEH/EACs cases with SqM, except one (high-grade adenosquamous carcinoma), had variably Cdx2-expressed cells. In particular, most foci of MOs in AEH/EACs are intensely positive, while sporadic immunoreactivity for Cdx2 was seen in foci of SCs. Neoplastic glandular cells in the backgrounds of SqM were variably Cdx2 positive in itself. Glandular cells were sporadically positive in six AEH/EACs without SqM. None of NBEs and AEH/EACs revealed positive for Pdx1.

Conclusion: We should note there are aberrant and distinct Cdx2 expressions for MO foci in NBEs. In four cases with MOs, Cdx2-positive cells are sporadically seen in glandular

epithelium. In other words, morular formation itself seems to be made by intraluminal proliferation of Cdx2-positive glandular cells. Cdx2 expressions in MOs of NBEs and AEH/EACs tend to be very similar to that of nuclear beta-catenin (data not shown). Pdx1 does not seem to be related to NBEs and AEH/EACs.

#### P 778

#### CLINICO-MORPHOLOGICAL CHARACTERISTICS OF CANCER ARISING IN ENDOMETRIAL POLYPS DERIZHANOVA Irina, VOLOSHIN Vladimir, VOLOSHINA Nina

Aims: to study morphogenesis of the carcinoma, arising in polyps of the uterus mucous membrane (EP).

The data of the clinical observations were analyzed. Endometrium scraps and the excised uterus and appendages from 16 patients with histological proved cancer in EP were subjected to common histological and histochemical examinations, the receptors of estrogen (ER) and progesterone (PR), as well as the markers of Ki67 (MIB-1) proliferation and p53, bcl-2 apoptosis being simultaneously clearly seen.

Endometrioid adenocarcinoma (EA) was diagnosed in 13 of these patients. The patients age averaged 52,2 years, most of whom had their menstrual cycle still alive. 84,6 % of these patients suffered adiposity, 76,9 % - had hypertension, 61,5 % - diabetes. In all 13 patients EA was accompanied with adenomyosis, uterus leiomyoma and hyperplasia of the ovarian theca. In 10 cases EA in O1a stage was well differentiated (G1). In 3 patients of O1b stage of EA it was of grade 2 (G2). EA was found to develop in polyps with diffuse (8) or focal adenomatosis (5), epithelium metaplasia and dysplasia I - III stage. Diffuse (5) or focal (5) simple (6) and complex (4) hyperplasia was seen in surrounding endometrium. The receptors of sexual hormones in proliferating epithelium polyps and EA were exhibited in abundance. As epithelium atypia grew, the ER content, PR in particular, was noted to decrease. The expression of Ki67, p53 was gradually intensifying.

Serous (SA) adenocarcinoma (G2) of T1c, O2 stages was found in 67-75-year - old women with the episodes of bleeding during menopause (3 cases). The tumor was embedded in adeno-fibrous polyps accompanied by endometrium cyst atrophy. No attributes adenomatosis and epithelium metaplasia were revealed. PR in the tumor was not revealed either. Poorly positive reaction to ER was demonstrated in some single epithelium cells, SA stroma and in the surrounding polyp. Expression Ki67 and p53 in cells was positively higher, compared to that of in the polyp surrounding epithelium and in atrophic endometrium, with bcl-2 being reduced.

Conclusion: EA (81,3 %) developed in the polyps followed by adenomatosis, metaplasia, epithelium atypia and corresponded to estrogen-dependent neoplasia. SA (18,7%) embedded in adeno-fibrous polyps accompanied by the atrophy of glandular epithelium and were characterized by a high proliferation activity and corresponded to estrogen-independent carcinoma.

#### P 779

#### CARCINOMA ARISING FROM ENDOMETRIOSIS OF THE ROUND LIGAMENT

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**Introduction** Endometriosis of the extraperitoneal part of the round ligament is very rare, from 0.4 to 0.6 % in the literature, mostly on the right side. Carcinoma arising from this endometriosis is an extremely rare event. Only a few cases have been reported. We present a case of endometrioid adenocarcinoma arising from endometriosis of the left round ligament.

**Case report** A 41-year-old woman, gravida III, para I, presented a left inguinal mass. Hysterectomy with bilateral salpingo-oophorectomy and a tumorectomy were performed under the clinical diagnosis of ovarian carcinoma and liguinal metastasis. The left inguinal tumor, a grey-whitish solid mass, arised from the extraperitoneal part of the round ligament. Frozen sections and imprint cytology suggested a epithelial malignant tumor. Macro- and microscopical examination revealed that uterus and bilateral adnexa were within normal limit. However the inguinal tumor was diagnosed as endometrioid adenocarcinoma(G3) associated with endometriosis. It was suggested that the tumor was arising from endometriosis of the extraperitoneal round ligament. Thereafter chemotherapy was performed. The patient is currently 2 years free of disease after operation.

**Conclusion** Extraperitoneal endometriosis is extremely rare moreover on the left side, malignancies arising from that should be considered in the differential diagnosis in women with an inguinal mass of reproductive age.

**P 780**  
**CELLULAR RETINOL BINDING PROTEIN-1 IS VARIABLY EXPRESSED IN ENDOMETRIAL CARCINOMA AND CORRELATES WITH HISTOLOGICAL GRADING**

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Cellular retinol binding protein-1 (CRBP-1) contributes to maintain the differentiated state of the endometrium through regulation of retinol bioavailability of endometrial epithelial cells. Purpose of the study was the determination of CRBP-1 expression by immunohistochemistry in a consecutive series of 8 atypical hyperplasias and 42 endometrial carcinomas. Methods used: 40 endometrioid (well-differentiated =14; moderately differentiated =18; poorly differentiated n=8) and two serous adenocarcinomas were stained using a polyclonal antibody to CRBP-1. Staining was graded according to the percentage of tumor cells stained: intense (>75% positive), intermediate (25-75% positive) and weak or absent (<25% positive). The slides were examined independently by two pathologists, with an intervariability of less than 5%.

**Summary of the results:** CRBP-1 staining was mainly cytoplasmic. A high expression of CRBP-1 was observed in atypical hyperplasia and well-differentiated adenocarcinoma, with no significant differences between these two groups. The positivity was the highest in areas of squamous differentiation. A significant decrease of CRBP-1 immunostaining was detected with the progressive increase of histological grade of endometrial carcinomas. In fact, a comparison of CRBP-1 immunodetection and the degree of tumor differentiation revealed significant statistic differences (G1 vs G2: P<0.005, G1 vs G3: P<0.005 and G2 vs G3: P<0.005, respectively). The decrease of CRBP-1 expression in moderately and poorly differentiated carcinomas was parallel to that of estrogen and

progesteron receptor positivity. In areas of different degree of differentiation in the same tumor, a variable CRBP-1 expression was maintained. Serous carcinomas were almost CRBP-1 negative. In 39.5% of cases (75% of them well-to moderately differentiated carcinomas), nuclear staining also observed. In contrast to cytoplasmic staining, nuclear CRBP-1 positivity was focal, and positive nuclei alternated with negative nuclei even in the same neoplastic glandular structure. **Conclusions:** Cytoplasmic staining for CRBP-1 can be considered a reliable method to support histological grading of endometrial carcinoma. Further studies are needed to define the biological role of these different patterns of CRBP-1 expression in endometrial neoplasms.

**P 781**  
**STRONG IMPACT OF VASCULAR PROLIFERATION, VASCULAR MATURATION AND LYMPHANGIOGENESIS ON PROGRESSION OF ENDOMETRIAL CANCER**

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**Introduction:**

In endometrial carcinoma, studies have reported reduced survival in tumors with high microvessel density. However, MVD might have limitations as a prognostic factor. The biological and clinical significance of lymphangiogenesis has not been much studied, predominantly due to the lack of specific lymphatic markers. Metastatic spread to regional lymph nodes is an important prognostic indicator, but lymphangiogenesis has not been previously studied in these tumors.

**Purpose of the study:**

To examine the significance of other angiogenic markers in endometrial cancer, especially vascular proliferation (by Ki-67/Factor-VIII staining) and vascular maturation (by  $\alpha$ -SMA/Factor-VIII staining), as well as lymph vessel density (LVD) in a large and population-based series of endometrial carcinoma (n=281), with long and complete follow-up.

**Methods used:**

Paraffin-embedded tumor tissue was examined immunohistochemically by using double staining for Ki-67/F-VIII (vascular proliferation) and SMA/F-VIII (vascular maturation). To assess lymphatic vessel density (LVD), we used the LYVE-1 antibody. Tumor sections were scanned at low magnification to identify areas with the highest amount of vessels ("hot spots"), either stained by double-staining or LYVE-1. A panel of angiogenic factors (VEGF-A, VEGF-C, VEGF-D and bFGF) was also examined in this series, by Tissue Microarray analysis. The information was related to other molecular markers, clinico-pathologic data and patient prognosis.

**Summary of the results:**

Median vascular proliferation index (VPI) was 3.9%, and high VPI was associated with features of aggressive tumors like presence of necrosis, high FIGO stage, and decreased survival in multivariate analysis. The prognostic impact was superior to that of microvessel density. Median vascular maturation index (VMI) was 35%, and low VMI was significantly associated with vascular invasion and poor prognosis in univariate analysis. Peri-tumoral lymphatic vessels were found in 39.5% of the cases, and high LVD-pt was significantly associated with features of aggressive tumors and decreased survival.

**Conclusion:**

Other tissue-based angiogenic features than microvessel density, in particular vascular proliferation, should be evaluated as a part of the angiogenic profile of human

tumors. Peri-tumoral lymphatic vessel density also seems to play a significant role in the progression and prognosis of endometrial carcinoma.

**P 782**

**MICROSATELLITE INSTABILITY AT TETRANUCLEOTIDE REPEAT IN TYPE I ENDOMETRIAL CARCINOMA**

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Defects in the DNA mismatch repair (MMR) system results in a mutator phenotype, which is manifested as microsatellite instability (MSI). Colon cancers exhibiting MSI could be divided into 2 forms: MSI-high(MSI-H) and MSI-low(MSI-L), based on the observed frequency of genomic mutations. Noncolonic human cancers also show two types of MSI. One group shows a high frequency of MSI at mono- and dinucleotide repeats similar to colon MSI-H type tumors. Another group rarely exhibits MSI in the mono- and dinucleotide repeats but does often exhibit MSI in the tri- or tetranucleotide repeats. This distinctive subtype of MSI is termed 'elevated microsatellite alterations at selected tetranucleotide repeat (EMAST)'. In contrast to MSI-H tumors, the underlying molecular mechanisms for MSI in MSI-L or noncolonic EMAST tumors have not been clarified.

In order to investigate the prevalence of MSI at tetranucleotide repeat in Type I endometrial carcinoma and the correlation between MSI status and alterations of MMR gene, such as hMLH1 and hMSH2, we examined the 3 mono-, 3 di- and 6 tetra-nucleotide repeat markers by PCR and denaturation gel electrophoresis and the expression of hMLH1 and hMSH2 protein by immunohistochemistry in 39 Type I endometrial carcinomas.

The presence of at least one new allele, consistent with MSI, was observed in 9 (23.1%) at mononucleotide repeat, in 6 (15.4%) at dinucleotide repeats, and in 23 (59.0%) at tetranucleotide repeats of 39 endometrial carcinomas. MSI was divided into high frequency of MSI (MSI-H), single EMAST and multiple EMAST by the number of affected loci. MSI-H was detected in 9 (23.1%) of 39 patients, and single EMAST and multiple EMAST were noted in 10 (25.6%) and 4 (10.3%) of 39 carcinomas, respectively. MSI status was not associated with clinicopathologic features, such as FIGO stage, histologic grade and depth of invasion. Of 39 carcinomas, 12 (30.8%) showed an absence of hMLH1 expression, 8 (20.5%) revealed an absence of hMSH2 expression, and 16 (41.0%) tumors demonstrated the absence of either hMLH1 or hMSH2 expression. Seven (77.8%) of 9 MSI-H tumors, 2 (20.0%) of 10 single EMAST tumors, and 3 (75.0%) of 4 multiple EMAST tumors revealed the absence of either hMLH1 or hMSH2 expression.

These results suggest that MSI-high and multiple EMAST endometrial carcinomas are highly associated with alterations of hMLH1 or hMSH2 and single EMAST tumors may be associated with another mechanism.

**P 783**

**THE ASSESSMENT OF THE EPITHELIOSTROMAL MICRO ANATOMICAL RELATIONS IN THE UTERUS EPITHELIUM METAPLASIA**

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Objectives. The transformation and evolution sometimes abnormal of the endometrium and endocervix epithelium frequently become a dilemma for the pathologist. It draws the

attention to the unknown problems of the metaplasia phenomena: the determined factors of the metaplasia genesis, the morph and functional epitheliostromal relations and finally its integration in neoplasia and displasia.

Purpose of the study. We proposed ourselves to identify and evaluate the potential of phenotype transformation of the epithelial cells and metaplastic epithelial structures inside uterus glands.

Materials and methods. We studied 400 cases of metaplasia of the uterus epithelium, investigated by histologic and histochemic visualization methods for the epithelium cells, fiber elements of the extra cellular matrix and glycosaminoglicans.

Results and conclusions. The epithelial and stromal structures of the endometrium and endocervix suffer cycle transformations that we integrated inside "uterus epithelium remodeling cycle". The extra cellular matrix integrates itself in this cycle but also in the processes of angiogenesis and apoptosis. Ortomeplasia imposes the evaluation of the epithelial transformations in ontogenesis and during normal and pathologic hormonal cycles. We discuss the integration of mataplasia in displasia and neoplasia pathomorphogenesis.

Key words: uterus, metaplasia, displasia, epithelium

**P 784**

**AN IMMUNOHISTOCHEMICAL COMPARISON BETWEEN LOW GRADE AND HIGH GRADE OVARIAN SEROUS CARCINOMAS: SIGNIFICANTLY HIGHER EXPRESSION OF P53, MIB1, BCL2, HER-2/NEU AND C-KIT IN HIGH GRADE NEOPLASMS**

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INTRODUCTION: Recently, a dualistic pathway of ovarian serous carcinogenesis has been proposed based on morphologic observations and molecular genetic analysis. In this scheme, low grade OSC arises in a stepwise fashion from a benign serous cystadenoma through a usual serous borderline tumour through a micropapillary variant of serous borderline tumour. In contrast, the more common high grade OSC arises de novo from the ovarian surface epithelium.

PURPOSE OF STUDY: Although the division of OSC into low and high grade variants is gaining greater acceptance, and although there is accumulating molecular genetic evidence for this, there is little published information regarding a comparison of protein expression between these two types of OSC. In this study, we have investigated the immunohistochemical expression of a wide range of proteins in cases of low grade (n=22) and high grade (n=47) OSC.

METHODS: Antibodies used were p53, MIB1, BCL2, WT1, HER-2/neu, C-KIT, osteopontin and survivin. For all antibodies, except MIB1, cases were scored as 0 (negative or occasional positive cells), 1+ (<10% cells positive), 2+ (10-25% cells positive), 3+ (26-50% cells positive), 4+ (51-75% cells positive) or 5+ (>75% cells positive). For MIB1, the percentage of positive nuclei was calculated. SUMMARY OF RESULTS: There was a statistically significant higher expression of p53, MIB1, BCL2, HER-2/neu and C-KIT in high grade compared to low grade OSC (p < 0.05). Thirty of 47 (64%) cases of high grade OSC exhibited 5+ staining with p53 compared to 4 of 22 (18%) low grade neoplasms. Twelve of 47 (26%) high grade OSC exhibited 5+ staining with BCL2 compared to one of 22 (5%) low grade OSC. The mean MIB1 proliferative index in high grade OSC was 55.4 % compared to 23.0 % in low grade OSC. Virtually all cases

of both low grade and high grade OSC exhibited diffuse nuclear positivity with WT1 and diffuse cytoplasmic positivity with survivin. Osteopontin expression was variable with no significant difference in expression between low grade and high grade OSC. CONCLUSION: The significant differences in protein expression between low grade and high grade OSC provides further support for a different underlying pathogenesis. In particular, the differences in p53 immunoreactivity are in keeping with the observation that p53 gene mutation is more common in high grade than low grade OSC.

#### P 785

##### OVARIAN ENDOMETRIOID TUMOR WITH YOLK SAC TUMOR COMPONENT.

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##### Case Report:

A 47 year old woman was admitted to our Hospital for abdominal pain. Physical examination showed, on palpation, a painful and firm Douglas sac. Transvaginal ecography revealed a 104 x 107 mm solid and cystic mass, located on the left ovary. The wall of the cysts appeared thick and irregular. Histologically, the mass showed endometrioid carcinoma areas intimately admixed with yolk sac tumor areas. Reticulo-microcystic, enteroid-glandular, and solid histological patterns were readily identified on the endodermal sinus component. FIGO stage of the tumor at this time was I-A.

Five months later a metastatic liver nodule was identified, with a significant rise of the serum alfa-fetoprotein. Histological examination of the liver nodule showed metastasis of the endodermal sinus tract component of the ovarian tumor.

##### Discussion:

Neoplasms that reproduce extraembryonic tissues (trophoblast or endodermal sinus tissue) not always have a germinal cell origin. These neoplasms may also be derived from somatic cells, usually from endoderm or mesoderm.

A differential diagnosis between an endometrioid tumor associated with an endodermal sinus tumor, and the endometrioid variant of the endodermal sinus tumor, should be established. Features that support the diagnosis of an endometrioid tumor associated with an endodermal sinus tumour are: the older age of the patient, the presence of endometriotic focus, and the presence of a biphasic immunohistochemical staining pattern. The biphasic immunohistochemical staining pattern is characterized by the presence of areas that are positive for EMA, estrogen and progesterone receptors (that represent the endometrioid component of the tumor), and areas that are positive for alfa-fetoprotein, and negative for EMA (that represent the endodermal sinus tumor component).

The response of this tumours to chemotherapy is poor and they are usually cis-platinum resistant. Poor prognostic should be expected

#### P 786

##### IMMUNOHISTOCHEMICAL DISTRIBUTION OF AN USEFUL ANTIBODIES PANEL FOR OVARIAN NEOPLASMS

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Introduction. Cancer of the ovary represents about 30% of all cancers of the female genital organs (WHO, 2003). In developed countries it is about as common as cancer of the

corpus uteri and invasive cancer of the cervix. About 70-75% of patients with ovarian cancer have tumor spread beyond the pelvis at the time of diagnosis.

Aim. Immunohistochemical study of the main epithelial tumors of the ovary-serous and mucinous adenocarcinomas-is a valuable adjunct the thorough histologic examination. Using a wide panel of antibodies, as ER (estrogen receptor), PGR (progesterone receptor), EGFR (epidermal growth factor receptor), cerbB2 (HER2NEU protooncogene), PCNA (proliferative cellular nucleolar antigen), CA125, Ki67, P53 (P53 oncoprotein) and CK7 (cytokeratin 7) we have tried to find a "happy combination" for an early and precisely diagnosis of ovarian epithelial cancers.

Material and methods. We investigated immunohistochemical a group of 60 patients with serous (n=45, 45.75%), mucinous (n=12, 20%) adenocarcinomas and borderline tumors (n=3; 5%). We used the trisodium methods of Hsu (1981) Avidin-Biotin-Peroxidase. A number of 19 patients have had peritoneal implants (31,6%) and tumors were bilateral in 7 cases (11,66%).

Results. The method used revealed that the ER was positive in 70% cases, PGR in 60%, PCNA in 61,66% and p53 in 63,33%, CA125 in 81,66% and the perimembrane pattern of cerbB2 in 63,33%. The majority of cases were the serous carcinomas.

Conclusions. The relevance of CA125-an useful antibody in ovarian cancer, also for metastatic disease-is limited because it is positive in many conditions (mesothelial proliferations, metastatic carcinomas, etc).

The ovarian serous carcinomas are an homogenous group from the standpoint of pathogenesis. The panel of antibodies was positive in more than 50% of serous carcinomas.

None of the antibodies or combination has a clearly established role for staging and prognosis of the tumor.

Only the three-tiered grading system has important prognostic and therapeutic implications.

#### P 787

##### DIFFERENTIAL EXPRESSION OF CADHERINS AND CATENINS ASSOCIATES WITH DISTINCT OVARIAN CANCER HISTOLOGICAL TYPES

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BACKGROUND: Ovarian carcinoma (OvC) is the leading cause of death among all gynecologic malignancies. In the last years, it has been demonstrated that alterations in the cadherin-catenin adhesion complexes are involved in tumor initiation, progression and metastasis. The aim of this study was to evaluate the immunoreactivity of the cadherins and the catenins in ovarian cancer and to correlate their expression with clinicopathological features.

DESIGN: Immunohistochemical staining was performed in 85 ovarian carcinomas: clear cell (CCs), n=31; serous (SCs), n=28; endometrioid (ECs), n=13; mucinous (MCs), n=13) using a tissue microarray. Monoclonal antibodies against E-cadherin (4A2C7, Zymed), P-cadherin, N-cadherin, cadherin-11, beta-catenin, gamma-catenin, and p120ctn (Transduction Labs) were applied using the LSAB method.

RESULTS: Overall, membranous expression of E-CD, N-CD, and P-CD was observed in 96%, 29.8%, and 8.4% of the OvCs analyzed, respectively. Cadherin-11 was rarely expressed in tumor cells but frequently in the stromal cells adjacent to the tumor nests. Each of the cadherins was

differentially expressed among the histological types of OvCs: E-CD preserved expression was frequent in MCs (61.5%) but very rare in SCs (3.6%). Serous tumors showed the higher incidence of N-CD (67.9%) and P-CD (21.4%) expression compared to the other histotypes. MCs and CCs rarely expressed N-CD or P-CD. With respect to catenins, significant differences were observed among the histological types: CC tumors and SCs showed more frequently reduced or absent expression of beta-catenin, gamma-catenin and p120 than other types. MCs were characterized by the frequent preservation of the three catenins. Abnormal nucleocytoplasmic beta-catenin expression was clearly associated with the EC histotype, since all cases showing this pattern (12%) were endometrioid tumors ( $p < 0.001$ ). Cytoplasmic localization of gamma-catenin and p120 was observed in 4.5% and 7% of OvCs respectively, but these expression patterns were not statistically associated to any histological type.

**CONCLUSIONS:** Our results suggest that each of the different histological types of OvC show a distinct and characteristic cadherin and catenin expression profile that could help in the differential diagnosis of these tumors and could explain differences in biological characteristics, such as the ability to dissemination.

**GRANTS:** FIS PI020342 and PI020355.

#### P 788

##### **STAGE I OVARIAN CARCINOMA. A DIFFERENT DISTRIBUTION OF HISTOLOGIC PATTERN**

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**INTRODUCTION.** Only a minority of ovarian carcinomas are diagnosed in Stage I. The histologic subtypes of ovarian carcinomas, despite their common epithelial origin, arise in different histogenetic settings being associated with different clinical and diagnostic implications.

**PURPOSE OF STUDY** is to identify histopathologic patterns of ovarian carcinoma that are likely to have an impact on early stage diagnosis.

**METHODS USED.** Histologic slides and clinical charts from 27 consecutive patients diagnosed from 2000 to 2004 at the MSSM with Stage I non-mucinous ovarian carcinoma were reviewed. The tumors were classified into serous papillary (OSPC), endometrioid (OEC) and clear cell (OCCC) carcinomas. Associated pathologic findings including ovarian endometriosis (OE), endometrial carcinoma (EC), endometrial hyperplastic polyps (EP) and clinical data (age of patients, history of breast cancer, presenting symptoms) were correlated with the histologic subtypes of the ovarian tumors.

**SUMMARY OF RESULTS.** The histologic subtypes of the ovarian carcinomas were: 15 OEC (55%), 8 OSPC (29%) and 4 OCCC (15%). In 12 patients the OEC arose in OE (44%) and their clinical presentation was pelvic mass due to endometriotic cysts. Concomitant EC was diagnosed in 7 patients (26%) and EP in two (0.07%). Four of these patients presented with vaginal bleeding. Of the patients diagnosed with OSPC three had histories of breast cancer, presenting with pelvic masses, one had concomitant EC presenting with vaginal bleeding and one was BRCA1 mutation positive, her ovarian carcinoma being diagnosed incidentally in the prophylactically removed ovaries. The remaining three presented with pelvic masses, of which one due to cystic endometriosis. The four patients with OCCC presented with pelvic masses, three arose in cystic endometriosis and one had concomitant EC with vaginal bleeding. The average age of the patients with OEC was 49, 4, with OSPC 58, 9 and with OCCC 67 years.

**CONCLUSION.** OEC and OCCC are more commonly diagnosed in Stage I because of their frequent association with symptomatic OC and OE. OSPC, the most prevalent ovarian

carcinoma overall, is less commonly diagnosed in Stage I, more often, however, in patients with breast cancer histories who are closely followed. Our findings suggest that screening of patients with OE, endometrial pathology and history of breast cancer can contribute to diagnose ovarian cancer in earlier stages.

#### P 789

##### **RELATIONSHIP BETWEEN P53-ASSOCIATED PROTEINS AND ESTROGEN RECEPTOR (ER) STATUS IN OVARIAN SEROUS NEOPLASMS**

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**Objective.** This study was undertaken to evaluate the relationship between p53-associated proteins and ER status, and the methylation status of the ER CpG island in ovarian serous tumors.

**Methods.** We studied the immunoexpression of p14ARF, MDM2, and p53, and relationship between those protein expression and ER $\alpha$  in 158 cases of ovarian serous tumors, which comprised benign (n=23), borderline (n=41), and malignant tumors (n=94). We also investigated the methylation status of ER with correlation of ER status.

**Results.** The aberrant expression of p14ARF, MDM2, p53, and p53-associated proteins were observed in 19.6% (31/158), 47.5% (75/158), 39.9% (63/158), and 70.3% (111/158) of cases, respectively. The expression of MDM2 was significantly higher in borderline serous ovarian tumors than in benign ( $P=0.04$ ) and malignant tumors ( $P < 0.01$ ). p53 expression in borderline tumors was not frequent and p14ARF expressional loss was mainly observed in carcinoma. Significant differences in the expressions of MDM2 ( $P=0.01$ ), p14ARF ( $P=0.04$ ), and p53-associated proteins ( $P=0.02$ ) were observed between ER-positive and ER-negative cases. Methylation specific PCR for the ER CpG island revealed methylation bands in 48.3% (14/29) of borderline tumors, and in 36.4% (12/33) of malignant tumors. Methylation statuses of ER were not significantly related to ER status.

**Conclusions.** This study shows a significant association between ER status and p53-associated proteins, but no relationship between ER status and ER CpG island methylation. This study indicates that alteration of p53-associated proteins may affect tumor behavior in ovarian serous neoplasms, but ER methylation is probably not an important cause of ER loss.

#### P 790

##### **AN IMMUNOHISTOCHEMICAL STUDY OF THE EXPRESSION OF ADHESION MOLECULES IN OVARIAN SEROUS NEOPLASMS**

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To clarify possible roles of adhesion molecules including E-cadherin, beta, gamma-catenin, CD44s, CD44v6, CD56, and CD99 in ovarian tumor development and progression, an immunohistochemical study was undertaken for 23 cases of benign, 41 cases of borderline, and 94 cases of malignant ovarian serous neoplasms using tissue microarray (TMA). Significantly reduced expression of E-cadherin, and overexpression of CD44s, CD56, and CD99 were more frequently observed in malignant tumors than benign and borderline tumors. Reduced expression of E-cadherin was

also correlated with high tumor grade ( $P=0.03$ ), presence of peritoneal seeding ( $P=0.03$ ), and low overall survival rate ( $P=0.02$ ). Overexpression of CD44s was significantly associated with high tumor grade ( $P=0.04$ ), advanced stage ( $P=0.03$ ), and low overall survival rate ( $P=0.02$ ). CD56 was increasingly expressed in the case of advanced stage ( $P=0.008$ ) and peritoneal seeding ( $P=0.002$ ). Among all clinicopathologic factors, E-cadherin expression, CD44s expression and stage were correlated with overall survival in univariate study. The results of immunohistochemical analysis suggest that E-cadherin, CD44s, and CD56 may be useful prognostic markers for serous neoplasm of the ovary.

#### P 791

##### EARLY STAGE EPITHELIAL OVARIAN CARCINOMAS: A STUDY OF MORPHOLOGIC PROGNOSTIC FACTORS

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There is limited experience with the early stage ovarian carcinoma. We intended to evaluate the prognostic factors that are commonly used, and reevaluated the diagnosis in the light of new knowledge especially related with borderline neoplasia.

For this purpose we examined 111 patients' slides chosen from 156 clinical early stage patients from the files of gynecologic oncology between 1984-2001, we eliminated the patients if we didn't have that patients' slides in our laboratory, if no absolute data and/or microscopic features concerning that it's a primary ovarian carcinoma was present and if patients' disease was reported as higher stage after the operation. We reviewed the slides, blinded to the clinical outcome, using current diagnostic criteria and graded the neoplasm according to the system suggested by Shimizu and noted the presence or absence of endometriosis. Statistical analysis for survival was done by Kaplan Meier Analysis and group comparisons were made with Log Rank test.

86 patients were stage 1 and 25 patients were stage 2. Distribution of histologic subtypes were as follows; 16 serous papillary carcinoma (%14.4), 14 mucinous carcinoma (%12.6), 16 endometrioid carcinoma (%14.4), 9 clear cell carcinoma (%8.1), 2 mixed carcinoma, 1 transitional carcinoma, 1 indifferantiated carcinoma, 26 serous borderline (%26), 17 mucinous borderline (%17), 3 endometrioid borderline (% 2.7), 2 serous cystadenoma, 3 mucinous cystadenoma, and 1 brenner tumor. There was statistically significant difference in survival between stage 1 and stage 2 patients ( $p=0.0188$ ) and endometrioid type had a better prognosis than clear cell ( $p=0.0105$ ) and mucinous type ( $p=0.0321$ ). In contrast there was no statistically significant difference in survival between mitotic scores, architecture scores, cytology scores and tumor grades. Endometriosis was present in 19 (%17.1) patients and endometriosis mostly related with endometrioid and clear cell type carcinoma ( $p<0.05$ ).

As a result pathologist must be familiar with the borderline tumor when dealing with early stage ovarian carcinoma, because borderline tumors can be diagnosed as carcinoma erroneously especially by non-gynecologic pathologist. Stage is the only significant prognostic factor in the early stage ovarian carcinoma. In contrast to wide spread knowledge, "clear cell carcinoma" histologic type is not a poor prognostic factor and tumor grade has no effect on survival for early stage carcinomas.

#### P 792

##### EXPRESSION AND PROGNOSTIC VALUE OF MATRIX METALLOPROTEINASE 7 AND ITS RELATION TO BETA-CATENIN EXPRESSION IN EPITHELIAL OVARIAN CANCER

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Introduction: Matrix metalloproteinase 7 (MMP-7) is a small member of MMP-family, which degrades various components of extracellular matrix. It has been shown that epithelial ovarian tumor cells produce MMP-7. Expression of MMP-7 has been shown to be regulated by beta-catenin.

Purpose of the study: To evaluate the prognostic significance of MMP-7 expression in human epithelial ovarian cancer. The relation between MMP-7 and beta-catenin expression was also studied.

Methods: Two hundred eighty four patients were diagnosed at Kuopio University Hospital and Jyväskylä Central Hospital, Finland, between 1976 and 1992. The median follow-up time was 29 months. The FIGO standards were used for staging. For histological typing and grading, the WHO classification was used. For immunohistochemical analyses, monoclonal antibodies for human MMP-7 and beta-catenin were used. Specimens were analysed by three independent observers and the intensity of staining and percentage area of positive tumor cells in the section were scored.

Results: Median percentage of MMP-7 expression was found to be 40%. A high percentage area (>40 %) of MMP-7 expression in tumor cells was significantly correlated with positive nuclear expression of beta-catenin ( $p=0.01$ ). A low percentage area (< 40 %) of MMP-7 expression was correlated with high histologic grade of a tumor ( $p=0.003$ ), advanced stage ( $p=0.002$ ) and large primary residual tumor ( $p=0.005$ ). A high percentage area of intense MMP-7 expression in tumor cells was found to be a prognostic factor predicting favorable disease related and recurrence free survival ( $p=0.0003$  and  $0.0052$ , respectively). Also in multivariate analysis a high percentage area of intense MMP-7 expression in tumor cells retained its prognostic power.

Conclusion: The low expression of MMP-7 in epithelial ovarian cancer cells is associated with high histologic grade, advanced stage and large residual tumor and the high MMP-7 expression with positive nuclear expression of beta-catenin. According to our results, the expression of MMP-7 seems to have prognostic significance in epithelial ovarian cancer.

#### P 793

##### CLINICOPATHOLOGICAL STUDY OF METALLOTHIONEIN IMMUNOHISTOCHEMICAL EXPRESSION, IN BENIGN, BORDERLINE AND MALIGNANT OVARIAN EPITHELIAL TUMORS.

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Metallothioneins (MTs) are a family of cystein-rich metal-binding proteins, which are expressed in normal cells during fetal and postnatal life but also in a variety of human neoplasms. MT expression in human tumors has been linked to resistance to anticancer drugs and differentiation and progression in some types of tumors. This study examined the immunohistochemical expression of MTs in benign,

borderline and malignant tumors of ovarian surface epithelium and the possible correlations with clinicopathological parameters and survival. A total of 87 cases with diagnosis of ovarian surface epithelial tumors were included. Specifically, 21 cases of benign cystadenomas (11 serous and 10 mucinous), 14 borderline (low malignant potential tumors, 8 mucinous and 6 serous) and 52 cases of ovarian cancer were analysed.

Immunohistochemical expression of MT (cut-off level >10% of tumor cells) was clearly associated with malignancy. A statistically significant correlation was found between the expression of MT in cancer cases and benign tumors ( $p < 0.0001$ ) and cancer cases and borderline tumors ( $p = 0.003$ ). In cancer cases was observed a difference between grade I and III ( $p = 0.002$ ). There was no correlation of MT overexpression with survival in the small number of ovarian carcinoma patients where it was analysed. MT constitutes a marker that characterizes aggressiveness and a high malignant potential in ovarian epithelial tumors. In diagnostic problems MT may help distinguish between benign, borderline and malignant tumors.

#### P 794

##### **INTRAOPERATIVE FROZEN SECTION DIAGNOSIS OF OVARIAN MASSES: HOW ACCURATE ARE WE?**

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In ovarian masses, intraoperative frozen section diagnosis is requested either to rule out malignancy or to verify the borderline or malignant tumor before proceeding with radical surgery.

A retrospective study was conducted to determine the accuracy of frozen section diagnosis in ovarian masses.

We reviewed the frozen section diagnosis and permanent pathological diagnosis of 227 resected ovarian masses from May 1999 to December 2004. Frozen and permanent section diagnoses were divided into 3 groups: benign, borderline and malignant.

The accuracy, sensitivity, specificity, positive and negative predictive value of frozen section were studied.

Final histological diagnosis was benign in 173 (76.3%) cases, borderline in 8 (3.5%) cases and malignant in 46 (20.2%) cases. The sensitivity for malignant, borderline and benign cases were 93.4%, 87.5% and 99.4% respectively. There were one false positive and four false negative cases. The specificity for malignancy was 100%, for borderline 98.6% and 98% for the benign masses. The positive predictive value of a malignant frozen section diagnosis was 100%; the negative predictive value of a benign frozen section diagnosis was 98%.

Most of the false negative cases were belong to mucinous tumor group. Two mucinous adenocarcinomas were incorrectly diagnosed as borderline and a borderline mucinous tumor diagnosed as benign. False positive case was also a mucinous tumor having frozen section diagnosis of borderline mucinous tumor turned out to be a benign mucinous cystadenoma in permanent sections.

In conclusion, although we have limited number of borderline cases, the accuracy of frozen section diagnosis was high in benign, borderline and malignant cases.

Mucinous tumors constituted the main diagnostic problems in frozen section evaluation. Large masses with wide range of tissue types from benign to malignant underline the sampling errors leading to false frozen section diagnosis. Surgeons and the pathologists should be aware of the limitations of frozen section diagnosis particularly in mucinous tumors of the ovary.

#### P 795

##### **OVARIAN WOLFFIAN TUMOUR: A CASE REPORT**

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**Introduction:** Ovarian wolffian tumors are defined according to OMS as tumor of presumptive wolffian origin characterized by a variety of epithelial patterns. This epithelial tumour may show diffuse solid tubular, hollow tubular and sieve-like patterns and a combination of the various patterns may occur. Although more common in the broad ligament, this tumour may occur in the ovary.

**Aims:** To review clinical data, histologic features, immunohistochemical profile and to study the differential of the ovarian wolffian tumor mainly from the endometrioid carcinoma of the fallopian tube.

**Materials and methods:** A 71-year-old woman presented with left ovarian tumour. Gross and microscopic findings were noted. Immunohistochemical stains for alpha inhibin were performed.

**Results:** Macroscopically, the tumour had 90x70x40 mm, lobulated, well circumscribed margins and was white and yellow. Histologic examination showed a well circumscribed islands or lobules of glandular and ductal epithelium in a back to back pattern. The lobules exhibited focal or extensive hyalinisation with obliteration of the epithelial component. The nuclei appeared moderately atypical and uniform. The central most part of the nodules appeared necrotic. The cells tumors were positive for alpha-inhibin.

**Discussion and conclusion:** The immunoreactivity of tumour cells with alpha inhibin and absence of squamous differentiation and destructive stromal invasion supported the wolffian nature of the neoplasm. Because of moderately nuclear atypia and striking mitotic activity, this tumor was considered as low grade malignancy.

#### P 796

##### **THE RELATIVE FREQUENCY OF OVARIAN TUMORS AND THEIR AGE DISTRIBUTION OVER A 20 YEAR PERIOD IN SHARIATI TEACHING HOSPITAL, TEHRAN, IRAN.**

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**Abstract:**

**Objective:**

The evaluation of the relative frequency of ovarian tumors and their age distribution over a 20 year period in Shariati Teaching Hospital, Tehran, Iran.

**Method:**

A retrospective descriptive study was performed on all pathologic reports of ovarian tumors, from 1983 to 2003 in Shariati Teaching Hospital in Tehran, Iran. Then the relative frequency and their age distribution was evaluated.

**Results:**

493 ovarian neoplasia had been recruited. Surface epithelial tumors were the most frequent tumors with 283 cases (57.4%) followed by germ cell tumors with 152 cases (30.83%) and sex cord stromal tumors with 31 cases (6.28%). Metastatic tumors were present in 24 cases (4.86%) and the remaining 3 cases were 2 cases of luteoma and 1 case of chondroma. The most frequent benign neoplasia of the ovary was a dermoid cyst (mature cystic teratoma) (39.53%) followed by serocystadenoma (29.94%) and mucinous cyst adenoma (14.82%).

The most frequent ovarian malignancy was malignant serous tumors (53.69%) and then metastatic tumors (16.1%) and

mucinous cyst adeno carcinoma (14.09%). The most frequent age group was the 21-30 years old (111 cases).

Conclusion:

In this study the surface epithelial tumors were less than other studies, and the germ cell tumors were more so. It may have been due to the younger age of our patients or genetic factors. Metastatic tumors were more frequent probably due to the late diagnosis of malignancies.

Between sex-card stromal tumors, the most common tumor was fibrothecoma. Whereas in other studies granulosa – theca cell tumors were the most frequent. Genetic and racial factors may have played a role in this.

Key words:

Ovarian tumors, benign ovarian neoplasia, malignant ovarian neoplasia, Iran.

#### P 797

##### **IMPORTANCE OF IMMUNOHISTOLOGICAL ER, PR EXPRESSION IN TUMOR FOR ENDOMETROID OVARIAN CARCINOMA HORMONOTHERAPY**

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#### I. Introduction.

Endometroid ovarian carcinoma makes up 20% of ovarian carcinomas. Hormonotherapy of endometroid ovarian carcinoma equally with surgical treatment and chemotherapy improves patient's survivability and quality of their lives.

The aim of this investigation is to specify the hormonotherapeutic indications in patients with endometroid ovarian carcinoma taking into account the level of tumor's differentiation, receptors content to steroid hormones.

#### II. Materials and methods.

We examined 38 endometroid ovarian carcinoma patients. The immunohistochemical analysis for estrogen/progesterone receptors (ER/PR), was performed using formalin-fixed. The dependence between the level of tumor's differentiation and estrogen/progesterone receptors content in it was found.

#### III. Results.

Endometroid ovarian carcinoma is characterized by the high level of estrogen – 66.7% and progesterone – 98.8% in women in menopause (61-67 years old) with G I-II.

The absence of estrogen receptors expression, and the high level of progesterone receptors expression were noted in 32% of women in menopause with G I-II. The low estrogen/progesterone receptors content in women in menopause and their absence were noted in 86% of endometroid ovarian carcinoma women in childbearing age (27-42 years old) with G III-IV. Prescription of Depo-Provera 500mg once a day during 3 weeks together with single dosing of Carboplatin and Mitotaks resulted to the tumor decrease up to 2-3 sm, revealed after Second Look, comparatively with initial data of patients with III level of endometroid ovarian carcinoma with G I-II.

#### IV. Conclusion.

In complex treatment of endometroid ovarian carcinoma patients the hormonotherapy was used effectively in every case of finding out the high level of estrogen/progesterone receptors expression in tumor's cells.

#### P 798

##### **LOCALIZATION OF ABCC2 (MRP2, CMOAT) IN NUCLEAR MEMBRANE OF OVARIAN CARCINOMA CELLS CORRELATES WITH RESISTANCE TO CISPLATIN AND CLINICAL OUTCOME**

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Because the majority of ovarian cancer patients are diagnosed in an advanced stage of the disease, first-line therapy commonly consists of surgical resection, followed by platinum drug-based chemotherapeutic regimens. Unfortunately, second-line chemotherapeutic treatment merely achieve response rates of about 20%. These clinical observations indicate that long-term prognosis of ovarian carcinoma depends on intrinsic and acquired drug resistance. In vitro experiments demonstrated that multidrug resistance-associated protein-2 (ABCC2) overexpression can confer resistance to platinum-containing anticancer drugs like cisplatin and carboplatin to cancer cell lines including ovarian carcinoma cells

In the present study we analyzed the expression and cellular localization of ABCC2 in ovarian carcinoma-derived cell lines exhibiting different levels of resistance against cisplatin and in a group of post-surgically platinum-containing drug treated ovarian cancer specimens taken from first-look laparotomies and from secondary cytoreductions in the same group of patients. ABCC2 expression was correlated in dependence on cellular localization with sensitivity against cisplatin or clinical outcome.

The analyses were performed on samples of ovarian cancer originating from 73 laparotomies and on 11 ovarian cancer cell lines. To check specificities of antibodies by reproducing the cellular localization of ABCC2 described in other studies, immunohistochemical reactions were performed on two tissue microarrays (TMAs).

The performed immunohistochemical reactions on the level of light and electron microscope showed, that ABCC2 expression in nuclear envelopes of studied cells significantly correlates with cisplatin resistance. In tumor samples we have shown, that nuclear ABCC2 expression is specific for cases of shorter overall- and progression-free survival.

This study for the first time demonstrates, that ABCC2 can be expressed in nuclear envelope and this localization is specific for cisplatin-resistant ovarian carcinoma cases.

#### P 799

##### **GRANULOSA CELL TUMORS FREQUENTLY EXPRESS EGFR (HER-1), HER-3, AND HER-4: POTENTIAL RELEVANCE TO NOVEL ANTI-HER THERAPIES**

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Granulosa cell tumors (GCT) of the ovary are rare sex cord-stromal tumors. Up to 50% of patients develop recurrences which sometimes occur as late as 30 years following the initial surgical treatment. Although some recurrent tumors have been treated successfully by re-operation, radiation therapy and/or chemotherapy, the prognosis is usually poor.

The aim of this study was to analyze immunohistochemically the expressions of EGFR (Her-1), Her-2, Her-3, and Her-4 in 40 tumors comprising 38 adult type GCTs (AGCT) and 2 juvenile type GCT (JGCT). Thirty one cases (77,5%) were positive for at least one of the receptors EGFR (Her-1), Her-3, and Her-4. Twenty six out of 40 (65%) GCTs showed positive reaction for EGFR (Her-1). Eight tumors (20%) were exclusively positive for EGFR (Her-1). None of 40 cases

showed a positive reaction for Her-2. Positive reactions for Her-3 and Her-4 were observed in 18 (45%) and 23 (57,5%) tumors. Only one case (2.5%) was exclusively positive for Her-4. Four tumors (10%) showed positivity for Her-3 and Her-4 but were negative for EGFR (HER-1). While one of the two JGCTs was negative for all members of the Her-family, one showed reactivity for EGFR (Her-1), Her-3, and Her-4.

These findings may provide the basis for clinical trials using monoclonal antibodies against extracellular domain of EGFR (such as cetuximab) or small molecule inhibitors of EGFR (such as gefitinib). Furthermore, the expression of Her-4 in GCTs raises the intriguing possibility of treatment with Heregulin-beta2/PE40, a ligand toxin with known anti-proliferative effects on granulosa cell lines.

#### P 800

##### **GRANULOSA-CELL TUMOURS OF THE OVARY: A STUDY OF 16 CASES**

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##### **Introduction:**

The granulosa-cell tumors of ovary (TG) are the most known malignant tumours of the mesenchyme and sexual cords; they are rare, accounting for 2 to 5% of the whole of the malignant tumours of the ovary. These tumours are of two types; the adult type and the juvenile type.

**Purpose:** Through our results and after a review of the literature, we reported the epidemiological, anatomoclinical and therapeutical aspects of the TG of the ovary.

##### **Methods used:**

We made a retrospective study of 16 cases of TG that were examined at the laboratory of pathological anatomy and cytology of the CHU Habib Bourguiba of Sfax, over 10 years period (1994-2003).

**Results :** The average age of our patients was 46 years for the adult type with extremes of age of 20 and 70 years and 35 years for the juvenile type; 19% of our patients were nulliparous, 31 % were menopausal women and an inductive treatment of ovulation was reported in 19 % of the cases; clinical symptomatology was dominated by the abdominopelvic mass and endometrial signs related to hyperoestrinism like disturbances of the menstrual cycle and post menopausal metrorrhagia. The tumour was unilateral in 94 % of the cases and bilateral in 6 % of the cases. Macroscopically, the tumor size was often important varying from 6 to 27 cm with a solido-cystic aspect in 50 % of the cases; histologically, the adult type was found in 94 % of the cases and the juvenile type in 6 % of the cases. The immunostaining was positive for the mesenchymatous markers (vimentine); variable positivity for the chromogranine, the NSE and the PS100 was observed. Association with endometrial lesions was noted in 44 % of the cases with simple hyperplasia without atypia in 25 % of the cases, polyp in 12,5 % of the cases and adenocarcinoma in 6 % of the cases. The treatment was surgical in all cases; only the patient with juvenile type was treated by chemotherapy. No relapse nor recurrence was noted after a follow up of 30 months for the adult type contrary to the juvenile type who developed multilocalized metastasis.

**Conclusion :** granulosa-cell tumors are an interesting entity which has several epidemiological and histological characteristics and a potential to aggressive behaviour.

#### P 801

##### **ADULT TYPE GRANULOSA CELL TUMOR – MORPHOLOGICAL FEATURES**

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Granulosa cell tumors (GCT) account for about 5% of all ovarian tumors. They are usually unilateral and variable in size. Adult granulosa cell tumors (AGCT) account for approximately 1-2% of all ovarian tumors and 95% of all GCT. They occur more often in postmenopausal women, with a peak incidence between 50 and 55 years. 6 cases of AGCT were diagnosed in the Clinical Hospital of Obstetrics and Gynecology Iasi, in a 7 years period. The age of the patients ranged between 35 and 67 years, 4 of them (66.66%) being postmenopausal. The macroscopical appearance showed that all were unilateral tumors – 4 of them (66.66%) solid and 2 cystic (33.33%). After paraffin-embedding and usual stainings, we observed the granulosa cells as small, cuboidal to polygonal cells, arranged in anastomosing cords, and gland-like structures filled with an acidophilic material recalling immature follicles (Call-Exner bodies). Two tumors presented microfollicular and macrofollicular patterns. Three tumors (50%) presented also a thecoma component formed by sheets of cuboidal to polygonal cells (specifically called granulosa-theca cell tumors). In two of them both granulosa and theca cells presented aspects of luteinization (luteinized granulosa-theca cell tumors). Only one tumor (16.66%) was associated with simple hyperplasia of the endometrium (suggesting an active endocrine tumor). 4 of them (66.66%) presented areas of malignant transformation, 3 of them being associated with tumoral necrosis, 1 with calcifications, and 2 with vascular invasion. Although considered as benign tumors, only 5-25% of them being malignant, with an indolent course and only local recurrences, in the reported cases the tumors were mainly malignant (66.66%), two of them being highly aggressive, as vascular invasion was noted, and one of them was also advanced, with histologically confirmed implants of abdominal peritoneal surfaces.

#### P 802

##### **SALPINGITIS WITH CHLAMYDIA TRACHOMATIS; POSSIBLE ROLE IN SECONDARY STERILITY**

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In etiopathogenesis of secondary sterility, salpingitis with various etiology plays an important role. The main infections of the tube in etiopathogenesis of sterility, except gonorrhoea, are Chlamydia Trachomatis, Mycoplasma Hominis and in post-partum streptococci and staphylococci.

This work is to demonstrate the role of Chlamydia Trachomatis infection in this matter.

A retrospective study was performed on 450 cases (women with age between 20 and 35 years) with secondary sterility, from which 160 cases (35,5%) had positive serology for IgM and 40 cases (9%) had positive cultures for C. Trachomatis from uterine cervix. Laparoscopic examination with prelevation of biopsies in 290 cases revealed in the majority of cases (280) chronic salpingitis with important sclerosis with obstruction of the tubal lumen and adhesions between fallopian tube and ovary and pelvic peritoneum. In five cases the histopathologic examination revealed the pattern of “salpingitis isthmica nodosa”.

This high incidence of infection with important process of fibrosis demonstrates the main role of Chlamydia Trachomatis in the pathogenesis of sterility with tubal origin.

#### P 803

## ANDROGENS AND PROGESTERONE IN PREECLAMPSIA

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Hypertensive disorders of pregnancy are from the most common ones. As the pathogenesis of this disease is not clear yet and as there are so many reasons to prove the role of sex hormones in this disease, so this study tries to investigate the change of serum progesterone, total testosterone, free testosterone and dehydroepiandrosterone sulfate (DHEAS) levels in the patients with preeclampsia. Nineteen patients with preeclampsia who were referred to Kosar hospital of Urmia were compared gestational age and body mass index. All subjects were primigravid women. They were also compared considering those four mentioned hormones. T-test was used as statistical way of analysis.  $P < 0.05$  was considered as significant. Progesterone and free testosterone levels were significantly lower in patients with preeclampsia than in the control group ( $p < 0.05$ ). There were no significant differences in total testosterone and DHEAS. There were also no significant differences in maternal age, gestational age and body mass index. Sex hormone binding globulin can increase in preeclampsia, so that it can decrease the amount of free testosterone in serum. On the other hand progesterone can relax smooth muscle of vessels resulting decreasing of blood pressure. So it can be said that decreasing of progesterone in preeclampsia may indicate a role for it in the pathogenesis of preeclampsia

### P 804

#### THE ROLE OF P63 IN HYDROPIC ABORTION, PARTIAL HYDATIDIFORM MOLE, COMPLETE HYDATIDIFORM MOLE AND CHORIOCARCINOMA

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Gestational trophoblastic diseases (GTD) are a group of interrelated diseases of trophoblastic tissue that include partial hydatidiform mole, complete hydatidiform mole, invasive mole, choriocarcinoma, and placental site trophoblastic tumor. Choriocarcinomas are clearly malignant neoplastic lesions but hydatidiform moles are just abnormal placental tissues with potential to malignant change. Thus, the identification of biological markers that allow differentiate these pathologies in doubtful cases are important in clinical practice. P63 is a p53 homologue that is associated to several malignancies. In normal placentas p63 is expressed in cytotrophoblast cells. The role of p63 in gestational trophoblastic diseases, however, merits further investigation. This work was carried out to study the expression of p63 in hydropic abortion (HA) and gestational trophoblastic diseases, including partial hydatidiform mole (PM), complete hydatidiform mole (CM), and choriocarcinoma (CC). Immunohistochemistry with the antibody p63 (clone 4A4) was performed in 40 formalin-fixed paraffin-embedded samples of placenta tissue. The cases were selected to represent HA (n=12), PM (n=11), CM (n=12), and CC (n=5). The distribution of p63 was quantitatively assessed in cytotrophoblast cells and recorded as: + (0-25% of cells stained by p63), ++ (26-50%), +++ (51-75%), and ++++ (75-100%). The intensity of immunostaining was also evaluated as weak, moderate, or strong. Statistical analysis was determined by the Fisher test. Contrary to the other diagnoses, none of the choriocarcinomas analyzed exhibited positive cells for p63

(CC x CM,  $P=0.0036$ ; CC x PM,  $P=0.0002$ ; and CC x HA,  $P=0.0104$ ). There were no statistical relationship between CM x PM ( $P=0.5008$ ); CM x HA ( $P=0.2825$ ); and PM x HA ( $P=0.0444$ ). Concerning the intensity of immunostaining, there was statistical difference only between PM and HA ( $P=0.0045$ ). The lack of p63 expression in choriocarcinomas indicates that this protein may be helpful in distinguishing CC from other GTD in challenging cases. We also verified that the intensity of p63 expression was much stronger in PM than in HA. Since sometimes is difficult to differentiate an abortion from partial hydatidiform mole because villous blood vessels may be not easily identified within villous with exuberant edema, this find may have clinical importance.

### P 805

#### EXPRESSION OF EGFR IN MOLE HYDATIDIFORMS, EXAGGARATED PLACENTAL SITES AND NORMAL PLACENTAS AND KI 67 IN MOLE

#### HYDATIDIFORMS AND NORMAL PLACENTAS

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**PURPOSE:**The aim of this study is to find out differences between expression of EGFR in complete, incomplete mole hydatidiforms, exaggareted placental sites and Ki 67 labeling index (LI) in complete, incomplete mole hydatidiforms and normal placentas.

**METHODS:**Parafin embedded sections of 18 incomplete, 7 complete hydatidiform moles, 19 exaggareted placental sites and 10 placentas (control group) were studied immunohistochemically for EGFR and Ki 67. Statistical analysis of the results were made using student t or ANOVA with post huc Tukey test.

**RESULTS:**Ki 67 was detected immunohistochemically in villous cytotrophoblasts and extravillous intermediate trophoblast of complete, incomplete hydatidiform moles, and only in villous cytotrophoblasts of control group. Ki67 LI of villous cytotrophoblasts differed significantly between complete and incomplete hydatidiform moles ( $p < 0.01$ ). Ki67 LIs of hydatidiform moles were significantly higher than control group ( $p < 0.001$ ).

Immunostaining of Ki67 in extravillous intermediate trophoblasts were not different between complete and incomplete hydatidiform moles ( $p = 0.58$ ).

EGFR was detected in syncytiotrophoblast and cytotrophoblasts of complete and incomplete hydatidiform moles, and control group. Staining of villous cytotrophoblasts differed significantly between complete hydatidiform moles and control group ( $p < 0.001$ ) and incomplete hydatidiform moles and control group ( $p < 0.001$ ) but staining of villous cytotrophoblasts was not differed significantly between complete and incomplete hydatidiform moles ( $p=0.10$ ).

EGFR expression of syncytiotrophoblast differed significantly between complete, incomplete hydatidiform moles and control group ( $p < 0.001$ ). However the difference of syncytiotrophoblasts immunopositivity between complete and incomplete moles was not significant ( $p > 0.05$ ).

EGFR immunostaining was not detected either in extravillous intermediate trophoblasts of mole hydatidiformes or exaggareted placental site.

**CONCLUSION:**Expression of EGFR in syncytiotrophoblast and cytotrophoblasts is important in the pathogenesis of complete and incomplete hydatidiform moles. Negative staining of EGFR in exaggareted placental sites may show us that EGFR-related family of oncogens does not play a role in the pathogenesis of exaggareted placental site. Ki67 LI can be a useful marker in differentiating complete and incomplete moles.

**P 806****EFFECT OF OBSTRUCTIVE CHOLESTASIS ON GONADOTROPINS AND GERM CELLS APOPTOSIS IN THE ADULT MALE RATS**

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Obstructive cholestasis is associated with overproduction of endogenous opioids (EOP), nitric oxide (NO) and cytokines in the blood streams. These consequences can be affected sex hormones. Since proper fertility will be resulting of physiological balance of sex hormones so that we investigated the relationship between obstructive cholestasis and sex hormones and apoptotic germ cells in adult male rats.

To study this, we used three groups of animals: Control (No-surgery), Sham (surgical control), and cholestatic (surgical ligation of the bile duct). After 3 weeks all animal were killed by ether, and serum concentrations of inhibin B, FSH and LH were determined by ELISA and Radioimmunoassay respectively, testicular germinal cells apoptosis was evaluated by DNA fragmentation detected by in situ terminal deoxynucleotidyl Transfrase-mediated dUTP nick end labeling (TUNEL).

Results: The findings of this study were shown that LH and FSH levels were significantly decreased in cholestatic compared to control and sham groups ( $p < 0.05$ ). However, inhibin B level was significantly higher in cholestasis than control and sham ( $p < 0.05$ ). On the other hand, we could not be shown any significant difference in germinal cells apoptotic index between cholestatic and other groups ( $P > 0.19$ ).

These findings have been shown which as obstructive cholestasis was although decreased the levels of serum gonadotropins but it has no significant effector testicular germinal cells apoptosis. We were speculated that testicular germinal cell apoptosis was not only dependent on gonadotropin hormones but also other factors may involved.

**P 807****MULTICENTRIC FIBROADENOMA WITH FLAT EPITHELIAL ATYPIA**

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Introduction: Fibroadenoma of the breast represents a benign biphasic tumour, which occurs most frequently under 30 years old. The epithelial component may show a wide spectrum of typical hyperplasia but very rare, this hyperplasia presents atypia. We present the case of an 18 years old woman, with 5 fibroadenomas in the left breast, two of them presenting flat epithelial atypia.

Material and method: The five tumours (25, 35, 55, 40, 45 mm diameter) were evaluated on hematoxylin and eosin-stained sections. Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue, using Actin, p 53, ki 67, ER, PR.

Results: All the five tumours had classic fibroadenoma's pattern. Two of them presented the replacement of the normal epithelium by a single or stratified epithelium (up to 3-5 cell layers), columnar type. The epithelial cells showed mildly atypia. The acini and ducts were distended and contained an

eosinophilic material. They were surrounded by myoepithelial cells, which were Actin-positive. The epithelial cells were ki 67, p 53, ER and PR-positive.

Conclusions: The presence of multicentric fibroadenoma, two of them with flat epithelial atypia increases the risk of developing cancer in this patient. Clinical and mamographic follow-up of this patient is necessary in the future. Six months later the patient developed three more fibroadenoma in the same breast, one of them with flat epithelial atypia.

**P 808****LOCALISING NYBR-1 - A LASER-CONFOCAL AND IMMUNO-ELECTRON MICROSCOPIC STUDY**

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Background: NY-BR-1 is a breast differentiation antigen recently discovered by SEREX technology in a breast cancer patient. Here we report on the localisation of this antigen in breast cancer cell lines as determined by confocal laser microscopy and immuno-electron microscopy. Methods: NY-BR-1 transfected breast cancer cell lines were cultured on glass cover slips, fixed, permeabilised and labelled with an anti-NY-BR-1 antibody. Further examinations involved confocal laser microscopy or transmission electron microscopy. Results: NY-BR-1 is associated to cytoplasmic vesicles preferentially close to the nucleus and follows the architecture of microtubules. In metaphase nuclei, NY-BR-1 co-localises with the chromosomes. Conclusions: NY-BR-1 is associated to microtubules, to vesicles and to chromatin. This suggests a function of the protein in cytoplasmic trafficking and/or chromosomal regulation in mitotic nuclei.

**P 809**

Introduction: Breast cancer is a significant public health problem worldwide. Plenty of mammographic screening programs resulted in the detection of breast carcinomas at early stage. The presence of microcalcifications in mammogram not only correlates with breast cancer in many cases, but also sometimes is the only early sign of this disease. Nowadays, Computer Aided Diagnosis (CAD) systems may help radiologists detecting and classifying clustered microcalcifications, which is vital for proper treatment.

Objectives: The objective of this work was to evaluate the performance of the computerized classification method for automatically detected clusters of microcalcifications.

Materials and Methods: In our research we used 106 images from Digital Database for Screening Mammography (DDSM), among which 67 presented malignant and 39 benign cases. Our experiments were carried out in two steps: automatic detection and automatic classification. For the detection, previously developed morphological method has been used. Before the classification task was conducted, the whole set of mammograms was divided into two groups: training and testing sets, including the same number of images. Only images from training set were used to train the Support Vector Machine (SVM) classifier. The performance of the classifier has been tested on images from testing set.

Results: Automatic classification of clustered microcalcifications in mammograms is a final stage of CAD

system which helps the radiologist in diagnosis. According to ROC analysis the presented algorithms yielded Az=95,23%. Conclusions: The SVM classifier used in experiments gave very good results and will be used in further research on complex CAD system.

#### P 810

##### **PRODUCTION AND CHARACTERIZATION OF ANTIBODIES TO ESTROGEN RECEPTOR-BETA ISOFORMS EFFECTIVE FOR USE ON ROUTINELY-PROCESSED HUMAN TISSUES**

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**Introduction-aim:** Estrogen receptor  $\alpha$  (ER $\alpha$ ) expression is a well established prognostic and predictive marker for breast cancer. Recent data indicate that ER $\beta$ , a recently described estrogen receptor type mainly expressed through two isoforms, ER $\beta$ 1 and ER $\beta$ 2, may also play a role in human breast cancer progression. Clinical studies suggest that a correlation may exist between breast cancer responsiveness to tamoxifen and ER $\beta$ 1 or ER $\beta$ 2 expression. The lack of suitable antibodies has been a preventing factor for the elucidation of the role of ER $\beta$  isoforms in breast cancer prognosis and treatment. We aimed to produce monoclonal antibodies (mAb) to ER $\beta$ 1 and polyclonal antisera (pAb) to ER $\beta$ 2 effective for the immunostaining of routinely processed human tissues.

**Methods:** An appropriately designed synthetic peptide (ER $\beta$ 1 aminoacids 517-530) and an appropriate GST-fusion protein were used as immunogens for the generation of mouse anti-ER $\beta$ 1 mAbs and rabbit anti-ER $\beta$ 2 pAbs, respectively. Following well established techniques, a number of specific ?R $\beta$ 1 mAbs and ER $\beta$ 2 pAbs have been produced as determined by Western blotting.

**Results:** One anti-ER $\beta$ 1 mAb and one anti-ER $\beta$ 2 pAb proved to be effective for use on formalin-fixed, paraffin embedded human tissues. Both antibodies produced intense nuclear staining in the majority of epithelial cells in normal breast, while in archival breast carcinoma tissues the percentage of ER $\alpha$  expressing neoplastic cells as well as the intensity of immunostaining varied considerably between tumours. Expression of ER $\beta$  was also observed in lymphocytes, fibroblasts, endothelial cells as well as squamous cells of the nipple epidermis.

**Conclusions:** The newly produced anti ?R $\beta$ -antibodies may prove useful for studying the expression of ER $\beta$  isoforms at the protein level in normal and malignant breast tissues in an effort to assess their significance in mammary carcinogenesis and their value as prognostic and predictive markers in human breast cancer.

#### P 811

##### **FOUR SYNCHRONOUS FEMALE GENITAL MALIGNANCIES: THE OVARY, CERVIX, ENDOMETRIUM AND FALLOPIAN TUBE**

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The coexistence of multiple primary carcinomas in the female genital tract, and especially the common occurrence of synchronous ovarian and endometrial adenocarcinoma is well-known. While the etiology of this phenomenon remains unclear, it has been postulated that tissues of a common

embryologic origin when simultaneously exposed to certain carcinogens may develop synchronous neoplasms.

We present the unique case of a 63 year-old woman with coexistent adenocarcinoma of the ovary, endometrium, cervix and fallopian tube; all of which we believe to be separate primaries, supported by the fact that all four lesions are of low grade and stage and exist together with neighboring preinvasive lesions. To our knowledge, four synchronous primaries involving each aspect of the female genital tract has not previously been reported.

#### P 812

##### **THE STUDY OF THE ULTRASTRUCTURE AND HORMONAL VARIATION OF CORPUS LUTEUM IN FEMALE RATS FED ANETHUM GRAVEOLENS EXTRACT**

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Anethum graveolens L.(Dill) as a medicinal herb, used as a remedy for inducer of menstruation and increase mother's milk production. Using too much dill leads to sexual weaknesses and decrease spermatogenesis. This investigation was performed on 54 Wistar female rats with regular estrous cycle divided into 6 groups: control, sham, low dose of aqueous extract (0.045 gr/kg), high dose of aqueous extract (.045 gr/kg), low dose of ethanol extract (0.5 gr/kg) and high dose of ethanol extract (5 gr/kg). The above mentioned doses of extracts were orally administrated by feeding needle for 10 days. Blood samples were taken at the following steps: 1- Blood sampling from tail vessels before the beginning of experiment, 2- Blood sampling from dorsal aorta after the end of experiment. Estrogen and progesterone hormones were measured by Elisa and RIA methods, respectively. In order to ultrastructural study of the corpus luteum, ovaries were dissected. The data was analyzed by the ANOVA test to determine the significant difference at  $p < 0.05$ .

The results indicated no significant difference in serum estradiol concentration, but the serum concentration of progesterone hormone increased significantly in high dose of aqueous and ethanol extract groups. Ultrastructural studies showed the increase of mitochondria, SER, RER and polyribosome in granulosa lutein cells of the high dose of aqueous and ethanol extracts administrated rats.

It may be concluded that high dose of aqueous and ethanol extract of Anethum graveolens L. act as LH hormone, which induced modifications on structure and synthetic activity of the granulosa lutein cells and lead to prolongation of luteal phase and increase progesterone hormone concentrations.

#### P 813

##### **ENDOMETRIAL AVB3 INTEGRIN AND OSTEOPONTIN EXPRESSION IN FERTILE WOMEN AND INFERTILE PATIENTS**

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**Background:** Osteopontin (OPN) is a glycosylated protein originally identified as a constituent of bone matrix that mediates cell adhesion and cellular signaling by binding to integrin heterodimers. Osteopontin is the ligand of one of the putative embryo implantation factor, avb3 integrin, and recent evidence has suggested that osteopontin and its receptor avb3 integrin are coexpressed in the human endometrium around the time of the "implantation window". The aim of this study was to evaluate investigate the endometrial expression of OPN and avb3 integrin as well as its temporal relationship

throughout the luteal phase in both normal fertile women and infertile patients.

**Design:** We studied 10 fertile controls and 88 infertile patients. All women underwent two endometrial biopsies during a single menstrual cycle (a midluteal biopsy on postovulatory day +7 to +8 and another biopsy four days later). Biopsies were evaluated according to the criteria of Noyes et al. avb3 integrin was detected in frozen tissue and OPN in formalin-fixed, paraffin-embedded material using the EnVision system. Immunohistochemistry results were correlated with the histological dating, cause of infertility (endometriosis, unexplained infertility or male infertility) and fecundity.

**Results:** OPN and avb3 integrin endometrial expression followed a similar pattern, with glandular expression increasing from histological day +3, when 37.5% of endometria expressed OPN and 0% expressed avb3 integrin, to day 9, when 100% co-expressed both factors. Co-expression of OPN and avb3 integrin was less frequently observed in out-of-phase than in-phase endometria (7.4% vs. 56.5%,  $p < 0.05$ ). However, no differences in the co-expression of OPN and avb3 integrin were observed between fertile and infertile women or according to the different causes of infertility. No relationship was observed between endometrial expression of these factors and fecundity outcome for subsequently untreated infertile patients.

**Conclusions:** Integrin avb3 and osteopontin expression are markers of endometrial maturation but their significance in terms of endometrial receptivity for embryo implantation in the clinical setting remains to be shown.

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#### P 814

##### ANALYSIS OF GENE EXPRESSION IN LIPOSARCOMA OF MIXED TYPE

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**Abstract :** Liposarcomas of mixed type are rare. We experienced a case of liposarcoma composed of pleomorphic and well differentiated components. To identify molecular alterations in this tumor, gene expression in pleomorphic liposarcoma (PL), well differentiated liposarcoma (WDL), and normal adipose tissue (NA) was examined using cDNA microarray representing 17,000 genes. The cDNA microarray analysis showed that 6 genes in WDL, not in PL, were upregulated more than 5-fold compared to NA, and 40 genes in PL, not in WD, were upregulated. Other 6 genes in both WDL and PL, were downregulated more than 5-fold compared to NA. The differentially expressed genes included those associated with signal transduction, transcription, cell cycle, enzyme, structural protein, immune system and others. Our experimental data demonstrated that multiple genes are differentially expressed in liposarcoma of mixed type. It is suggested that these genes are involved with the differences in morphological characteristics and carcinogenesis of liposarcoma.

#### P 815

##### CLINICO-MORPHOLOGICAL AND CYTOGENETIC STUDY OF SOFT TISSUE LEIOMYOSARCOMAS

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**Introduction:** Leiomyosarcomas of soft part (SPLsa) are relatively rare neoplasms which estimate less than 10% of all malignant soft tissue tumours. What is more they were a heterogeneous group of neoplasms divided according to their localization. Thus the pathologists' experience concerning these lesions is limited.

**Purpose of the study:** The aims of study are: morphological and cytogenetic characteristics of neoplasm and the estimation of prognostic parameters for patients treated because of separate groups of SPLsa.

**Materials and methods:** Clinical records of 37 patients treated for SPLsa were reviewed. Formalin-fixed, paraffin embedded material from 44 tumours was available for routine examination in light microscopy. In 7 cases we had at our disposal the primary tumours as well as recurrent or metastatic ones. Two pathologists independently analyzed the slides stained with hematoxylin-eosin, Masson's trichrome and/or stained immunohistochemically. Immunohistochemical reactions were carried out using the avidin-biotin complex immunoperoxidase technique (LSAB2+ kit) or ultraviolet labeled polymer. Small fragments of fresh tumour tissue from 24 cases were taken for ultrastructural and flow cytometric studies. Additionally, in 12 cases, short-term in vitro cultures from tumour material were used to obtain metaphases for cytogenetic analysis.

**Results:** Patients included 21 female and 16 men. The tumours localized in soft tissues of extremities (31 cases), retroperitoneal space (4 cases), head and neck region, abdominal cavity and thoracic or abdominal wall (2 cases each) The metastatic tumours were situated in lungs and subcutaneous tissue. Histologically, all tumours consisted of long fascicles which were built of the spindle cells with cigar-shaped nuclei and deeply staining eosinophilic cytoplasm. Masson's trichrome dyed the cell cytoplasm red. In 18 cases the additional MFH-like component of tumour was observed (so-called pleomorphic leiomyosarcoma) The tumour cell were stained positively for smooth actin (29 cases), and H-caldesmon (35 cases). Additionally the positive reaction to S100-protein, CD57 and CD68 were noticed in 6, 1, and 7 cases, respectively. Mitotic activity varies from 4 to 150 figures per 10HPF. 24 patients live from 15 to 155 months after tumour resection. For 8 patients who died the mean survival reached 30 months.

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#### P 816

##### SMALL ROUND CELL MYOEPITHELIOMA OF SOFT TISSUE: CLINICOPATHOLOGIC STUDY OF 3 CASES EXPANDING THE MORPHOLOGICAL SPECTRUM OF MALIGNANT MYOEPITHELIOMA.

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**Background:**

Myoepitheliomas and mixed tumors (« pleomorphic adenomas ») are well-characterized in salivary glands but have only recently been recognized as primary soft tissue neoplasms. The plasticity of myoepithelial cells is responsible for a broad morphological spectrum. Tumors are usually composed of epithelioid, spindle or plasmacytoid cells,

arranged in nests or intersecting cords within an abundant chondromyxoid or hyalinized matrix.

Design:

We recently encountered a few cases of malignant myoepithelial tumors of soft tissue with an unusual small round cell component, mimicking other small round cell malignant neoplasms. We present the clinicopathologic features of 3 cases of this rare morphological variant.

Results:

There were 2 male and 1 female patients aged 4-21 years at the time of resection. Tumors were located in the mediastinum, elbow and leg (within a nerve). Histologically, 2 tumors had infiltrative margins; the intraneural tumor was well circumscribed. All tumors were composed predominantly (60-95% of tumor tissue) of sheets of dyscohesive small round cells, with scant eosinophilic or clear cytoplasm and round hyperchromatic nuclei. All cases showed a high mitotic index (>10 mitoses / 10 high power fields). In all tumors, foci composed of epithelioid cells, arranged in a reticular growth pattern within a chondromyxoid or hyalinized matrix, were identified in the surgical resection specimen.

In all cases, tumor cells expressed at least focally cytokeratins (AE1-AE3), epithelial membran antigen, S-100 protein, smooth muscle actin, calponin, and GFAP.

Two tumors recurred locally and one metastasized in an inguinal lymph node, after 2 years.

Summary & Conclusion:

We present 3 cases of malignant myoepithelioma of soft tissue showing a previously unreported small round cell appearance. All tumors affected young patients and the 2 cases with available follow-up behaved aggressively. The diagnosis was based on the identification of areas showing classical morphological features of myoepithelial differentiation, and on the co-expression of S-100, GFAP, myogenic and epithelial markers.

Our study expands the morphological spectrum of myoepithelial tumors. The differential diagnosis of small round cell myoepithelioma includes metastatic carcinoma, poorly differentiated synovial sarcoma, PNET/Ewing Sarcoma, mesenchymal chondrosarcoma and desmoplastic small round cell tumor.

#### P 817

##### **MALIGNANT PERIPHERAL NEUROEPITHELIOMA 'PERIPHERAL NEUROBLASTOMA' A CASE REPORT**

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Peripheral neuroepithelioma is a rare malignant tumour of primitive peripheral non autonomic nervous system. The tumour can occur at any age involving soft tissue mainly of lower extremities. Other sites of occurrence are the shoulder-thoracic region and the head and neck. It is a very aggressive tumour with early metastasis mainly to the lung.

We report a case of 16 years old female who was presented with rapidly enlarging soft mass of labia majora. Physical examination revealed a 15x10 cm oval lobulated firm mass covered by normal skin on the right side of labia majora. There was no lymphadenopathy. The remainder of the physical examination of the patient in general, was within normal, so were routine laboratory tests.

The mass was excised followed by administration of chemotherapy and radiotherapy. The tumour was very cellular with wide areas of necrosis and viability around vessels. The cells are small and rounded arranged all over in Homer-Wright rosettes. Vascular invasion was noticed at the periphery of the tumour with retained rosettes formation of the intravascular clumps. However, 3 months after excision, the

patient developed secondaries in the lung, with pleural effusion, but no local recurrence.

This is a rare case of Peripheral neuroepithelioma. This tumour is classified under peripheral neuroectodermal tumours (PNET). Histologically, it is reminiscent to neuroblastoma, but the two tumours vary in clinical aspect, behaviour, prognosis, and response to treatment.

#### P 818

##### **PLEXIFORM CELLULAR SCHWANNOMA: THE HISTOLOGICAL, IMMUNOHISTOCHEMICAL, ULTRASTRUCTURAL AND CYTOGENETIC STUDIES OF A RARE CASE WITH A LONG FOLLOW-UP.**

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Introduction: Cellular variant of schwannoma is defined as the hypercellular tumor composed exclusively or predominantly of Antoni A tissue, and devoid of formed Verocay bodies. The cellular schwannomas growing in a plexiform or multinodular pattern are extremely rare and their diagnosis controversial. We found only 15 described cases of plexiform cellular schwannoma (PCS). The longest clinical observation in all those cases was about 13 years.

Aims: The aims of the study are: presentation of the case of a very rare neoplasm with very long follow-up and the analysis of criteria of malignancy in peripheral nerve tumours.

Case report: A 1-year-old girl underwent surgery in a case of a deeply localized lateral neck tumour. After 4, 18 and 20 years she developed 3 recurrences treated by incomplete excisions. Since the last one, she has been living for 3 years with loco regional, slowly growing tumour. No malignant transformation nor metastases was observed.

Materials and methods: Formalin-fixed, paraffin embedded material from primary and all recurrent tumours were available for routine examinations in light microscopy. Immunohistochemical (IHC), ultrastructural, flow cytometric and cytogenetic studies were made on the tissue samples from the third recurrent tumour.

Results: Gross examination showed tumor of 11cm in diameter, composed of multiple, variable in size nodules. The histological examination showed plexiform tumor infiltrating thyroid gland and soft tissue. At higher magnification the tumor was composed of uniform spindle cells, arranged in fascicles of high cellular density. Their nuclei were of low polymorphism. Mitotic activity was 2/10 HPF, neither atypical mitotic figures nor necrosis were found. IHC stainings for S100 and CD57 were strongly positive, and reaction to p53 was negative. MIB index reached 2.0. Evaluation in electron microscopy showed neoplasmic well differentiated Schwann cells. The tumor cells were diploid and S phase fraction reached 2.2. The karyotype of tumour cells was normal.

Discussion: PCS is a entity that resemble MPNST. In our opinion multinodularity, infiltrative tumor borders and local tissue destruction are designative for local recurrence but not for capacity to metastases. Low or moderate polymorphism of cells, low mitotic activity and MIB-1-index, low S phase fraction, and negative reaction to p53 protein, as well as normal karyotype can prove against malignancy and metastatic potential of the tumor.

#### P 819

##### **ECTOPIC MYXOPAPILLARY EPENDYMOMA: AN UNCOMMON CASE ARISING IN THE PELVIS**

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Myxopapillary ependymoma is a well recognized variant of ependymoma, which usually arises from the filum terminale. However, ectopic presentation in the penis and around male and female genital tracts is extremely rare. We recently encountered a case arising in the pelvis of an elder Japanese female without any relationship to the spine, which was diagnosed as a myxopapillary ependymoma on basis of the histological appearance. The patient was a 69-year-old female, who was referred complaining of irregular genital bleeding in September 2003. Computed tomography and MR imaging revealed a mass lesion, which was measured approximately 8cm in the largest dimension and was located between the rectum and posterior wall of the vagina, but no relationship to the spine was confirmed. Two months later from initial presentation, transvaginal biopsy was performed along with resection of the uterus and left appendages, but failed to reach any specific diagnosis. In the middle of November 2003, the tumor was surgically removed. Microscopic examination revealed a lobulated mass lesion with dense fibrous septa, in which ovoid or polyhedral neoplastic cells were arranged in trabecular and reticular patterns against the background of loose myxoid stroma. In some areas, plump spindle cells showed solid growth with poorly organized fascicles. Intra- and extracellular hyaline globules were identified. Immunohistochemically, neoplastic cells showed diffuse positive reaction for vimentin and S100 protein, and focally positive for GFAP. Actins, desmin, CD34, cytokeratins, EMA, and Glut1 were completely negative. Follow-up observation detected a recurrent lesion three months later after surgical removal. But the lesion did not show aggressive growth and the patient is alive and well for a year. Myxopapillary ependymoma is not a highly lethal tumor and is designated as WHO grade I. Post-operative course in the present case would support the diagnosis. We present its histological features in detail and the literature is briefly reviewed.

#### P 820

**BENIGN TRITON TUMOR: ABOUT ONE CASE**  
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Benign Triton tumor of the hand: about one case

Introduction: The neuromuscular hamartoma or benign triton tumor is a developmental lesion composed of mature elements of both striated muscle and nerve.

Observation: A 22 years old woman presented a nodular mass of 1.5 cm in his finger; appeared since the birth. The excision was complete. Microscopic examination showed multiples nodules of fascicles striated muscles and nerves.

Discussion:

The benign triton tumor is rare. Large nerves are the most involved. Hand's location is uncommon. The histological diagnosis has sometimes proved to be difficult. The differential diagnosis can be made with fibromatosis and rhabdomyoma.

The pathogenesis still unclear and could be the result of incorporation of mesenchymal tissue into nerve sheaths during embryogenesis.

#### P 821

**UNUSUAL PRESENTATION OF EWING SARCOMA IN A PATIENT WITH PLEXIFORM NEUROFIBROMA**  
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Plexiform neurofibromas (PNF) are benign tumours of the peripheral nerves which mostly develop in patients with neurofibromatosis type 1 (NF1). Approximately in 10 % of NF1 patients, a malignant peripheral nerve sheath tumour (MPNST) emerges from a preexisting PNF. Ewing sarcoma, which is the second most common soft tissue malignancy in children has not been described in NF1 patients.

A 4-year-old female child presented with right thigh pain, swelling and with café au lait spots. MRI showed a large multilobulated mass. The plexiform tumour measured 50 cm. Immunohistochemical and architectural findings supported the diagnosis of PNF. Five years later, a mass measuring 16 cm in diameter occurred in the soft tissue of left shoulder. Tru-cut biopsy revealed a high-grade, primitive small round cell malignancy. The neoplastic cells were diffusely immunoreactive for MIC-2. The cells were nonreactive for S100 protein, desmin, leukocyte common antigen, and keratin. The tumour was diagnosed as Ewing sarcoma. Initial treatment consisted of 3 cycles of chemotherapy (consisting of ifosfamide-etoposide alternating with vincristine, adriamycin, actinomycin-D and cyclophosphamide) (IE/VAC) every three weeks. Thorax computerised tomography revealed small lesions in both lungs, which were disappeared after 3 cycles of chemotherapy. After 3 cycles of chemotherapy the mass, which regressed radiologically more than 50%, was resected. The histopathological examination revealed 80 % of necrosis. Although the patient is now free of disease, she is going to have chemotherapy until 18 cycles will have finished.

Ewing sarcoma has not been described in NF1 patients. The immunohistochemical findings and response to chemotherapy justified our diagnosis. The t(11;22) (q24;q12) is the most detectable genetic changes in Ewing sarcoma, whereas the inactivation of the CDKN2A (9p21) is the second most common genetic alteration. It is noteworthy that the inactivation of CDKN2A is also responsible for the malignant transformation of PNF to MPNST. But there was no preexisting PNF in the left shoulder of our patient. Therefore, we can suggest that there may be a common genetic alteration on the gene regulating CDKN2A, which may be responsible for developing both tumour in our case.

#### P 822

**HIGH NUCLEAR SURVIVIN EXPRESSION IS ASSOCIATED WITH METASTATIC DISEASE IN EWING SARCOMA**

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Introduction: Ewing's sarcoma (ES) is a small round blue cell tumour with a high incidence of metastasis and poor survival. Metastatic disease at diagnostic is the key adverse prognostic factor with a significant influence on relapse-free survival.

Recently, the overexpression of surviving, apoptotic inhibitor, has been shown to be correlated with unfavorable clinical outcome in several types of tumours.

**Purpose:** to assess the prognostic value of the immunohistochemical expression of survivin in ES of bone. This is the first study to evaluate the expression of survivin on ES.

**Methods:** Biopsy tissues of 24 consecutive patients with ES were assessed retrospectively for the immunohistochemical expression of survivin (monoclonal D-8, SantaCruz Biotechnology) by the streptavidin-biotin peroxidase procedure. For survivin, 1000 cells were counted and the percentage of positively stained nuclei was computed. A statistical analysis using the non-parametric Mann-Whitney test was performed. Clinical data and follow-up were obtained by the patient charts.

**Results:** the series comprised 17 males for 7 females with a mean age of 17, 4 yrs (range 1-45 yrs). Metastases were present in 11 patients at diagnosis. Treatment consisted of chemotherapy, surgery and/or radiotherapy in all the patients. 8 patients developed metastasis and/or local recurrence after treatment. 5 patients died of the disease, 4 patients were alive with disease and 15 were alive without disease at last follow-up (mean follow-up : 26,8 months). Survivin immunostaining was only nuclear. The mean expression was statistically higher in ES with metastases (range : 0-26% ; mean 14,8 %) than in ES without metastasis at diagnosis (range : 0-23,7% ; mean 6,67 %) (p=0,023).

**Comments:** This preliminary retrospective study shows that a high level of nuclear survivin expression implied a more aggressive biological behaviour of ES of bone (p<0,05). So, survivin seems to be involved in the critical steps of cancer onset and may be associated with unfavorable clinical outcome.

#### **P 823**

##### **ANALYSIS OF SEQUENTIAL SECONDARY CYTOGENETIC CHANGES IN EWING'S SARCOMA: A XENOTRANSPLANTATION MODEL**

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**Background:** Ewing's sarcoma (ES) is characterized by the specific cytogenetic event t(11;22)(q21;q24) and its variants, although minor cytogenetic aberrations have also been described, the most frequent being trisomy 8, trisomy 12 and the derivative chromosome der(1;16). Their prognostic relevance in ES is still unclear.

**Purpose:** Our aim was to evaluate the occurrence, frequency and changes in the pattern of secondary numerical aberrations in a dynamic model of ES.

**Methods:** seven tumors were transplanted into athymic nude mice and sequentially maintained through serial passages, during a period of time ranging from 18 months to 6 years. Fresh samples from original tumors and their consecutive passages were cultured and karyotyped, and analyzed by means of comparative genomic hybridization (CGH), FISH, and image analysis (cytometry and morphometry). For CGH, tumor DNA and normal DNA from healthy donors were amplified, differentially labelled and cohybridized. FISH analysis with centromere-specific probes was carried out for chromosomes 8 and 12. Image analysis was performed with Isis 2.50 software (Metasystems, Germany).

**Results:** a good correlation between karyotypic changes and CGH and FISH results was found in all cases. All cases harbored the specific change t(11;22). The total mean number of numerical aberrations detected by CGH in the original tumors and their subsequent passages was low (1.9). Gain of chromosome 8, whether complete or restricted to the long

arm, was the most frequent numerical aberration found by CGH (4 out of 7 tumors). Its appearance ranged from 0 to 26 months. In these cases, trisomy or tetrasomy of chromosome 8 was detected by FISH analysis. In two cell lines, gain of chromosome 8 was associated with gain of chromosome 12. Gain of 1q, loss of 16q (2/7 each) and other minor events were also detected by CGH.

**Conclusion:** gain of chromosome 8, whether associated or not with gain of chromosome 12, is the most frequent secondary change in ES. The cytogenetic heterogeneity in the pattern of occurrence of this numerical aberration could be related to the different proliferative advantage observed in these tumors.

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#### **P 824**

##### **CD117 EXPRESSION IN MALIGNANT TUMORS, OTHER THAN GIST**

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CD117, the c-kit proto-oncogene product is a transmembrane receptor for stem cell factor, normally expressed by interstitial cells of Cajal of the gastrointestinal tract and by many others cells like: mast cells, melanocytes, germ cells, subsets of hematopoietic cells; it is expressed also by their malignant tumors. The positivity for CD117 in a tumor does not necessarily indicate a mutation of the gene. The problem to be solved is if a targeted therapy against CD117, may be useful only in GIST or equally in other CD117 positive tumors; for this reason it is necessary to assess a large number of such tumors.

We performed a triserial indirect immunohistochemical method on formalin fixed, paraffin embedded sections for detecting CD 117 (Policlonal, DAKO, Denmark), CD34 (Monoclonal, BIOGENEX, USA) and Nestin (Monoclonal, Santa Cruz, USA).

We found a IHC positivity for CD117 in cases of : malignant melanomas (MM) ( 10), seminomas (6), disgerminomas ( 3 ), granulosa cell tumors (GCT) (2), phyllodes tumors of breast (BPT) (5), solitary malignant fibrous tumor (SMFT)(1), gastric glomeric tumor (GGT) (1). The MM and the BPT presented also a positivity for Nestin; the SMFT and GGT were positive also for CD34.

We concluded that at least a part of various tumors expressing CD117 may be considered as extra GISTs. It remain to establish if all the CD 117 positive tumors could benefit a specific therapy.

#### **P 825**

##### **RECURRENT AMPLIFICATION WITHIN CHROMOSOME 6P DETECTED BY FISH IN OSTEOSARCOMA**

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Osteosarcoma (OS) is characterized by complex karyotype, high frequency of cytogenetic aberrations and gene amplification. Chromosomal instability (CIN) provides genetic diversity for OS tumour progression, but both the mechanisms that lead to CIN and the biological basis of the conferred selective advantage of genomic aberrations in this tumour are poorly understood. CGH studies have identified frequent amplification and rearrangement involving the central genomic region of 6p. A number of genes associated with oncogenesis such as RUNX2, TNF $\alpha$  and VEGF cluster to this region and their increased expression by amplification would be predicted to be associated with tumor progression. Moreover, the chromosomal domain hypothesis suggests that genes with a functional relationship may be closely linked within subchromosomal regions of the nucleus. Thus the location of a gene within a chromosomal territory is thought to influence access to the proliferative machinery responsible for specific nuclear functions, such as replication, transcription, splicing and repair. Segmental duplications within this region may facilitate amplification by homologous recombination. The aim of this study was to evaluate, by FISH technique, the frequency and distribution of 6p amplification in osteosarcoma. Fluorescence in situ hybridization (FISH) was performed on a 77 core tissue microarray of primary osteosarcoma using chromosome enumeration probe (CEP) for the centromeric region of chromosome 6 (SpectrumOrange, Vysis Inc., Downers Grove, IL). Hybridization signals were scored among 100 non-overlapping tumor cell nuclei using the following scoring criteria: disomy  $\leq 2$  chromosome copies in  $> 90\%$  of the cells, low trisomy 3 copies in  $> 10\%$  but  $< 40\%$  of the cells, high trisomy 3 copies in  $> 40\%$  of the cells, low polysomy  $> 4$  copies in  $> 10\%$  but  $< 40\%$  of the cells, high polysomy  $> 4$  copies in  $> 40\%$  of the cells. Numerical aberrations of chromosome 6 were observed in 7 out of 8 tumors. Low polysomy was detected in 4 cases, low trisomy in 3 and disomy in 1 case. An increase in the number of chromosome 6 may frequently occur in osteosarcoma. Further studies at molecular and clinical level must be carried out to identify the gene alterations reflected by polysomy 6 or amplification within 6p. Such studies may facilitate the detection of specific tumours subtypes and genes directly associated with OS-tumour progression and clinical response.

**P 826**  
**THE RESULTS OF PREOPERATIVE CHEMOTHERAPY AND/OR RADIOTHERAPY IN NONMETASTATIC HIGH-GRADE OSTEOSARCOMA OF THE EXTREMITIES**

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Purpose: To assess the role of neoadjuvant chemotherapy and adding of radiotherapy to the chemotherapy in patients with nonmetastatic high-grade osteosarcoma of the extremities and to compare the response of therapies on local control, tumor necrosis rate and overall survival rate.

Methods and materials: Between 1990 and 2002, 61 extremity-localized nonmetastatic high grade osteosarcoma patients were treated with radiotherapy in our hospital. Thirty-eight patients were male and 23 patients were female. Median age was 17 years (11-66 years). All patients were treated with neoadjuvant chemotherapy. Chemotherapy schedule that was consisted of epirubicin, cisplatin and ifosfamide  $\pm$  high dose methotrexate was given before surgery. In order to increase the chance of limb sparing surgery, preoperative radiotherapy was added to patients who refused amputation or whose limb-sparing surgery would have been difficult because of borderline respectability. Radiotherapy was applied usually between the second and the third cycle of chemotherapy. Radiotherapy was given 35 Gy in 10 fractions to 44 patients. Remaining 2 patients were treated with 46 Gy with 2 Gy/day. Chemotherapy was given 3-6 cycles after surgery. In the radiotherapy group tumor size was between 3-32 cm (median 10 cm). In the chemotherapy group tumor size was between 4-20 cm (median 10 cm). Results: Forty-four patients out of 46 patients who were treated with radiotherapy, had limb-sparing surgery. Thirteen patients out of 15 patients who were treated with only chemotherapy had limb-sparing surgery and 26 patients (56.5%) in radiotherapy group, 7 patients (46.7%) in the chemotherapy group had tumor necrosis rate  $\geq 90\%$  ( $p=0.05$ ). In the radiotherapy group the 5-year local control, disease-free and actuarial survival rates were 98, 38 and 52, respectively. In chemotherapy group the 5-year local control, disease-free and actuarial survival rates were 92, 41 and 31, respectively.

Conclusion: Preoperative radiotherapy helps to increase the tumor necrosis rate and the chance of extremity sparing surgery when combined with chemotherapy. Though local control and overall survival rates were higher in patients with treated radiotherapy and chemotherapy, this difference was not statistically significant. It is difficult to make definite conclusions, because this was a nonrandomized and retrospectively analyzed study and the quality and the quantity of the patients were not the same in two groups.

**P 827**  
**EXPRESSION OF E-CADHERIN, P-CADHERIN, BETA-CATENIN, APC AND C-ERBB-2 IN CONVENTIONAL OSTEOSARCOMAS OF EXTREMITIES**  
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Background: Several series of osteosarcomas (OS) have been reported in the literature, but debate still exists regarding the clinical prognostic factors for this disease. The WNT proteins form a large family of secreted signaling proteins implicated in the proliferation and morphology of several tumors. The prognostic importance of WNT pathway and c-erbB-2 expression in OS has not been totally clarified.

Methods: The present study included 97 extremities conventional OS cases (70 osteoblastic, 20 chondroblastic and 7 fibroblastic) retrieved from our files. Cases were stained immunohistochemically using antibodies against E-cadherin, P-cadherin, beta-catenin, APC and c-erbB-2. Expression of the markers was correlated with clinical data and local recurrence, distant metastasis, free-disease survival, and overall survival.

Results: Expression of E-cadherin was not observed in all cases of OS. However, expression of P-cadherin, could be observed in 94 cases (96.9%). All the three patients with loss of P-cadherin expression had overall survival less than 8 months. Positive APC staining was observed in 65 cases (67%). APC was more expressed in patients under 18yo ( $p=0.025$ ). In patients treated with chemotherapy, positive immunoeexpression of APC correlated with better survival. Patients APC positive had 69% of five year survival against

APC negative patients with 34% of five year survival. Only 3 cases showed immunoeexpression of beta-catenin. Expression of c-erbB-2 was observed in 16 cases and did not show any correlation with clinical parameters and tumor outcome.

Conclusion: P-cadherin is the adhesion molecule expressed in extremities OS and loss of its expression is an indicative of worse prognosis. However it is a very infrequent event and further studies should be performed to validate this finding. Downregulation of APC was an important prognostic factor in patients treated with chemotherapy. Expression of beta-catenin and c-erbB-2 are infrequent events in OS, and did not correlate with clinical behaviour.

#### P 828

##### EVIDENCE OF GENETIC ALTERATIONS IN CHROMOSOME 11, 9, AND 2 IN EMBRYONAL AND ALVEOLAR RHABDOMYOSARCOMAS

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Introduction: Rhabdomyosarcomas (RMS) are most common soft-tissue sarcomas of childhood. They are subclassified into two major subtypes - embryonal RMS (E-RMS) and alveolar RMS (A-RMS). Various chromosomal alterations have been described in both subtypes. Inactivation of tumor suppressor genes frequently occurs due to loss of heterozygosity (LOH). Mutations of one allele may be followed by a loss of the remaining wild-type allele.

Purpose: To study LOH in RMS in microsatellite loci at mismatch repair genes, at loci close to the genes coding p15 and p16, and at loci at 11p with three probable suppressor gene regions which may be involved in pathogenesis of RMS. Methods: We used microsatellite polymorphic markers from the region 9p (tumor suppressor genes p15, p16), and from mismatch repair genes hPMS1, hMSH2 and hMLH1. The main attention was paid to telomeric region on 11p. We analyzed 11 patients with E-RMS and with 17 A-RMS. In all patients the presence of LOH was studied using frozen samples from tumor tissue taken at diagnosis. Each tumor sample was compared with constitutional DNA of the same patient obtained from peripheral blood or bone marrow aspirate.

Results: In the study 6/9 (67 %) informative E-RMS patients and 8/15 (53 %) informative A-RMS patients demonstrated LOH on at least one of the microsatellite loci in 11p region. The frequency of LOH for hMSH2 was 4/6 (67%) in E-RMS, and 2/11 (18 %) of informative A-RMS. For hPMS1 the result was 1/5 (20%) and 2/6 (33 %) of informative E-RMS and A-RMS, respectively. LOH in the region 9p was not detected in any of nine informative E-RMS and it was found in 5/14 (36%) A-RMS.

Conclusion: Our study demonstrates a frequent loss of heterozygosity on the short arm of chromosome 11 in both E-RMS and A-RMS and a relatively high frequency of LOH in 9p in A-RMS. High frequency of LOH in the locus D2S123 (hMSH2) in E-RMS may be caused by a relatively high portion of non-informative (homozygous) cases. At this stage of understanding it is impossible to speculate whether the described LOH are involved at early stages of the tumor development or whether they are secondary events of the tumor progression.

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#### P 829

##### RHABDOMYOSARCOMA OF THE SPINAL CORD: A CASE WITH IMMUNOHISTOCHEMICAL, ULTRASTRUCTURAL AND CYTOGENETIC STUDY

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#### INTRODUCTION

Although primary Central Nervous System (CNS) rhabdomyosarcoma has been described, it is very rare and is thought most often to arise from the meninges. We report a case of a spinal cord tumour showing rhabdomyoblastic differentiation on light microscopy, immunohistochemistry and electron microscopy. Cytogenetic analysis showed an unusual karyotype, which has not previously been reported in rhabdomyosarcoma.

#### METHODS

Case report

#### RESULTS

A 14 year old boy was found to have a contrast-enhancing spinal cord tumour at the level of the conus. At surgery the tumour appeared invasive and was not attached to the dura. Microscopy revealed a malignant tumour composed of rounded and spindled cells, with widespread rhabdomyosarcomatous differentiation, including strap cells. Although entrapped nerve fibres were identified within the infiltrative tumour, no evidence of a coexistent glial or other CNS neoplasm was found. Immunophenotyping confirmed positivity for vimentin, desmin and myoglobin.

Ultrastructural examination confirmed the presence of thick and thin cytoplasmic filaments, with clear evidence of sarcomere formation in places. Z bands were readily seen. Admixed myelinated neural cells were also seen, but there was no evidence of ependymal differentiation.

Cytogenetic analysis revealed an abnormal karyotype in 19 of 20 metaphases, containing a derivative chromosome 5 resulting from a t(5;18) translocation. The karyotype was: 45,XY,der(5)t(5;18)(p?15.3,p?11.2),-18 [19]/46,XY [1].

#### CONCLUSION

As only a small amount of tissue was available for study, we cannot entirely discount the possibility that this tumour represents myosarcomatous differentiation within a composite mixed or multiphasic malignant tumour. However, in the absence of any other evidence to the contrary, we present this as a case of apparent primary CNS rhabdomyosarcoma, with a novel cytogenetic aberration.

#### P 830

##### RHABDOMYOSARCOMA: CLINICOPATHOLOGIC REVIEW OF 36 CASES

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INTRODUCTION:Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescent periods and can seldomly be seen adults too. Despite all research, a single prognostic classification for modelling the treatment is still needed. The clinicopathological features of our recorded rhabdomyosarcoma cases are reviewed and the major prognostic factors are discussed.

MATERIALS AND METHOD: Thirtysix RMS cases diagnosed in our department between 2000 and 2005 were reevaluated with respect to age, sex, localization, histological type, immunohistochemical stains, therapy, recurrences, metastasis, survival time and correlations between these entities were studied.

RESULTS and CONCLUSION:Eleven Percent of our total malignant tumor material in this period was diagnosed as

sarcoma, among which 1,8% was RMS. The male / female ratio was almost equal and no significant difference was observed in survival between sexes ( $p>0,41$ ). Cases below 10 years of age were 57%, between 10-20 were 23% and over 20 were 20%. Embryonal RMS was the frequent histological type and had the best prognosis ( $p<0,002$ ). Thirtyseven percent were located on head and neck region, 25% were intraabdominal. Desmin, HHF35, and Myogenin positivity of different intensities were observed in all cases. Fortyeight percent of the patients were at the stage of III or IV (IRS clinical classification) at the time of diagnosis. All of the cases were treated by chemotherapy (VAC) and radiotherapy whenever was needed. Some of the cases were inoperable, resection was done pre- or posttherapeutically in most of the cases. According to the clinical follow-ups; 31% of the cases showed relapse or metastasis. The survival time was longer in early ages ( $p<0,02$ ). Most of the childhood cases were at the head and neck localization. If it was intracranial and parameningeal the survival time gets shorter and if there was spreading to cerebrospinal fluid this time was too short. Histologic type may have an influence on prognosis and in the presence of alveolar component aggressive course was expected, but it may be difficult to subclassify the tumor in a small biopsy specimen due to common use of FNAB or trucut biopsies because of the sample size and mixed nature of the tumor.

#### P 831

#### SPINDLE CELL RHABDOMYOSARCOMA IN ADULTS

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Spindle cell rhabdomyosarcoma (SCRMS), a rare variant of embryonal rhabdomyosarcoma, occurs mainly in the group of pediatric patients and is characterized by a predilection to male sex and paratesticular and head and neck location as well as very favourable prognosis which is superior to that of classical embryonal rhabdomyosarcoma. SCRMS is rare in adults and, to the best of our knowledge, only seven cases have been reported to date in patients older than 18 years.

To further elucidate the features of SCRMS occurring in adults we report additional three cases of this rare entity.

The tumours were identified in a 24-year-old woman, a 35-year-old man and a 39-year-old woman, and arose in the vulva, muscles of the calf and subcutaneous fatty tissue of the leg, respectively. They were all treated by surgery and chemotherapy. The first patient developed uncontrollable local recurrence in the pelvic soft tissues and died due to metastatic disease 22 months after the first surgery; the second patient developed inguinal lymph node metastases 24 months after surgery and was then lost to follow up; the third patient is alive with no evidence of disease 12 months after surgery.

Microscopically, the tumours were composed mainly of fascicles of relatively monomorphic spindle cells with eosinophilic cytoplasm and rare rounded or strap-shaped rhabdomyoblasts. Cross-striations could be identified in one case only. Immunohistochemically, all three tumors were positive for muscle specific actin (HHF35), desmin, myoglobin and MyoD1; two tumors were focally positive for alpha smooth-muscle specific actin, and none was reactive for S-100 protein.

The analysis of the data of the ten reported cases, including the three presented here, indicates that, as opposed to the pediatric group, SCRMS in adults shows no predilection to particular anatomic site or male sex, and is not associated with favourable outcome. In fact, its prognosis in adults seems to be equally poor as that of other histologic types of rhabdomyosarcoma.

#### P 832

#### HER-2 ONCOGENE AMPLIFICATION IN SYNOVIAL SARCOMA

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#### Background

The treatment regimens for synovial sarcoma (SS) is very limited, though this type of sarcoma seems to be more heterogeneous as it has been traditionally considered. The present study investigates the Her-2 oncogene status of 20 cases of SSs, whether the Her-2 amplification can be considered as an independent prognostic factor.

#### Materials and methods

Her-2 oncogene amplification was determined on smears (in each case frozen material was used from our tumor bank) using Fluorescence in Situ Hybridization technique (dual color FISH with centromeric probe of chromosome 17 and unique specific probe for Her-2 oncogene). Beside this, the DNA ploidy status was measured using image analysis. We had 5 biphasic and 15 monophasic SSs, patient age ranged from 13 to 68 years (mean, 39.8). Tumor size was larger than 5 cm in each case. Follow up ranged from 6 to 78 months (mean, 38.5 months). For statistical analysis the chi-squared-test was used.

#### Results

Her-2 oncogene amplification was found in three cases (15.0%) of 20 SSs, but so called massive amplification which is characteristic of some subset of breast carcinoma, was not observed. Her-2 oncogene amplification was significantly associated with a lower risk of developing metastasis ( $P<0.05$ ) (none of 3 amplified cases had metastases), while no association was found concerning recurrences ( $P<0.5$ ). 6 cases proved to be aneuploid and 14 were diploid but no association was found between Her-2 amplification status and ploidy and between ploidy status and metastases or recurrences.

#### Conclusions

Her-2 oncogene amplification is a rare event among SSs, but this small subset of SS has a better overall prognosis. Beside this, there may be a theoretically new treatment possibility with Trastusumab for Her-2 amplified cases of SSs.

#### P 833

#### A PULMONARY METASTASIS OF SYNOVIAL SARCOMA APPEARING 18 YEARS AFTER TREATMENT OF THE PRIMARY NEOPLASM

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A 57 years-old woman presented with weakness and coughing. Investigations revealed a mass in the left lower pulmonary lobe, which was resected. The histological examination suggested a diagnosis of synovial sarcoma. Anamnestic data found out a primary neoplasm treated 18 years ago, only by surgery. The patient is well doing a year after operation of the lung.

Methods: Tissue was examined by routine histological slides, stained by H.E. and immunohistochemically. A revision of the primary tumor was made.

Results: The bought tumors showed biphasic pattern. The pulmonary mass showed a presence of sheets and cords of polygonal epithelial cells with quite rare split like spaces. The epithelial component was intimately intermixed with a spindle-cell component, composed of uniform spindle cells with hyper chromatic nuclei. Immunohistochemical analysis showed widespread labeling for cytokeratins and local labeling for vimentin in the epithelial component, and positive labeling for epithelial membrane antigen in the epithelial and spindle-cell components.

Conclusion: This is a rare case of synovial sarcoma treated with surgery only, metastasing in the lung, after being indolent 18 years.

#### P 834

##### **MYXOID MONOPHASIC SYNOVIAL SARCOMA OF THE FOOT: A CASE REPORT**

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Introduction and aims: Focal myxoid change is a well recognized feature of synovial sarcoma, but the presence of a predominately myxoid stroma is rare. We described a case of myxoid monophasic synovial sarcoma in which marked myxoid change initially obscured the diagnosis, leading confusion principally with malignant peripheral nerve sheath tumor.

Case report: A 16-year-old adolescent presented with left foot tumour. Roentgenographic studies demonstrated tumor with irregular margin and evidence of bone destruction. Histologically, the tumour had an alternating hypercellular and hypocellular foci. The areas of hypercellularity consisted of spindle cells arranged in fascicular growth. The cells had bland round or ovoid nuclei with a slightly wavy appearance. Because of nuclear hyperchromasia, the evaluation of mitotic activity is very difficult. Areas of hypocellularity contained individual spindle cells in a myxoid stroma. Neither calcifications nor foci of glandular differentiation were noted. Immunohistochemical stains for cytokeratin, S100 protein, desmin, myogenin and CD34 were negative, while a focal positivity for epithelial membrane antigen was noted. By reverse transcriptase polymerase chain reaction, a SSX1 transcript of synovial sarcoma was found.

Discussion and conclusion: Clinical differences between myxoid synovial sarcoma and conventional synovial sarcoma can't be evaluated because of too limited cases in the literature. Recognition of this rare histological variant of synovial sarcoma is important because it can be easily mistaken for other myxoid spindle cell neoplasms, potentially resulting in suboptimal therapy.

#### P 835

##### **MORTALITY OF TUBERCULOSIS IN RHEUMATOID ARTHRITIS**

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The risk of tuberculosis is higher in RA, or other autoimmune diseases, than in a normal population.

The aim of this study was to determine

1. the mortality due to tuberculosis (Tb), or miliary disseminated tuberculosis (mTb) in RA,
2. the clinically missed diagnosis of Tb, or mTb in RA,
3. the main complications associated with Tb, or mTb in RA

4. the link between Tb, or mTb and coexistent major complications, such as systemic vasculitis (SV), or secondary AA amyloidosis (AAa), lethal septic infection (SI), or associated diseases like malignant tumours (mTu), diabetes mellitus (DM), and atherosclerosis (Ath) in RA.

##### Patients and Methods

A randomized (non-selected) autopsy population of 234 in-patients with RA was studied. RA was confirmed clinically according to the criteria of the ACR.

Post-primary tuberculosis, (with, or without miliary dissemination) and the major complications of rheumatoid arthritis (SV, AAa, SI) associated to Tb, or mTb, and accompanying diseases (mTu, DM, Ath) were confirmed histologically.

A possible link between tuberculosis, or disseminated miliary tuberculosis and coexistent complications, or associated diseases was determined statistically by Chi<sup>2</sup>-tests.

##### Results

1. Post-primary fibrous, or fibrocaceous tuberculosis accompanied RA in 27 (11.54 %) of 234 cases.

Eight (3.4%, 29.6 rel%) of 27 post-primary fibrous, or fibrocaceous tuberculosis cases were complicated by disseminated miliary tuberculosis.

Three patients (1.28 %) died of circulatory failure due to Tb complicated by miliary dissemination.

2. Only one case of Tb with mTb and lethal outcome was clinically recognized.

3. There was a significant and positive correlation between vasculitis and tuberculosis (Chi<sup>2</sup>=4.16 p<0.04), or mTb (Chi<sup>2</sup>=3.86 p<0.04); furthermore between granulomatous type of vasculitis and mTb (Chi<sup>2</sup>=5.55 p<0.01).

##### Conclusions

Coexisting complications modify the basic disease (RA) and present atypical clinical manifestations. The age of the patients, the autoimmune character of the underlying disease, the steroid and/or immunosuppressive treatment may also play a role in missing the clinical diagnosis of miliary tuberculosis.

The correlation between Tb, or mTb and vasculitis may represent statistically an influence of Tb, or mTb on vasculitis.

The diagnosis of vasculitis of the granulomatous type should alert the surgical pathologist to initiate clinical exclusion of existing tuberculosis with miliary dissemination.

#### P 836

##### **CHRONOLOGIC SEQUENCE OF AA AMYLOIDOSIS IN RHEUMATOID ARTHRITIS - A RETROSPECTIVE CLINICOPATHOLOGIC STUDY OF 161 AUTOPSY PATIENTS**

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##### Statement

AA amyloidosis (AAa) is a progressive, cumulative process, involving in its early stages only a few structures in some organs, and increasingly more in the later stages of the disease.

During the progression of systemic secondary amyloidosis, amyloid A deposition begins in the organs and tissue structures that are frequently involved and later show marked deposition of amyloid. Where amyloid deposits are infrequent or less marked, deposition starts later (1).

The aim of this study was to determine

- (1) the frequency and extent of amyloid A deposits in various tissue structures,
- (2) the frequency and extent of AAa in different organs,

(3) the chronologic sequence of amyloid A deposition in various organs and tissue structures.

#### Patients and Methods

A randomized autopsy population of 161 in-patients.

Systemic AAa was observed in 34 (21.1%) of 161 cases (1).

AAa was diagnosed histologically.

#### Results

Amyloid deposits were frequent and massive in the wall of blood vessels, and along collagen and reticulin fibers (or, if present, on the basal lamina), mainly in the GI tract, heart, kidney, spleen, adrenal glands, and the liver.

Amyloid deposits were less frequent and in limited amount in skeletal muscles, synovial membranes, lymph nodes, peripheral nerves, bones, or skin.

The frequently involved structures and organs showed marked deposits of amyloid. In less frequently involved tissue structures and organs the amyloid deposits were less marked at death.

#### Discussion

The amyloid deposition starts in the wall of blood vessels (capillaries, arterioles, small arteries), followed by deposits on collagen fibers, basal membranes, and reticulin fibers (fat tissue). Deposition in the wall of medium size vessels starts later. The involvement of venules, small and medium size veins increases in case of stasis. Amyloidosis of peripheral nerves is a late, end-stage phenomenon.

The chronology of AA amyloid deposition in various organs is as follows: GI tract, heart, kidney, spleen, adrenal gland and liver. Other organs like pancreas, lung, thyroid gland, aorta, skeletal muscle, synovial membrane, lymph nodes, peripheral nerves, bones and skin are less frequently involved, and AA deposition starts later in them.

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#### P 837

##### **FOCAL MYOSITIS OF THE TEMPORAL MUSCLE**

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Focal myositis is a rare inflammatory disease of the skeletal muscle with histopathological features of interstitial myositis. Any skeletal muscle in the body can be affected. We present a 40-year-old female, previously in good health, with headache of one year duration and the three month history of a painful pseudotumor in the right temporal region. MRI pictures showed markedly thickened right temporal muscle with focally increased signal intensity on T2-weighted scan. Muscle biopsy revealed the characteristic histological findings of polymyositis. However, immunohistochemistry revealed the predominance of CD4 positive cells. In addition, immunofluorescence showed granular deposits of IgM and C3 in the walls of small endomysial and perimysial vessels. Therapy with nonsteroidal antiinflammatory drugs as well as with metilprednisolone was not effective, but responded to radiation therapy.

#### P 838

##### **MULTIPLE MICROSCOPIC RENAL ANGIOMYOLIPOMA INDICATING TUBEROUS SCLEROSIS**

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Renal manifestations are present in one half of patients with tuberous sclerosis (TS). They include angiomyolipomas, cysts, combination of both and renal cell carcinomas. They are usually detected by imaging techniques. Pure microscopic diagnosis is rather rare.

We report the case of a 36 years old patient, treated surgically for ureteropelvic junction obstruction with calyceal and pelvic dilation and atrophic renal parenchyma.

The histological pictures were unusual. The obstructive lesions were rather moderate when compared to the severity of the renal atrophy. These were associated with chronic ischemic damages, low grade papillary tumor and medial angiodysplasia of arteries. In addition, small multi-focal nodules suggested smooth muscle-cells proliferation. Immunohistochemistry screening for these clusters show positive staining for HMB45. As such, diagnosis of multiple microscopic angiomyolipomas is confirmed.

These microscopic features led to conduct a genetic survey. No cutaneous ophthalmologic or neurological tumors were detected. However, one of his daughters presented neonatal achromatic spots and hypsarythmia when she was 5 months old. The study of the two known genes (TSC1 and TSC2) is in process.

Microscopic renal angiomyolipomas are often predominantly composed of epithelioid smooth muscle cells. These could be associated with vascular affection. They must be recognised as they are highly evocative of TS. Their presence must lead to a full clinical check, complementary imaging analysis, genetic investigation and screening for TS genes mutation.

#### P 839

##### **A CASE OF PRIMARY RENAL MALIGNANT FIBROUS HISTIOCYTOMA AND ANGIOMYOLIPOMA**

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Primary renal malignant fibrous histiocytoma (RMFH) is a rare tumor of the kidney. Fiftytwo cases of RMFH have been reported in the literature. It is clinically and radiologically indistinguishable from a renal cell carcinoma.

We report a case of RMFH originating in the capsule of the upper pole of right kidney which was detected incidentally on routine physical examination of a 43-year-old man. The patient underwent right radical nephrectomy with a diagnosis of renal cell carcinoma clinically. The tumor was also seen preoperatively as a bulky mass around the vena cava and could not be resected totally. Gross examination of the specimen revealed two tumoral foci, one of which was a huge, yellowish white, rubbery, nodular mass measured 9x8.5x8.5 cm. Histologically, the tumor was mainly composed of inflammatory cells including lymphocytes, plasmacytes and histiocytes. There were also areas of plump spindle-epithelioid histiocytic cells densely arranged in whorl-like pattern. Because the differential diagnosis included inflammatory pseudotumor, sarcomatoid renal cell carcinoma and RMFH, immunohistochemical analyzes were performed for antibodies pancytokeratin, smooth muscle actin (SMA), desmin, CD31, CD34, CD68 and Ki67. Immunohistochemistry revealed diffuse positivity for CD68 and focal positivity for SMA. Ki67 proliferation index was detected to be 8-10%. The tumor cells were negative for other antibodies. Therefore, the tumor was diagnosed as RMFH.

The other tumor, 1 cm in diameter displaying similar macroscopical features to RMFH, was localised in the lower pole, diagnosed as angiomyolipoma histologically.

In conclusion, RMFH is a very rare tumor and it may lead to differential diagnostic difficulties in the reassessment of renal masses. To our knowledge this case is a unique example of RMFH because of its coexistence with angiomyolipoma.

#### P 840

### COLLECTING DUCT CARCINOMA OF THE KIDNEY: A REPORT OF FIVE CASES AND REVIEW OF THE LITERATURE

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#### INTRODUCTION

Collecting duct carcinoma of the kidney is a rare neoplasm centered in the renal medulla; these tumors are aggressive, often metastatic and commonly result in death. The clinicopathological features of five cases are here presented to add to the approximately 100 cases reported so far in the literature.

#### CASES

Clinical presentation in the majority of cases has been hematuria, less frequently loin or back pain, or urinary. Upper tract imaging often suggests urothelial carcinoma of the renal pelvis. We report five illustrating cases.

#### PATHOLOGY

Macroscopic: CDC is usually located in the central region of the kidney and often involves hilar structures. It ranges widely in size from 0,5 to 12cm. The typical CDC is firm and gray-white with irregular infiltration borders; areas of necrosis, and rarely cystic elements may be present.

Microscopic: Typical CDC has a tubular or tubulo-papillary growth pattern. Irregular and disorganised tubules or ducts within a desmoplastic stroma infiltrate and expand the interstitial tissue of both the cortex and medulla, with scattered normal tubules and glomeruli within the malignant tissue. Vascular and lymphatic space invasion is common. Tumor cells are seen both infiltrating into stroma and evoking desmoplasia. Their cytoplasm appears granular and lightly eosinophilic; nuclear grade according to the grading system of Furhman et al. is typically grade 3 or 4. Cells with a hobnail pattern are often present.

Immunohistochemical studies on these carcinomas have been variable; the expected profile would be positive for low molecular weight keratin, for EMA, Ulex europaeus agglutinin and peanut lectin.

#### DISCUSSION

CDC shows a male predominance (ratio of about 2:1). There is a wide age range with a mean of 55 years, and it has been noted a strong family history of cancer. CDC are the most aggressive carcinomas of the renal tubular epithelium. Approximately 35% of patients have metastases at presentation. Approximately two thirds of patients with collecting duct carcinoma have died of the cancer within two years of diagnosis.

CDC are unlikely to be confused histologically with other renal cell carcinomas (including papillary renal cell carcinoma, urothelial carcinoma with glandular differentiation, adenocarcinoma arising from the urothelium of renal pelvis, and metastatic carcinomas). Accurate classification is important because CDC has a considerably worse prognosis than other renal neoplasms.

#### P 841

### AN UNUSUAL CASE OF UNILATERAL PYELOURETERAL IDIOPATHIC RETROPERITONEAL FIBROSIS SIMULATING RENAL MALIGNANCY

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#### INTRODUCTION

Idiopathic Retroperitoneal Fibrosis (IRF) or Ormond's disease is a relatively uncommon disorder, with a reported incidence of 1 in 200000 inhabitants, with a male: female ratio of 2-3:1. It typically presents insidiously, in the fifth and sixth decades of life and it is rare in children. It is most commonly localized around aorta and iliac vessels, it usually spreads laterally to involve the inferior vena cava and one or both ureters which are displaced medially. It can cause obstructive uropathy and renal failure.

#### CASE REPORT

A 85 year old man presented with fatigue, anorexia and weight loss, lasting about three months. History was significant for diabetes mellitus type 2 and hypertension. Computerized tomography (CT) showed a deformation of the right kidney due to a low density area which involved the renal hilum and was not clearly separated from the perirenal fat. On magnetic resonance imaging (MRI) the soft tissue mass appeared isointense with muscle on T1 weighted images and hyperintense on T2 weighted images. Excretory urography revealed proximal hydronephrosis and complete obstruction of the ureter. Radiological studies suggested a malignant tumor of the renal pelvis.

#### RESULTS

Lumbar incision revealed a dense hard grayish-white mass encasing the upper part of the ureter and extending to the hilum of the right kidney. Right nephrectomy was performed. Microscopic examination revealed a fibrous proliferation, broad anastomosing bands of hyalinized collagen and a prominent inflammatory infiltrate composed of mainly of lymphocytes and plasma cells often containing germinal centers, there were also some macrophages and eosinophils. Multiple histological sections were performed to rule out a malignant neoplasia with desmoplastic reaction. The diagnosis of IRF was made after secondary conditions both benign and malignant were ruled out. The postoperative course was uneventful.

#### DISCUSSION AND CONCLUSIONS

IRF renal involvement is generally bilateral and typically involves the middle third of both ureters. In our case renal pelvis and proximal part of right ureter were diffusely and deeply infiltrated by an inflammatory fibrous process. Originally sarcomatous or carcinomatous neoplasia were strongly considered in the clinical differential diagnosis and treated it with nephrectomy. The correct histologic diagnosis of IRF with multiple histological sections, allows urologist to choose less aggressive options of treatment.

#### P 842

### NEPHROGENIC ADENOMA A PITFALL DIAGNOSIS WITH AMACR (P504S) STAINING

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Introduction : Nephrogenic adenoma is a rare distinctive benign lesion typically found in areas of urothelial mucosa after urinary injury. Microscopically, the lesion usually confined to the urothelial mucosa, exhibits a range of patterns with variable proportions of papillary, microcystic, solid nested, or tubular patterns. The cells lining the tubules, cysts, and papillae are predominantly cuboidal to low columnar with a small amount of cytoplasm, and with sometimes prominent nucleoli. In such cases when the tubular pattern is exclusive it could mimics prostatic carcinoma. Aim of the study : To characterise the morphology and immunoprofile of nephrogenic adenoma. Materials and methods : 23 cases of NA from surgical pathological files of two institution, were selected. Immunostaining was performed with Urinary and

prostate markers CK5/6 (Dako), CK7 (Dako), 34&E12 (Dako), PSA (Dako), PSAP (Dako), P63/p504s (PIN cocktail, Menarini Dg) antibodies.

Results : Histological features displayed a papillary pattern, associated with a tubular component in most cases. The tubes are lined by one layer of small cuboids cells with nucleolar nuclei, surrounded by an inflammatory stroma. In case of exclusive tubular pattern, infiltration could mimic a small acinar proliferation suspicious of prostatic cancer. No mitosis, necrosis or cytonuclear atypia was seen except in 1 case. Immunostaining study revealed a 34&E12, CK 5/6 and CK7 positivity, with a diffuse cytoplasmic positivity with P504s. Furthermore only in urethral cases a weak and focal positivity for PSA and PSAP was observed. In all cases P63 was negative.

Discussion : One must be aware of the range of appearances of nephrogenic adenoma to avoid its misinterpretation. In exclusive tubular pattern with muscular involvement nephrogenic adenoma could mimic prostate carcinoma. In such cases one must remind that the over expression of AMACR (P504s) in such small glandular tubular structure may be misinterpreted as prostatic carcinoma. The presence of inflammatory stroma surrounding the small atypical tubes, and the positivity of CK 5/6, CK7 and 34&E12 positivity would restore the true diagnosis of such benign lesion.

#### P 843

##### **INTERSTITIAL KIDNEY FIBROSIS IN AN EXPERIMENTAL MODEL OF UNILATERAL URETERAL OBSTRUCTION (UO)**

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#### INTRODUCTION

Interstitial fibrosis is a final outcome of many chronic renal diseases, including obstructive nephropathy. Fibrosis considers fibroblast proliferation and intercellular matrix deposition, leading to characteristic tissue changes resulting in chronic renal failure. Angiotensin II is one of the most important factors in the progression of renal diseases.

#### OBJECTIVE

The objective of this study is to quantify the degree of interstitial fibrosis in rat kidney after experimental UO and administration of an angiotensin converting enzyme (ACE) inhibitor – cilazapril.

#### MATERIAL AND METHODS

Male Wistar rats, weigh 210-300 g, were used in this experimental model. There were 4 experimental groups, 6 rats each: 1. sham-operated (sham-UO); 2. UO (left ureter was ligated); 3. sham-UO+cilazapril 10 mg/kg orally q.d. (Cilazil, Pliva, Zagreb, Croatia); 4. UO+cilazapril 10 mg/kg orally. Rats were sacrificed on the 10th day after the operation. The interstitial kidney fibrosis quantification was performed on Gomory stain slides using semiquantitative scale (0- no reticulin increase; 1- rare foci of reticulin multiplication; 2- fine reticulin net around tubules; 3- dense reticulin net around tubules; 4- reticulin foci in more than 50% of HPF; 5- diffuse fibrosis) and morphometric system ISSA (Vamstec, Zagreb, Croatia). Morphometric quantification was performed using computer grid (13x10 fields) by counting the number of grid fields containing interstitial fibrosis. Six, randomly selected, microscopic fields (400x) were reviewed for each case.

#### RESULTS

Interstitial fibrosis developed in all animals after UO. Number of grid field containing fibrosis per HPF in UO

group was significantly higher than in sham-operated group ( $p<0,05$ ) and in sham-operated+cilazapril group ( $p<0,01$ ). Fibrosis level obtained by semiquantitative analysis did not showed statistically significant difference between experimental groups. However, there was significant correlation between semiquantitative and morphometric method (Pearson's coefficient  $r=0,71$ ,  $p<0,0001$ ).

#### CONCLUSION

Interstitial kidney fibrosis developed after the UO does not decrease after the ACE-inhibitor administration. The level of interstitial fibrosis can be determined using several methods, such as semiquantitative scale and morphometric quantification. The morphometric quantification is more sensitive method than semiquantitative scale.

#### P 844

##### **LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE RENAL PELVIS: A CASE REPORT AND REVIEW OF THE LITERATURE**

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[Introduction] Lymphoepithelioma-like carcinoma (LELC) shares the same morphologic features with undifferentiated nasopharyngeal carcinoma and is very rare in the urinary tract, especially the renal pelvis. We report the third documented case of LELC of the renal pelvis and review the literature to elucidate its clinicopathologic features.

[Case report] The tumor was found in a 58-year-old woman suffered from left flank pain and gross hematuria. The ultrasound study showed left hydronephrosis with a renal pelvic tumor; therefore, left nephroureterectomy was performed. Grossly, the tumor was fungating and fragile within the dilated renal pelvis. Microscopically, it was composed of sheets or ill-defined nests of syncytial undifferentiated cells exhibiting prominent nucleoli and mixed with extensive lymphoplasmacytic infiltrations. Immunohistochemically, the tumor cells were positive for cytokeratin, but negative for leukocyte common antigen and vimentin. No Epstein-Barr viral (EBV) transcripts were detected by in-situ hybridization for EBER-1. The tumor was limited within the pelvis with no renal parenchymal invasion. No evidence of conventional transitional cell carcinoma was seen after thorough sampling, although focal dysplasia was noted in the urothelium elsewhere. The patient remains well without malignancy 2 years after surgery.

[Conclusion] Reviewing the literature, all three cases of LELC of the renal pelvis occurred in the elderly with a good prognosis. They were not associated with EBV latent infection, unlike LELCs in salivary glands, lungs, or stomach. These clinicopathologic features were almost identical to those seen in the cases of LELC of the urinary bladder. Accordingly, urothelial LELCs should be separated from high-grade urothelial carcinoma and be classified as a distinct entity with a favorable outcome, although they were composed of undifferentiated tumor cells.

#### P 845

##### **MIXED EPITHELIAL AND STROMAL TUMOUR OF THE KIDNEY IN MALE-CASE REPORT MIXED EPITHELIAL AND STROMAL TUMOUR OF THE KIDNEY IN MALE-CASE REPORT MIXED EPITHELIAL AND STROMAL TUMOUR OF THE KIDNEY IN MALE-CASE REPORT MIXED EPITHELIAL AND STROMAL TUMOUR OF THE KIDNE**

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**Introduction and aim:** We present the case of an 60 year old man with no history of hormonal treatment and urological diseases except renal lithiasis. He was hospitalized for left lobar pain and intermittent hematuria. Echography and NMR revealed a kidney mass. A total left nephrectomy was performed. The left kidney showed a polar tumor mass. Macroscopically examination of the tumour on cut section showed a solid white tissue with numerous cystic formations of several sizes.

**Material and methods:** Fragments of the tumor were fixed in formalin 10%, embedded in paraffin and sections were stained with HE. Immunohistochemical tests have been done: ACT, CD 34, Progesterone receptors, Estrogen receptors, CD 117, CD 56, HMB 45, Androgen receptors, NK 1

**Results:** The tumor was composed of a mixture of a spindle cell proliferation intermingled epithelial elements that formed microcyst and macrocystic dilatation. No hemorrhages, areas, necrosis, cytologic atypia or mitotic activity were present. Immunohistochemical analyses revealed: ACT, CD 34, Progesterone receptors expression in tumoral areas were positive and CD 117, HMB 45, Androgen receptors, Estrogen receptors, CD 56, NK 1 were negatives

**Conclusions:** The morphological appearance and immunohistochemical results enabled us to diagnose this case as a mixed epithelial and stromal tumour of the kidney. This is a rare entity in male (few cases previously reported) but our case presents the particularity of no hormonal treatment and positive Progesterone expression.

#### P 846

### MUCINOUS TUBULAR AND SPINDLE RENAL CELL CARCINOMA :

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#### INTRODUCTION

The mucinous tubular and spindle renal cell carcinoma (MTSCC) is a very rare neoplasm, recently described as a distinct subtype of renal cell carcinoma (RCC). This low-grade polymorphic renal epithelial neoplasm with mucinous, tubular and spindle cell features may have striking morphologic similarities to the more common papillary RCC. Herein, we report an additional case of MTSCC with immunohistochemical study and summarise the present knowledge of this newly recognised entity.

#### CASE REPORT

A 52-years-old woman with uncontrolled hypertension underwent a renal ultrasonography that disclosed a 6.4-cm-sized mass involving the left kidney. A radical nephrectomy was performed. The gross pathology revealed a well-circumscribed whitish solid tumour. Microscopy showed a proliferation of regular cuboidal cells often arranged in long cords and tightly packed tubules and spindle cells component forming small sheets with low grade nuclear cytology. The stroma showed focally a mucinous material and clumps of foamy histiocytes. The mucinous material reacts strongly with alcian blue. The tumour's cells exhibit positive reactions for vimentin, epithelial membrane antigen (EMA), high-molecular-weight cytokeratin (CK), CK7 and CK18. The diagnosis of mucinous tubular and spindle renal cell carcinoma was made. The patient is free of disease since more than 12 months.

#### DISCUSSION

Clinically, female predominance is reported with a mean age of 53 years. MTSCC often presents as asymptomatic mass. The MTSCC have a distinctive histologic appearance

composed of cuboidal and spindle cells with mucinous extracellular matrix with some degree of morphologic variability. Some authors have reported small foci of clear cells. These pathologic features may be reminiscent of papillary RCC with sarcomatoid features or metanephric adenoma. Most MTSCC are immunoreactive for vimentin, EMA, CK cocktail, CK 7 and Ulex Europaeus agglutinin-1 but usually not for CD10. Ultrastructurally, focal short microvilli are generally observed with sometimes junctional complexes and basal lamina. MTSCC exhibit multiple losses of chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22 with no VHL deletions. The prognosis is commonly favourable. However, few cases of local recurrence or metastasis are reported. Morphologic, immunohistochemical and ultrastructure features indicate differentiation toward distal nephron segments has been suggested as the MTSCC phenotype but this view is debatable.

#### P 847

### BILATERAL RENAL ADENOMATOSIS AND ONCOCYTOSIS WITH MIXED ONCOCYTIC AND PAPILLARY TUMOURS

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Adenomatosis and oncocytosis are rarely described in the kidney. We report a case of 66-year-old man with bilateral renal adenomatosis and oncocytosis found at autopsy. Both kidneys were occupied by innumerable tumours, the majority of them fulfilled the criteria of renal papillary adenomas. Three tumours showed mixed solid alveolar and papillary patterns composed of oncocytic cells and small cells of papillary adenoma type. Transitional features between patterns were clearly visible. Moreover, apart from dysplastic tubules and microadenomas nearly all histological sections revealed multiple foci of oncocytic transformation of tubular epithelium and microoncocytomas. Oncocytic features of cells were confirmed ultrastructurally. Results of immunohistochemical examination showed that papillary adenomas might be of distal nephron origin.

To our knowledge this is the first case of bilateral renal adenomatosis and oncocytosis described. Mixed oncocytic and papillary tumours are not considered distinct entity. In this case they probably represent collision tumours but taking into account distal nephron origin of described papillary adenomas, oncocytic transformation of papillary tumour cells cannot be excluded.

#### P 848

### COMPARISON OF HEP PAR 1 AND MITOCHONDRIAL ANTIGEN EXPRESSION IN RENAL ONCOCYTOMAS: IS IT MITOCHONDRIA WHICH HEP PAR 1 REACTS WITH?

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**Introduction:** Monoclonal antibody, Hep Par 1 has been used to identify tissues of hepatocytic origin and those with hepatoid differentiation. The characteristic diffuse cytoplasmic granular immunostaining pattern suggests an organelle, possibly mitochondrial localisation of the antigen. We investigated Hep Par 1 immunoreactivity in the renal oncocytomas which were proved to be rich in mitochondria and compared the mitochondrial antigen (Mab-2) expression of this tumor with Hep Par 1 expression.

**Material and Methods:** Thirty two cases of renal oncocytoma were included in this study. Staining intensity (+, ++, +++)

and staining area (0-25%, 25-50%, 50-75%, 75-100%) were noted for Hep Par 1 and Mab-2.

Results: We detected the mitochondrial antigen in 75-100% of tumoral areas with (++) to (+++) staining intensity whereas none of the tumors showed the coarse granular staining which is characteristic to Hep Par 1 immunohistochemistry. Only two oncocytomas displayed diffuse cytoplasmic immunoreactivity which is moderately intense while eleven showed faint cytoplasmic staining in <25% of the cells. Twenty tumors showed no immunoreactivity. We did not detect any correlation between the immunoreactivity profiles of Hep Par 1 and Mab-2 in renal oncocytomas.

Conclusion: These findings suggest that there is no relationship between the mitochondria and the antigen which Hep Par 1 antibody reacts with.

#### P 849

##### IMMUNOHISTOCHEMICAL EVALUATION OF CATENINS IN RENAL CELL CARCINOMA

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SUMMARY: Catenins have prognostic value in several human tumors.

AIMS: The aim of this study was to determine the expression of catenins in Renal cell carcinoma (RCC) and their relationship with tumor morphology and TNM status. METHODS: 28 cases of RCC, 2 metastasis and 2 samples of normal kidney tissue were studied using streptavidin-biotin-complex technique. Catenins expression levels were semiquantitatively scored. RESULTS: Alpha-, beta and gamma catenin were present in most cases of RCC (43%, 64% i 50%, respectively). Sarcomatoid type of RCC and T3 RCCs displayed higher beta-catenin level than T1/T2 tumours. There is no correlation between cell type and level of alpha or gamma catenin expression ( $p > 0.05$ ). Beta catenin is predominantly expressed in sarcomatoid type of RCC and this correlation showed statistical significance ( $p < 0.05$ ). Tumors of grade 1 more often showed alpha and beta catenin expression (84%; 100%) then tumors of grade 2/3 (46%; 50%), while there was no differences between gamma catenin distribution in this two groups of tumors (G1-65%; G2/3-41%). Alpha and gamma catenin were expressed more often in tumors of stage T3 (57%; 71%) then in T1/T2 tumors (38% for both catenins) and that difference has statistically significant association ( $p < 0.05$ ). On the contrary, tumors of lower stage (T1/T2) showed beta catenin expression more often than tumors of higher stage (62%; 57%), but this expression did not show significant correlation ( $p > 0.05$ ) with tumor stage. CONCLUSIONS: Our results indicate that there is different expression of cell adhesion molecules (alpha, beta and gamma catenin) in RCC, which depends on histological type, tumor stage and grade.

#### P 850

##### IMMUNOHISTOCHEMICAL EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND MICROVESSEL DENSITY IN RENAL CELL CARCINOMA: PROGNOSTIC VALUE AND RELATION TO CLINICOPATHOLOGIC PARAMETERS.

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INTRODUCTION AND OBJECTIVE: Epidermal growth factor receptor and tumoral angiogenesis have been proposed as potential prognostic parameters in renal cell carcinoma. The aim of this study is to detect the immunohistochemical

expression of epidermal growth factor receptor (EGFR) and microvessel density (MVD) and to relate to prognosis and clinicopathologic parameters.

METHODS: Immunohistochemical stains using EGFR and CD 34 monoclonal antibodies were used in 55 renal cell carcinomas diagnosed subsequent to radical nephrectomy or nephron sparing surgery between 1987 and 2001. The MVD was detected by counting the number of microvessels stained by CD 34 in the most vascularised area of tumor.

RESULTS: Univariate analysis showed only pathological stage of tumor has highly significant association with cancer specific survival ( $p < 0.01$ ). EGFR immunostaining was present in 22 of 55 (40%) RCCs and mean number of counting vessel was 53.31. EGFR immunoreactivity and MVD were not exhibited prognostic significance with respect to survival and clinicopathologic parameters such as stage and grade of tumors.

CONCLUSIONS: The expression of EGFR and MVD seems not to provide additional prognostic information in RCCs. Our results show that the best predictors of cancer specific survival is pathologic stage of tumor in RCCs.

#### P 851

##### EXPRESSION OF CLAUDIN 1,2,3,4 AND 7 IN NORMAL AND NEOPLASTIC KIDNEY

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Introduction: Claudins (CLDNs) are tight junctional (TJ) proteins expressed in epithelial and endothelial cells. They participate in the formation of barriers between different tissue compartments by regulating the efflux of molecules through TJ complexes.

Purpose: Our aim was to detect the difference between the expression of CLDNs in the embryonal and adult kidneys. Further, the expression of CLDNs in benign and malignant tumours of the kidney was also studied.

Method: CLDN 1,2,3,4,7 were studied by immunohistochemistry in formalin fixed, paraffin embedded samples of 11 normal embryonal and adult kidneys, and 18 kidney tumours [5 oncocytomas, 5 conventional renal cell carcinomas (CRCC), 5 papillary renal cell carcinomas (PRCC) and 3 chromophobe renal carcinomas (CRC)]. A semiquantitative evaluation was used.

Results: The expression of CLDNs was found to be similar in embryonal and adult kidneys. CLDN1 was expressed only in the Bowman's capsule, CLDN2 in the proximal tubules, CLDN3 in the distal tubules, CLDN4, 7 in the distal tubules, the collecting duct and the pylon. In oncocytomas only CLDN7 showed a weak, focal, positive reaction, while the other CLDNs were negative. Similarly, CLDN7 was the only CLDN detected in CRC, though with much stronger reaction. In CRCCs intensive expression of CLDN4, 7, and a weak reaction for CLDN2 were observed, while CLDN1 and 3 were negative. In contrast, CLDN1 was expressed in PRCCs, while reaction for the other CLDNs was similar to that observed in CRCCs.

Conclusions: We described a characteristic CLDN pattern in the kidney, which is preserved through the embryonal development till the adult stage. Marked differences were noted among the tumors probably representing different cellular origin, which might be used in differential diagnosis too.

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#### P 852

**RELATIONSHIP BETWEEN VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IMMUNOSTAINING PATTERN, VEGF PLASMA LEVELS, TUMOR NECROSIS AND USUAL PROGNOSTIC VARIABLES IN RENAL CELL CARCINOMAS**

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**Objectives:** VEGF has been proposed as an important prognostic marker in RCC. However no optimal method of VEGF measurement has been defined so far. The objective of this study was to compare different VEGF measurement modalities to usual clinical and pathological variables in order to better define links between tumor aggressiveness and VEGF blood release.

**Methods.** 50 clear cell carcinomas were prospectively analysed for VEGF levels by enzyme-linked immunosorbent assay in peripheral blood and in renal vein during nephrectomy (VEGFrv). In addition, detection of VEGF expression was performed in tumor tissue by immunohistochemistry (VEGFt) and three different types of VEGF immunostaining were defined: membranous (type 1), cytoplasmic (type 2) or both: (type 3). The results were correlated together as well as to the following clinical and pathological variables: TNM stage, Fuhrman grade, tumor size, ECOG performance status, presence of tumor necrosis on pathological analysis, haemoglobin, platelet and leucocyte counts. Mann-Whitney and Kruskal-Wallis tests were used for comparison.

**Results :** VEGF immunostaining types and tumor necrosis were both correlated to T Stage (p:0.02) and Fuhrman grade (p:0.001 and 0.002 respectively). Tumor necrosis and VEGF immunostaining type were strongly correlated together: 5.3%, 62.2% and 80% of tumors with type 1, 2 and 3 VEGF immunostaining displayed necrotic lesions respectively (p<0.0001). VEGFpv (p:0.003), VEGFrv (p:0.03), VEGFt (p<0.001), platelet count (p:0.02) and haemoglobin (p:0.01) were correlated to VEGF immunostaining type. For example median VEGFpv was 99 (12-582), 144 (20-437) and 301pg/ml (230-1087) in type 1, 2 and 3 immunostaining type respectively. Similarly the presence of tumor necrosis was associated with increased VEGFpv (p:0.03), VEGFrv (p:0.0001), VEGFt (p:0.001), and platelet count (p:0.001) as well as with decreased haemoglobin level (p:0.02). Finally both VEGF immunostaining profile and tumor necrosis were associated with early cancer recurrence (p:0.003).

**Conclusion.** We presented here a particular VEGF tumor immunostaining pattern which is related to high levels of circulating VEGF and higher tumor stages. Moreover relationship between tumor necrosis and VEGF both in tissue and in peripheral blood is an additional evidence of the importance of the VHL/hypoxia inducible pathway in RCC.

**P 853**

**CORRELATION BETWEEN RENAL ARTERY CHANGES AND TUMOR NECROSIS IN PATIENTS WITH RENAL CELL CARCINOMA**

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**INTRODUCTION:** Different lesions might involve main renal artery such as arteriosclerosis, fibromuscular dysplasia

(FMD) and some other conditions (arteritis, radiation injury and congenital malformations). Renal cell carcinoma (RCC) represents 2% of all human malignancies and over 90% of all malignancies of the kidney. Necrosis, cysts, hemorrhage and calcification are commonly present.

**PURPOSE OF THE STUDY:** Aim of the study was to correlate the presence and extent of necrosis in RCC and renal artery status.

**METHODS:** We analyzed a consecutive series of 112 patients (M: F=71:41) with RCC who underwent nephrectomy in the period from 2003 to 2004. Patients were aging from 35-82 years (mean 60.5) and the tumor size was 2-20 cm (mean 6.1). Necrosis was found in 88 (78.6%) cases of RCC of which 63 had less than 50% necrosis and 25 tumors contained more than 50% necrotic areas. Specimens were routinely fixed, embedded in paraffin, cut and stained with hematoxylin and eosin, Mallory trichrome method and orcein.

**RESULTS:** Renal arteries of 50 (44.6%) patients (M: F=36:14) showed no changes (Group I). These patients were in age from 38-78 years (mean 57.8) and tumors measured from 2.5-17 cm (mean 7.4). FMD (Group II) was found in 41 (36.6%) patients (M:F=17:24) ranging in age from 35-81 years (mean 59.1), and the tumor size was 2-19 cm (mean 8.2 cm). Atherosclerotic changes of renal arteries (Group III) were observed in 21 (18.8%) patients (M:F=18:3), aged from 42-82 years (mean 67.2) with tumors from 2-20 cm (mean 6.7).

In the first group necrosis was not found in 11 (22.0%) cases, 30 (60.0%) had less than 50% necrosis and 9 (18.0%) tumors were necrotic in more than 50%. In the second group, 7 (17.1%) cases were without tumor necrosis, 24 (58.5%) had less than 50% necrosis and 10 (24.4%) tumors were more than 50% necrotic. In the third group, 6 (28.6%) tumors did not show necrosis, 10 (47.6%) were less than 50% necrotic and 5 (23.8%) had more than 50% necrosis. Within the second group RCC of patients with FMD type I, 3 cases were less than 50% necrotic, while 4 cases were more than 50% necrotic, and only one was without necrosis. In the group of patients with FMD type IIb, 5 tumors were without necrosis, 21 had less than 50% necrosis and 4 were more than 50% necrotic.

**CONCLUSION:** Renal artery changes are very common in patients with RCC. Necrosis of the tumor was more common in patients with associated FMD and atherosclerotic changes of renal artery. However, t

**P 854**

**SARCOMATOID RENAL CELL CARCINOMA. REPORT OF TWO CASES WITH IMMUNOCHISTOCHEMICAL STUDY**

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**Introduction:** Sarcomatoid Renal Cell Carcinoma (SRCC) is an uncommon subtype of Renal Cell Carcinoma (RCC) with a poor prognosis. The origin of the sarcomatoid component is controversial: some authors consider it to be a dedifferentiation of the epithelial component while others believe that this tumor is a true carcinosarcoma.

**Purpose-Method:** In the present report two cases of this rare entity were studied using an extensive immunohistochemical panel of antibodies: cytokeratin 7, cytokeratin 20, cytokeratin 8, cytokeratin 18, cytokeratin 19,

p53, Bcl-2, Ki-67, AFP, PLAP, EMA, CEA, Vimentin Cyclin D1 and S-100.

The expression of these antibodies was evaluated in the sarcomatoid component.

Results: The first case showed no epithelial differentiation and in the second case the epithelial component was conventional RCC.

The two cases were intense and diffusely positive against vimentin. The second case did not show any positivity for any of the cytokeratin antibodies. The first case showed positivity for cytokeratin 8, cytokeratin 19, EMA, CEA, Cyclin D1, P53 overexpression and Ki-67 high labeling index.

Conclusion: Our results suggest that SRCC includes two different entities that should be separated: a sarcomatoid carcinoma and a mixed tumor with a true sarcomatous component.

#### P 855

##### CELL CYCLE REGULATORY FACTORS IN RENAL CELL CARCINOMA

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Introduction and aims: In this study we analysed the immunohistochemical expression of p53, Bcl-2, Rb and PCNA and the relation of this expression to apoptotic status, clinicopathological characteristics and prognosis in renal cell carcinoma specimens.

Material and methods: The expression of p53, Bcl-2, Rb and PCNA was studied by immunohistochemical methods in paraffin-embedded nephrectomy specimens from 22 patients with no history of chemotherapy, radiotherapy, or immunotherapy. The apoptotic status was determined by flow-cytometry following propidium iodide incorporation.

Results: The p53 protein expression was recognized with a very low intensity (1-8%) in most of the cases but not in those with sarcomatoid features where it was elevated (30%). The expression of Bcl-2 was recognized in only 17% of cases also with low intensity; it was associated with the high tumoral grade and medium apoptotic intensity. Half of the tumor specimens were Rb positive, its absence correlated with a high grade and a high mitotic index. PCNA was detected in 86% of cases and showed a heterogeneous expression and correlated with the high grade of tumor. In 2 cases PCNA expression was absent and was correlated with a very differentiated tumor tissue.

Conclusions: Our observations suggest that the high apoptotic intensity of these tumors seems to be not p53 dependent. Thus, the low p53 expression could sustain the high degree of resistance to chemotherapy and radiation of renal cell carcinomas. Also, our results emphasize that high grade differentiated tumor cells induce Bcl-2 expression in order to stop apoptotic process. In renal cell carcinomas Rb protein seems to act like an antiproliferative factor but not like an antiapoptotic one. We observed that PCNA expression increase while apoptotic intensity decrease. In our study the expression of p53, bcl-2 had no independent prognostic value and suggests that they would play a minimal role in helping to further stratify patients at high risk for disease progression or recurrence. Rb and PCNA seem to be a good objective and quantitative marker of biological malignant potential in renal cell carcinoma.

#### P 856

##### CYSTIC RENAL CELL CARCINOMAS IN ADULTS: IS PREOPERATIVE RECOGNITION OF

##### MULTILOCULAR CYSTIC RENAL CELL CARCINOMA POSSIBLE?

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Purposes: To correlate clinicopathological and imaging features of multilocular cystic renal cell carcinomas in order to propose preoperative criteria for therapeutic modalities.

Materials and Methods: Twenty four clear renal cell carcinomas with a chiefly cystic component were identified from 1993 to 2002. For each case, histological slides and available imaging studies were retrieved. Two tumor groups were defined: multilocular cystic renal cell carcinomas (MCRCC) and clear renal cell carcinomas with cystic change (CRCC) either by intrinsic growth or necrotic degeneration. Radiological correlation using CT scan and MRI studies was performed considering criteria such as mural nodule, cyst wall thickness, septa.

Results: On imaging study, MCRCC presented as a multilocular cystic mass lacking mural nodule, with regular thin cyst wall and septa. On pathologic study, MCRCC presented as complex, multilocular cystic carcinomas with septa covered by low nuclear grade clear renal tumor cells without grossly expansible nodule. They were all staged pT1 with a free clinical course. On the opposite, CRCC were described on imaging study with a mural nodule (5 mm or higher), thick, irregular cyst wall and irregular septa. On pathologic study, CRCC were characterized by a grossly expansible nodule within the septa and/or the cyst wall. Nuclear grade and TNM stage were higher in CRCC.

Conclusions: Preoperative recognition of MCRCC is possible when using strict CT and/or MRI criteria. The current study confirms the low malignant potential of MCRCC. A nephron sparing surgery should be proposed when MCRCC is suspected.

#### P 857

##### MORPHOMETRICAL ANALYSIS OF RENAL ARTERIES IN PATIENTS WITH RENAL CELL CARCINOMA

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Introduction: The presence of tumor necrosis in renal cell carcinoma (RCC) is very common and was correlated with different features including tumor size, cell type, nuclear grade as well as microvessel density and the expression of different markers. However, there are no data about the relationship between renal artery changes including atherosclerosis and fibromuscular dysplasia (FMD) and the extent of necrosis in RCC.

Purpose of the study: to analyze renal arteries in patients with RCC especially regarding the thickness of medial layer and circumference of the artery using image analyzer.

Methods: We analyzed a consecutive series of 57 (35 male and 22 female) patients ranging in age from 35-79 years (mean 58.9 years) who underwent nephrectomy due to RCC in the year 2003. The patients had RCC measuring from 2-16 cm (mean 7.1 cm). Renal arteries were cut during nephrectomy up to 2 cm from renal hilus. Specimens were routinely fixed, embedded in paraffin, cut and stained with hematoxylin and eosin, Mallory trichrome method and orcein. Results: The thickness of the media of 40 patients measured from 167.86-1177.21  $\mu$ m (mean 571.35). Renal arteries of 17 patients (11 male, 6 female) were not measured due to

technical problems. The mean thickness of media in patients with FMD was 567.33  $\mu\text{m}$ , those with arteriosclerosis 637.18  $\mu\text{m}$  and with no renal artery changes 551.11  $\mu\text{m}$ . There was a difference between the thicknesses of media in different types of FMD (FMD type I = 590.83  $\mu\text{m}$ , FMD type IIA was 476.92  $\mu\text{m}$ ). Renal arteries of patients with arteriosclerosis were larger in comparison to arteries with no changes (4.2 mm: 3.5 mm), and control arteries obtained at autopsy, however this difference was not statistically significant.

Conclusion: Renal arteries of patients with RCC and atherosclerotic changes have thicker media and larger diameter in comparison with controls, and those with FMD.

The cause of such changes and their relationship to the degree of tumor necrosis and other changes in renal arteries should be further analyzed.

#### P 858

##### RENAL SINUS INVASION IN RENAL CELL CARCINOMAS.

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The renal sinus (RS) is the fatty region located near the renal cortex and not delineated from it by a fibrous capsule. The large quantity of vessels promotes a hematogenous and lymphogenous dissemination of the cancer cells at the tumor invasion to this zone. However, the present TNM staging system for renal cell carcinomas (RCC) does not take into the RS involvement.

The aim of research was to estimate the frequency of the RS invasion in RCC and its connection with tumor stage, grade and the histological form.

118 RCC were investigated (86 clear cell carcinomas, 13 papillary carcinomas, 11 chromophobe cell carcinomas, 8 oncocytomas). The tumor size of 51 renal carcinomas did not exceed 7cm; 67 RCC were over 7cm. The RS invasion was revealed in 81 cases RCC (68,6 %): 66 (76,7 %) clear cell carcinomas, 7 (53,8 %) papillary renal carcinomas, 8 (72,7%) chromophobe cell cancer. Oncocytomas did not invade into the RS. The RS involvement correlated with a nuclear grade of RCC. The RS invasion was 10 of 30 a low nuclear grade tumours (Fuhrman's grade 1-2) (33,3%), 55 of 71 a moderate nuclear grade tumours (Fuhrman's grade 2-3) (77,5%) and all of 17 a high nuclear grade tumours (Fuhrman's grade 3-4). However, the RS invasion did not depend on the tumor size. The RS involvement was marked at 39 (33,1 %) of patients at the tumor size up to 7 cm, and in 42 (35,6 %) cases at the tumor size over 7 cm. Tumor localization was the prior feature than tumor size. Among the RCC up to 7cm, the RS involvement was 19 (65,5%) of the 29 lateral location tumours, and 20 (90,9%) of the 22 medial location tumours. Besides fatty tissue, 44 (37,3 %) RCC invaded into the RS veins and lymphatic vessels. 11 (9,3 %) kidney cancer invaded into the lumen of the main renal vein. In 25 (21,2 %) RCC, the RS invasion combined with intrarenal dissemination of tumor. At the absence of the RS invasion, metastases were lacking.

The RS involvement should be regarded as a risk factor of the tumor dissemination. The prognostic value of the RS invasion in RCC is necessary to take into future revision of the TNM staging system for the tumors of this localization.

#### P 859

##### INCIDENTALLY DIAGNOSED RENAL CELL CARCINOMA: CLINICOPATHOLOGICAL FEATURES.

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In the last 20 years, with the increasing use of abdominal ultrasound and CT scans, many renal tumours are being diagnosed incidentally during examinations for other conditions.

Our study aimed to compare the clinicopathological features of renal cell carcinomas (RCC) discovered incidentally with those of symptomatic tumours. We retrospectively analyzed 149 patients subjected to radical nephrectomy.

61 tumours (40,93%) were diagnosed incidentally. No significant difference was detected between both groups in regards to middle age, sex, and histological type. A greater incidence of tumours in stage T1a was found in incidental RCC (59,21% vs 12,5%), while advanced forms (stage T3) were more frequent in symptomatic RCC (24,59% vs 67,04%). 33 (54,1)% incidental RCC were a low nuclear grade (Fuhrman's grade 1 or 2) and only 3 (5%) tumours were high grade. 2 (3,3)% incidental RCC were N1, and none were M1, whereas 15 (17%) symptomatic RCC were N1-2, and 9 (10,2%) were M1. Survival after 5 years was of 69,1% for symptomatic patients in comparison to 85,7% for the incidental group, which implies high statistical significance (log rank = 0.0018).

The patients with incidentally discovered RCC had a prognostically more favorable tumor size, stage and grade.

#### P 860

##### ASSOCIATION OF CXCR4 CHEMOKINE RECEPTOR EXPRESSION WITH MMP AND TIMP MRNA TRANSCRIPTION IN CLEAR-CELL RENAL CELL CARCINOMA

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Introduction: CXCR4 chemokine receptor mediated signalling is of importance for the metastatic process in a variety of human cancers. We have recently found increased CXCR4 expression levels in clear-cell renal cell carcinoma (ccRCC) compared with normal renal tissue. This study also revealed an association between strong CXCR4 expression and poor tumour-specific survival indicating the importance of this chemokine receptor in renal tumour biology (ccRCC; Nature, 425, 2003). Interestingly, it has been shown in vitro that ligand stimulation of CXCR4 leads to alterations in the expression levels of certain Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Matrix Metalloproteinases (TIMPs). Since deregulated expression of MMPs and TIMPs is one of the pre-requisites for tumour growth and tissue invasion in many human cancers including ccRCC one might consequently speculate that increased CXCR4 signalling contributes to tumour progression and metastatic dissemination by influencing the expression of MMPs and TIMPs.

Aims: In order to analyse the association of CXCR4 and MMP/TIMP expression in ccRCC we have correlated the mRNA abundance of certain MMPs and TIMPs with the CXCR4 expression status in primary tumour samples.

Methods: mRNA expression levels of MMP2, MMP9, TIMP1, and TIMP2 were determined in 29 primary ccRCC and 7 normal renal tissues with known CXCR4 expression levels by RT-PCR using the LightCycler system. The abundance of MMP/TIMP transcripts were correlated with the CXCR4 mRNA expression status of the given tissue samples.

Results: Twenty-six of 29 ccRCCs showed an increase of more than 2-fold in the expression level of CXCR4. The average increase in CXCR4 expression was 7.3-fold (range 2.3-18.8-fold). Expression of TIMP1 and TIMP2 did not correlate with CXCR4 expression levels. In contrast, mRNA expression of MMP2 and MMP9 was significantly higher in tumours with strong CXCR4 expression ( $p=0.04$  and  $p=0.01$ , respectively).

Conclusions: CXCR4 up-regulation in ccRCC is associated with increased mRNA expression levels of MMP2 and MMP9. Our results provide first evidence for the involvement

of the CXCR4-MMP/TIMP pathway in renal cancer progression.

#### P 861

##### **CIGARETTE SMOKING, VON HIPPEL-LINDAU GENE MUTATIONS AND SPORADIC RENAL CELL CARCINOMA**

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#### INTRODUCTION

Cigarette smoking is a known risk factor for renal cell carcinoma (RCC) with a modestly increased rate ratio (RR) and a positive dose-response relation. Cigarette smoke metabolites can cause mutations in human DNA.

Von Hippel-Lindau (VHL) gene mutations are considered a primary event in the carcinogenesis of RCC.

#### PURPOSE

We investigated whether smoking is associated with mutations in the VHL gene in sporadic RCC.

#### METHODS

The Netherlands Cohort Study on diet and cancer (NLCS) includes 120,852 persons, who completed a self-administered questionnaire on smoking habits and other factors. After 11.3 years of follow-up, 337 cases and a random sample of 5,000 persons (subcohort) were used in a case-cohort approach. Collected tumor DNA (N=235) was analyzed for VHL gene mutations. RRs and corresponding 95% confidence intervals (CI) were estimated using Cox proportional hazard models, adjusting for age, sex and body mass index.

#### RESULTS

For men, RRs for total RCC were 1.52 (95% CI: 0.89-2.59) and 2.07 (95% CI: 1.20-3.56) for ex- and current smokers compared to never smokers, respectively. Estimates for women equaled 0.95 (95% CI: 0.57-1.59) and 1.37 (95% CI: 0.87-2.16), respectively. For men, RRs for current smokers compared to never smokers were 2.34 (0.79-6.94) and 2.95 (0.65-13.28), for VHL gene mutated and VHL wildtype tumors, respectively. For women, these estimates were 0.82 (0.35-1.93) and 2.04 (0.94-4.45), respectively. Results have to be interpreted cautiously because of small numbers.

#### CONCLUSION

Smoking was associated with RCC risk of men, but not specifically with VHL gene mutations, irrespective of sex, suggesting that smoking may cause RCC independent of VHL gene mutations.

#### P 862

##### **C-KIT EXPRESSION IN RENAL EPITHELIAL TUMORS**

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C-kit encodes the membrane-bound tyrosine kinase KIT, whose expression has been identified in several types of

human neoplasms. The overexpression of c-kit in chromophobe renal cell carcinoma and oncocytoma has been proposed as a possible specific hallmark of these neoplasms. The aim of this study is to establish immunohistochemical expression of KIT in a series of renal epithelial neoplasms and the normal renal tissues.

In this study we analysed KIT expression in 45 surgical specimens of clear cell, papillary, and chromophobe renal cell carcinomas and oncocytomas with the adjacent normal renal tissue. There were focal sarcomatoid differentiation in 6 cases. The extent of immunoreactivity was categorized as focal, moderate, and diffuse. Only membranous staining was considered positive.

All cases of chromophobe renal cell carcinomas (n=6) and two of 3 cases of oncocytomas showed diffuse membranous staining. Three of 33 cases of clear cell renal cell carcinomas were focally positive for KIT. One case with sarcomatoid differentiation showed focal positivity in the sarcomatoid areas. None of 3 papillary carcinomas were positive. In non-neoplastic kidney tissue adjacent to the tumor, strong KIT expression was detected in the cytoplasm of the renal tubules. The results suggest that KIT plays a role in the tumorigenesis of chromophobe renal cell carcinoma and oncocytoma, and could be a useful marker for differential diagnosis of different renal cell carcinomas. Overexpression of KIT in some subtypes of renal tumors indicates a potential therapeutic role for tyrosine kinase inhibitors in managing patients with these tumors.

#### P 863

##### **EPITHELIAL CELL ADHESION MOLECULE (EPCAM) EXPRESSION IN RENAL CELL CARCINOMAS (RCC). A TISSUE MICROARRAY (TMA) STUDY**

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Introduction: EpCam is a widely expressed adhesion molecule in normal epithelium of different organs and in epithelial neoplasms. Due to its membrane distribution, specific immunotherapies are now being used with promising results. Advances in the treatment of patients with RCC are utmost importance to improve the prognosis. The aim of this study was to study the expression of EpCam in RCC using TMA.

Methods: We studied retrospectively EpCam expression of renal tumors and matched normal renal tissue using a TMA. Different histologic subtypes of RCC were investigated, including 109 clear cell, 38 papillary RCC, 10 papillary adenomas, 50 chromophobe, 31 oncocytomas, 2 collecting duct carcinomas, and 2 mucinous tubular-spindle cell carcinomas. Primary clear RCC and corresponding metastases (n=32) were also studied. A mouse mAb anti-EpCam (clone VU-1D9, Novocastra, dilution 1:50) was applied after trypsin pretreatment. The immunoreactivity was correlated with clinicopathological data.

Results: EpCam is expressed in the distal nephron on normal renal tissue. EpCam overexpression was found in 17.4% of clear RCC (4.6% strong, 12.8% moderate). A total of 59 cases were negative. There was significant association between EpCam overexpression and distant metastases at diagnosis (pM stage) (p=0.03), and overall stage (p=0.039). Overall, 18.8% of metastases of clear RCC did show EpCam overexpression. EpCam overexpression was a significant univariate predictor of increased survival in clear RCC (p=0.035). Twelve (31.6%) of 38 papillary RCC showed EpCam overexpression. There was no different EpCam expression in type 1 and 2 papillary RCC. Three of 10 papillary adenomas showed EpCam overexpression. Fourty-

six (92%) chromophobe RCC showed EpCam overexpression (88% strong, 4% moderate), whereas only one (3.2%) oncocytoma revealed moderate EpCam in sparsely clustered cells ( $p < 0.05$ ). All cases of collecting duct and mucinous tubular-spindle cell carcinomas were negatives.

Conclusions: In clear RCCs, EpCam overexpression could be used as a prognostic marker (associated with improved survival). EpCam represents a potential target for immunotherapy (mAbs or vaccines) in the adjuvant and palliative treatment of RCCs. EpCam immunohistochemistry proves to be a useful tool in the differential diagnosis between chromophobe RCC and oncocytoma, because of the different staining patterns of these two entities.

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#### P 864

### GRANULOMATOUS REACTION IN PRIMARY ADULT RENAL NEOPLASMS: A CLINICOPATHOLOGICAL AND MOLECULAR STUDY OF 17 CASES

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Introduction: Granulomatous reactions (GR) within lymph nodes draining malignant neoplasms are well known. More rarely, GR may occur within the stroma of malignancies. The occurrence of GR in renal neoplasms appears to be much more rare (few cases reported).

Methods: Retrospective analysis of 488 renal cell carcinomas (RCC) and 25 renal angiomyolipomas (AML), diagnosed between 1994 and 2005, was performed. Clinicopathological data were reviewed. In selected cases, sections were stained with PAS, Grocott, and Ziehl-Neelsen. Immunohistochemical studies were performed using mAb against CD20, CD3, CD4, CD8, and CD68. Paraffin sections and/or frozen tissue were subjected to molecular analysis: PCR method was used to detect the presence of *M. tuberculosis* complex DNA.

Results: Among 488 RCC studied, 16 cases (3.27%) with GR were detected. Thirteen patients were male (81.3%), and three were female, with a mean age of 60.4 yrs (range: 34-77 yrs). No patient from our serie had any history of sarcoidosis or other granulomatous disease. The histological type of RCC were clear cell (n=12), papillary (n=1), chromophobe (n=1), and unclassifiable (n=2). Mean tumor size was 4.85 cm (range: 2.2-10 cm). Eleven of the 16 cases were TNM stage I or II. One case of GR was detected among 25 AML reviewed. GR was observed within the tumor stroma in all cases (focal: 7 cases, and diffuse 10 cases) and 3 cases had also GR within the normal kidney. One case demonstrated GR within lymph node metastasis. GR was characterized by groups of epithelioid cells and multinucleated giant cells (strongly positive for CD68), and small lymphocytes (predominantly CD3 although scattered CD20 positive were observed) without central necrosis in 14 cases (sarcoid-like). In 3 cases GR had central caseating necrosis: Mycobacteria were identified using Ziehl-Neelsen in 2 cases, and infection by *M. tuberculosis* complex were proved in 2 cases using PCR. Clinical follow-up was complete in 16 patients (median: 8.18 mos.; range: 1.83-109.13 mos): twelve patients were alive without clinically detectable tumor and granulomatous process.

Conclusions: The occurrence of granulomas within the stroma of renal neoplasms is extremely rare. Sarcoid-like GR in renal neoplasms could be an immunological response to tumor antigens. In patients with no previous diagnosis of tuberculosis, the discovery of caseating granulomas after nephrectomy for RCC is exceptional

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#### P 865

### STRONG COEXPRESSION OF OSTEOPONTIN AND VASCULAR ENDOTHELIAL GROWTH FACTOR CORRELATES WITH SURVIVAL IN CLEAR CELL RENAL-CELL CARCINOMA

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INTRODUCTION: Osteopontin (OPN) is a highly phosphorylated protein with numerous functions, including roles in bone remodelling, cell-adhesion, cell-mediated immunity, cell signalling, and angiogenesis. It has been recognized as a transformation-associated protein and as a potential prognostic factor for various human cancers. Several lines of experiments have implicated OPN in angiogenesis, and it has been shown that vascular endothelial growth factor may induce expression of OPN. Also, it has been shown that osteopontin overproduced by tumour cells acts as a potent angiogenic factor contributing to tumour growth.

PURPOSE OF THE STUDY: The aim of this study was to analyse the relationship between the OPN and vascular endothelial growth factor (VEGF) expression in clear cell renal-cell carcinoma. Also, we have analysed the association of VEGF and OPN coexpression with disease outcome.

METHODS: A total of 81 clear cell renal-cell carcinomas were immunohistochemically analyzed for the OPN and VEGF expression and scored in a semi-quantitative manner, by combining tumour epithelial proportion score and intensity score. Statistical analysis was performed by nonparametric Spearman's correlation coefficient method. Survival probabilities were estimated by the univariate Kaplan-Meier method and survival curves were compared by the log-rank test.

RESULTS: Renal-cell carcinomas showed heterogeneous staining pattern for both OPN and VEGF, ranging from focal expression of low intensity to strong and diffuse cytoplasmic staining of tumor cells. We found statistically significant correlation between OPN and VEGF expression at the level  $p = 0.0026$ , ( $r_s = 0.3304$ ). Also, the survival of patients with strong OPN and VEGF coexpression was significantly worse compared to those with either low or without OPN and VEGF coexpression ( $p < 0.003$ ).

CONCLUSION: Our results show the upregulation of OPN and VEGF in clear cell renal-cell carcinomas. The correlation between their expression and the association of their strong coexpression with poor disease outcome support the hypothesis that cooperation of OPN and VEGF may facilitate tumor progression in human cancer.

#### P 866

### CD34 EXPRESSION IN RENAL CELL CARCINOMA IS EXCEPTIONAL BUT MAY BE FOUND IN HIGH GRADE TUMORS

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Background:

CD34 is a transmembrane protein expressed by primitive hematopoietic and mesenchymal cells, endothelial cells, and a variety of other cell types. Its expression in epithelial cells and carcinomas is exceptional, and has never been reported in renal cell carcinoma (RCC). The observation of CD117 expression in some subtypes of RCC prompted us to investigate the expression of CD34, as both molecules are often coexpressed in other proliferations, most notably gastrointestinal stromal tumors.

Design:

A total of 138 renal epithelial tumors (104 clear cell, 17 chromophilic, and 2

chromophobe carcinomas; 1 collecting duct carcinoma; 14 oncocytomas) were used to construct two different TMA blocks with 200 cores each. Consecutive sections of both were stained with H E and with antibodies (Ab) for CD34 (QBEND, Biomedica, CA), and CD117 (DakoCytomation, Denmark). Representative sections of the positive tumors were stained with Ab for CD34, CD117, CD10, Cam 5.2, AE1-AE3, keratin 7, keratin 34 E12, vimentin, and HMB45. The immunohistochemical reaction was visualized with the Envision system (DakoCytomation, Denmark).

#### Results:

Only two out of 138 tumors showed intense and diffuse CD34 expression but were negative for the CD117 Ab. In standard sections, both tumors showed strong and diffuse membranous staining of epithelial cells with CD34 Ab, and were in addition positive with Ab for CD10, Cam 5.2, AE1-AE3, and vimentin. Keratins 7 and 34 E12, and HMB45 were negative. Both cases were morphologically and immunohistochemically classified as clear cell RCC. Case 1 is a 53 year-old man with a Fuhrman grade 4, stage T1 N0 M0 tumor, that subsequently developed lymph node metastases, and that is alive with residual disease 15 years after surgery. Case 2 is a 70 year-old woman with a grade 4, T3a N1 M0 tumor, and she is alive without disease 4 years after surgery.

#### Conclusion:

CD34, although exceptionally, may be expressed in clear cell RCC, without CD117 coexpression. The two cases in this series suggest that this feature may be observed in a subset of high grade tumors progressing to or presenting with high stage disease.

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#### P 867

##### TISSUE MICROARRAY OVEREXPRESSION OF CYCLIN D1 IN RENAL EPITHELIAL TUMORS

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#### ABSTRACT

Background: Cyclins have an essential role in cell cycle control. Cyclin D is involved in the G1/S transition. The prevalence, biological meaning and prognostic impact of Cyclin D1 (CyD1) expression in renal cell carcinoma (RCC) are controversial. Some studies suggest a favorable role, but other series do not support this conclusion. This is a descriptive study of the immunohistochemical (IHC) overexpression of CyD1 in a RCC/oncocytoma tissue microarray (TMA), and its related clinicopathological variables in different tumor subtypes.

Design: A total of 138 renal epithelial tumors (104 clear cell (CCRCC), 17 papillary chromophilic (PCRCC), and 2 chromophobe carcinomas; 1 collecting duct carcinoma; 14 oncocytomas) were used to construct two different TMA blocks, 200 cores each. Consecutive sections were stained with an antibody for CyD1(DSC-6, DakoCytomation, Glostrup, Denmark). The IHC reaction was visualized with the Envision system (DakoCytomation, Glostrup, Denmark). CyD1 overexpression was defined as a 3+ intensity in more than 20% of the tumor cell nuclei.

Results: We found CyD1 overexpression in 9 (65%) of 14 oncocytomas, 23 (22%) of 104 CCRCCs, and 5 (30%) of 17 PCRCCs. From the 23 CyD1 overexpressing CCRCCs, 19 (82%) were low stage (T1-2), 17 (74%) were low grade (G1-2), and 18 (78%) were alive without disease. In the overall group of CCRCCs, 68% of the tumors were low stage, 57% were low grade, and 66% were alive without tumor. From the 5 CyD1 overexpressing PCRCCs, 4 were low stage, 1 was low grade, and all were alive without disease. In comparison,

65% of all the PCRCCs were low stage and low grade, and 70% were alive without disease.

Conclusion: 1.CyD1 is not uniformly overexpressed in all oncocytomas. 2.The proportion of CCRCCs overexpressing CyD1 is slightly lower than that of PCRCCs. 3.In both tumor types, this feature seems to be associated with more favorable prognostic variables than their respective overall groups. There is a paradoxically increased proportion of high grade cases in overexpressing PCRCC, that is not associated with higher stages or more adverse outcome. 4.CyD1 overexpression is relatively uncommon in RCC, and although it is associated with better outcome, it does not appear to be a useful tool in the prognosis of these tumors.

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#### P 868

##### TISSUE MICROARRAY OVEREXPRESSION OF P53 AND PAB 240 IN RENAL CELL CARCINOMA

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Background: The prognostic impact of p53 overexpression in renal cell carcinoma (RCC) is not fully understood. In different series, with diverse immunohistochemical (IHC) and evaluation protocols, variable p53 overexpression is reported, although association with poor outcome is usually suggested. We investigated the IHC overexpression of p53 in a RCC tissue microarray (TMA), using strictly standardized methods. We also studied expression of Pab-240, an antibody (Ab) that recognizes a p53 epitope exposed in many mutant forms of this protein. Expression of this Ab in RCC has not been previously reported.

Design: 138 renal epithelial tumors (104 clear cell (CRCC), 17 papillary chromophilic (PRCC), 2 chromophobe, and 1 collecting duct carcinoma; 14 oncocytomas) were used to construct two different TMA blocks (200 cores each). Ab for p53 (DO7, Novocastra, UK), and mutated p53 (Pab 240, DakoCytomation, Denmark) were used. A standardized IHC protocol included mutated cell lines as controls. p53 overexpression was defined as a 3+ intensity in >20% of the nuclei. Pab 240 was investigated in p53+ and - cases.

Results: p53 overexpression was observed in 12% (17 of 138 tumors): 30% (5 of 17) of PRCC and 12% (12 of 104) of CRCC. From those, 2 CRCC and 2 PRCC were positive with Pab 240, and the remaining cases in the TMA were negative. From all TMA cases, CRCC were high grade in 43%, high stage in 32% and progressed in 33% of cases. In contrast, 92% (11) of the p53 overexpressing CRCCs were high grade (3 G3 and 8 G4) and 75% (9) were high stage (6 T3 and 3 T4) and died with tumor. Similarly, p53 negative PRCCs were high grade in 20%, high stage in 30% and progressed in 20% of the cases, while 80% (4) of the overexpressing PRCCs were high grade (3 G3, 1 G4) and 40% (2) were high stage (T3,T4), progressing tumors.

Conclusion: 1. In both CRCC and PRCC, p53 overexpression is associated with a marked increase in grade, stage, and tumor progression. 3. Negativity of Pab 240 does not exclude non-productive mutations, but suggests that p53 overexpression is probably not associated with mutation. 3. p53 overexpression is uncommon in RCC, and is more often observed in PRCC than in CRCC. A large number of RCCs should be examined both by IHC and molecular methods to establish the prognostic and pathogenetic role of TP53 in renal cancer. (Funded by Fondo de Investigacion Sanitaria, FIS 99/0736, Spain.)

#### P 869

##### TESTICULAR GERM CELL TUMOR PATHOLOGY REPORTING

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#### Introduction.

Accurate pathology report is crucial in providing information regarding prognostic factors and possible further treatment.

Purpose of the study.

To compare the standard of pathology reporting for testicular tumors with the checklist provided by the College of American Pathologists (CAP) and to evaluate potential variation in reporting by hospital type.

#### Methods.

We reviewed 211 pathology reports for adherence to recommendations of the CAP and calculated the overall percentage of each item. We classified hospitals by teaching and non-teaching hospitals and used the chi-squared test to determine whether statistically significant difference existed by hospital type (teaching and non-teaching).

#### Results.

Adherence to CAP recommendations was 100% for description of histologic type, 87% for tumor size and 82% for laterality. All other criteria were reported in <80% of cases. Overall, teaching hospitals demonstrated greater adherence to CAP recommendations when compared with non-teaching hospitals ( $p < 0.05$ ).

#### Conclusions.

Pathology reports of testicular germ cell tumors omitted some critical items. The variability in reporting is based on hospital type.

#### P 870

##### **MORPHOLOGICAL VARIANTS OF A V. TESTICULARIS SINISTRA FORMATION IN CHILDREN WITH VARICOCELE**

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There is no clear conception about the venous system, generated from pl. pampinifomis in a retroperitoneal space in patients with varicocele now. We evaluated the incision biopsies of vessels received during a high ligation from 20 boys from 8 to 15 years old. The biopsies were fixed and paraffinized. Light microscopy, immunohistochemistry was performed with labeled, specific anti-VEGF and CD 34 antibodies.

There were arterial vessels, surrounded with groups of muscular type veins and a plenty of engorged capillaries and postcapillary venules in biopsies. We found three morphological variants of the muscularis type veins. Type I was presented by veins of a large diameter with gaping lumens and thick walls composed of three layers. The most thin internal layer contained endothelial and longitudinal smooth muscle cells; a medium layer included circularly directed smooth muscle cells; and the thickest external layer was composed of smooth muscle cell bundles directed longitudinally and separated from each other by collagen fibers. This morphological variant of veins was considered as the basic trunk of v. testicularis.

Type II veins had a irregular configuration of a lumen. Their walls were thinner, than these of type I veins and were composed of three layers. The thick longitudinal external layer had fragments of collagen and elastic fibers. The flabby valves or fragments of the valves were found in some veins. Dilated vessels with fibrous walls were present.

Type III veins had a small diameter and thick walls with well defined three layers. The external muscular longitudinal layer was much thicker than the medium and internal layers both. The characteristic feature of these vessels was a present of

small "pillows" generated from longitudinal smooth muscle cells of the internal layer. These "pillows" filled the lumens of some veins. This is considered to be an adaptive response to prevent a retrograde blood flow.

Engorged capillaries are most likely the newly formed vessels ensuring improved outflow of blood. This is confirmed by expression of the angiogenic factor VEGF and CD34 on endothelium cells.

The morphological analysis of vessels within the incision biopsies revealed multiple character of v. testicularis sinistra formation in pediatric patients with varicocele, represented by three morphological variants of muscularis type veins. First of them is principal. We showed the possible variants of compensatory drainage of blood from the pl. pampinifomis system.

#### P 871

##### **EXPRESSION OF KLF-6 TRANSCRIPTIONAL FACTOR IN CIS AND MALIGNANT TESTICULAR GERM CELL TUMORS.**

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**INTRODUCTION.** KLF-6 (Kruppel-like factor 6) is a transcriptional factor that has been shown to be mutated in a significant number of prostate and colon carcinomas. KLF-6 is a candidate tumor suppressor gene.

**PURPOSE OF THE STUDY.** KLF-6 immunoexpression was explored in 21 seminomas, 12 embryonal carcinomas, 7 immature teratomas, 4 mature teratomas, 6 yolk sac tumors, and 27 combined tumors, including carcinoma in situ (CIS) as well as 4 normal adult testes.

**METHODS.** A polyclonal anti-KLF-6 antibody (Santa Cruz) was used at 1:400 dilution. Streptavidine peroxidase Zymed kit was employed. The immunoreaction was revealed with DAB. KLF-6 western blotting was done in 13 cases, including 2 normal testis.

**RESULTS.** Variable KLF-6 expression was observed in the cytoplasm of spermatogonia in human normal testis. However a significant increased expression was shown in the cytoplasm of CIS cells. This intense stain was constantly seen in intratubular and infiltrative seminoma. In mature teratoma, KLF-6 the expression of KLF-6 is higher in the epithelial component than in the mesenchymal tissues, although KLF-6 is always present in the immature neuroepithelial teratomatous differentiation. Interestingly, KLF-6 expression is lost in undifferentiated embryonal carcinoma, the most aggressive of the testicular cancers studied. No specific KLF-6 stain in the different patterns of yolk sac tumors was observed. KLF-6 expression is significantly higher by Western blotting and densitometric measuring in the cancer cases than in the normal cases.

**CONCLUSION.** The overexpression of KLF-6 in all types of testicular germ cells tumors and even in CIS cells suggests that KLF-6 is a transcriptional factor that may be implicated in the development and progression of testicular malignant tumors.

#### P 872

##### **SEMINIFEROUS TUBULES WITH AN ABNORMAL CONFIGURATION: A NEW LESION OF THE TESTICULAR DYSGENESIS SYNDROME.**

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**INTRODUCTION.** The entity of "Testicular dysgenesis syndrome" includes several heterogenic pathologies as undescended testis, hypospadias, testis cancer and infertility. Associations of different testicular histological lesions have been observed in the testicular dysgenesis syndrome including undifferentiated seminiferous tubules, partial Sertoli-cell-only tubules, testicular microliths, CIS cells and Leydig-cell micronodules.

**MATERIAL AND METHODS.** In a retrospective revision of 450 prepubertal and adult patients with infertility, cryptorchidism, intersex and testicular cancer from the files of La Paz University Hospital, 15 cases with seminiferous tubules with abnormal configuration were observed.

**RESULTS.** In all 15 cases a spectrum of different patterns of malformative configuration of the seminiferous tubules abnormally were seen. The morphological anomalies of the seminiferous tubules included frequent ramifications, annular configuration, culde sac dichotomy ending and changes in diameter in different segments, with dilatations alternating with hypoplastic stenosed tubular segments. Different grades of impairment of the spermatogenesis was observed in this variants of abnormally configured tubules. This new lesion was found in: 7 cryptorchidic testis (5 with ambiguous external genitalia), 3 patients with descended testes that consulted on infertility, 2 patients with testicular microliths and 3 in the testicular biopsy contralateral to the testis excised by testicular cancer.

**CONCLUSIONS.** Seminiferous tubules with abnormal configuration was present both in infantile and adult testis which suggest a primary lesion. The lesion we describe should have been begin at that moment of the fetal development, because the abnormal tubules resemble the first stages of the formation of sexual cords in normal fetus. However, the absence of remodeling of these sexual cords will cause the persistence of seminiferous tubules with an irregular caliber, frequent anastomosis and an irregular spermatogenesis varying from normal to completely absent. We suggest that the seminiferous tubules with abnormal configuration are characteristic lesions founded in testicular dysgenesis syndrome.

**P 873**  
**THE EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) ON HISTOPATHOLOGICAL CHANGES IN TESTICULAR ISCHEMIA-REPERFUSION INJURY**

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**Purpose:** Testicular torsion causes an enhanced formation of reactive oxygen species which contributes to the pathophysiology of ischemia-reperfusion injury in testis. We evaluated here the effect of caffeic acid phenethyl ester (CAPE), a new antioxidant and anti-inflammatory agent on histopathological changes in testicular ischemia-reperfusion injury.

**Materials and Methods:** Adult male Wistar rats were divided into 6 groups of 5 each: Control group1 (n=5), sham operation group2 (n=5), torsion/detorsion (T/D) group3 (n=5),

T/D+Saline group4 (n=5), T/D+CAPE group5 (n=5) and T/D+CAPE group6 (n=5). One group served to determine baseline values of histopathological parameters, 1 that underwent sham operation served as a control, others were subjected to left unilateral torsion (2 h) and detorsion (4 h) periods. Rats were sacrificed and unilateral orchiectomy was performed. Testis tissues were put into Bouin's solution. After routine tissue processing myeloperoxidase (MPO) and inducible nitric oxide synthase (iNOS) immunohistochemical methods were studied from paraffin embedded tissues.

**Results:** Treating rats with CAPE (applied at 10 µmol/kg, 30 min. prior to T/D) attenuated the testicular injury and as well as the tissue levels of MPO. At the same time testis tissue showed an increase in iNOS activity.

**Conclusion:** Our results suggest that CAPE treatment have a protective role on testicular T/D and this effect may be due to inhibiting the neutrophil mediated cellular injury.

**P 874**  
**CANCERS OF THE TESTIS: A RETROSPECTIVE STUDY OF 29 CASES**

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**Introduction:**

Cancer of the testis (CT) is rare, accounting for about 1 % of all cancers in men; in Europe, the annual incidence rates range between 7,3 (West) and 4,6 (south) per 100.000; in Africa, the annual incidence rates are very low (less than 1 per 100.000). It is the commonest cancer in men under 45 years old. Cryptorchidism is the only confirmed risk factor. The majority of CT are testicular germ cell tumours which are divided into seminoma and non seminoma types for treatment planning because seminomatous testicular cancers are more sensitive to radiotherapy.

**Objectives:**

The purpose of this study is to discuss the clinic and pathologic characteristics, the course and the prognosis of CT. **Methods and materials:**

We report a retrospective study of 29 cases of CT diagnosed in the department of pathology of the Habib Bourguiba hospital-Sfax-Tunisia over a five years period (2000-2004).

**Results:**

The mean age of our patients was 40 years (extremes 1 and 83 years). Undescended or maldescended testis are found in 80% of cases; all of CT are clinically detectable at initial presentation (diffuse pelvic mass 50%, inguinal swelling 30%; hardness scrotal mass: 20%). The surgical procedure to make the diagnosis was a radical orchidectomy in all cases. Pure seminoma was the most frequent histological type (30%) followed by mixed germ cell tumours (20%); 18% of patients have a testicular lymphoma and are old mens. Only four cases of paratesticular tumours are found and are a rhabdomyosarcoma type in three cases. After testicular surgery, the management was depended on type and stage of disease. The mean follow up was 47 months, 20% of patients with mixed germ cell tumours developed a pelvic recurrence versus any relapse for pure seminoma; a paratesticular rhabdomyosarcoma represent the most aggressive pathologic variant and early visceral metastases are observed in all cases.

**Conclusion:**

Cancer of the testis is a relatively rare disease; the majority are testicular germ cell tumours who occur most frequently in young men and have a good prognosis: it is highly treatable, often curable cancer; many germ cell tumours produce tumoral markers (AFP, HCG, LDH) who are useful in the diagnosis and staging of disease, to monitor the therapeutic response and to detect tumour recurrence.

**P 875**

### CASE OF ABSOLUTE RELAPSE OF "BURNT-OUT" TESTICULAR TUMOR OF LECITHAL SAC

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We report the case of a 25-year-old man with testicular tumor of lecitthal sac having as initial clinical indication a retroperitoneal tumor.

Macroscopically, the retroperitoneal tumor consisted of numerous grey, elastic- friable pieces of tissue. The maximum dimensions of the greatest piece were 6x4,5x3,5 cm.

Microscopically, the tumor was solid, dedifferentiated and metastatic. From the immunohistochemical examination performed with a great range of antibodies (CEA, EMA, LCA, UCHL1, CD 20, Vimentin, SMA, HMB-45, CD 56,HCG, PLAP, CD68, Ulex europaeus, CD 34, Desmin, S-100, MIC-2, pancytokeratin AE1/AE3, Synaptophysin) we concluded that the tumor cells were positive only for low grade molecular weight cytokeratin (AE1).

On ultrasonography numerous punctate ultrasonic lesions were found in the left testis. Later on, left testectomy was operated. The histological examination of the testis revealed a small scar with calcification and numerous testicular tubules with decations of intratubular neoplasia of germinal cells. Further estimation of the retroperitoneal tumor showed that the tumor cells were positive for AFP.

In conclusion, we can report that in case of a retroperitoneal metastatic tumor we should examine testis meticulously.

#### P 876

### ADENOCARCINOMA ORIGINATING FROM A MATURE TERATOMA OF THE TESTIS; CASE REPORT

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Malignant transformation in testicular teratomas has been reported very rarely in the literature. Although testicular teratomas in childhood are regarded as benign neoplasms, if these tumors are left untreated until advanced ages, the risk of malignant transformation may be in question.

We report a case of differentiated adenocarcinoma originated from colonic glands existing in primary testicular teratoma.

#### P 877

### PURE SEMINOMA IN A MALE PHENOTYPE 46,XY TRUE HERMAPHRODITE

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### INTRODUCTION

True hermaphroditism (TH) is a rare cause of intersexuality characterized by the development of ovarian and testicular tissue in the same individual. The most frequent karyotype is 46,XX followed by various forms of mosaicism. The karyotype 46,XY is extremely rare, present in only 7% of cases. Gonadal tumours are reported in 4.6% of patients with TH, most of them are gonadoblastoma. Herein, we report a case of a pure seminoma occurring in a male phenotype 46,XY true hermaphrodite.

### PATIENT AND METHODS

A 32-year-old man presented with pelvic pain, constipation and important weight loss. Physical examination revealed bilateral cryptorchidism and a firm pelvic mass, without gynecomastia.  $\alpha$ -human chorionic gonadotropin,  $\alpha$ -fetoprotein and carcinoembryonic antigen had normal levels in serum. The computerized tomography showed a large retrovesical tumor of unknown origin. The ultrasonography guided biopsies concluded to a pure seminoma. At surgery, an uterus, two tubes, two gonads, a prostate and two seminal vesicles were identified. The tumour (7 cm), adherent to the uterine cervix and to the prostate, was almost entirely excised.

### RESULTS

The pathological examination confirmed the diagnosis of pure seminoma. The gonads were consistent with two ovotestis one of them was massively unfiltered. Cytogenetic evaluation of peripheral leukocytes revealed a 46,XY karyotype with a sex-determining region of the Y chromosome (SRY gene) detected by PCR. The patient received a complement of radiotherapy (35 Gy). He was free of disease six months later.

### CONCLUSION

TH is a complex and heterogeneous condition. SRY gene seems to play a major role. Further studies are needed to better characterize its pathogenesis. Few cases of tumours occurring in the gonads of true hermaphrodites have been reported. Our report is peculiar by the arising of a pure seminoma in the ovotestis of a male phenotype 46,XY true hermaphrodite.

#### P 878

### HISTOLOGICAL INHOMOGENEITY IN MULTIPLE TESTICULAR BIOPSIES

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### Introduction:

Histological evaluation of testicular biopsy is an important method in management of male infertility.

Materials and methods: Multiple testicular biopsies of 143 infertile men were taken using atraumatic microsurgical method. Samples were fixed for 24 hours in Bouin's fixative or buffered formaline. After paraffin embedding 3-4  $\mu$ m slides were cut and stained by HE. Data of histological reports were examined concerning inhomogeneity using three different methods.

### Results:

1. Etiological inhomogeneity: 43 of the 86 azoospermic, 22 of the 29 OATS and 21 of the 28 oligozoospermic patient showed different etiology in the biopsies.

2. Spermatogenetic activity: 24 of the azoospermic individuals presented mature spermatozoa only in some of the samples. 13 men in the OATS group showed spermatogenesis only in some of the biopsies. 10 of the oligozoospermic patients was proven inhomogenous concerning spermatogenetic activity.

3. Statistical inhomogeneity: The number of mature spermatozoa counted in 10 round shaped tubules was evaluated. In the azoospermic group an average of 59,4 +/- 92,3 was found. In the OATS an oligozoospermic group it was 122 +/-120,4 and 152,7 +/-130,5, respectively.

### Discussion:

Our data proved that function of testicular tissue shows marked local differences in etiology and spermatogenic activity either. Taking of single biopsy might give a false impression of spermatozoa content and alterations. Histological evaluation of multiple biopsies helps andrologist to plan further therapy.

#### P 879

### IMMUNOHISTOCHEMICAL EVALUATION OF THE STEM CELL FACTOR RECEPTOR (SCFR, CD-117, C-KIT) EXPRESSION IN TESTICULAR GERM CELL TUMORS

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**BACKGROUND AND OBJECTIVE :** Testicular germ cell tumors consist 95% of all malignant tumors arising in the testis and most of them are sensitive to chemotherapy. However 10-20% of those develop resistance to chemotherapy. Recently, targeted therapy drugs are in use for the management of chemoresistant malignancies. Imatinib (STI571) is one of them and targets c-Kit among other molecules. Overexpression or constitutive activation of c-Kit by mutations have been associated with the development and progression of various malignancies. In this context we investigated the expression of c-Kit in testicular germ cell tumors in order to evaluate the incidence of its expression in various tumor types along with the possible use of c-Kit inhibitors as a tool for therapeutic intervention.

**MATERIALS AND METHODS:** In this study, 169 archival tumor samples were analyzed immunohistochemically, using a polyclonal rabbit anti-human antibody against c-Kit (DAKO). From them 61 were seminomatous (49 pure, 11 anaplastic, 1 spermatocytic) and 108 non-seminomatous tumors (36 malignant teratoma undifferentiated, 15 malignant teratoma trophoblastic, 21 malignant teratoma intermediate, 32 malignant teratoma combined, 5 other). c-Kit expression was considered as positive, if >10% of tumor cells displayed membranous staining.

**RESULTS:** 77.5% (n=38) of pure seminoma exhibited a positive immunostaining for c-Kit, while only 18.2% (n=2) of anaplastic were identified as positive (p<0.001). Out of 32 cases with a combined tumor, 17 showed a positive c-Kit reactivity and 15 a negative one, while only 2 non-seminomatous tumors had a positive staining. Although c-Kit expression is strongly correlated with seminomatous histological features (p<0.001), there is no correlation with stage (p=0.19) or chemotherapy treatment response (p=0.11). Overall, 4 (1 seminoma, 3 combined tumors) out of 22 chemoresistant cases revealed a positive expression for c-Kit and one of them had a positive response to Imatinib treatment in combination with chemotherapy.

**CONCLUSIONS:** c-Kit is expressed in the majority of seminomas and in seminomatous components of the combined tumors, in a minority of anaplastic seminomas and rarely in non-seminomatous tumors. The recent development of c-Kit inhibitors may offer a possibility of cure in chemoresistant testicular germ cell tumors overexpressing this transmembrane receptor.

#### P 880

### BLOOD VESSELS IN TESTICULAR BIOPSIES OF INFERTILE MEN

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#### INTRODUCTION

Infertile men were shown to have reduced lumen of the testicular blood vessels. The reduced lumen is mostly due to the process called “hyalinization”, pathological accumulation of glycoprotein substance within the blood vessel wall. Hyalinization could be found in the case of cryptorchidism, immune orchitis, Klinefelter’s syndrome and in the significant number of patients with idiopathic infertility. Microangiographic data on the testicular arterioles in severe cases of male infertility demonstrated irregular blood flow, damage of the blood vessel wall and reduced spermatogenesis within the seminiferous tubules.

#### AIM

There is a lack of electron microscopic, immunohistochemical and morphometric studies dealing with the structure of testicular blood vessels in patients with the non-obstructive azoospermia. Therefore, the aim of our study was: a) to identify the most frequent blood vessel type affected in this group of infertile patients; b) to describe blood vessels changes both at light and electron microscopic level; c) to perform an immunohistochemical and morphometric (stereological) analysis of small blood vessels of the testicular biopsies of infertile men.

#### MATERIALS & METHODS

48 couples that consulted andrologist at the Urology Clinic (University of Zagreb, Medical School) for infertility in the period 1997-2003 were included in the current study. The infertility problem was due to the male factor. In 40 infertile men, a non-obstructive azoospermia could be diagnosed. 8 patients had obstructive azoospermia with completely preserved testicular tissue and served as controls. Whenever possible, a bilateral biopsy was performed. Testicular biopsies were examined by light and electron microscopy, immunohistochemistry (IHC) and stereology.

#### RESULTS

Interstitial blood vessels in the control group displayed regular architecture. In infertile group, there was a change in the morphology of some arterioles and venules. In the subendothelial layer of these vessels, an accumulation of a hyaline could be observed. Observed by the transmission electron microscope, the thickened subendothelial layer had an accumulated material of a moderate electron density. IHC and stereological analysis pointed out a significantly increased number, volume, length and curvature of capillary network in the infertile group of patients.

#### CONCLUSION

In the testis of infertile men, there is a pathological change of arterioles, venules and capillaries.

#### P 881

### PRIMARY MALIGNANT LYMPHOMA OF THE TESTIS

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**Introduction and aim:** Primary testicular lymphomas are rare entities representing 1-2% of non-Hodgkin lymphoma (NHML) and 1-7% of malignant testicular tumors and they are the most common testicular tumor in men older than 50 years of age. This study included 8 cases of inpatients

diagnosed by echography and NMR) with testicular tumors. The age of patients was between 46 and 81 (with a mean of 52). The tumors were unilateral, with disease limited to testicle and accompanied by pain. Orchestomy was performed as first therapeutic and diagnostic purpose. All patients were clinically staged according to the Ann Arbor criteria and received a doxorubicin based chemotherapy regimen (CHOP, MTX, CVP, and Leukeran).. A standard chemotherapy protocol has not been used because of reduced number of patients.

Material and method: Tumor fragments were fixed in 10% formalin, paraffin embedded, sectioned and standard H.E. stained. Immunohistochemistry for L26, Alphafetoprotein, NK1, CD30, and CLA was performed.

Results: Microscopy revealed in all cases a stromal proliferation with medium size cells, monomorphic shape and prominent nucleoli. Alphafetoprotein was positive in seminal tubes and negative in tumor, NK1 in small lymphocyte and negative in tumor and L26 diffuse positive in tumor. We were able to follow up only four patients.

Conclusions: The diagnosis was of NHML in 6 cases and for 2 secondary involvement of hematopoietic malignancy (myeloid sarcoma and leukemia). Lymphoma cases were typed using REAL classification as small and large B cell lymphoma. Unfavorable evolution with 6 months relapse and one death prove a more aggressive evolution of primitive testicular lymphoma.

**P 882**  
**SEMINOMA WITH A MICROCYSTIC AND TUBULAR GROWTH PATTERN**

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A 63year old gentleman presented with a large right testicular mass, which was subsequently resected. On macroscopic examination, the testis was extensively replaced by a cream coloured tumour, which focally had a nodular appearance. On histological examination, the tumour was composed of polygonal cells with clear cytoplasm and a stippled chromatin pattern. Much of the tumour had a solid growth pattern but, in large areas, showed florid microcystic change and tubule formation. The spaces were generally small and closely packed. Granulomata were present and there was a mild lymphocytic inflammatory infiltrate. There was no evidence of intra-tubular germ cell neoplasia in the residual tubules. On immunohistochemical staining, the tumour cells were negative for PLAP. The tumour cells were immunoreactive for CD117 (c-kit).

Seminomas with microcystic and tubular growth patterns are unusual and should not be confused with a yolk sac tumour, but the cytological features are helpful in distinguishing these two entities. It is not yet known if these tumours are associated with an adverse prognosis compared to the classical seminoma.

**P 883**  
**ISOLATED POLYARTERITIS OF THE MALE REPRODUCTIVE SYSTEM COINCIDING/ASSOCIATED WITH A MALIGNANT GERM CELL TUMOUR OF THE TESTIS: A CASE REPORT**

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Polyarteritis nodosa (PAN) is a necrotizing vasculitis affecting primarily small and medium-sized arteries. PAN presents mostly as a systemic disease with poor prognosis, rarely in an isolated form with a usually favorable outcome. Both forms may affect the male reproductive system. The etiology of this

inflammatory disease is still unclear. However, PAN has been described in the contexture of various diseases such as systemic lupus erythematoses, rheumatoid arthritis, hepatitis B and C, human immunodeficiency virus (HIV) infections and neoplasms. Therefore it has been speculated that triggering events may be identified in most cases in the future. We report for the first time a localized PAN of the reproductive system coinciding/associated with a mixed germ cell tumour of the testis in a 21-year-old man. One year after surgery he is without evidence of recurrence of either the tumour or PAN.

**P 884**  
**SURVEYING 177 TESTIS BIOPSIES OF INFERTILE MALES AND COMPARING THE RESULTS WITH OTHER DIAGNOSTIC MODALITIES**

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Background : Recently infertility becomes more common. In half of the infertile couples, men are completely or relatively responsible. Therapeutic approaches to infertile males impose great expenses on the family. Present study deals with sensitive clinical or paraclinical methods, other than testis biopsy, that could provide informative data regarding the absence or presence of sperms.

Material and Methods : Medical files of 177 infertile men were reviewed. Then findings of physical examination, hormonal studies, past medical history and testis aspiration were compared with hystopathological findings of testis biopsies. All these patients underwent testis biopsy following the initial studies. Spermatid count per tubule was considered to be the major criteria of fertility in patients' biopsies.

Result : of 177 patients, 95 (53.5%) had no chance of fertility due to the absence of mature spermatid or spermatozoa in their biopsies. Testis aspiration has a sensitivity of 86%, specificity of 85% and positive predictive value of 93% to detect sperm.

Conclusion : Our result revealed that physical examination, serum hormone assay and testis needle aspiration can not substitute hystopathological studies of the testis biopsy to prove infertility.

**P 885**  
**CANCER OF THE PENIS.PATHOLOGICAL ASPECTS OF SEVEN CASES OBSERVED IN CAMEROON(CENTRAL AFRICA)**

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BACKGROUND:Cancer of the penis is an uncommon malignancy in developed countries,but the incidence is as high as 17% of all male cancers in some undeveloped countries. The most important etiologic factor is the presence of an intact foreskin but this is still unknown.

METHODS/ We described the pathological aspects of seven cases of penile cancers observed in Cameroon, an undeveloped country of 15000000 inhabitants situated in Central Africa, within a period of twenty years (1984-2004). Human Papilloma Virus(HPV) DNA detection and typing were carried out on paraffin-embedded specimens of our cases by Polymerase Chain Reaction.

**RESULTS**

The patients aged 43 to 75 years and were circumcised. Four of the seven cases were observed in 2004 an one of this four patient was HIV(Hman immunodeficient Virus) positive and another was diabetic. All patients consulted late with

metastatic disease. The pathological aspect was squamous cell carcinoma. HPV DNA was detected in four cases.

**CONCLUSION/** Penile cancer is still rare in Cameroon were only 10% of cancers are confirmed histologically. The etiology is unclear. The HIV should be investigated as an etiologic cofactor.

#### P 886

##### **AMYLOID TUMOR OF THE RETROPERITONEUM: REPORT OF A CASE.**

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A case of primary localized multinodular amyloid tumor of the retroperitoneum is reported. A previously healthy 40 year old man presented with non specific symptoms of lower abdominal pain and a tumour mass in the inguinal region. The clinical diagnosis (based mainly on echography and computed tomography, the serology being normal) was that of a very large tumor mass, of 20/8/6 cm, occupying the right retroperitoneal space, from the kidney to the inguinal region. The surgical excision revealed a multilobular tumor mass with an osseous consistency, apparently well circumscribed but infiltrating the adipose tissue and pressing the ureter. The patient needed an ureterostoma. Subsequent histology showed nodular amyloid deposits associated with osseous metaplasia, giant cell reaction and an apparently normal lymphocyte and plasma cell infiltrate. Further standard tinctorial tests, like a positive dimethyl benzaldehyde method for triptophan and permanganate-resistant congophilia, revealed the lesion to be a primary AL type immunoamyloid. Immunohistochemical investigations did not reveal any immunoglobulin light chain restriction, being  $\epsilon$  and  $\bar{\epsilon}$  intense positive, at the site of plasma cells but also of the amyloid deposits. These observations indicate that the amyloid nodules are produced by the plasma cells, which are of polyclonal rather than monoclonal origin, implying an inflammatory process rather than a solitary extramedullary plasmacytoma. The patient is well two years after the surgical excision, without recurrences.

#### P 887

##### **RNA QUALITY AND TISSUE PRESERVATION OF DIFFERENT FIXATIVES IN PARAFFIN EMBEDDED TISSUE**

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**Introduction** Several publications reported that RNA recovered from paraffin embedded tissue could be used as a substrate for PCR. Formalin fixed, paraffin embedded histopathology archives contain an inestimable collection, which could be analyzed to investigate changes in mRNA expression in pathological processes. It would be also reasonable to decide for future tissue conservation of

pathology samples which fixative to use to satisfy both histological and molecular biological needs.

**Aim** Three different fixation methods were compared by histology, immunohistochemistry and Real-Time RT-PCR.

**Materials and Methods** 24 hours' fixation with RNAlater™, acetone and formalin, followed by paraffin embedding, were compared on human endometrium samples. Hematoxylin-eosin staining and immunohistochemistry with mono- and polyclonal antibodies were performed. RNA was extracted and the transcription of different size amplicons (121, 225, 406 bp for GAPDH; 166, 310, 536 bp for beta globin) were compared by Real-Time RT-PCR.

**Results** The most adequate tissue preservation was found with formalin fixation, while there were no significant differences in the 3 fixative's yields for various size amplicons with Real-Time PCR (GAPDH 121 bp threshold cycle number for RNA later 28,4±3,3, acetone 26,9±2,7, formalin 27,8±3,0). PCR product quantity decreased with length regularly, longer than 225 bp amplicons gave poorer results.

**Conclusion** Acetone and formalin fixatives followed by paraffin embedding can also be used for RNA studies besides histological and immunohistochemical use, with the limitation of using short amplicons for paraffin embedded material. Longer amplicons gave less reliable results because of RNA fragmentation, while shorter amplicons can represent better real tissue composition. Our findings confirmed the importance of suitably designed primers' on the success of RNA recovery. Further investigations are necessary to define the correct use and validity of paraffin RNA for gene expression studies.

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#### P 888

##### **MOLECULAR ANALYSIS OF GENOMIC LOW COPY NUMBER DNA EXTRACTED FROM LASER-MICRODISSECTED CELLS**

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**INTRODUCTION.** Tissue microdissection techniques, allowing a correlation between the topologic organization of the cells and the molecular analysis of nucleic acid extracted, represent a key technique in molecular pathology. In fact, the presence of a wide spectrum of cell types in tissue samples may complicate the analysis of a particular cellular population. Laser-assisted microdissection is an advanced procedure to cut small tissue fragments as well as single cells by an ultraviolet laser beam in order to select a specific cell population of interest from a heterogeneous sample, under direct microscopic visualization. In the present report we evaluate the sensitivity of this method in order to perform genomic analysis from low copy number DNA.

**METHODS.** Laser-microdissection was performed using a Leica AS LMD system (Leica Microsystems, Germany) on smears of aploid (spermatozoa) and diploid (lymphocytes) cells, on sections of tissues routinely formalin-fixed and paraffin-embedded, on cryostatic sections obtained at surgery and post-fixed with cold methanol. The samples were stained with specific procedures (Haematoxylin-Eosin, Giemsa, Papanicolau, Picroindigocarmine-Nuclear fast red). For each sample, an increasing number of cells from 1 to 100 was harvested in different PCR tubes. DNA extraction was performed by Chelex™ 100 (Biorad), QIAmp DNA Micro Kit (Qiagen) and DNA IQ™ System (Promega Corp.). The DNA extracted from each sample was amplified to identify a specific genetic profile by the most common microsatellite loci (BAT25, BAT26, BAT40, D2S123, D5S346, D17S250) as well as multiple Short Tandem Repeat (STR) typing

(AmpFLSTR Identifier, PE Biosystems) of forensic interest. PCR products were separated by capillary electrophoresis with 3100 AB Prism Genetic Analyzer; and analyzed by the Genescan and Genotyper Softwares v 3.7.

**RESULTS.** We have obtained sufficient DNA for amplification and STR typing starting from 10 aloid cells while amplification for microsatellite loci was achieved with 5 diploid cells obtained from routinely processed tissue samples. Moreover, some differences in relation to the integrity of the extracted DNA was found with the specified staining procedures.

**CONCLUSIONS.** The documented possibility to perform a genomic analysis of low copy number DNA from few cells harvested by laser microdissection represents a valid aid in order to solve diagnostic and forensic problems in the medical practice.

#### **P 889**

##### **ASPECTS OF DIGITAL MICROSCOPY IN HUNGARY**

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**AIMS:** Although telepathology techniques have been accepted as global method, there are serious doubts to implement cyber age tools in professional practice. Traditional static telepathology has proven biased due to the low explicit/implicit ratio of transmitted information; dynamic telepathology can not be automated using remote controlled conventional microscopes. Our aim is to present concept and realizations of digital microscopy in Hungary.

**METHODS:** Selected histopathological samples were captured with a bright field transmitted light microscope and in house developed slide scanner. Images of biopsies have been joined in order to create digital slides using different scanning methods. Electric slides have been stored on slide server, then diagnosed at remote stations via Internet.

**RESULTS:** The value of bias of transferred digital information can be reduced to zero digitizing series of histopathology slides. We tested the following systems:

1. Manual digitizing of the specimen via x and y axes using RGB microscopic camera and frame grabber is extremely laborious and inappropriate in the routine practice.
2. Automatic scanning of slides applying traditional microscope, which is equipped with motorized stage digitizes the entirety of selected slides but not able for fully automated capturing of large slide series.
3. Histological slide scanner without conventional microscope but with network connected slide server, containing software controlled stage and auto-focus, enable creation and storage digital slide series and remote diagnostics on routine way.

**CONCLUSION:** Basic questions of digital slide's application in the routine practice are the way of slide scanning, the speed of archive's access and image quality of digital slides. Application of slide scanner, slide server accessed via TCP/IP protocol dissolves bias experienced in telepathology practice and enables a new agenda for expert discussions, consultations, archiving, education and quality assurance.

#### **P 890**

##### **THE ADVANCES IN SCANNING FLUORESCENT MICROSCOPY, (AUTOMATED SLIDE HANDLING, METAL-HALIDE ILLUMINATION, SOFTWARE FEATURES) MEANS SIGNIFICANT ADVANTAGE FOR ROUTINE APPLICATIONS**

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**Background:** we reported recently about the application of single slide fluorescent scanning technology on a routine motorised microscopy. Several technical limitations became known as scanning speed, slide identification, scanning area determination., Internet access to fluorescent digital slides, application on histological sections.

**Aims:** Development and evaluation of an automated new fluorescent slide scanner (Hi-Scope, 3DHISTECH Ltd, Budapest) and software enhancements for supporting high volume automated analysis.

**Materials and methods:** For automated slide handling slides, coverslips and slide boxes(50 slides) were developed. Hi-Scope (3DHISTECH Ltd, Budapest Hungary) features a vertical fluorescent illumination pathway of a commercial microscope, automated slide box movement (upto 6), slide loading and barcoded identification, edge fluorescent labelled coverslip localication and laser triangulometry based focus point determination. Automated scanning software features multichannel slide digitisation depending on the staining intensity upto 1 sec for three channels. Coverslips are fixed using Pro-long media (MolecularProbes, USA). Illumination was enhanced using a metal-halide fluorescent light source. Cytometric calibration and standardisation was done using beads Coulter beads.

**Results:** The Hi-scope system's slide box and slide loading safety was proven in a three month tests. Mechanical slide loading errors were found occasionally after loading 300-400 slides. Focus determination worked without error. CV of the density measurements on the calibration beads were 3.9 %. The determination of area of interest using prelabelled coverslips was automatically performed without failures. Resolution of the system is 0.3 um<sup>2</sup>

/ pixel. Digitisation of a field of view took upto 0.1 sec in FITC, 0.1 sec in DAPI, upto 0 sec in the Rhodamine channel. This way a cytospin area could be scanned in three channels in less then 4 minutes.

**Conclusions:** Hi-Scope can contribute to high volume fluorescent slide scanning. The produced image quality is acceptable for routine use. Based on these results development of routine applications can be started.

#### **P 891**

##### **WORKING EXPERIENCE WITH DIGITAL SLIDE AND VIRTUAL MICROSCOPY-BASED ROUTINE AND TELEPATHOLOGY EVALUATION OF BIOPSY SPECIMENS**

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##### **INTRODUCTION**

Automated scanning of histological sections and visualisation of the histological image by virtual microscopy is becoming increasingly important in routine diagnostic pathology.

##### **PURPOSE OF THE STUDY**

The aim of present study is to test Zeiss Mirax slide scanner solution (Carl Zeiss, Jena) as virtual microscope system about its usability in routine diagnosis of biopsy specimens as well as in telepathology.

##### **METHODS**

Altogether 1500 biopsy sections were scanned and examined on screen until the submission of this abstract. The established diagnoses were compared to those which were given after light microscopic examination. The study was carried out by four experienced pathologists, both on site and at a remote workstation equipped with the software including the facility of remote access for digital slides.

##### **RESULTS**

Accurate diagnosis could be established in all cases using the virtual microscope, if the sections and the staining were of

good quality and 8  $\mu$  thin or less, both on site and from the remote workstation. The images taken by use of 20x objective were suitable for routine diagnosis. The scanning of an average sized section took 5-7 minutes at this magnification. Three hundred sections/day can be scanned without difficulty. Further digital magnification of the images did not improve the quality of structures. A period of 3-4 weeks is needed even for experienced pathologists to get used to microscopic images on the screen. The system enables the establishment of collections and the storage of histological images, first of all for gradual and postgradual education.

#### CONCLUSION

The Mirax virtual microscope system is valid for use in diagnostic histopathology and telepathology.

#### P 892

##### THE EFFECT OF DIFFERENT THICKNESS OF THE SAME TISSUE SECTIONS ON THE COLORIMETRIC DETERMINATION OF COLLAGEN CONTENT

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In clinical and experimental studies, it is important to measure the collagen content quantitatively in tissues for study in the development of fibrotic changes. Fibrosis has been estimated by histomorphometric or qualitative methods. Histomorphometric methods are time consuming and qualitative analysis inaccurate. A simple and reproducible method has been reported by López-de-León and Rojkind routinely to estimate collagen content in formalin-fixed paraffin-embedded sections. The method is based on the selective binding of Sirius Red and Fast Green FCF to collagen and noncollagenous proteins, respectively. In this method 15  $\mu$ m thick- sections are frequently used. But studying with 15  $\mu$ m thick- sections have some difficulties in process. The aim of this study is to evaluate the reliability of the colorimetric collagen measurement method with different tissue sections and staining procedures.

40 bile duct ligated-female Wistar Albino rats (200  $\pm$  30 gr) were used in this study. 3, 6, 9 and 15  $\mu$ m-thick double sections were obtained for each liver section.

Collagen content was measured 320 liver-tissue sections for using two different staining procedures. First staining procedure was based on the method published by Lopez-de León and Rojkind. After the liver sections were deparaffinized, all of them covered with saturated solution of picric acid in distilled water that contained 0,1% Sirius Red and 0,1% Fast Green FCF and incubated 30 minutes in a rotary shaker. Sections were rinsed several times with distilled water until the fluid was colourless. 1 ml of 0,1 N NaOH in absolute methanol was then added and mixed until all the colour was eluted from the section. Eluted colour was read immediately in Jasco V 50 UV-VIS spectrophotometer at 540 and 605 nm, i.e. Second staining procedure was based on Gascon Barré and et al. In this procedure, sections were first stained with 0,04% Fast Green FCF and with both dyes. Different from Lopez-de León and Rojkind's procedure 1 ml of 0,05 N NaOH in 50% aqueous methanol was added for obtaining eluted colour. Eluted colour was read immediately in Jasco V 50 UV-VIS spectrophotometer at 530 and 605 nm.

Collagen contents of four different thickness of the same tissues showed a high correlation with each other (r mean= 0,9896, p<0.01). The results of the different staining procedures are highly correlated (r mean= 0,9638, p<0.01).

This method is not affected by tissue thickness and different staining procedures.

#### P 893

##### LOW RESOLUTION VIRTUAL SLIDES: AN INEXPENSIVE AND SWIFT SOLUTION FOR BOTH

##### EDUCATIONAL PURPOSES AND AUTOMATIC QUANTITATIVE PATHOLOGY

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Whole histological slide digitization, allowing "virtual slide" examination and analysis, has several potential applications for pathologists.

It may be useful first for teaching purposes, using web-based interactive self-instructive methods. It is expected to provide, in the near future, adequate solutions for telepathology exchanges. It also potentially offers the best response to the difficult problem of tissue heterogeneity for quantification of prognostic markers in oncology.

Nowadays, virtual slides are obtained using costly and more or less rapid devices, yielding to images which can barely be manipulated and which cannot be automatically processed, due to their huge size. The issue of disk storage capacity will probably be solved in the near future, but the development of efficient, rapid and economic methods, for fully automatic analysis of images of more than 10 Gigabytes, will not be achieved before several years.

Purpose : In this context, we propose an inexpensive, swift and multipurpose approach, using 4,000 dpi scanned images of the whole histological slide, ranging from 15 to 70 Megabytes, which can be either stored in a database or automatically processed for tissue marker quantification.

Methods : Thanks to a simple calibrated professional slide scanner, we were able to build a user-friendly website dedicated to breast pathology, including around 250 cases (scanner image associated to relevant microscopical details).

Furthermore, dedicated image analysis routines have been developed, one allowing automatic quantification of tissue markers on whole slides (cytoplasmic or nuclear immunostaining, immunolabeled blood vessels or stroma), the other performing automatic labeling and analysis of Tissue Micro Arrays. Superimposition and image matching of serial immunostained sections, as well as analysis of staining heterogeneity, are also available.

Conclusion : We herein propose to take advantage of an inexpensive and swift image acquisition device, affordable by each pathology, biology or research department, for low resolution virtual slide production, examination and analysis.

#### P 894

##### TOWARDS COHORT BASED FROZEN TISSUE BIOBANKS: LOGISTICS AND RNA QUALITY

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New sophisticated tools for molecular profiling generally require collection of fresh frozen tissue as a source of high quality DNA and RNA. Unfortunately, most frozen tissue biobanks represent a selected fraction of surgically removed cancer cases. There is usually an over-representation of large, well-demarcated tumors while sampling of small, diffuse tumors is avoided due to an appropriate concern about diagnostic safety. To address this issue, we have implemented a prospective biobank allowing for "clinical cohort-based" collection of cancer tissues.

Novel logistics for managing non-fixed specimens has been established. Resected tumors and biopsies are sent on ice to the Department of Pathology. Selected pieces of representative diseased and normal tissue are cut out, blocked in cryogel, and snap frozen. High quality "tape-transfer" frozen sections are produced from each case and the sections are included in the routine histopathological examination. In our experience safe evaluation of small lesions and even resection margins can be done on these slides. In this way a non-selected collection of cases can be obtained from a treated patient cohort. Digital images of the frozen sections are linked to the biobank register. Thus, the cellular composition of the tissue can be assessed before profiling experiments and microdissection.

Difficulties associated with the fragile nature of RNA prompted us to examine the effects of transport time and transport conditions. Structural RNA integrity was checked by microchip electrophoresis in a Bioanalyzer, and gene expression patterns were analysed using real-time PCR. In an experimental set-up, tonsil tissue was cut into cubes and some pieces were snap frozen immediately after surgery. The remaining pieces were left at room temperature, kept on wet ice, in normal saline or in a commercial RNA-stabilising buffer. Surprisingly, the RNA remained quite stable in the tonsil specimens under all conditions and time points tested. Even after 16 h at room temperature only slight RNA degradation was revealed by the electropherograms. However, gene expression levels at different transport time points were most stable during transport on ice.

Our study shows that non-fixed tissue from most tumors can be collected safely in a routine pathology setting and that RNA degradation is a minor problem during handling of fresh tissue before biobanking.

#### P 895

##### SOFTWARE DEVELOPMENT FOR FRACTAL ANALYSIS OF HISTOPATHOLOGIC IMAGES

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**Aim:** Considering the multiple applications of fractal analysis in the field of histopathology, we developed "Fractalyser", a specialized fractal analysis software program for Windows operating system.

**Material and Methods:** The application was developed in Visual Basic 6.0 (SP5) (Microsoft Corp.) environment. For user friendliness the program was designed with multiple document interface and Windows XP compatibility. "Fractalyser" can open, edit and analyze the most common image file types (bmp, jpg, gif). Images and graphs created by the program can be saved as bmp files, while numerical results can be saved in a text file. The program has some basic image processing functions such as invert, horizontal and vertical flip, grayscale conversion and crop. The user can precisely select the area of interest applying parameterized threshold or edge detection algorithms in the color image, while getting real time graphical feedback on the process. The produced binary image, which is projected as a red layer on the original image for user facilitation, can be further optimized before fractal analysis with 'dilation', 'erosion' or several noise reduction filters. The estimation of the fractal dimension of the image is carried out by using the "box-counting" algorithm. The box size range as well as the number of grid offsets are specified by the user. The program provides detailed numerical results, as well as a graphic presentation of the data and the least-squares linear regression results. The correlation coefficient and the coefficient of determination are also estimated.

**Results:** The accuracy of the application was assessed by analyzing images with theoretically known fractal dimension. The errors ranged from 0.0 to 5.8%, being conversely proportional to the number of grid offsets. Repeated comparisons of the statistical results of the program with the corresponding data of a commercially available statistical package (Statistica v.6.0, StatSoft Inc.) confirmed their validity and precision.

**Conclusion:** As it has been observed in thorough testing, the application presented here constitutes a stable, relatively fast, reliable and easy to use tool for fractal analysis. "Fractalyser" is provided as freeware to researchers after request via e-mail to pavlopoulos@medscape.com.

#### P 896

##### THE PATHOLOGIST AND THE ELECTRONIC HEALTH RECORD.THE ROLE OF THE IHE (INTEGRATING THE HEALTHCARE ENTERPRISE) INITIATIVE.

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The challenging aim of the Electronic Health Record (EHR) is to gather current and previous medical data, including images, coming from several experts. In pathology, the necessary technology for digital imaging is available allowing pathologists to enrich the EHR. The challenge now is to define specific healthcare needs and workflows and to agree on technical protocols and file formats for "virtual slides" and reports exchange. The goal of the Integrating the Healthcare Enterprise (IHE) initiative is precisely to stimulate integration of healthcare information resources to improve clinical care. IHE promotes implementation and use of established data standards. Although the ADICAP (Association for the Development of Informatics in Cytology and Pathology) proposed a European de facto standard for image folders in pathology, the most used standards in medical imaging are HL7 and DICOM standards.

The objective of the presented work, done in the framework of a national IHE initiative promoted by ADICAP, consisted identifying the main integration profiles that could be addressed in pathology, taking advantage of the work done in radiology and biology, and to define the main requirements for virtual slides and reports communication according to both the principles of the ADICAP and the DICOM modes.

ADICAP, with the collaboration of the GMSIH (Group promoting the Modernization of Hospital Information Systems in France), recruited national IHE initiative participants to work on the Pathology Technical Framework: 7 pathologists and haematologists, 4 IT professionals, 2 professional associations and 12 vendors. Three working sessions were organized to define the pathologists' needs and to propose Integration Profiles. Furthermore, a DICOM module was developed to produce DICOM Visible Light objects and DICOM Structured Reports and tested at the Georges Pompidou Hospital.

The integration profiles for pathology were defined and presented at the 2005 IHE-Europe workshop in Noordwijkkerhout (Netherlands). The images and reports produced by the DICOM module were stored into the Picture Archiving and Communicating System (PACS) of the Georges Pompidou Hospital and available within the EHR.

In conclusion, the national IHE initiative in pathology produced a first definition of useful integration profiles in pathology. On that basis, a first version of the Pathology Technical Framework could be proposed at an European level. Next steps are to support testing, demonstration and educational activities to promote the deployment of this framework by vendors and users.

P 897

### QUALITY ASSURANCE IN PATHOLOGY : AUDITS ON LABORATORY WORKFLOW AND INDIVIDUAL PRACTICE

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In 1998, on behalf of the AFAQAP, standards and guidelines of quality management for individual Pathologists or in Pathology department were published. AFAQAP Committees have elaborated and conducted numerous voluntary audits, mainly on diagnostic performance or technical quality in the past 10 years. In 2002 and 2004 clinical governance was introduced in France, as in other countries, to ensure that each doctor was involved in a continuing professional development (CPD) program. The pathologists always had a duty to patients but that duty now becomes a legal obligation. Clinical audit are performed against standards is the process by which pathologists can gather the evidence to improve patient care. ANAES (National Healthcare Accreditation and Evaluation Agency) /HAS (Main Healthcare Authority) has developed a strict methodology for the certification of clinical audit. To ensure high quality of clinical audit, it has to fit in the audit cycle : choose a frequent topic, identify agreed relevant standards, collect data, compare to standards, plan changes, re-evaluate. The AFAQAP intends to help pathologists (either individual or in pathology department) to meet their duty of quality by providing a selection of audits. To ensure high quality for pathologists 2 kinds of clinical audit are necessary, based either on laboratory work organization (preanalytic, analytic or postanalytic processes, risks assessment ...) or on individual professional performance (applying evidence-based practice, clinical standards). In 2004, Committee 4 in relation with HAS developed 2 audits,

? "laboratory work" testing the pre-analytic process of the specimen (collection, transport, reception and registration). Are procedures written, available knowledgeable to persons involved in those activities? Is the personal safety appropriate (adequate space? formaldehyde vapor ...)? Are the patient and specimen identification satisfactory?...

? "individual practice" testing histopathological reports on tissue samples. Is every specimen identity maintained at all times? Are gross examination performed in accordance with guidelines? Do gross and microscopic findings support pathological diagnosis or hypothesis? Are standards systems of grading and staging used in cancer cases?...

More than 90 voluntary pathologists underwent those 2 self auto-evaluations. Statistical analysis in on progress.

Many other countries, such as Great Britain or US, are involved in CPD program. Quality programs should be maintained under pathologists responsibility. Europe is an opportunity to exchange experiences.

P 898

### THE NEW FIXATIVE RCL2, A NON TOXIC ALTERNATIVE TO CRYOPRESERVATION.

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Introduction

Fixed specimens are suitable for most of diagnosis and immunostainings. However, identifying tumour determinants of drug efficacy looks like a new Holy Grail quest and the conservation of the cellular components becomes a key step in the specimen management.

Purpose

We present a new fixative fluid developed in our laboratory that allows extraction of RNA, DNA and proteins from paraffin embedded specimens.

Methods

The quality of tissue samples fixed and paraffin embedded according to our technique was evaluated on:

- the tissue morphology (H&E staining)
- the immunostaining results of more than 20 antibodies
- the FISH (PathVysion Vysis™) results
- the mRNA quality using a 2100 bioanalyzer (Agilent™), and quantity using RQ-PCR
- the DNA size and quality using agarose gel electrophoresis and three different long range PCR of 600bp , 800bp and 2600bp followed by DNA sequencing
- the protein quality using the bioanalyzer and by Western blotting of a nuclear protein (estrogen receptor), a cytoplasmic protein (actin F) and a mitochondrial protein (Bcl-2).

Results

The tissue morphology was very good, close to that obtained using AFA or Bouin's fluids. The immunostaining results were similar to those obtained on formalin fixed specimens. We succeeded in FISH technique (PathVysion Vysis™) without modification of the procedure designed for formalin fixed specimens. The mRNA quality was very good with 28S/18S ratio rising above 1.5. The results of RQ-PCR were always similar to those of the frozen samples of the same specimen. The DNA extracted was of very large size (>> 10 kbp) and all the long range PCR worked without modification of the procedures designed for frozen samples. The sequencing of the amplification products was also performed easily without procedure modification. While the protein profiles were slightly different from those observed in the frozen samples of the same specimen, the Western blotting results were similar for the proteins tested.

Conclusion

This new fixative fluid allows the extraction of high quality mRNA, DNA and good quality proteins from paraffin embedded tissue samples. Furthermore, the tissue morphology and immunoreactivity are very well preserved. It can be a good alternative for tissue preservation when freezing is difficult such as for small biopsy or in private laboratory.

Moreover, it is worthy of note that this fixative fluid does not contain any toxic product.

P 899

### EUROPEAN TISSUE ARRAY : A USEFUL TOOL FOR QUALITY CONTROL OF IMMUNOHISTOCHEMISTRY. A PROGRAM OF THE EORTC GI GROUP

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Background: Numerous studies are based on immunohistochemistry (IHC) results, but IHC is not standardized and quality control is not yet widely established. In addition, important variability may be due to fixation time

and tissue heterogeneity. Therefore, the EORTC GI group designed a quality control tissue array (TA), in order to evaluate the suitability of a given antigen/antibody test before use in clinical trials. Here we report testing of the TA with routine antibodies.

**Methods:** A 216-spot-TA was designed using paraffin-embedded tissue from 14 colorectal cancers coming from 4 European centers. Immediately after surgery, specimen from tumor rim or tumor center were formalin-fixed individually overnight, for >24 h, or for >48 h, respectively. After evaluation by H&E staining, 3 cores from each specimen were assembled in a tissue array, i.e. 18 cores per tumor. Sections were distributed to 3 pathology departments for IHC, each using one or more of nuclear (p53, Ki67) or cytoplasmic antibodies (CEA, CK20, Cox2) in routine staining protocols. Blinded reading of immunoreaction was performed manually using a visual grading system based on the percentage of positively stained malignant cells.

**Results:** IHC was successful in the 3 laboratories. The staining pattern varied considerably between cores from individual specimen (e.g. Ki67: from 10-90% pos. nuclei; CEA: 50-100% pos. cells, CK20: 10-60% pos. cells) with moderate differences for staining intensity (variation from weak to moderate or moderate to strong). If average staining intensity or staining pattern for the 3 cores per location were calculated, there was no difference between tumor center and rim ( $p=0.62$  in paired t-tests for all comparisons). Most importantly, fixation time had no impact on the expression levels of the five antigens ( $p$  values from 0.12 to 0.93).

**Conclusions:** We observed a high heterogeneity in the staining from core to core for each tumor. However, we couldn't demonstrate an impact of location (rim or center) or fixation time on the expression levels for the five tested antibodies. This quality control TA is a useful tool to ensure the reliability of a given antibody and staining procedure before its use in translational research in multicenter clinical trials.

#### P 900

##### **THE HOPE TECHNIQUE OFFERS A COMPLETE PANEL OF MOLECULAR APPLICATIONS IN PARAFFIN-EMBEDDED MATERIALS**

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**Introduction:** Asservation of materials for pathologic diagnostics is mostly achieved by formalin-fixation and paraffin embedding. This results in good preservation of histologic details, but to a large degree prevents the application of modern molecular techniques due to the bad condition of nucleic acids and proteins if compared to fresh or frozen specimens, which show a comparably low preservation of histomorphologic details.

**Purpose of the study:** To establish an alternative fixation technique to overcome with these problems.

**Methods used:** We have shown that the novel HOPE-technique allows for both, well preserved 'formalin-like' morphology and excellent preservation of nucleic acids and proteins. In this study we present protocols for In situ hybridization, Immunohistochemistry, Western-blot, Northern-blot and Transcription-microarrays.

**Summary of the results:** All of these methods can be unambiguously performed with HOPE-fixed materials.

**Conclusions reached:** These results open up new perspectives for molecular approaches in paraffin-embedded probes providing a novel powerful tool for modern pathology.

#### P 901

##### **ICON-TMA® FOR QUALITY CONTROL OF IMMUNOHISTOCHEMISTRY**

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Immunohistochemistry (IHC) has become an essential adjunct in daily histopathological diagnosis. However, quality control is difficult and becoming critically important considering the need for standardization and quantification of immunoreactivity.

To help solving these problems we developed small size tissue microarrays (TMAs) with 2 to 4 punch biopsies from well characterized specimens of sizes between 0.6 and 2 mm diameter. In its simple variant these internal control (iCon) TMAs consist of 2 tissue cylinders, one being clearly positive and the other clearly negative for a specific immunohistochemical marker, e.g. cytokeratins or hormone receptors. In its more elaborate format, the iCon-TMAs carry additional tissue spots providing a scale for quantitatively assessable markers, e.g. providing examples of immunoreactivity scores 0, 1+, 2+, 3+. The specimen with the yet unknown immunoreactivity is then applied to the iCon-TMA section and both are simultaneously analyzed.

A successful IHC analysis could almost be judged by the macroscopical inspection of the two +/- spots representing the positive and negative sample of the iCon TMA. While the spot with positive control provided an internal standard for strong immunoreactivity, the negative one provided an estimate to differentiate weak positivity from unspecific background staining. The technique proved to be particularly useful for markers that were not necessarily present in the uncharacterized specimen.

iCon-TMAs provided a convenient measure for internal quality control and may become an indispensable tool for standardization and quantification of gene expression by immunohistochemistry or related techniques like mRNA in situ hybridization.

#### P 902

##### **PRESERVED COUPLING OF OXYDATIVE PHOSPHORYLATION BUT DECREASED MITOCHONDRIAL RESPIRATORY CAPACITY IN IL-1B TREATED HUMAN**

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The peritoneal mesothelium acts as a bioactive cellular membrane regulating serosal responses to injury, infection and neoplastic diseases. Inflammation of the serosal surfaces induces an "activated" mesothelial cell phenotype. In the present study we simulated activation of cultured human peritoneal mesothelial cells (HPMC, isolated from human omentum majus) by treatment with the pro-inflammatory cytokine interleukin-1beta (IL-1beta). Respiratory activity of suspended cells was analysed by high-resolution respirometry to assess changes in respiratory capacity and coupling of oxidative phosphorylation in activated HPMC. Citrate synthase (CS) and lactate dehydrogenase (LDH) activities

were determined by spectrophotometry. IL-1beta activation for 48 hours resulted in a significant decline of respiratory capacity ( $P < 0.05$ ), without affecting integrity of the inner mitochondrial membrane or cell viability. Treatment of HPMC with IL-1beta resulted in a decrease of CS activity ( $P < 0.05$ ) and an increase of LDH activity ( $P < 0.05$ ). The present data indicate that activation of peritoneal mesothelial cells with IL-1beta is associated with a decrease of oxidative phosphorylation and mitochondrial content that appears to be compensated by an increase in glycolytic capacity.

#### P 903

##### THE IMPORTANCE OF HISTOPATHOLOGICAL/NECROPTIC EXAMINATION IN COWDEN'S DISEASE

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Cowden's disease (CD) is a rare autosomal dominant inherited disease characterized by multiple hamartomas with ectodermal, mesodermal and endodermal origin, the most constant features being the mucocutaneous lesions (almost pathognomonic). It associates an increased risk of malignancies (especially breast and thyroid) and, sometimes, due to the presence of multiple tumors in different organs, an overestimation of the stage is possible ("metastatic cancer")

We present the case of a 82-years-old female recently investigated for progressive alteration of the general status and loss of weight; a supposition of possible metastatic malignant melanoma was made – based on the presence of innumerable pigmented cutaneous lesions (with diffuse distribution including face and palmoplantar localization) and CT findings (many tumors in the skull, meninges, lungs and liver).

The current episode consisted in hemiplegia in right limbs; during admission, an acute ischemia of the left leg developed; considering the neurological status and the "terminal malignant disease" no surgical treatment was considered. Autopsy revealed recent left sylvian infarct, recent thrombotic occlusion of the left external iliac artery and numerous tumors (oncocytic meningioma, intraosseous cranial, pulmonary and hepatic hemangioma, pulmonary hamartoma, bilateral adrenal adenomas, gastric leiomyoma, ovarian serous papillifer cystadenofibroma, pigmented seborrheic keratosis); previous hysterectomy for leiomyomas. CD was concluded (1996 International CD Consortium criteria).

Despite the simplicity of the case, we want to emphasize the risks of clinically misinterpretation of CD as metastatic cancer (without histopathologically proven malignancy) and a subsequent therapeutic redrawn. Only histopathologic/autopsy examination establishes the correct nature of these tumors and a precise diagnosis.

#### P 904

##### PATHOGENIC CONSEQUENCES OF ALV INFECTION IN CHICKENS INOCULATED IN MID EMBRYOGENESIS

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Introduction: Avian leukosis viruses (ALV) in addition to their oncogenic potential are responsible for induction of a syndrome called wasting disease (WD). Clinical signs of WD are progressive weight loss, anaemia and occasionally

diarrhoea as well. Histopathological findings include depletion of lymphoid tissue and immunosuppression.

The aim of this study was to determine the rate and severity of WD induced in chickens by various ALV strains and to evaluate pathological and immunological consequences of the disease.

Material and Methods: For the experiments outbred Brown-Leghorn chickens (genotype C/E) were used. Virus was inoculated in mid embryogenesis into the chorioallantoic vein. ALV viruses of subgroups A–D were tested. Signs of the wasting were monitored during the first 2 weeks after hatching. Lymphatic organs were thoroughly examined immunohistochemically.

Results: When comparing ALVs of different subgroups for their ability to induce WD, viruses of subgroup C (namely td B77-C) were found to cause the most prominent changes. Chickens infected with viruses of subgroup A did not differ significantly from the control birds. WD can be induced only by intraembryonic inoculation of the virus and in a mild form also when inoculated on the 1st day after hatching. The lowest limit for WD induction was determined as 102 infectious units of the virus. In diseased chickens, severe B cell depletion in primary and secondary lymphatic organs was found. Thymus atrophy seems to be partially compensated by a moderate increase of the T lymphocyte number in T-dependent regions. Lymphocyte depletion is accompanied by prominent activation of the monocyte-macrophage system. While defects of both humoral and cellular immunity were found in chickens infected by ALV-C, only the second one was injured by viruses of subgroup A. A significant increase of peripheral CD8+ T lymphocytes was produced by viruses of subgroup C.

Conclusion: These results indicate that inoculation of chicken embryos by ALVs provides a suitable experimental model for study of retroviruses-induced impairment of immunity.

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#### P 905

##### MORPHOLOGICAL EVALUATION OF THE HEALING OF BONE DEFECTS IN RABBITS FILLED WITH A COMPOSITE MADE OF POLYLACTIDE/POLYGLYCOLIDE COPOLYMER AND CARBON FIBERS [P(LLA/GLA)+CF]

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Biodegradable polylactide/polyglycolide copolymers are applied widely in medicine for joining broken bones. Their disadvantage is a small mechanical endurance. It can be increased by creating copolymer and carbon fiber composite. The aim of the study was morphological evaluation of the healing process of mandibular bone defects in rabbits filled with a composite made of polylactide/polyglycolide copolymer and carbon fibers [P(LLA/GLA)+CF].

The study was performed on 30 New Zealand rabbits. After the soft tissue was prepared, the mandibular bone was exposed. The hole here a composite implant was inserted, had been drilled with a 3.2 mm bur. The wound was sutured. Moreover, the composite cylinders were inserted into

subcutaneous and intramuscular pockets. The animals were divided into 7 groups, 4 rabbits in each group. The groups were euthanized after 1, 2, 3, 6, 12, 24 and 52 weeks and bones were fixed in 10% solution of neutralized formalin. Bone tissue was decalcified in Romeis fluid with decalcifying agent PW23. After the typical procedure, the samples were emerged in paraplast. The blocks were sliced in a microtome. The 6 microtone slices were deparaffinized and dyed routinely with hematoxylin and eosin. The obtained preparations were evaluated in a light microscope with magnifications from 40 to 400x. After 1 week, clearly formed canal around the implant was visible in the mandible. The features of active band of bone tissue in the form of numerous young, immature bone trabeculas surrounded by osteoblasts. Numerous, immature and mature bone trabeculas covered with osteoblasts were present after two weeks. Three weeks later and in the following periods, mature bone trabeculas without osteoblastic activity were observed. A connective tissue capsule was formed around the implant in the subcutaneous tissue. Mature, cicatrizing, fibrous connective tissue, growing among the injured bundle of muscle fibers was visible in the muscle tissue around the implant. The study showed good tolerance of the applied P(LLA/GLA)+CF composite implants in the animal body, no inflammatory reactions and an increased time of bone tissue regeneration around implants.

#### P 906

##### EVALUATION OF RATS STRIATED MUSCULAR TISSUE RESPONSE TO TINICO IMPLANTS

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Rapid progress of medicine connected with reconstructive surgery leads us to searching for the new materials which would substitute, temporarily or permanently, harmed tissues.

The TiNiCo alloy with the shape-memory effect was taken upon the study and implanted to rats of Sprague-Dawley breed. After the implantation period lasting 12, 24 or 52 weeks the implants were removed and the surrounding tissues subjected to histopathological examination.

The morphologic features of inflammation (lymphocytic, granulocytic, eosinophilic, plasmocytic infiltrations), necrosis, fibrosis (fibrous capsulae formation), degeneration, giant cells and granulomas presence in the tissue samples were evaluated. The vascular proliferation and nuclear changes were estimated as well. The TiNiCo alloy and Titanium placed in the tissues accordingly to the ASTM F 981-99 standards cause low biologic tissue activity.

The investigated materials do not trigger any tissue response that could suggest their cytotoxicity or carcinogenic properties. The achieved results indicate high biocompatibility of TiNiCo alloys.

Key words: tissue response, rat, shape-memory effect alloy, TiNiCo implant, biocompatibility, Titanium

#### P 907

##### A HIGH AUTOPSY RATE IN A TEACHING HOSPITAL IN BRAZIL

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Autopsy is an invaluable diagnostic tool in medical practice since postmortem studies provide relevant information in patients with complex pathologies. In academic hospitals, autopsy also contributes to research and teaching by supplying a rich material to these purposes. In our Institution, autopsy is performed in 85% of patients that die during hospitalization (75% among adults and 90% among fetal and pediatric patients). A high autopsy rate allows accurate epidemiological studies and quality control. As a teaching Hospital, it is also important to know if death of patients varies along the year, days and months in an attempt to identify if there are medical and non-medical factors such as the activity of less trained residents and undergraduate students influencing it. We analyzed all autopsies performed in a University Teaching Hospital in Brazil during 52 consecutive weeks. The following data were retrieved from individual autopsy records: gender, age, time of death (hour of the day, day of the week and month) and the main cause of death. There were 1419 autopsies (79% adults and 21% pediatrics, 60% male and 40% female). Those performed during working days summed up to 67.5%, the remainder 32.5% were performed during weekends or holidays. They were more frequent during the nocturne than diurnal period (52.6% and 47.4%, respectively). The causes of death distributed among the ICD-10 categories were: cardiovascular diseases 21.3%, infectious diseases 19.2%, neoplasms 12.8%, perinatal conditions 10.8%, respiratory diseases 6.6%, gastrointestinal diseases 6%, congenital anomalies 4.7%, CNS diseases 3.8%, genito-urinary diseases 1.8%, and others 13%. In our Hospital a high autopsy rate is maintained due to readiness of results, performance of autopsy during the 24 hours of the day, very short interval between death and autopsy and a team composed by an experienced pathologist, a resident and a mortuary assistant. There was coexistence of diseases typical of both industrialized and developing countries, indicating the epidemiological transition in our country. The regular distribution of deaths along the day, weeks and months indicate that it is not affected by the inexperience of training students and residents probably due to adequate supervision.

#### P 908

##### UNUSUAL SITES OF FUNGAL INFECTION

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Aspergillus is a filamentous fungus commonly isolated from soil, plant debris, and indoor air environment, includes over 185 species, and *Aspergillus fumigatus* is the most commonly isolated. Immunosuppression is the major factor predisposing to development of opportunistic infections. There are several reported cases that describe female genital tract infections with opportunistic fungi, *Candida* and *Aspergillus* are most commonly found in fungal endometritis and are generally requiring some type of predisposing condition like a use of Intrauterine Device

Coccidioidomycosis is a systemic infection caused by *Coccidioides immitis*, a dimorphic fungus endemic in the south of the USA and in the north of Mexico. The disease usually affects the lungs. Primary cutaneous infection is extremely rare.

Blastomycosis is a disease caused by the dimorphic fungi *Blastomyces dermatitidis*, and is acquired via inhalation of the conidia, which transform into the yeast form once in the lungs, however at least 50% of primary infections are

asymptomatic and most of the cases become manifest during a chronic phase that may affect the lungs, skin, bones, genitourinary tract and other reticuloendothelial organs. Involvement of genitourinary tract is seen in 10% of cases and affects the prostate and epididymis.

We present 5 cases of non-usual sites infections with this 3 fungi: 2 cases of aspergillosis in a curettage secondary to spontaneous abortion, 2 cases of primary cutaneous coccidioidomycosis localized in the nose and ear, and a case of blastomycosis localized on the prostate, in the 5 cases the patients did not reveal the presence of a primary infection in any other system. It is important to have in mind this unusual sites of primary infection by fungi because we can mislead the opportunity to bring the patient an opportune treatment with anti-fungal drugs.

#### P 909

##### **UNKNOWN PRIMARY CANCER: IMMUNOHISTOCHEMICAL ALGORITHMIC APPROACH FOR EPITHELIAL NEOPLASMS WITH MULTIPLE METASTASES.**

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**Introduction:** The exact diagnosis of unknown primary cancer (UPC) is a common problem for the surgical pathologist especially in cases with multiple metastases at different sites. The typical feature of a UPC with > 2 metastatic sites at the initial clinical presentation is a rapid progression of the disease, often less than three months. This requires rapid diagnosis in these patients. The aim of this study was to develop an optimal algorithm of immunohistochemical investigation of UPC with multiple metastatic sites.

**Materials and methods:** Histopathological and immunohistochemical investigations were performed in thirty two cases with UPC with > 2 metastatic sites, mainly in lymph nodes. A broad immunohistochemical panel and high sensitivity polymer detection system were performed on formalin fixed paraffin embedded tissues from each case.

**Results:** Because the metastatic tumors were mainly poorly differentiated carcinomas or poorly differentiated adenocarcinomas, the initial step of immunohistochemical investigation was to determine lines of cell differentiation. For this purpose we used the panel consisting of p63, CK8, HMW, CD56 (N-CAM) and calretinin. For adenocarcinomas (CK8+, HMW+/-, p63-, CD56-, calretinin-) the second step was to identify a primary site. To perform this we investigated expression of CK7 and CK20, and 'tissue specific' transcription factors - TTF1, Cdx2, WT1, ER and PR. In addition we investigated expression of some specific cell products, such as CA-125, PSA, GCDPF-15. Approximately in thirty percent of performed cases the results of histopathological and immunohistochemical investigations were the same. In seventy percent of cases the results of immunohistochemical investigations gave important additional information for further management of the patients or radically changed the interpretation of histopathological date.

**Conclusion:** Although the morphological examinations is the basis of diagnosis in surgical pathology, immunohistochemistry is a powerful ancillary method, particularly in cases with multiple metastases of unknown primary site.

#### P 910

##### **POLY(ADP-RIBOSYLATION) MODULATES THE EXPRESSION OF TUMOR-RELATED GENES DURING CARCINOGENESIS**

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**PARP-1**, an enzyme that catalyzes the attachment of ADP ribose units to target proteins, acts as a component of enhancer/promoter regulatory complexes. In a previous study we have shown that *parp-1* knockout mice exhibit resistance to chemical carcinogenesis and this was related to a decreased NF- $\kappa$ B activation. **Objective:** Our aim was evaluated the effect of *parp-1* inhibitor on skin carcinogenesis in a model of *parp-1* knockout mice. **Material and Methods:** We used cDNA expression arrays analysis, Real-Time PCR, image analysis and morphological conventional techniques in this study.

**Results:** We show that pharmacological inhibition of PARP-1 with DPQ (3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone) results in a strong delay in tumor formation and in a dramatic reduction in tumor size and in skin morphological changes and multiplicity during DMBA+TPA-induced skin carcinogenesis. PARP inhibition also affected AP-1 activation but not NF- $\kappa$ B. Using cDNA expression arrays analysis, a substantial difference in key tumor-related gene expression was found between chemically-induced mice untreated or not with PARP inhibitor and also between wild type and *parp-1* knockout mice. Most important differences were found in gene expression, mainly for *nfkbi2*, *s100a9*, *hif1alfa* and other genes all of them involved in carcinogenesis and inflammation. These results were corroborated by Real-Time PCR. In summary, this study shows that inhibition of PARP on itself is able to control tumor growth and PARP inhibition or genetic deletion of PARP-1 modulates tumor promotion through their ability to cooperate with the activation AP-1 and/or NF- $\kappa$ B and to regulate the expression of genes related with tumor promotion/progression.

#### P 911

##### **ABSENCE OF MYCOBACTERIUM TUBERCULOSIS PCR DETECTION FROM LYMPH NODES'SARCOIDOSIS GRANULOMAS ISOLATED BY LASER CAPTURE MICRODISSECTION.**

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Previous recent works have shown the usefulness of polymerase chain reaction (PCR) performed in granulomas isolated by laser capture microdissection (LCM) for improving the detection of *M. tuberculosis* in formalin-fixed paraffin-embedded tissues. Some studies have demonstrated that mycobacterial DNA can be sporadically detected by means of PCR amplification performed on formalin-fixed paraffin-embedded tissues in granulomatous diseases other than tuberculosis, such as sarcoidosis and Crohn's disease. The aim of the present work was to investigate if mycobacterial DNA could be detected by PCR in granulomas isolated by LCM from lymph nodes' sarcoidosis. The PCR DNA amplification method was used to detect *M. tuberculosis* in granulomas microdissected from one section stained by haematoxylin and eosin from a formalin-fixed paraffin-embedded specimen. The results were compared to those obtained from PCR performed from tuberculosis granulomas

with positive Ziehl staining. Twenty-nine formalin-fixed and paraffin-embedded samples from 29 patients with sarcoidosis and 24 similar conditioned samples obtained from 24 lymph nodes' tuberculosis were investigated by this combined method. After staining with hematoxylin and eosin, granulomas were microdissected using a LCM microscope (PixCell II, Arcturus Engineering). For the PCR reaction, we used specific sequences of the 16S ribosomal RNA gene to *M. tuberculosis*. Results: PCR from microdissected sarcoidosis granulomas procured from one 5 mm section of each specimen was negative in the 29 studied cases, whereas PCR from microdissected tuberculosis granulomas was positive in the 24 studied cases. In conclusion, our work showed that mycobacterial DNA is not detected in lymph nodes' sarcoidosis when using PCR on laser microdissected granulomas from formalin-fixed paraffin-embedded specimen.

#### P 912

#### **HYPERMETHYLATION PATTERNS IN THE FHIT REGULATORY REGION ARE TISSUE SPECIFIC** GULER G 1,2\*, ILIOPOULOS D 2,5\*, HAN Shuang-Yin 2, FONG Louise YY 2, LUBET Ronald A 3, GRUBBS Clinton J 4, HUEBNER K 2,5

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DNA hypermethylation is associated with decreased gene expression of tumor suppressor genes. In a previous study we observed decreased Fhit expression and Fhit promoter region methylation in rodent tumors induced by various carcinogens and noted differential methylation patterns in DNA from tissues of different organs; i.e., the 5' regulatory regions in the promoter, exon 1 and intron 1 were differentially methylated, depending on the tissue of organ. Because different carcinogens were used for induction of tumors of the different organs, we could not conclude that the methylation patterns were tissue-specific. In order to determine if 1) rat Fhit methylation status is related with expression levels and 2) rat Fhit methylation patterns were tissue or carcinogen-specific, by examining Fhit methylation status and expression levels in DMBA and MNU-induced benign and malignant mammary tumors. Fhit intron 1 was methylated in 3/9 DMBA and all (5/5) MNU-induced benign mammary tumors in association with reduced Fhit expression levels; Fhit promoter and intron 1 were methylated in all DMBA and MNU-induced tumors in association with highly reduced Fhit expression levels. Treatment of rat cancer cells in vitro with the DNA methyltransferase inhibitor, 5'-Aza-2'-deoxycytidine, for 4 days, increased Fhit expression and altered the methylation status. Before treatment both promoter and intron 1 regions were methylated; after treatment only intron 1 remained methylated. Thus there is an overall association of rat Fhit regulatory region methylation with its expression and hypermethylation patterns were tissue and not carcinogen-specific.

\*These authors contributed equally to this work

#### P 913

#### **HIGH MOBILITY GROUP (HMG) PROTEINS IN MALIGNANT TUMOURS - FROM DIAGNOSTIC MARKER TO INDEPENDENT PROGNOSTIC FACTOR?**

Introduction: High mobility group proteins are structural transcription factors with further involvement in enhancosomes and hormone-receptors. Because of their ability to boost proliferation, infiltration and metastasis of tumour-cells, they are sometimes referred to as kinases. In the normal adult tissue they are either not or only very weak expressed.

Purpose: In previous publications it was shown that HMGA1 and A2 are expressed in many neoplastic tissues and mark either malignancy or bad prognosis. Because there were no data concerning Breast- and Non-Small-Cell-Lung-Carcinoma (NSCLC) we started investigations concerning these frequent tumours.

Methods: Breast- and NSCLC tissue were investigated concerning HMG expression on RNA-Level (using Real-Time PCR) and Protein level (using Immunohistochemistry). First data are compared concerning immunohistological and Real-Time PCR results. Furthermore, first data of HMGA1 and HMGA2 expression in these frequent tumours are shown. Results and Conclusions: In breast-carcinoma HMGA1 expression is significantly correlated with Grading, Hormone-Receptors and HER-2.

In NSCLC HMGA2 is highly expressed in appr. 50% of squamous cell carcinoma and 20% of adenocarcinoma. There seems to be no correlation with any other morphological marker, but, as first data suggest, with disease free and overall-survival.

#### P 914

#### **PILOMATRIX CARCINOMA: A CHALLENGING DIAGNOSIS OF BASALOID CELL TUMORS**

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Pilomatrix carcinoma (PC) is a rare locally aggressive tumor of hair matrix that has a predilection for head and neck. There is male to female ratio of 2:1. The mean age of affected patients is 48 with a reported range from age 2 to 88. We report a case of PC treated by Mohs surgery that initially diagnosed as basal cell carcinoma (BCC) which is the main and most challenging differential diagnosis.

A 35-years old man was evaluated of a solitary, slow growing nodule on his dorsum of the nose. The nodule has been present for a few years without any pain and tenderness. The nodule was ulcerated by patient manipulation. On physical examination a 2cm firm nodule with telangiectasia and central depression on surface was noted on the dorsum of the nose. From initial biopsy specimens, It has been reported lobulated island of basaloid cells connected to the epidermis with a final diagnosis of BCC that involve surgical margins and depth. Mohs surgery carried out for an incomplete excised and recurrent BCC of the nose. Frozen section revealed island of tumors in deep dermis without connection to epidermis. Permanent slides showed sheets and nests of neoplastic basaloid cells in deep dermis, hypodermis and between muscles with varying degree of central necrosis. The neoplastic cells had hyper chromatic nuclei, moderate pleomorphism and numerous mitosis, some of which were atypical. The mitotic rate was 75 per 10 high power fields (35/mm<sup>2</sup>). The basaloid nest went abrupt transition to central necrotic areas with pycnotic and shadow cells. Using immunohistochemical techniques, Ki-67 showed proliferation rate of 50%. Bcl-2 was expressed in 3% and P53 in 36% of the tumor cells. A large portion of neoplastic cells expressed Low molecular cytokeratin (CK19). S100 and CEA were negative, all findings confirming the diagnosis of PC.

In summary, although the histopathological diagnosis of PC is simple, in the presence of epidermal connection as our case

in first biopsies, it will be problematic. However with more careful study, numerous mitosis and central necrosis can be seen which are rare in BCC.

#### P 915

##### **ROLE OF NON-HISTONE PROTEINS IN EARLY DIAGNOSIS OF NORMAL AND SKIN LESIONS**

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#### Abstract:

Objective: Evaluation of the diagnostic value of silver nucleolar organizer Region assessment in skin diseases is important. According to this reason we want to study the Argyrophilic Nucleolar Organizer Region (Ag-NOR) count and Subjective Ag-NOR Pattern Assessment (SAPA) score in normal skin, Squamous Cell Carcinoma (SCC), Basal Cell Carcinoma (BCC) and Solar Keratosis (SK).

Method: The study groups considered of 69 cases (18 SCC, 18 SK, 17 BCC and 16 Normal skin) was studied by H&E staining and silver staining for Ag-NOR.

Results: The mean count was in SCC 2.92, in SK 2.29 in BCC 1.59 and 0.93 in normal skin (p value <0.001).

The SAPA score was in SCC 7.94 in SK 7.61 in BCC 6.35 and in normal skin 5.00 (p value <0.001).

Conclusion: Ag-NOR count and SAPA score in normal and pathological state of skin has positive results.

Key Words: Ag-NOR, SCC, BCC, SK, Skin.

#### P 916

##### **HISTOPATHOLOGIC STUDY OF ROSACEA**

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#### Background :

Rosacea is a common but ill-defined disease and Demodex follicularum has been reported in rosacea in a number of clinical studies. As the Demodex mite is also present in many healthy individuals, the mite may have a pathogenic role only when it is present in high densities.

In this study the possible role of demodex follicularum and the importance of mite density had investigated using an elliptical incisional skin biopsy technique.

#### Methods :

Thirty-nine patients with rosacea, twenty-five female, 14 male, and 41 age and sex matched healthy persons entered the study. With the incisional skin biopsy of face we studied six section of each sample. We studied the number of follicles, mite density in them, epidermal changes, solar elastosis, inflammatory cells in the dermis and their pattern, granuloma formation and vascular changes.

#### Result :

Varying degree of vasodilatation, solar elastosis and inflammatory infiltrate were found in all patients. The infiltrate were lympho-histiocytic in 29 cases, lympho-plasmacytic in 6 cases and neuropilic, specially around hair follicles in 2 cases, and eosinophilic infiltration mixed with mononuclear cells in 2 cases. The number of D. follicularum was statistically significant. Between male and female there was not important difference, 0.929 to 1.200, P value 0.81; .

There was important difference in the number of mite in rosacea related to age, it was 0.545 in under 40 year group and 1.308 over 40 year age, P value 0.047. But it was not meaningful in non rosacea group 0.118 mite per follicle under 40 and 0.509 mite per follicle over 40 year, P value 0.53.

There was granuloma to appendages in 3 of cases and granuloma around hair follicle in 2 of cases. One of the later group revealed degenerated demodex cuticle.

#### Conclusion :

Rosacea is a multifactorial disease, and high densities of demodex is important, in the form of hypersensitivity reaction to antigens or granuloma formation in response to its cuticle or destructed hair follicles. The individual properties may modify the severity of inflammatory response to demodex.

#### P 917

##### **ANALYSIS OF CYCLOOXYGENASE-2 EXPRESSION AS A POSSIBLE MARKER OF VITALITY IN CUTANEOUS WOUNDS**

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#### Introduction

The histologic differential diagnosis between vital and postmortem cutaneous wounds is difficult when the injury is inflicted close to the time of death. Ancillary techniques such as immunohistochemistry may aid to the diagnosis of vitality through the analysis of molecules implied in the initial inflammatory reaction. Cyclooxygenase-2 (COX-2), an enzyme rapidly synthesized in response to different insults to the skin, is essential for prostaglandin biosynthesis, which is an important process for cutaneous wound healing.

#### Purpose

Our objective has been to investigate COX-2 expression in vital and postmortem cutaneous wounds to check its possible application as a marker of vitality.

#### Materials and methods

41 samples from human incised cutaneous wounds were investigated comprising: 16 vital injuries aged 3 minutes to 8 hours (mean:125 minutes), 14 inflicted postmortem (collected after a period of 1-3 hours), and 11 scars from sutured surgical incisions whose age was 0.5 to 11 months. Formalin-fixed paraffin-embedded sections were immunostained through the labelled streptavidin-biotin technique using a monoclonal antibody anti COX-2 (DakoCytomation).

#### Results

All of the samples showed normal immunoreactivity for COX-2 in eccrine glands with faint staining in sebaceous glands and the basal layer of the epidermis. Differences in staining were not observed when comparing the injury edge with the opposite margin in every specimen, nor between vital and postmortem wounds. Scars with inflammatory reaction in the dermis (aged 0.5 to 3 months) showed overexpression of COX-2 in histiocytes and mononuclear cells.

#### Conclusions

Immunohistochemical analysis of COX-2 has not been useful to differentiate vital and postmortem injuries. Overexpression of COX-2 could only be detected in the histiocytic inflammatory phase of wound healing. For these reasons it is not a good marker to diagnose vitality in difficult injuries, aged just a few hours.

#### P 918

##### **EXPRESSION OF E-CADHERIN AND CATENINS IN VITAL AND POSTMORTEM CUTANEOUS WOUNDS**

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## Introduction

The differential diagnosis between vital and postmortem wounds is often difficult for the pathologist, specially when the injury is produced close to time of death. Immunohistochemical detection of different molecules involved in inflammatory reaction and healing process have been proposed to aid to the diagnosis. Re-epithelialization is a key process in the wound healing and it begins early after the injury. E-cadherin and catenins, critical for adhesion between keratinocytes, play an important role in the process. The diagnostic value of their expression as markers of vitality has not been well established.

## Purpose

Our objective was to analyse the expression of E-cadherin and catenins (alpha, beta and gamma) in vital and postmortem wounds as possible markers of vitality.

## Materials and methods

The study comprised 42 human incised wounds (32 vital and 10 postmortem) and 3 cutaneous scars. The vital wounds were obtained from abdominal surgical incisions with post infliction intervals between 55 minutes and 8 hours. Two of the scars were 2 months old and the other was 5 months old. The postmortem specimens were obtained from autopsy abdominal incisions and were collected after a mean period of 105 minutes (range 15-180).

Formalin-fixed paraffin-embedded sections were immunostained following the labelled streptavidin-biotin technique with monoclonal antibodies against E-cadherin (BioGenex) and alpha, beta and gamma catenins (Transduction Laboratories). Staining of the wound edge was compared to that of the opposite margin of the sample.

## Results

Differences in the staining between the two margins of the specimen were noted in 11 cases (9 vital and 2 postmortem). The staining was increased in the wound edge in 7 cases and in the control edge in 4. The differences corresponded to E-cadherin in 5 cases, alpha-catenin and gamma-catenin in 3. No differences were appreciated for beta-catenin.

## Conclusions

Immunohistochemical analyses of E-cadherin, alpha, beta and gamma catenins are not useful for the differential diagnosis between vital and postmortem wounds. This could be related to a low sensitivity of immunohistochemical techniques to detect the subtle changes of levels of these proteins at the beginning of the inflammatory reaction.

## P 919

### GENERALIZED LICHEN AMYLOIDOSIS: A CASE REPORT

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Lichen amyloidosis is a type of cutaneous amyloidosis, caused by the extracellular deposition of amyloid composed primarily of degenerated keratin filaments. Generalized lichen amyloidosis is a rare form of the disease and characterized by pruritic discrete hyperkeratotic scaly papules. We report a case of lichen amyloidosis with extensive distribution in a 33-year-old female.

## P 920

### SUBACUTE RADIODERMATITIS

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Introduction : Radiodermatitis is a rare cutaneous disorder induced by radiation used in diagnostic and interventional procedures. The histologic characteristics of this entity have been poorly described.

Case report : A 74-year-old man with a history of coronary disease presented with a 10-centimeters, pruritic, fixed, well demarcated erythematous patch of the back, which had begun four weeks after a coronary artery stenting. A skin biopsy was performed showing interface dermatitis. Given the history of radiation exposure and the clinical course of the lesion, the histological findings were felt to be consistent with subacute radiodermatitis.

Discussion : Skin damages in subacute radiodermatitis involve epidermis and dermis. The main histological findings are : interface dermatitis with keratinocyte necrosis, clefting along the dermal-epidermal junction and variable perivascular lympho-histiocytic infiltrate with no eosinophils. Key differentiating factors from graft-versus-host disease and fixed drug reaction include history, clinical morphology, type and distribution of cutaneous infiltrate.

Conclusion : With the increased use of radiation for diagnostic and interventional technique, this entity should be known and discussed by both clinicians and pathologists, when an interface dermatitis is observed.

## P 921

### IMMUNOHISTOCHEMICAL STUDY OF SKIN LESIONS AFTER THERAPEUTIC IRRADIATION

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Introduction. We focused on skin alterations after cobalt-therapy knowing that exposure to overdoses of radiation could induce cancerous lesions.

Purpose of the study. We studied the expression of S100 protein in order to assess melanoblastic cells, dendritic cells and nerves, Vascular Endothelial Growth Factor (VEGF) for its mitogenic effects on endothelial cells and that of cytochrome c to evaluate apoptosis as a phenomenon involved in regulation of cell number using immunohistochemistry.

Materials and methods. Samples of breast integument irradiated for therapeutic reasons and control non-irradiated ones were collected after tumor excision and then processed for paraffin embedding. Serial sections were rehydrated and incubated with each required primary antibodies and then the immunoreaction was amplified using avidin – biotin or PAP (peroxidase - antiperoxidase) technique.

Results. After irradiation we noted an increased number of melanoblastic cells in the epidermal basal layer and also in the deep intermediate one revealed by the S100 immunolabeling. The same antibody marked also an increased number of Langerhans cells with the same distribution. The latest were also increased in the papillary dermis with a pericapillary disposition from the irradiated skin compared to control samples.

VEGF were found positive in few stromal cells and negative in endothelial cells from normal integument. In the irradiated dermis the number of VEGF positive fibroblast cells was increased and same the endothelial cells of capillaries from the papillary dermis. Vessel walls of arterioles and venules were entirely negative.

Epidermal germinative layer displayed few cytochrome c positive cells disposed with an insular pattern. Some cells from vessel walls were also positive for the apoptotic marker. Conclusions. Irradiated skin has a non specific cellular reaction similar to that from the inflammatory process. The most obvious changes were found in the upper dermis layer

where mitotic effect and increase of permeability induced by VEGF seem to imbalance apoptotic post irradiated response.

#### P 922

##### CD 30 EXPRESSION IN TWO CASES OF NODULAR SCABIES

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CD30 antigen, protein of the tumor necrosis factor receptor family is a marker of lymphocyte activation. Its expression in large atypical cells has been observed in non neoplastic cutaneous inflammatory infiltrates. We report two cases of nodular scabies with CD30-positive large cells.

A 74-year-old woman presented with a nine-month history of generalized pruritus. On examination there were well demarcated papuloerythematous prurigo-like lesions on the back and the buttocks. The second patient, a 30-year old man, presented with a persistent pruritus after scabies treated six months before. Clinically, papulonodules of the external genitalia and the buttocks suggested nodular scabies. Histopathological examination of the two cutaneous biopsies revealed a prominent perivascular inflammatory infiltrate in the papillary and reticular dermis. It contained lymphocytes, histiocytes, few eosinophils and many large lymphoid cells with cleaved nuclei and one or more large nucleoli resembling Reed-Sternberg cells. Many mitoses were found in one case. Immunohistochemical analysis revealed that most cells of the infiltrate including large cells were CD3 positive. The large cells expressed CD30 in a membranous pattern with accentuation of the Golgi apparatus. We suspected a primary cutaneous CD30-positive lymphoproliferation. Nevertheless the demonstration of *Sarcoptes Scabiei* after skin scraping and the improvement of cutaneous lesions with treatment for scabies led us to the diagnosis of nodular scabies.

The presence of large CD30-positive atypical cells has been reported in cutaneous inflammatory infiltrates of scabies with long standing lesions. No CD30 expression has been found in early lesions (less than two months). Atypical CD30-positive lymphoid cells have also been observed in activated lymphocytes in a variety of inflammatory reactions rich in neutrophils and eosinophils especially in insect bites, infectious conditions including bacterial, viral (herpes virus and molluscum contagiosum) and fungal, hidradenitis suppurativa, Sweet syndrome, drug eruptions...

CD30 expression in cutaneous infiltrates is not uncommon in non neoplastic inflammatory conditions and doesn't imply a diagnosis of primary cutaneous CD30-positive lymphoproliferative disorder, particularly lymphomatoid papulosis: others parameters (clinical feature, evolution) are required to establish this definitive diagnosis.

#### P 923

##### MERKEL CELL CARCINOMA – CASE REPORT

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Introduction: Merkel cell carcinoma (MCC), first described by Tang and Toker in 1972., is a rare highly malignant skin tumour of neuroendocrine origin. Pathognomonic immunohistochemical markers for the diagnosis are epithelial (CK20), as well as neuroendocrine (chromogranine) markers.

Case report: A 69 years old woman, showed dark-red nodules, located in the lateral side of the her right leg. Although the mass was large measuring 28 cm, there was no evidence of nodal involvement. It consisted of small epithelial cells (small cell type) which formed solid cell complexes, arranged in characteristic trabecular patterns. The tumour cells were

monomorphic, with oval nucleus, numerous mitotic figures, and massive necrosis. These cells infiltrated subcutaneous fat. The diagnosis MCC was confirmed through the histological and immunohistochemical analysis: Merkel cell tumour expressed epithelial (CK20) as well as neuroendocrine (chromogranine) markers.

Conclusion: In addition to histological/immunohistochemical characteristics for MCC diagnosis, the clinical data that indicate on primary skin tumour, provide a great help to pathologist in establishing the diagnosis of Merkel cell carcinoma.

#### P 924

##### ASSOCIATION OF MERKEL CELL CARCINOMA AND BOWEN'S DISEASE

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##### Introduction

Merkel cell carcinoma (MCC) is an uncommon neuroendocrine malignant neoplasm whose origin has not been well established. Little is known about its association with other malignant skin diseases, although there have been some reports associating it with basal cell carcinoma in-situ or infiltrating squamous cell carcinoma and malignant melanoma.

##### Case report

An 86-year-old woman was referred to the hospital complaining of a 10-cm tumour located in her left forearm. The tumour was completely excised.

Histopathological examination showed dermal nests of small and medium-sized, round, basophilic cells arranged in a trabecular or diffuse pattern, extending deeply through the dermis to the subcutaneous fat tissue. In some sections, the keratinocytes located in the overlying epidermis were atypical and immature in appearance, exhibiting hyperchromatic nuclei and pleomorphism. Occasionally, dyskeratotic cells and areas of pagetoid appearance were also observed.

Dermal tumour cells were positive for neuron-specific enolase, synaptophysin, chromogranin A, CK 20 and CK AE1-AE3. The neoplastic epidermal cells were negative for neuron-specific enolase, synaptophysin, chromogranin A and CK20, but strongly positive for CKAE1-3. The diagnosis of Merkel cell carcinoma associated with Bowen's disease was established. The patient died two years later of an unrelated disease.

##### Comments

Histopathological examination and immunohistochemistry clearly demonstrate that this patient has two different but synchronous neoplasms arising in the same place. The reason for the association of both neoplasms remains unknown. Although the induction of a second neoplasm by the first tumour cannot be excluded, the most probable explanation is that we are dealing with a collision tumour, well casual or because they share some common etiopathogenic agents or risk factors that have not yet been identified.

#### P 925

##### CYCLO-OXYGENASE 2 EXPRESSION IN MERKEL CELL CARCINOMA

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**Introduction:** Merkel cell carcinoma (MCC) is an aggressive neuroendocrine tumor of the skin associated with a high rate of recurrence and metastasis and only partial response to current radio- and chemotherapy regimens. The cyclooxygenase 2 (COX2) enzyme is involved in the development and progression of many tumors, including large bowel, breast and lung cancers. COX2 inhibitors have been shown to block tumor growth. The aim of this study was to examine COX2 expression in 26 primary MCCs and 5 of their lymph node metastases.

**Materials and methods:** Formalin-fixed paraffin-embedded tissues were stained immunohistochemically with monoclonal antibody directed against COX2. The percentage and intensity of staining were analyzed semiquantitatively using a three-tiered system.

**Results:** Immunopositivity for COX2 was found in 20 out of the 26 primary tumors (76%), including 16 (80%) with widespread positivity. Staining intensity was mostly weak (9 tumors-45%) or moderate (6 tumors-30%). Three of the 5 metastases (71%) showed similar staining. Tumors with prominent mitotic activity were associated with more widespread COX2 immunopositivity. No association was found between COX2 expression and outcome.

**Conclusions:** Most MCCs express COX2. Expression is related to one parameter of aggressive behavior- a high mitotic rate- but not to any others. Owing to the large number of MCCs that express COX2, the possibility of treating patients with COX2 inhibitors should be considered.

#### **P 926**

##### **MYXOID NEUROTHEKEOMA: IMMUNOHISTOCHEMICAL ANALYSIS OF A CASE**

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**Introduction:** The myxoid variant of neurothekeoma is a benign, myxomatous neoplasm, that most probably originates from the peripheral nerve sheath. Recently it has been proposed that the so-called cellular and mixed (intermediate) variants of neurothekeomas warrant a separate classification, since they show no evidence of neurosustentacular differentiation.

**Method:** Herein, we describe the clinical, histopathologic and immunohistochemical features of a case of myxoid neurothekeoma, in a 53-year-old woman.

**Results:** The patient presented with a subcutaneous tumor, measuring 1.5 cm in maximal dimension at her lower back (sacroccocygeal area). The preoperative diagnosis was a lipoma. On macroscopic examination, a circumscribed mass with a rubbery to hard consistency and gray-white cut surface was noted. On microscopic examination, the tumor was centered to the dermis, and extended to the subcutaneous fat, without extending to the epidermis. At low-power magnification it had a multinodular architecture, with varying-sized nodules of moderate cellularity, separated by dense bands of collagen. The neoplastic cells were small, stellate- or oval-shaped, with bland nucleus, pale eosinophilic cytoplasm and very rare mitoses. They were loosely arranged within Alcian blue-positive myxomatous stroma. Upon immunohistochemical examination, the neoplastic cells showed strong nuclear and cytoplasmic staining for S100 protein. In addition they exhibited weak immunoreactivity for NSE (Neuron Specific Enolase) and CD57 (Leu-7). Based on the morphologic and immunohistochemical features the diagnosis of myxoid neurothekeoma was made. The tumor had been excised completely. The patient is well 1 year after excision, without evidence of local recurrence.

**Conclusion:** Myxoid neurothekeoma is a benign neoplasm of probable Schwannian origin. Dermatologists should be aware of this neoplasm, whose complete surgical excision appears to be curative.

#### **P 927**

##### **INTRAPARENCHYMAL MELANOCYTIC NEVUS IN AXILLARY LYMPH NODE**

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##### **INTRODUCTION**

Benign melanocytic cells within lymph nodes is a well-documented but unusual finding. Stewart and Copeland firstly reported it in 1931. Focal aggregates of nevocytes are occasionally found in the fibrous capsule and trabeculae of lymph nodes, whereas an intraparenchymal location is exceptional. Only 20 cases have previously been reported. Non-malignant cellular inclusions can be misinterpreted as metastases from a melanoma or carcinoma.

We report here an intraparenchymal nodal nevus within an axillary node removed from a patient with breast cancer and nodal metastases.

##### **CASE REPORT**

A 64-year-old woman underwent a left radical modified mastectomy with axillary lymphadenectomy for a 6cm breast carcinoma in the external quadrants. Tissue specimens removed were fixed in 10% buffered formalin and routinely processed for light microscopy. Histopathological examination showed a multicentric infiltrating pleomorphic lobular carcinoma with histological grade 3 and an associated in situ component. Metastases were found in 5 out of the 24 nodes identified.

Clusters of medium size cells were detected within the nodal parenchyma, as well as in the capsule and trabeculae of one of the metastases-free lymph nodes. These cells showed a pale cytoplasm, with little or no melanin pigment and indistinct cell borders. Nuclei with diffuse chromatin pattern were centrally placed. Cytological atypia, mitosis, and prominent nucleoli were not observed. When we compared this aggregate with the primary carcinoma cells they showed morphological differences. The query cells were positive for S-100 but negative for CEA, Keratins, HMB-45, Ki-67 and hormone receptors.

##### **CONCLUSION**

Melanocytic nevi within lymph node parenchyma is a very unusual finding.

Nevus cell aggregates could be easily mistaken for metastatic melanoma or carcinoma. A differential diagnosis between nodal nevi and metastases requires a careful morphological examination and supportive immunohistochemical studies, mainly in patients with a primary tumor without obvious features of malignancy.

This case highlights the rarity of nevus cell aggregates within the parenchyma of lymph nodes, and alerts the pathologist to avoid a misdiagnosis of malignancy.

#### **P 928**

##### **EZH2 EXPRESSION IS ASSOCIATED WITH INCREASED PROLIFERATION RATE AND AGGRESSIVE TUMOR SUBGROUPS IN MELANOMA AND CANCER OF THE ENDOMETRIUM, PROSTATE, AND BREAST**

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#### Introduction:

The Enhancer of Zeste Homolog 2 (EZH2) is a member of the polycomb group of genes (PcG), which are important for transcriptional regulation through nucleosome modification, chromatin remodelling and interaction with other transcription factors. EZH2 serves as a histone methyl transferase (HMT), and disruption of EZH2 expression may lead to dysregulation of genes critical for the G2/M transition. Further, EZH2 is controlled by E2F transcription factors, which is downstream of the retinoblastoma protein (Rb), and is also involved in p53 regulated cell cycle control. EZH2 is previously shown to be overexpressed in prostate, and breast cancer, and increased expression evaluated by in situ mRNA was found in 34% of human cancers in a recent study.

#### Purpose:

The aim of our study was to evaluate the expression of EZH2 in a broader range of human cancers, with special attention to key features such as tumor cell proliferation, tumor extent, and patient outcome. This is especially relevant since the polycomb group proteins have recently been suggested as candidates for targeted therapy.

#### Patients and methods:

In our study of 696 patients, we examined EZH2 expression by immunohistochemistry using high-throughput Tissue Microarray (TMA), and its association with clinical features and other tumor markers in malignant melanoma and cancers of the endometrium, prostate and breast.

#### Results:

Strong EZH2 expression was significantly associated with increased tumor cell proliferation in all four cancer types. Associations were also found between EZH2 and important clinico-pathological variables, such as tumor thickness and level of invasion, histologic grade, locally invasive growth, and extent of the disease. Further, EZH2 expression showed significant prognostic impact in melanomas, prostate and endometrial carcinomas in univariate survival analyses, and revealed independent prognostic importance in carcinomas of the endometrium and prostate.

#### Conclusion:

Our present findings point at EZH2 as a novel prognostic marker in malignant melanoma and endometrial cancer, and extend previous findings on prostate and breast cancer. The fact that EZH2 might identify aggressive subgroups in several cancers may be of significant practical interest, since the polycomb group proteins have recently been suggested as candidates for targeted therapy.

#### P 929

##### **TMA: CADHERINS & CATENINS STUDY IN DIFFERENT STAGES PROGRESSION OF CUTANEOUS MALIGNANT MELANOMA**

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#### INTRODUCTION

Cutaneous malignant melanoma, the most aggressive common skin tumor, is characterized by a multifactorial etiology. The incidence and mortality of this tumor have increased over the last several decades.

Cadherins comprise a growing family of surface membrane glycoproteins that mediate cell-cell adhesion. The extracellular domains of cadherins serve as zippers connecting neighboring cells, whereas the cytoplasmic tails are linked noncovalently to the actin cytoskeleton via catenins.

#### PURPOSE OF STUDY

The aim of our study is to determine the protein expression patterns of some catenins and cadherins using tissue microarrays (TMA), in 191 malignant melanomas samples from 162 patients in different histological progression phases. The study tries to identify a set of stage-specific markers, and allows a predictor model to be developed that identifies groups of patients with increased risk of death.

#### METHODS USED

Primary radial and vertical phases, and metastatic malignant melanoma were used to construct a TMA's from a series of 162 patients diagnosed at Ramon y Cajal University Hospital, Madrid, between 1978 and 2002. There was no history of familial incidence. We also included normal tissues as internal controls, with a 1.5mm diameter needle.

Antibodies used for these study were E-Cadherin, N-Cadherin, Cadherin 11,  $\beta$ catenin,  $\beta$ catenin and p120.

#### RESULTS

The serie compriseS 162 patients diagnosed of cutaneous malignant melanoma. Forty (20.7%) radial growth phase malignant melanomas, 128 vertical ones (66.3%), and 24 (12.4%) cutaneous or ganglionar metastases. Patients with radial growth phase malignant melanomas never had metastases, and vertical growth phase malignant melanomas had metastases in 42.9% of cases.

In general, all immunostaining showed a membranous pattern, except for  $\beta$ catenin which also expressed on the nucleous. E-cadherin and  $\beta$  catenin expression showed notably decrease with melanoma progression. The expression pattern of  $\beta$ -catenin was very similar to p-120 expression. Both proteins showed moderate decrease with tumor progression. The expression pattern of Cadherin11 was absent.

#### CONCLUSIONS

The current study demonstrates the suitability analysis of TMA technique in the study of a classical model of human tumor progression. This study also demonstrates qualitative changes in the expression of some catenins and cadherins in different stages of malignant melanoma progression.

#### P 930

##### **THE CORRELATION OF ANGIOGENESIS WITH METASTASIS IN PRIMARY CUTANEOUS MELANOMA: A COMPARATIVE ANALYSIS OF MICROVESSEL DENSITY, EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND BASIC FIBROBLASTIC GROWTH FACTOR**

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Tumor cells supply oxygen and nutrients necessary for growth by the recruitment of new blood vessels. Angiogenesis is not only a must in tumor growth but in metastasis as well. However, the prognostic significance of angiogenesis in malignant melanoma is controversial. In order to find out the correlation of angiogenesis with metastasis in primary cutaneous melanoma (PCMM), we studied the microvessel density, the expression of vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (bFGF) in 22 cases of PCMM with metastasis at presentation (metastatic group) and 28 cases of PCMM without any metastasis for 24 months or more (nonmetastatic group). Microvessels were stained with CD31/PECAM-1 antibody and counted. Besides the proportion of VEGF expression in tumor cells, lymphocytes infiltrating the tumor (TIL) and lymphocytes at the periphery of the tumor, as well as, the proportion of bFGF expression in tumor cell cytoplasm, nuclei and intra- and peritumoral vessels were assessed. An increased microvessel density was detected in the metastatic group [15-33

(24.09±5.55) vs. 2-24 (12.96±6.02)]. Moreover, enhanced expression of VEGF in tumor cells and peritumoral lymphocytes (Chi-square  $p=0.038$  and  $p=0.018$ ) and bFGF in peritumoral vessels (Chi-square  $p=0.013$ ) correlated with the simultaneous presence of melanoma metastasis in PCMM. In conclusion microvessel density, as well as, the expression of both VEGF and bFGF might be informative concerning the progression of melanoma.

### P 931

#### THE MITOCHONDRIAL SIGNATURE OF MELANOMAS PROVIDES A MARKER OF PROGNOSIS

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**Introduction:** A large body of clinical data documents that most human carcinomas contain reduced levels of the catalytic subunit of the mitochondrial H+ATP synthase (B-F1-ATPase). In colon and lung cancer this alteration correlates with a poor patient prognosis.

**Purpose of the study:** In this study we have investigated the mitochondrial phenotype of melanomas as a prognostic marker of disease progression.

**Material and methods:** A set of twenty-one nevi, forty-one melanomas and thirteen metastases were examined for quantitative differences in protein expression of the mitochondrial B-F1-ATPase and Hsp60 markers by immunohistochemical techniques. Quantification of the expression level of the markers was carried out in digital images acquired at 20x magnification with a Leica DC100 camera and processed with the Leica QWin software. Correlations of the expression level of the protein markers were established with the clinicopathological information of the melanomas and the follow-up data of the patients.

**Results:** The expression B-F1-ATPase and Hsp60 was significantly higher in melanomas and metastases than in nevocellular nevi. However, with advanced disease, the expression of both mitochondrial markers was significantly diminished in the melanomas. Cluster analysis by the expression level of the mitochondrial markers allowed the identification of a group of melanomas that predicted a worse prognosis for the patients. The good prognosis group had an expression level of B-F1-ATPase that was 50% higher ( $P<0.001$ ) than that found in melanomas of worse prognosis. Kaplan-Meier survival analysis showed that the expression level of B-F1-ATPase significantly correlated ( $P=0.024$ ) with overall survival of the patients.

**Conclusion:** The alteration of the mitochondrial proteome of melanomas provides a relevant marker to add in the prognosis of these patients.

### P 932

#### IMMUNOHISTOCHEMICAL EXPRESSION OF MSH-2 AND MLH-1 EN MALIGNANT MELANOMAS: CORRELATION WITH TUMOR STAGE AND PROGNOSIS

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**Introduction:** Defects in DNA mismatch-repair genes MLH-1 and MSH-2 first described in hereditary nonpolyposis colon cancer, have been postulated to be responsible for malignant transformation in many types of tumors, including malignant melanomas.

**Purpose of the study:** The main aim of this study was to investigate the expression of these genes in malignant melanomas in relation to tumor stage and prognosis.

**Methods:** We examined the protein expression pattern of MLH-1 and MSH-2 by immunohistochemistry in 20 cutaneous melanomas. Formalin-fixed paraffin-embedded sections were immunostained following the labelled streptavidin-biotin technique (LSAB20, DAKO) using monoclonal antibodies against MLH-1 (1:10, clone G168-15, BD Biosciences) and MSH2 (1:200, clone G219-1129, BD Biosciences). For epitope retrieval slides were pretreated with citrate buffer for 5 minutes in a pressure cooker.

Association of expression of MSH-2 and MLH-1 with clinical stage (pT1, pT2, pT3 and pT4) and prognosis (alive or died) was evaluated.

**Results:** The study cohort comprised 12 females and 8 males ranging in age from 17 to 74 years (average 55 years). The 20 patients in the study cohort had malignant melanomas with the following pT distribution: pT1:35%, pT2:20%; pT3:10% and pT4:35%.

Relation between tumor stage and detection of MLH-1 was: pT1:50%, pT2:42%, pT3:37% and pT4:48%.

Relation between tumor stage and presence of MSH-2 was: pT1:81%, pT2: 82%, pT3: 75% and pT4:44%.

Relation between prognosis and positivity for MLH-1 was: alive: 46.6%; died:46.4%.

Relation between prognosis and detection of MSH-2 was: alive: 80%; died:45.7%.

The immunohistochemical expression of MSH-2 was significantly correlated with tumor stage ( $p=0.01$ ) and prognosis ( $p=0.002$ ).

**Conclusions:** The analysis of mismatch-repair genes MLH1 and MSH-2 can be studied by immunohistochemistry. Our study demonstrates that expression of MSH-2 has statistical correlation with tumor stage and prognosis. Further studies with larger series should be performed in order to confirm our results.

### P 933

#### VEGF AND VEGF-C IMMUNOHISTOCHEMISTRY IN HUMAN CUTANEOUS NEVI AND MALIGNANT MELANOMAS

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Vascular endothelial growth factors (VEGFs) are a family of secreted endothelial cell-specific cytokines that act as endothelial cell mitogens. They play a hierarchical role in regulating physiologic and pathologic angiogenesis. These factors are involved in vasculogenesis and vasculogenic sprouting which are crucial for tumour development and metastasis. VEGFs also act as vascular permeability factors. VEGFs are overexpressed in many skin disorders characterized by angiogenesis and increased vascular permeability. In order to determine their possible role in melanocytic tumour progression, VEGF (immunoreactive for VEGF-A and VEGF-C) immunohistochemical expression were evaluated in 107 biopsy specimens of human skin including 28 superficial spreading melanomas (SSM), 40 nodular melanomas (NM) and 39 common nevi. Primary mouse monoclonal antibody VEGF (Santa Cruz Biotechnology, clone C-1, diluted 1:50) and goat polyclonal antibody VEGF-C (Santa Cruz Biotechnology, clone N-19, diluted 1:50) were used for the analysis. VEGF was expressed

markedly not only in malignant melanoma cells but also in half the cases of common nevi. In malignant lesions staining of stromal cells - fibrocytes, endothelial and inflammatory cells predominated. VEGF-C staining was found mainly in malignant lesions but less frequently in cells of common nevi. Stromal staining in malignant lesions was significantly higher in comparison with the stroma microenvironment surrounding moles. Our results indicate that VEGFs expression may regulate skin vessel function under benign as well malignant conditions and therefore cannot be used to discriminate between malignant melanoma and benign melanocytic lesions. Nevertheless strong up-regulation of the above mentioned factors may contribute to the progression of melanoma.

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#### P 934

##### **IMMUNOHISTOCHEMICAL ANALYSES OF KI67, P53 AND BCL-2 EXPRESSION IN CUTANEOUS MELANOCYTIC LESIONS**

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**INTRODUCTION:** The clinical behaviour of melanoma is often unpredictable using clinical and histological criteria. Differentiation between naevi, especially dysplastic naevi and melanomas, can sometimes be difficult to assess by conventional histopathological analysis. Malignant transformation of melanocytes is a multistep process characterized by distinct histopathological stages.

**AIM:** To analyse the differences in proliferative activity and apoptosis between intradermal naevi (IN), dysplastic naevi (DN) and cutaneous melanoma (CM).

**MATERIALS AND METHODS:** 45 IN, 45 DN and 45 CM (thickness less than 1.5 mm) were immunohistochemically stained for Ki-67, which was used as a proliferative marker, p53 as an apoptotic and bcl-2 as an antiapoptotic marker. The number of positive cells was counted on 200 melanocytes.

**RESULTS:** The median values of Ki-67 positive cells in IN, DN and CM were 2.5, 6.0 and 12.16, respectively. Statistical analysis showed a significant difference in proliferative activity comparing IN, DN and CM group ( $p=0.000$ ) with a significant positive correlation ( $r=0.659$ ,  $p=0.000$ ) between the proliferative activity and lesion malignancy. The p53 overexpression was present in 3.3 IN, 50.0 DN and 22.6 CM cells. Statistical analysis showed that the observed difference in p53 overexpression comparing IN with both DN and CM is statistically significant ( $p=0.000$ ), but showed no significant difference between DN and CM. The median values of bcl-2 in IN, DN and CM were 49.2, 42.3 and 26.7. Statistical analysis showed a significant difference between IN and DN comparing to CM ( $p=0.000$ ).

**CONCLUSION:** Our preliminary results show a progressive growth of proliferative activity from benign to malignant melanocytic lesions. The strongest expression of p53 in DN suggests that its overexpression could be the first step in the loss of cell cycle regulation and possible malignant transformation of melanocytes. Lack of bcl-2 expression correlates with progression of malignant melanoma and it could be related with poor prognosis.

#### P 935

##### **CUTANEOUS B-CELL LYMPHOMA IN AN ELEVEN YEAR OLD IRANIAN GIRL**

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Primary cutaneous B-cell lymphoma (PCBCL) is an uncommon neoplasm of skin giving rise to anywhere between 4.5 to 20 percent of all primary cutaneous non-Hodgkin's lymphomas (Zackheim HS, et al. J Am Acad Dermatol 2000; 43:793-96). Although this type of lymphoma was primarily reported in older individuals, the occurrence of this neoplasm has also been observed in children. We would like to report a unique case study performed on an 11-year old Iranian female suffering from a slowly expanding round tumour on her left lower leg.

The 11-year old Iranian girl was seen in our clinic with a 2-year history of asymptomatic tumours on her left lower leg. Physical examination revealed two firm tumoral masses on her lower leg, the larger measuring 8 cm x 8 cm. No hepatosplenomegaly or lymphadenopathy was detected. The patient otherwise felt well and a review of systems was negative. Laboratory studies, X-ray of leg, CT scan of chest, abdomen and pelvic and sonography were all normal. Smear for leishmaniasis, culture for deep mycosis and atypical mycobacterium were all negative. Histopathologic examination of multiple ellipse biopsy specimens from the larger lesion demonstrated dense diffuse infiltration of small blue round cells in superficial and deep dermis in 'botton heavy' pattern with destruction of hair follicles and eccrine glands. The neoplastic cells have round hyper chromatic atypical nuclei and scant cytoplasm of small to intermediate size without any rosette formation. No differentiation is observed. Mitosis is inconspicuous. No well-formed germinal centers were evident and tingible body macrophages were not seen. Using immunohistochemical techniques, a large portion of neoplastic cells expressed common leukocyte antigen confirming the lymphoid origin of the tumour with demonstrated lambda light chain restriction. Stains with CD19 were invariably positive. Stain with HMB45, S100, cytokeratin20, CD5, CD3 were all negative; the latter excluded any T-cell lymphoma.

In summary, PCBCL is an entity that can be suspected clinically, detected and confirmed pathologically. To the best of our knowledge this is amongst the first rare reports of PCBCL without dissemination in a very young girl.

#### P 936

##### **HISTOPATOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL CORELATIONS IN CUTANEOUS LEIOMYOSARCOMAS (CLMS)**

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**Introduction:** Cutaneous leiomyosarcomas (CLMS) is a malignant tumour who may be derived from arrector pili muscles or subcutaneous blood vessel walls and is more common in a deep location.

**Materials and methods:** The authors make a retrospective study of 10 parafin - embedded cases diagnosed histopathological like leiomyosarcomas and confirmed by immunohistochemical reactions. The immunohistochemical tests are : Actin, Desmin, Mioglobulin, Caldesmon, Vimentin, S-100 protein, citokeratin. IHC study was carried on formalin

fixed, paraffin - embedded tissue using the avidin-biotin peroxidase complex.

Discussion and conclusion: Histopathological ( with hematoxylin and eosin stain), CLMS is composed by numerous interlacing bundles of smooth muscle cells with eosinophilic cytoplasm and large , sometimes monstrous , hyperchromatic , blunt-ended nucleus.

Their pleomorphism and presence of mitotic activity , particularly abnormal and greater , constituting an important criteria for malignancy. Sometimes areas of myxoid degenerescensy may occur.

Usually CLMS can occurs at any age but in childhood are rare ; both sexes are affected , with a slight predilection for males ; is present tendency to local recurrence . Most of the tumors appear solitary ( nodules ulcerated or not) and the usual site is the limbs

( especially the legs).

#### P 937

##### **MALIGNANT ECCRINE SPIRADENOMA (SPIRADENOCARCINOMA) : A CASE REPORT**

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Introduction : Eccrine spiradenocarcinoma is a rare dermal appendage carcinoma believed to arise from transformation of a long-standing benign spiradenoma. This tumor demonstrates highly malignant biologic behavior with a high recurrence rate, frequent lymph node metastases, and overall poor survival.

Observation: We report the clinicopathologic and immunohistochemical features of a spiradenocarcinoma occurring in the forehead of a 73-year-old man. The lesion had been present for 10 years; recently, it had increased in size and had bled occasionally. Cervical lymph nodes were inconspicuous on palpation and full body examination was within normal ranges. Sections cut from excision specimen revealed a lobulated tumor with illdefined borders and cavity-like structures. The proliferation, located in the dermis without obvious continuity with the epidermis, consisted of multiple lobules of epithelial cells surrounded by connective tissue and contained ducts and cystic spaces. The tumor cells were polygonal in shape with scant pale cytoplasm and round slightly hyperchromatic nuclei. Mitoses were up to four per high-power field. Areas of necrosis were seen. In certain foci, especially at the periphery of lobules, sheets, nests and glandular structures were surrounded by abundant stroma and infiltrated skeletal muscle infiltration and perineural tissue. Immunohistochemical studies for markers of proliferation (Ki-67, Mib1, proliferating cell nuclear antigen: PCNA) revealed 80% of tumor cells staining positive for PCNA and 20% for Ki-67. One year after the excision of the nodule, the patient is free of signs and symptoms of recurrence or metastasis. No complementary treatment had been administered.

Conclusion: Spiradenocarcinoma is an exceedingly rare malignant neoplasm with ductal differentiation. Many authors consider it to be an eccrine neoplasm, and others favor apocrine differentiation. It originate from previous spiradenoma that remained unaltered for many years, but de novo spiradenocarcinomas had already been reported.

The authors report a new case and review the literature about spiradenocarcinoma, and discuss briefly some histogenetic concepts relevant to this neoplasm.

#### P 938

##### **IMMUNOHISTOCHEMICAL STUDY OF MUC1, MUC2, AND MUC5AC EXPRESSION IN MALIGNANT SWEAT GLAND NEOPLASMS**

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Introduction: Malignant sweat gland neoplasms are rare skin tumors. MUCs are glycoproteins associated with carcinogenesis and cell adhesion. MUC1 is suggestive to inhibit cell to cell adhesion. In the present study, we examine the immunohistochemical expression of MUC1, MUC2, and MUC5AC in malignant sweat gland neoplasms.

Materials and Methods: Formalin fixed, paraffin embedded tissues of nineteen cases of malignant sweat gland neoplasms were immunohistochemically studied. Intensity and percentage of staining were evaluated.

Results: Patients included eleven females and eight males, mean age 60 yrs (age range: 24-84 yrs). Three cases had extramammary Paget's disease. The head and neck was involved in 6 cases, the trunk in 5 cases, the genital regions in 4 cases, the thigh in 3 cases. The histologic feature showed sweat gland adenocarcinomas (n=9), sweat duct carcinoma (n=4), porocarcinoma (n=2), microcystic adnexal carcinoma (n=3). 18 cases were immunopositive for MUC1. One porocarcinoma case was negative for MUC1. All 19 cases of malignant sweat gland neoplasms were immunonegative for MUC2 and MUC5AC. The intraepidermal component of extramammary Paget's diseases in 3 cases was positive for MUC5AC. Follow up data was available in 12 cases.

Conclusion: Malignant sweat gland neoplasms are associated with expression of MUC1 and not associated with MUC2 and MUC5AC expression. These observations suggest that MUC1 may play a role in cancer cell invasion malignant sweat gland neoplasms.

#### P 939

##### **HYALINE-CELL RICH MALIGNANT CHONDROID SYRINGOMA WITH OVERT MORPHOLOGICAL FEATURES OF MALIGNANCY. A CASE REPORT**

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Malignant chondroid syringoma (MCS) is malignant mixed tumor of the skin. It is extremely rare neoplasm. We describe a case of MCS which was characterized by predominant hyaline-cell population and distinctive morphological signs of malignancy. A 62-year-old female presented with subcutaneous mass 5 cm in diameter on the lateral side of the left forearm. After a 15-year period of slow growth, the lump suddenly increased in size. The surgically excised neoplasm was evaluated by routine histology and immunohistochemistry. Histopathologically, the tumour consisted mainly of broad sheets of pleomorphic plasmacytoid-hyaline cells with rare mitoses. A part of the tumor showed the features of benign mixed tumor, where uniform slightly eosinophilic cells formed irregular small nests, partly with tubular and cystic lumina, in the myxoid and hyaline matrix. Large areas of necrosis were surrounded with small undifferentiated cells with high mitotic rate: 7/10HPF. Invasion of the capsule and satellite nodules in subcutaneous adipose tissue were found. The hyaline cells were strongly and diffusely immunopositive for CK, EMA, CAM 5.2, CEA, and partially for GFAP antibody. S-100, aSMA, desmin, calponin and bcl-2 immunostaining was negative. In areas with high mitotic activity mean p53 protein index was 21%. Although the ductal differentiation was vague, we favour the diagnosis of MCS over myoepithelial

carcinoma due to the lack of immunoeexpression of myogenic markers and S-100 protein.

#### P 940

##### SYRINGOID ECCRINE CARCINOMA

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Syringoid eccrine carcinoma is a rare variant of adnexal carcinoma thought to derive from eccrine sweat glands. It's often clinically underestimate which lead to local recurrences. We received a lesion developed on the forehead of a 27 year old woman. Clinically a lipoma was suspected. The lesion measured 1'1 x 0'7 cm and was excised reaching focally the muscular layer. On microscopic examination the epidermis was unremarkable, and a proliferation of small, low density, glandular structures was seen all over the dermis. A few of them configured small keratinous cysts especially in superficial areas. The lumina were lined by two layers of cuboidal cells, and contained a granular eosinophilic material. There was a slight pleomorphism in nuclei, mainly in the inner layer, with small single nucleoli. Mitotic activity was very scant, lower than 1 per 10 high power fields. Some of the glands had a thin projection, giving a comma shaped appearance. The stroma had a fibrous configuration, with dense collagenous pseudonodular disposition centred by the glands. Perineural invasion was occasionally seen, and deep dermic infiltration, although not reaching the muscular layer. Immunohistochemical study showed in the glandular cells expression for keratin (AE1-AE3); epithelial membrane antigen, carcinoembryonic antigen (CEA), and S-100 protein. Cytokeratin 7 was focally positive, and Cytokeratin 20 negative.

This neoplasm shares certain similitude with the more common eccrine microcystic carcinoma. In fact many reports use those terms as synonyms, but syringoid carcinoma lacks the basaloid nest differentiation of microcystic carcinoma, has the typical comma shaped tubules, and has a less prominent perineural invasion. Confusion extends when using the other synonymous term, sclerosing duct carcinoma, generally referred to microcystic carcinoma, but present also in our case with a prominent concentrically peritubular stromal reaction.

#### P 941

##### THE INTERPRETATION OF BENIGN BIOLOGIC BEHAVIOR FOR KERATOACANTHOMA VERSUS CUTANEOUS SQUAMOUS CELL CARCINOMA, USING A PANEL OF ANTIBODIES AGAINST:CYCLIND1,P53,P21RAS,EGFR

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##### Introduction:

The keratoacanthoma (KA) is a relatively common tumor which most often occurs on the sun – exposed areas of light skinned individuals of middle age and older.

This tumor is regressing spontaneously, so the recognition of the true nature of this tumor is of considerable practical and biological important

Its etiology is unclear, although ultraviolet light, viruses, oncogenic chemicals, and epidermal growth factor have been considered.

- EGFR : is membrane growth factor receptor encoded by the Cerb 1 gene
- P21 ras: is a signal transducing (cytoplasmic) oncoprotein with GTP as activity. Ras mutative abolishes GTP as activity and results in constitutive stimulation of the way.

- P 53 Nuclear protein playing an important protective role in the response to DNA damage induced by ionizing radiation chemical carcinogens and viruses.
- Cyclin D 1: CDK Complex regulates the RB protein ( PRB) . Mutational gene causes hyperphosphorylation of RB

protein .with consultative stimulation of the pathway

##### Material and methods :

- 24 cases of Keratoacanthoma
- 26 cases of SCC
- Immunostaining of EGFR, P21 ras, P53, Cyclin D 1 oncoprotein
- Scores of Positive staining : diffuse basal and suprabasal staining. Focal basal and suprabasal staining

##### Results

- The expression of P53 (mutant gene) showed a great difference between SCC and Keratoacanthoma ( 20/26 – 80% in SCC versus 4/24 – 16% in KA) ( P<0.01) -2/4 of KA . Cases were reevaluated as SCC ( KA –like SCC ) .
- The expression of P21 ras was more evident in KA versus SCC (14/24 -58 % in KA versus 5/26- 19% in SCC ( P < 0.05).
- EGFR was negative in both all cases of KA and SCC.
- Cyclin D1 showed more evident ( focal expression ) in SCC ( 10/26 -38% in SCC versus 4/24 – 16 % in KA )

##### Conclusions:

- - Our results refer to the decisive role of P53 mutation in the development of SCC and Its invaluable role in setting down a convincing diagnosis of SCC.
- - P21 ras was more definitive in KA
- - EGFR : is expressed more in reactive and benign epidermal growths , and has no role in both KA and SCC.
- - P53 mutation is critical and decisive in the development of cutaneous malignancy.

#### P 942

##### APOPTOSIS AND PROLIFERATION IN SQUAMOUS CELL CARCINOMA AND KERATOACANTHOMA

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BACKGROUND: The controversy of distinguishing keratoacanthomas (KAs) from a well-differentiated squamous cell carcinoma (wdSCCs) is still present. Model of cutaneous carcinogenesis focus on the regulation of keratinocyte proliferation, differentiation, apoptosis and/or inability to undergo growth arrest.

OBJECTIVE: We investigated the expression of proliferation and apoptosis markers in wdSCC, poorly differentiated squamous cell carcinoma (pdSCC), proliferating keratoacanthoma (pKA) and regressing keratoacanthoma (rKA) in order to distinguish a factors important in the progression of SCC and the regression of KA.

METHODS: 120 tumors (30 of each group) were analyzed for p53 protein, Bcl-2, Bak and Ki-67.

RESULTS: The percentage of p53 positive tumor cells was 73.20% in pKA, 46.62% in rKA, 69.32% in wdSCC and 78.96% in pdSCC. Ki67 was found in 35.13% in pKA, 30.11% in rKA, 48.57% in wdSCC, and 65.68% in pdSCC. Bcl-2 protein was found in 3.07% of cells in pKA, 13.43% in rKA, 14.97% in wdSCC, and 4.69% in pdSCC. Pro-apoptotic protein Bak was expressed in 96.23% in pKA, 41.63% in rKA, 93.04% in wdSCC, and 54.69% in pdSCC.

CONCLUSION: Relatively slow proliferation rate, as compared to SCC, coupled with an intensive apoptosis seen in

pKA could lead to regression of this tumors. Growth and malignancy of pSCC could be a result of intensive proliferation of tumor cells. Proliferation in pSCC is mainly controlled by an intensive but still not sufficient apoptosis. This preliminary study suggest that balance in expression of proliferation and apoptosis related proteins could be important factor in determining a tumor progression or regression.

#### P 943

##### **AN INCIDENTAL FINDING OF COEXISTENT, CUTANEOUS KAPOSI'S SARCOMA AND LEISHMANIASIS IN AN HIV-INFECTED PATIENT.**

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Cutaneous lesions in Mediterranean visceral leishmaniasis are very unusual, except for the presence of *Leishmania* organisms in cutaneous Kaposi's sarcoma in patients infected by the Human Immunodeficiency Virus (HIV). A 40 year old male with a 15 year history of HIV presented with a one month history of multiple purple asymptomatic papules on his left forearm, feet, penile tip, buccal mucosa and splenomegaly. His current CD4 count was 39 cells/mm<sup>3</sup> and viral load, 10411. The patient lived in Southern Spain and had recently adopted a dog. Histological examination of one of the biopsied lesions on his forearm showed a dermal vascular proliferation suggestive of kaposi's sarcoma, with positive immunostaining for Human Herpes Virus 8 (HHV8). There was an associated peripheral infiltrate of plasma cells and histiocytes, the cytoplasm of which contained many small round *Leishmania* amastigotes. A large number of these Giemsa positive, PAS negative organisms were also seen extracellularly. The features of the skin biopsy were those of coexistent cutaneous leishmaniasis and Kaposi's sarcoma. The patient's splenomegaly was attributed to visceral leishmaniasis. Canines are the natural host of *Leishmania* donovani. Most cases of leishmaniasis in HIV-positive patients appear in the advanced stages of the disease (CD4<200mm<sup>3</sup>). *Leishmania* organisms are thought to reach the skin by dissemination during visceral disease, either after external infection or by reactivation of latent leishmaniasis. Kaposi's sarcoma is now classified as a low-grade malignancy but is regarded by many authors as a non-tumoural, reactive, entity. DNA sequences from HHV8 have been found in all types of Kaposi's sarcoma. The role of *Leishmania* in Kaposi's sarcoma remains a subject of discussion. The fact that the vascular tumour usually regresses after treatment of the parasitic infection, strengthens the proposal that it is a reactive process.

#### P 944

##### **DIFFERENTIAL PROX-1 AND CD 31 EXPRESSION IN MUCOSAE CUTANEOUS AND SOFT TISSUE VASCULAR LESIONS AND TUMOURS**

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The study of lymphatic vessels and lymphatic tumours has been hampered with difficulty, due to the overlapping morphological features between blood and lymphatic endothelial cells, as well as to the lack of specific lymphatic endothelial markers. Over the last few years, lymphatic vessels and lymphangiogenesis have received great attention owing to their putative implications in terms of metastatic dissemination and the promise of targets for lymphangiogenic therapy. Prox-1 is a nuclear transcription factor that plays a major role during embryonic lymphangiogenesis and is deemed to be a useful marker for differentiating lymphatic endothelial cells from the other blood vessels endothelial cells. Here we describe a double-immunostaining strategy for formalin-fixed paraffin-embedded tissues aiming to evaluate the distribution of Prox-1 and CD 31 - a cytoplasmic pan-endothelial marker - in a series of twenty eight mucosae, cutaneous and soft tissue vascular lesions and tumours (haemangiomas, n=12; spindle cell haemangioma, n=1; lymphangiomas, n=10; lymphangiectasia, n=1; Kaposi's sarcoma, n=4). Our results showed that in non lesional mucosae and skin, Prox-1 decorated exclusively the nuclei of endothelial cells in vascular structures with bona fide morphological features of lymphatic vessels. CD 31 stained endothelial cells of blood vessels of superficial and deep dermal plexuses and also lymphatics. Prox-1 stained almost all the benign lymphatic vascular lesions/tumours (93%) and was absent or only focally positive in 75% of blood vascular tumours. CD 31 showed positive staining in all blood vascular lesions/tumours, being focal in two lymphatic lesions/tumours. Kaposi's sarcomas were all positive for both CD 31 and Prox-1 markers. In conclusion, whilst not utterly specific in the tumoral setting, Prox-1 may be employed as an adjunct marker of lymphatic endothelial cells in routinely processed formalin-fixed paraffin-embedded samples, namely of non-tumoral vessels.

#### P 945

##### **EXPRESSION OF CYTOKERATIN SUBTYPES IN INTRAEPIDERMAL MALIGNANCIES: A GUIDE FOR DIFFERENTIATION**

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Among intraepidermal malignancies of epithelial origin, Bowen's disease, bowenoid actinic keratosis (BAK), intraepidermal malignant eccrine poroma (MEP) and Paget's disease may pose diagnostic difficulties. Considering these difficulties, the localization, histologic features, and immunohistochemical profiles of 24 cases of Bowen's disease, 21 cases of BAK, 18 cases of intraepidermal MEP and 11 cases of Paget's disease were analyzed. Using multivariate logistic regression test, multinuclear giant cells and solar degeneration were found to be the only histologic parameters of diagnostic help. On the other hand, bivariate tests, performed for each pair of diseases revealed that an immunohistochemical panel composed of four different cytokeratin (CK) subtypes could be of benefit in the differential diagnosis. A widespread positive reaction for CK 5/8, CK 7, CK 19, and negative reaction for CK 10, was a helpful feature in differentiation of Paget's disease from Bowen's disease and BAK. CK 10 expression could be considered in favor of Bowen's disease, when compared to BAK. The widespread expression of CK 5/8 and CK 7 and negative reaction for CK 10, was in favor of Paget's disease, compared to intraepidermal MEP. On the other hand, widespread expression of CK 19 was a common finding in intraepidermal MEP, in contrast to Bowen's disease. In conclusion, our findings suggest that an immunohistochemical panel including certain cytokeratin subtypes such as CK 5/8, CK 7, CK 10 and CK 19, can

provide significant hints in the differentiation of common intraepidermal malignancies, Bowen's disease, BAK, MEP and Paget's disease, especially in problematic cases.

**P 946**

**LOCALIZED AMYLOIDOSIS IN BASAL CELL CARCINOMA**

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**Introduction:** Amyloid can be found in some disease localized on lesion regions. One of these diseases is Basal Cell Carcinoma(BCC). The existence of amyloid deposits in basal cell carcinomas of the skin has been an established fact since 1930.

**Purpose:** In my study I purposed to investigate the presence of amyloid in 60 BCC tissue specimen and the amyloid's relationship between BCC's histopathologic types, mitotic rate, solar elastosis, degree of apoptosis and the age of the patients.

**Material and Methods:** Their ages ranged from 24 years to 92 years with a mean age of 56 years. The male to female ratio was 1.14. Excisional biopsy specimens were obtained from 60 patients were fixed in 10% buffered formalin and embedded in paraffin. Five micrometer thick sections were stained with hematoxylin and eosin and Congo Red.

**Results:** The histologic distribution of the amyloid deposits was fairly consistent. They were found in the fibrous stroma between clumps of tumor cells and in the connective tissue abutting the advancing front of the carcinoma. Amyloid was identified as amorphous, eosinophilic extracellular material which stained rose-pink with Congo Red.

**Conclusion:** Amyloid is found on the 55% of the BCC patients. The presence of amyloid didn't change with the age of the the patients, the tumours histopathologic types and apoptosis. A relationship between solar elastosis and mitotic rate quantity and the presence of amyloid is detected.

**P 947**

**MEASUREMENT OF TELOMERE LENGTHS IN SQUAMOUS CELL CARCINOMAS FROM BOTH RENAL TRANSPLANT RECIPIENTS AND NON-TRANSPLANT INDIVIDUALS USING AN ESTABLISHED FLUORESCENT IN SITU HYBRIDISATION METHOD FOR ARCHIVAL TUMOUR MATERIAL.**

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Telomeres are specialised repeats at the extreme ends of chromosomes that prevent chromosomal instability and that shorten in most normal somatic cells at each round of cell division. This is due to the inability of the replication machinery to fully maintain the ends of linear DNA molecules. Critically shortened telomeres cause cessation of cellular proliferation and it is significant that most human cancers have reactivated the ribonucleoprotein enzyme telomerase, which compensates for telomere shortening and maintains the telomere lengths.

We have performed telomere length measurements in formalin fixed paraffin embedded tissue sections of both normal skin and squamous cell carcinoma from renal transplant recipients (SCCRTR) and non-transplant individuals (SCCN). These analyses were undertaken to determine whether telomere length maintenance via telomerase plays a role in the almost 100-fold increased incidence and more biologically aggressive nature of the SCCs that develop in immunocompromised renal transplant patients, compared with the non-transplant population. Telomere lengths were determined using a modified

TELOmere Fluorescence In-Situ Hybridisation (TEL-FISH) technique recently developed for archival material. We examined TEL-FISH signals in 21 SCCRTR tumours, 25 SCCN lesions and in 10 normal skin specimens from both renal transplant and non-transplant patient groups. We then established a telomere length standard curve using FISH and Southern analysis of telomere lengths from three different human cell lines. Statistical analysis subsequently revealed that both SCCRTR and SCCN tumours have shorter telomeres than their respective normal skin controls, which is consistent with the telomere length profiles of many types of carcinoma. Furthermore, there is a statistically significant trend towards longer telomeres in the SCCRTR than in the SCCN tumours. This may be indicative of a central role for telomere lengthening and maintenance in the increased incidence and aggressiveness of SCC in renal transplant recipients.

**P 948**

**BASAL CELL CARCINOMAS OF THE SCROTUM**

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**Introduction:** Basal Cell Carcinoma(BCC) is the most common human tumor. However, its appearance on scrotal skin is rare with an estimated incidence of 0.1/100.000 cases per year. These tumors must be differentiated from the more common Squamous Cell Carcinoma(SCC).

**Purpose:** Their etiology remains unclear compared to the occupational predisposing factors reported in the literature for SCC in the same area. Wide surgical excision is recommended and prognosis is usually good. I report the clinical and histologic features of scrotal BCC and review the literature.

**Methods:** A 88-year-old man presented with a 6-month history of asymptomatic scrotal plaque. Physical examination lesion was 1.2cm. indiameter, erythematous and had irregular, ill defined borders. No adenopathy was present and the remainder of the skin examination was unremarkable. In case, it was no history of trauma, radiation therapy, or exposure to chemicals, or arsenic.

**Results:** Tissue from the incisional and then excisional biopsy were fixed in formalin and embedded in paraffin. Samples were stained with hematoxylin and eosin. Biopsy specimen showed superficial BCC. The lesion was excised with a margin of 1.8cm. of normal skin. Examination of the surgical specimen revealed a superficial BCC, which was completely excised. The patient has been free of disease for 24 months.

**Conclusion:** Scrotal carcinomas are uncommon, with an estimated annual incidence of 0.1/100.000. The majority are squamous lesions and cases of BCC of the scrotum have been documented in the world literature. In contrast to SCC, BCC is usually of low malignancy. Thorough clinical evaluation, followed by wide local excision may be most appropriate initial therapeutic approach to these lesions.

**P 949**

**EXPRESSION PROFILES OF P53 AND KI67 IN PREINVASIVE AND INVASIVE LESIONS OF BURN SCAR**

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**Introduction:** Burn scars with the reconstruction difficulties and developing tumor are still the lesions that cause problems. In the background of burn scars usually squamous cell carcinoma, basal cell carcinoma, malignant melanoma, skin adnexial tumors and soft tissue sarcomas can develop. In this study we investigated the presence of reactive atipia, dysplasia, melanocytic hyperplasia, melanocytic atypia in the

epidermis and the tumors that develop from burn scars and evaluated the relation of these findings with p53 and Ki67 immunohistochemically.

**Materials Methods:** The study subjects were 35 cases of burn scar from 1999 to 2005. Parafin blocks of all specimens were examined immunohistochemically with monoclonal antibodies p53 and Ki67.

**Results:** 21 cases were male and 14 female. Their ages varied from 9 to 80 years, with a mean age of 44.6 years. The causes of the initial burn injury were flame, 20; hot water, 4; lightning, 3; electrical, 1; chemical, 1; and hot milk, 1. The duration of burn ranged from 10 months to 57 years with a mean of 27.3 years. The lesions were located on the limbs in 22 cases, 8 on the head and neck, and 5 on the trunk.

On the histopathological examination we described squamous cell carcinoma in 10 cases, malignant melanoma acral lentiginous type in 1 case, basosquamous cell carcinoma in 1 case and pseudosarcomatous fasciitis in 1 case. In the cases that had no tumor, showed the following changes in the epidermis: low grade and high grade dysplasia in 2 cases, low grade dysplasia in 11 cases, melanocytic hyperplasia in 10 cases, atypical melanocytic proliferation in 3 cases, reactive atypia in 5 cases, and changes indefinite for dysplasia in 2 cases. In 2 cases there were no pathological finding in the epidermis. With Ki67 proliferation marker, in the cases with dysplasia, there was a continuous nuclear staining at the basal layer of the epidermis. The tumors showed high staining ratio with Ki67. In the cases with dysplasia or tumor, we established varying staining ratios with p53.

**Conclusion:** In this report we found out that Ki67 proliferation marker can be helpful in the differential diagnosis of the dysplasia in burn scars. We also suggest that p53 gene mutation can play a role in some of the tumors that arise in burn scars.

**P 950  
MULTIPLE CONDYLOMATOUS CARCINOMA  
(WARTY CARCINOMA): A CASE REPORT**

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**Introduction:** Condylomatous carcinoma is a variant of squamous cell carcinoma that has marked condylomatous features. Unlike verrucous carcinoma, condylomatous carcinoma demonstrate features of cytoplasmic vacuolization and nuclear changes resembling koilocytic atypia. It is frequently associated with HPV infection. This tumor has been reported in various sites, such as anogenital region, oral cavity, vagina, cervix and bladder. Two condylomatous carcinomas which arise in condyloma acuminata in the same patient are introduced.

**Case Report:** The patient was a 66 year old, male. He referred with genital and right axillary lesions, present for 15 years. The diameters of the tumors were 15cm and 2,7cm in the genital and axillar regions respectively. On histopathological examination, there were typical features of condyloma acuminatum at the periphery of each lesion. Each tumor has multiple papillary projections with a keratinized epithelial surface. Most of the tumor cells have cytoplasmic perinuclear clearing similar to koilocytosis.

**Conclusion:** To our knowledge, this is the first case of multiple condylomatous carcinoma on the skin. Light microscopic, immunohistochemical features and differential diagnosis of condylomatous carcinoma are presented here.

**P 951  
THE FIRST REPORT ON SARCOID OCCURRENCE  
IN CASPIAN PONY IN IRAN**

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Adibnishabouri

**INTRODUCTION:**

Sarcoid as the most common skin tumor, take place in different breeds, colors, and both genders of perisodactyla (horse, donkey, mule). The most common areas of such an occurrence are those areas with thin skin and less hair. Different forms of sarcoi consist occult, warty, fibroblastic, and mixed types. Treatment approaches are different, but recurrent state is the most important problem in this regard.

**Methods and materials:**

A five-year-old Caspian pony was observed with a warty mass in axillary site and around its prepuce in rakhsh equestrian section of Khorasan Jihad-e-agriculture educational complex and was examined. To confirm the diagnosis, the biopsy of the mass was conducted.

**Results and conclusions:**

Given the examination of the mass under study, the sarcoid warty type was identified. The histopathological examinations proved the sarcoid tumor. They include acantotic hyperplasia, proliferation of satellite and fusiform fibroblasts, vast ground substance with collagen fibers and mitotic form of cells.

Although sarcoid has been frequently reported in horse so far, the recent article is the first report of sarcoid occurrence in Caspian pony.

**Key words:** sarcoid, caspian pony.

**P 952  
THE NATURAL COMPOUND  
BUTYLIDENEPHTHALIDE STRONGLY SUPPRESSES  
HUMAN MALIGNANT BRAIN TUMOR GROWTH IN  
VITRO AND IN VIVO**

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Brain tumors are the third leading cause of cancer-related death in those aged 15-34 years old. In particular, glioblastoma multiforme (GBM) and malignant gliomas present the lowest one-year survival rates. Current anti-tumor drugs are not effective against GBM. The naturally occurring compound butylidenephthalide (BP) isolated from the chloroform extract of *Angelica sinensis* (AS-C) has been studied in treatment of angina. Presently, we report on the potency of BP in the growth suppression of malignant brain tumor cells without coincident fibroblast cytotoxicity. BP up-regulated the expression of CKI including p16 and p21 and down-regulated the expressions of cell cycle regulators. The result was a decrease of phosphorylated Rb proteins and the cell arrest at G0/G1 phase. The expression of p53 protein was increased by BP and correlated with the activation of apoptosis-associated proteins. In vivo, BP not only suppressed growth of subcutaneous rat and human brain tumors, but also shrank the volume of GBM tumors in situ. The post-BP survival period of the rats was significantly prolonged. These in vitro and in vivo anticancer effects indicate that BP has the potential as a new anti-brain tumor drug.

**P 953  
AN ANALYSIS OF CENTRAL NERVOUS SYSTEM  
TUMORS IN RASOOL AKRAM HOSPITAL IN IRAN**  
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Neoplasm of the nervous system and its adjacent structures are diverse group of lesions. They can be primary neoplasm in all degrees of differentiation derived from any of the many normal cellular constituents of this organ or secondary neoplasm metastatic from a variety of primary sites.

A series of 240 pathologically verified brain and spinal cord tumors from the neurosurgical department of Rasool Akram Hospital in Tehran, Iran were analyzed for the relative incidence as well as the distribution of the age and sex.

These data were compared with series from another countries. The male/female ratio was 119/121 and the age distribution was: <4 years old (y/o) (3.7%), 4-11 y/o (8.1%), 12-18 y/o (3.6%), 19-44 y/o (45%), 45-54 y/o (16%), 55-64 y/o (12%), 65-74 y/o (9.9%) and >75 y/o (1.3%). Percentage of representative tumors were meningioma (26.25%), glioma (25.4%), pituitary adenoma (11.6%), and neurinoma (13.7%). The incidence of glioma was lower and that of neurinoma was higher in Rasool Akram Hospital than those in other reports.

#### P 954

##### PATHOLOGIC CHARACTERISTICS OF CONVENTIONAL AND ANAPLASTIC PLEOMORPHIC XANTHOASTROCYTOMAS

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**Introduction:** Pleomorphic xanthoastrocytoma (PXA) is an uncommon CNS tumor characterized by a relatively favorable prognosis despite of ominous pathologic features. However, PXA may occasionally undergo anaplastic transformation. The aim of this study is to clarify pathologic characteristics of conventional and anaplastic PXAs.

**Cases:** According to the WHO classification, 8 PXAs were divided into conventional (5) and anaplastic (3) types. The age of 8 patients (7 females and 1 male) ranged from 16 to 79 years (median 45.5, mean 42.6). All tumors were located in supratentorial regions: frontal (3), occipital (2), parietal (1), frontotemporal (1), and sellar (1). Radiologic images, available in 5 patients, exhibited contrast-enhancement in 5 and cystic changes in 3.

**Results:** All tumors showed typical microscopic features of PXA, that is, pleomorphic tumor cells with xanthic changes, intranuclear pseudo-inclusion, marked desmoplasia, perivascular lymphoid cuff, numerous eosinophilic granular bodies, and occasional calcification. In anaplastic PXA, monotonous proliferation of atypical cells was noted and mitotic counts were 10, 11, and 15/10 HPF, respectively. Two also showed necrosis and microvascular proliferation. One was a combined tumor of conventional PXA and glioblastoma. Another PXA underwent anaplastic changes through two recurrences. PXA showed immunoreactivity for GFAP (100%), S-100 protein (100%), neurofilament proteins (88%), and synaptophysin (33%).

**Conclusions:** Anaplastic transformation may occur de novo or through recurrences in PXA probably more frequently than previously postulated. This anaplastic variant should be distinguished from glioblastoma by identifying areas showing conventional microscopic features. PXA often shows neuronal immunophenotype, which may arouse some controversies about its classification and histogenesis.

#### P 955

##### THE RHABDOID TUMOURS OF THE NERVE CENTRE : SEVEN CASE STUDIES

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**Introduction :**

The rhabdoid cerebral tumours were first identified by Beckwith and Palmer in 1978. Their frequency was estimated to be 2,1% of those affecting children under eighteen. Such tumours are equally characterised by a critically and speedy mortal development. Their historical genesis is still a controversial issue.

**Purpose statement:**

The aim of the present study is to discuss the various anatomically clinical aspects and examine the prognosis of this rare tumours.

**Methods and materials:**

In the course of this study, we will observe, examine and compare seven cases hospitalised in a period of eight years (1994-1997) in the sfax laboratory of anatomy and pathological cytology.

**Results:**

The average age of hospitalised patients is 22 years old among whom four being under fourteen and five males. The clinical symptomatology showed a predominance of the intracranial hypertension syndrom. Radiography through magnetic resonance reveal a heterogenous tumours processus localised respectively at the level of the spine (two cases), the insula (1 case) the temporal front (three cases) and the medulla (one case). The histological examination of those tumours has also showed a proliferation of giant cells whose cytoplasmic inclusion is hyalin based. These inclusion were rather positive for the vimentine and the keratin.

A tumours rhabdoid diagnosis was made and an adjuvant X-ray treatment was prescribed for five patients. The evolution was equally marked by a recurrence of rhabdoid tumours among three cases. Five patients were pronounced dead with a delay range between a week to eighteen months.

**Conclusion:**

The cerebral rhabdoid malignant tumour constitutes one of the most aggressive intracranial tumours. Still, there is hardly any efficient therapeutic protocol.

#### P 956

##### EXPRESSION OF TTF-1, CK7, AND CK20 IN BRAIN METASTASES

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**Introduction:** Metastatic carcinoma to the brain is a common problem but complicated by the histologic similarity to determine the primary origin. Thyroid transcription factor (TTF-1) is a tissue specific transcription expressed in the thyroid cancer and lung neoplasms. In the present study, we evaluate the role of TTF-1, CK7, and CK20 in identifying the primary source.

**Materials and Methods:** Forty-eight consecutive cases of histological proven brain metastases cases were retrieved from a four-year period. Formalin-fixed, paraffin-embedded sections of metastatic carcinoma to the brain were immunostained with TTF-1, CK7, and CK20.

**Results:** There were 24 females and 24 males ranging in age from 35 to 78 years (mean 59 years). 22 cases were immunopositive for TTF-1. TTF-1 staining was present in 20 (71%) of the 28 metastatic lung carcinomas. 26 cases were negative for TTF-1 and the sites of origin included: lung (n=8), colon (n=7), breast (n=3), liver (n=2), esophagus (n=1), nasopharynx (n=1), kidney (n=1), prostate (n=1), and unknown primary (n=2). CK7 staining was present in 26 (93%) of the 28 metastatic lung carcinomas. CK20 staining was negative in 24 (86%) of the 28 metastatic lung carcinomas. 28 metastatic lung carcinomas showed CK7(+)/TTF-1(+)/CK20(-) (n=17), CK7(+)/TTF-1(+)/CK20(+) (n=3), CK7(+)/TTF-1(-)/CK20(-) (n=5), CK7(+)/TTF-1(-)/CK20(+) (n=1), CK7(-)/TTF-1(-)/CK20(-) (n=2).

Conclusion: CK 7 and TTF-1 are helpful aid in brain metastases with lung origin. Positive staining is suggestive of lung primary. However, in our study, about 30% of metastatic lung carcinomas to the brain are negative staining for TTF-1.

**P 957**  
**HAEMANGIOBLASTOMA : REPORT OF 5 CASES**  
**AND REVIEW OF THE LITERATURE**

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Central nervous system haemangioblastoma is a histologically benign tumor that usually occurs in the cerebellum and represents 1,1 % to 2,4 % of all tumors of the central nervous system. Haemangioblastoma can present as sporadic lesions or as manifestations of Von Hippel-Lindau syndrome (VHL).

Five patients with haemangioblastoma are presented. They were 4 men and 1 woman. The age of patients at diagnosis ranged from 36 to 60 years. In 4 cases, the tumors were located in the cerebellum. One patient had intramedullary tumor. The younger patient had VHL syndrome. Histologically, the tumors were composed of small blood vessels and large vacuolated stromal cells. No deaths associated with surgery occurred.

Haemangioblastoma is a rare tumor, affects mostly young adults and has a favorable prognosis after surgery. However, patients with VHL syndrome are at risk of developing new lesions. They require lifelong follow-up

**P 958**  
**LARGE CELL/ANAPLASTIC MEDULLOBLASTOMA**  
**WITH LONG SURVIVAL - A CASE REPORT**

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Medulloblastomas (MBs) are malignant small cell embryonal tumors of the cerebellum found predominantly in children. Modern diagnostic methods, classification and treatment of MBs contribute to the progress in five-year survival. However, the clinical outcome and response to therapy is sometimes difficult to predict although, there are some increasing evidence that clinical outcome may depend on histological type of MB. Besides the histologically "classic" MBs there are two extremes of the histological spectrum that could correlate to their survival: MBs with extensive nodularity versus large cell/anaplastic (LC/A) MBs which are associated with better and worse clinical outcome, respectively.

We present a case of LC/A MB with unusual long survival. Our patient 26 years-old male was operated on for the first time at the age of 11-years because of cerebellar tumor without CSF dissemination. The tumor was totally resected and diagnosed as MB. The combined radiation and chemotherapy for pediatric MB was performed and the patient was free of disease without cognitive and hormonal disturbances almost fifteen years. He was followed-up with serial brain CT scans and MRI. In the meantime he graduated Secondary School and University School for Agriculture. At the age of 26-years because of recurrent cerebellar tumor the patient underwent to the second operation. However he died three months later due to CSF metastases.

We analyzed histologically and immunohistochemically surgical specimens of the first and recurrent tumor. Both tumors were similar and showed the features of LC/A MB. Histologically, they were predominantly composed of large highly pleomorphic cells that wrapped around one another. Their nuclei were also large, markedly atypical with irregular

shapes, often without prominent nucleoli. The mitotic and apoptotic rate were increased. There were zones of necrosis and vascular proliferation. In the vicinity of highly severe anaplastic foci of LC/A MB small zones of "classic" MB were present. Immunohistochemically, the cells expressed synaptophysin, NF and focally GFAP. The EMA, LCA, CK and SMA were negative.

The poor survival should be expected in our case with LC/A MB. However, patient age, absence of CSF metastases and total tumor resection may contribute to long survival of fifteen years. We concluded that determination of histological subtype of MBs is not sufficient for clinical outcome and that the correlation with clinical data is mandatory.

**P 959**  
**INTRACRANIAL LOW GRADE FIBROMYXOID**  
**SARCOMA (A CASE REPORT)**

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Intracranial low grade fibromyxoid sarcoma (A case report)

We report Low grade fibromyxoid sarcoma of brain (LGFS) a very rare tumor in the twelve's years old boy with history of temporal bulging from two months ago. MRI study was shown a large extra-axial mass with wide dural base at left frontal region with compress effect on brain tissue and subfalxian herniation and thinning of bone. Resected tumor was 10\*9\*5 cm with firm gray cut surface. In microscopic examination, proliferation of bland spindle cell in background of fibromyxoid stroma was seen. Cellularity varied from low to high. We considered all primary or metastatic spindle cell tumor, include: neurofibroma, schwannoma, perineurioma, fibromatosis, solitary fibrous tumor, myxofibrosarcoma, chordoma, and mono phasic synovial sarcoma. The tumor cells were negative for pancytokeratin, epithelial membrane antigen, S100, CD34, actin, GFAP and were reactive only for vimentin. Therefore, we have excluded all suggestive differential diagnosis and have established LGFS. In PubMed we have found only one case of intracranial LGFS which was reported by Paulus and colleague in 1991.

Key words: Intracranial sarcoma, Low grade fibromyxoid sarcoma

**P 960**  
**EFFICACY OF NEUROENDOSCOPIC BIOPSIES IN**  
**OBTAINING MATERIAL FOR**  
**HISTOPATHOLOGICAL EXAMINATIONS OF BRAIN**  
**TUMORS IN PEDIATRIC PATIENTS.**

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**Introduction**

Neuroendoscopic biopsies seem to be a valuable method in obtaining material for pathological examination in cases of intra- and periventricular brain tumors. The procedure is usually performed in patients in whom total microneurosurgical tumor resection is impossible or is to be delayed. In some cases neuroendoscopic technique facilitates removal of small intraventricular lesions.

Aims of the study were: 1. to evaluate the efficacy of endoscopy in obtaining representative tissue samples according to particular tumor locations; 2. to estimate the accuracy of pathological diagnosis in a subgroup of patients in whom pathology was verified by open or stereotactic

biopsies; 3. to define locations and growth characteristics of tumors where endoscopic biopsies were ineffective.

#### Material and methods

Between years 1996 and 2003 we performed 93 neuroendoscopic biopsies of brain tumors in children. The material was analysed according to the following locations of the lesions: tumors of the lateral ventricles, suprasellar tumors, pineal region and posterior third ventricle tumors, thalamic tumors (focal and diffuse), posterior fossa tumors and others.

#### Results

In 65/93 cases representative samples were obtained. In the remaining 28/93 patients the biopsies were not informative, the samples were too small or the tissue specimens were removed from the tumor margins. In 55 out of 93 patients open and/or stereotactic biopsies were also performed, moreover in 42 children we could compare the diagnoses established on the basis of neuroendoscopic biopsies with the diagnoses after open/stereotactic procedures. In 38/42 cases the diagnoses were the same. The biopsies of thalamic tumors were less reliable. Ineffective procedures in 3/8 lesions of lateral ventricles were caused by thin ependymal tumor spread, marked tissue necrosis or calcifications.

#### Conclusions

This study proved that neuroendoscopic biopsies were most effective in cases of suprasellar, pineal region and posterior third ventricle tumors as well as in patients with posterior fossa lesions. The biopsies of thalamic tumors were less reliable.

Stereotactic or open biopsy seem to be better method for thalamic lesions. Indications for neuroendoscopic procedures should be established on the basis of careful evaluation of neuroradiologic examinations to avoid false results.

#### P 961

### MALIGNANT PERIPHERAL NERVE SHEATH TUMOR (MPNST) WITH GIANT ROSETTES: THE FIRST CASE ASSOCIATED WITH SPINAL NERVES IN NEUROFIBROMATOSIS (NF1).

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**Introduction:** MPNST associated with NF1 and characterized by giant rosettes, complex immunohistochemical (IHC) profile, arising from spinal nerve-roots has not as yet been reported.

**Purpose:** To present the results of thorough analysis of the first case of MPNST with giant rosettes arising from spinal nerves and associated with NF1, and to discuss differential diagnostic aspects of the case.

**Methods:** conventional histological, IHC and electron microscopic (TEM) methods were used on formalin-fixed, paraffin embedded tissue.

**Findings:** The 24-y-old woman had had café-au-lait spots, several subcutaneous and one subpleural, indolent, slowly growing nodules, that did not cause clinical or cosmetic problems. She was admitted and urgently operated on because of subtotal „cauda-syndrome”. Partial resection of a necrotic, hemorrhagic, grossly encapsulated tumor, attached to a nerve root, located at the level of vertebrae ThXII-LII was accomplished. Severe, progressive caudal syndrome developed 4 months later and the patient died of ARDS. None of the neurofibromata were malignant. The caudal 18 cm long portion of the myelon was almost completely destroyed by a firm, hemorrhagic-necrotic, grayish-white neoplasm ensheathing nerve roots and the filum terminale without distant metastases. The spindle-celled tumor displayed focal regimentation and whorls. Antoni A or B areas and Verocay bodies were absent. The mitotic activity was marked (Mib-1

LI: 35-55%). Rich vascularity with endothelial cells which strongly reacted with CD31, CD34 and FVIII was present. The vascular smooth muscle cells were SM actin positive. None of these antibodies reacted with the neoplastic elements. The latter were intensively decorated by vimentin and S100, were focally positive with NSE, NF, GFAP. Desmin, panCK, FXIIIa, CK14, CAM5.2, synaptophysin and CD57 did not react with the tumor cells. The most striking feature was the high number of S100 and NSE positive giant rosettes (diameter = 100-350 µm). Homer-Wright- or ependymal rosettes and perivascular pseudorosettes were absent. EM showed schwannian differentiation, broad processes, incomplete basal lamina plus long spacing collagen and broad amianthoid fibers in the matrix.

**Differential diagnosis:** giant rosettes were described in one case of „neuroblastoma-like neurilemmoma” and rarely in fibromyxoid fibrosarcoma, but our results excluded these entities. A thorough search of PubMed and Google Scholar failed to disclose a similar case.

#### P 962

### CORRELATION OF THE HISTOLOGICAL FEATURES AND CLINICAL PICTURE IN VESTIBULAR SCHWANNOMAS

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The objective of the study was to evaluate relationships between histological features of vestibular Schwannomas and onset of early clinical symptoms.

The 125 patients, who had their first surgery for vestibular Schwannoma in the years 1988-2004, were analyzed retrospectively.

Conventional Schwannoma (including the “ancient” variety) and Cellular Schwannoma variant, according to the WHO 2000 classification, were diagnosed. The relationships of facial nerve paresis before the surgery and the histology of the tumour (including 15 morphological criteria), the tumour size and historical classification into Antoni A, B and mixed types, were statistically analysed.

The early onset of facial nerve paresis was most frequent (50%) in patients suffering from Cellular Schwannoma variant, less frequent in connection with Conventional (ancient) Schwannoma (37%), and the least frequent in Conventional Schwannoma (26%).

In the group of “small” tumours (up to 20mm), the incidence of facial nerve paresis was lower (25%) than in “medium sized” lesions (20-40mm), and in “large” tumours (larger than 40mm). In these two groups, the incidence of facial nerve paresis was nearly equal (36% and 38% respectively).

Facial nerve paresis before the surgery was observed with similar frequency (33% and 38%) in cases classified as Antoni A and A/B or as in Antoni B and B/A. These relationships were representative for tumours of different diameters.

Analysis of relationship between early onset of the facial nerve paresis and histological picture of vestibular Schwannomas revealed statistically significant correlation ( $\chi^2=6,56$ ;  $p<0,05$ ) of this symptom with low cellularity of the tumour. The paresis appeared sooner in lesions with low number of cells, rich in blood vessels, with effusions, oedema, other degenerative changes, necrosis in large tumours.

We observed as well statistically significant coexistence ( $\chi^2=6,63$ ;  $p<0,05$ ) of early onset of the facial nerve paresis and numerous Verocay’s bodies, plexiform

structures, coiled structures and nuclear palisades in small tumours.

Correlations of other morphological features and early onset of facial nerve paresis were not statistically significant.

#### P 963

##### **P 53, KI-67 AND BAX EXPRESSION AND THEIR RELATION WITH SURVIVAL IN GRADE II AND GRADE IV ASTROCYTOMAS**

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**Introduction:** Diffusely infiltrating astrocytomas are the most frequent intracranial neoplasms. Patient factors influencing patient survival includes the age and the general condition, tumor location and the adequacy of the surgical resection performed prior to conditional radiotherapy and/ or chemotherapy. Mutation of tumor suppressor genes, expression of apoptosis related genes and Ki-67 labeling index are the markers used to predict the patient survival.

**Purpose of the study:** In this study, we investigated immunohistochemical expression and the correlation between proapoptotic gene bax, Ki-67 labeling index and mutant p53 in glioblastoma multiforme (GBM) and grade II astrocytoma specimens with the patients' survival.

**Material and methods:** The immunohistochemical expression of p53, Ki-67 and bax, in the specimens of the 9 grade II, and 40 in GBM specimens who underwent resection for glial tumors in the Department of Neurosurgery were evaluated. Histological parameters were based on WHO criteria. Grade II and IV tumors were compared by Mann-Whitney U tests. Spearman's rho test was used to analyse the correlation between parameters. Karnovsky performance scale was used for the survival.

**Results:** 9 patients with grade II and 40 patients with grade IV tumors were evaluated. Median ages were 39 in grade II, 50 in grade IV tumors ( $p=0.009$ ). Median Ki-67 values were 4 in grade II and 14.5 in grade IV tumors ( $p=0.001$ ). No difference for bax, p53 or Karnovsky score was observed between groups. Correlation analysis showed no statistically significant relation between age, Karnovsky score and immunostaining parameters.

**Conclusion:** Value of the largely used immunohistochemical markers related with apoptosis or cell proliferation in prediction of the patient survival is controversial. It seems that adequate surgical resection is the gold standard for the patients with glial tumors. In this study we conclude that utilisation of these markers is not cost effective since we did not obtain statistically significant results.

#### P 964

##### **ANALYSIS OF PROTEIN EXPRESSION REGULATING THE PI3-K/PKB/AKT SIGNALING PATHWAY IN RELATION TO ASTROCYTOMA PATHOGENESIS**

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**Introduction:** The most frequent alterations in astrocytomas are mutations and deletions of the tumour suppressor genes PTEN, p53 and amplification of epidermal growth factor receptor (EGFR) and platelet derived growth factor receptor (PDGFR). Growth factor receptors activate phosphatidylin-

ositol-3-kinase (PI3-K) in the PI3-K/protein kinase B/Akt (PI3-K/PKB/Akt) pathway. PTEN dephosphorylates phosphatidylinositol-3 phosphate and inactivates its downstream target, PKB/Akt.

**Aim:** The aim of our study was to detect changes in: PTEN, the phosphorylated form of PKB/Akt and EGFR protein expression in astrocytoma tissue samples.

**Material and methods:** We used paraffin embedded astrocytoma tissue samples from 71 patients which were divided into low grade astrocytomas (grade I-II) 34 samples and high grade astrocytomas (grade III IV) 37 samples. Mouse monoclonal antibodies against PTEN (26H9, Cell Signaling), PKB/Akt phosphorylated on serin 473 (587F11, Cell Signaling) and EGFR (31G7, Zymed) were used, followed by standard indirect immunohistochemical method with Envision plus kit (DakoCytomation).

**Results:** EGFR was expressed mainly in membranes. EGFR protein expression was detected in 25 % of low grade and in 65 % of high grade astrocytomas with stronger intensity of staining in high grade compare to low grade tumours. The phosphorylated form of PKB/Akt was localised in both nucleus and cytoplasm. Protein expression of phosphorylated PKB/Akt was found in about 85 % in both groups of astrocytomas with similar intensity of staining. In the group of high grade tumours a slight increase in PKB/Akt phosphorylation in the nucleus was observed. PTEN was detected mainly in the cell cytoplasm but in a few cases it was seen in the nucleus. Generally, PTEN protein expression was lower compared to EGFR and the phosphorylated form of PKB/Akt expression. PTEN was detected in 30 % of all astrocytomas, in 20 % of high grade, and in 40 % of low grade tumours.

**Conclusion:** Protein expression of EGFR increases with astrocytoma grading. This may subsequently lead to activation of PI3 K/PKB/Akt survival pathway. A rather surprising finding was no difference in phosphorylation of PKB/Akt in either group of tumours. We expected high level of PKB/Akt phosphorylation in high grade astrocytomas. PTEN expression was altered in more than half the analysed astrocytomas, more frequently in high grade tumours.

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#### P 965

##### **GRANULAR CELL ASTROCYTOMA: A RARE MORPHOLOGIC VARIANT OF DIFFUSE ASTROCYTOMAS**

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**Aim:** To present a rare morphologic variant of diffuse astrocytoma, namely granular cell astrocytoma and to define the diagnostic criteria of this tumor.

**Introduction:** Granular cell astrocytoma is an infiltrative glioma consisting of astrocytes with prominent granular cytoplasm due to lysosomal accumulation. Morphologically may be confused with demyelinating disorders, infarctus and other granular cell tumors. Although it has not been set in the recent WHO classification, it is known a granular cell variant of diffuse astrocytoma.

##### **CASE REPORT**

58 years old male applied to our emergency room with a strong headache lasting for a month in lower stringency. In addition, tendency to sleep and walking disability were seen. Physical examination revealed only a left-side paresis prominently. A 6x4.5x3 cm-contrast enhancing mass with an oedematous halo was located in the cerebral right frontoparietal lobe with a striking shift on his CT scans. The cerebral mass was totally removed under urgent surgical circumstances. On histologic examination of the sample submitted during the operation

were seen numerous macrophage-like cells as well as hemorrhagic and ischemic changes. Owing to atypical cells observed, low grade neuroglial tumors were included in the differential diagnosis. The microscopic examination of the permanent sections revealed the cells with abundant granular and polygonal cytoplasm, round and peripheral nuclei. Morphologically fibrillar formations were also seen in scarcely separated spindle cells. Mitosis was observed, but there was no necrosis. The MIB-1 index was moderately high. Cytoplasm of the tumor cells showed strong PAS staining. Tumor cells were reactive for GFAP, S100, CD68 proteins immunohistochemically. The tumor was diagnosed as granular cell astrocytoma.

Conclusion: Because of the rarity and the morphologic similarity to some non-tumoral lesions of the cerebrum, practicing surgical pathologists should keep in mind this morphologic variant of diffuse astrocytoma.

#### P 966

##### **EVALUATION OF hTERT IMMUNOPOSITIVITY IN THE BRAIN AND ASTROCYTIC TUMORS.**

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Assessing the histological grade of astrocytic tumors can be problematic because these tumors are often quite heterogeneous in terms of histology and biomarker distribution. This problem becomes more pronounced in stereotactic biopsies where limited material needs to be evaluated. In this study, we asked whether hTERT expression, which has been reported to correlate with the histological grade of astrocytic tumors, could serve as a diagnostic aid when evaluating stereotactic biopsies. For this purpose, we used 45 astrocytic tumor specimens, 31 of these from stereotactic biopsies (16 low-grade [I and II], 29 high-grade [III and IV]) and 12 normal brain specimens, 6 from patients without cancer. hTERT expression was assessed by immunohistochemistry (IHC) with the NCL-TERT antibody, in situ hybridization (ISH) and relative quantification (RQ) with real time PCR on paraffin embedded tissues. With ISH and IHC, hTERT was identified in the majority of neoplastic cells in high-grade tumors, but also in single neurons and endothelial cells. IHC further showed focal hTERT positivity in neoplastic cells of low-grade tumors that were negative with ISH. These findings corresponded to the RQ values obtained from hTERT positive microdissected specimen areas ( $p < 0,001$ ) but not from respective whole tumor extracts. hTERT protein was specifically localized in the nucleus, in a lacy pattern with prominent nucleolar positivity (pattern A), or as dense granular staining throughout the nuclear area with or without recognizable nucleoli (pattern B). When positive, normal and neoplastic cells of all grades exhibited pattern A of hTERT protein distribution. Pattern B was observed rarely in neurons, focally in low-grade, and focally or diffuse in high-grade tumors. Rarely, cytoplasmic staining was observed. According to these data, IHC but not PCR seems to be a valuable tool for the investigation of hTERT in the brain, since astrocytic tumors heterogeneously express this protein. It will be interesting to evaluate the patterns of hTERT positivity in larger series of these tumors in order to determine the respective clinical impact. The limited but specific positivity observed in low-grade astrocytic tumors should be taken into account when assessing stereotactic biopsies with this marker. hTERT is expressed in normal neurons, a finding of potential important biological significance in the context of neuronal progenitor research.

#### P 967

##### **CHONDROID METAPLASIA IN GLIOMAS - AN IMMUNOHISTOCHEMICAL STUDY**

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The presence of cartilage in neuroepithelial brain tumors was rarely reported in the literature and there are many controversies concerning its origin. We present an immunohistochemical study of four cases of gliomas (two ependymomas and two astrocytomas) in which foci of hyaline cartilage with a formation of metaplastic bone were found. No other tissues suggesting diagnosis of teratoma were identified. Immunohistochemistry for extracellular matrix (ECM) components (tenascin, vitronectin, fibronectin, laminin, collagen types II, IV and VI) revealed that the type II collagen, which represents one of the major structural components of a cartilage, was present in these tumors in the wall of the blood vessels as well as in the intercellular spaces. Investigation of other ECM glycoproteins, tenascin and type VI collagen, which were also reported to be structural components of human cartilage revealed a similar observation. The abundant ECM production was in these particular tumors accompanied by areas of dystrophic calcifications. The anti-GFAP immunohistochemical reaction revealed the cartilage to be GFAP-positive in the astrocytomas and in one case of ependymoma. We interpret the cartilaginous islands as having their origin from neoplastic glial cells. A rare coexistence of the specific local conditions such as calcifications and a capacity for production of cartilaginous ECM might lead in such gliomas to the formation of chondroid areas even with metaplastic ossification.

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#### P 968

##### **EGFR AMPLIFICATION AND NFkB OVEREXPRESSION IN PRIMARY GLIOBLASTOMAS.**

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Glioblastoma multiforme (GBM) are the most frequent and malignant tumors of the human central nervous system. Within these tumors primary glioblastomas develop rapidly, with a short clinical history de novo, a mean age at diagnosis of 55 years, and with a genetic pathway showing EGFR gene amplification/overexpression, and only in exceptional cases TP53 mutations.

EGFR, a tyrosine kinase receptor, has been implicated in tumorigenesis and neoplastic progression in many tumors. EGFR has been reported to activate NFkB, a transcription factor that regulates genes involved in cell survival and oncogenesis.

We present 16 newly diagnosed GBM with initial symptoms detected between 7 days to 4 months before diagnosis. The mean age was 58 years old (ranging from 45 to 77 years). CT scans of the brain showed mass tumors in supratentorial localization, with a size from 2,5 to 4,5 cm large. Histopathological study revealed cellular heterogeneity, anaplasia and glial astrocytic differentiation. Hyperplastic and irregular vascularization, and necrosis have been observed. Evaluation of proliferation showed a mean of 6,5 mitosis per 10 HPF (ranging from 3 to 12) and a mean of 26,6% Ki-67 (MIB-1) labelling index. Neoplastic cells were GFAP

immunoreactive. Immunohistochemical expression of EGFR was also detected.

All cases showed trisomy/polysomy of chromosome 7 by FISH analysis. EGFR gene amplification and no mutations in TP53 gene (exons 5 to 8) were demonstrated in all cases.

Overexpression of NFkB was detected with EMSA (electrophoretic mobility shift assay) in 11 cases of these primary glioblastomas. Neoplastic cells showed positive expression of NFkB in all cases by immunohistochemistry.

These data suggest a relationship between EGFR activation and the NFkB transduction pathway during the neoplastic progression of primary glioblastomas.

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The recipient of the grant was Miguel Cerdá-Nicolás (jose.m.cerda@uv.es)

#### P 969

##### **HYPOXIA-INDUCIBLE FACTOR 1ALPHA (HIF-1) AND CARBONIC ANHYDRASE IX (CA 9) EXPRESSIONS IN GLIOBLASTOMA MULTIFORM ARE PREDICTIVE OF RESPONSE TO RADIATION THERAPY.**

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**Background:** Tumor hypoxia is known to be associated with resistance to radiotherapy. Hypoxia induces the expression of hypoxia-inducible factor-1alpha (HIF-1), allowing up-regulation of the expression of downstream genes such as carbonic anhydrase IX (CA 9).

**Methods:** We examined the expression of HIF-1 and CA 9 by immunohistochemistry in glioblastoma multiform (GBM) biopsies, and investigated their relationship with response to radiation therapy. The response to irradiation was assessed by comparing contrast-enhanced magnetic resonance images obtained before and six weeks after the completion of radiotherapy. Assessment of odds ratio were based on the logistic regression model with stepwise adjustment. The multivariate model included HIF-1 and CA 9 coded on a semi quantitative scale according to the positive tumor cell percentage (0 = no expression; + = 1-10%; ++ = 11-50%; +++ = 51-100%), and age.

**Results:** Fifty six consecutive patients with inoperable glioblastoma treated with radiotherapy (59.4 Gy in 1.8 Gy/fraction) at the Pitié-Salpêtrière hospital between 1995 and 2002, were included in this study (median age: 56 years, range, 30 to 67 years). HIF-1 was expressed in 33 of 56 (59%), and CA 9 in 38 of 52 (73%) of glioblastomas. Tumor HIF-1 expression correlated significantly with that of CA 9 (Kappa = 0.23, p = 0.003). The response rate to radiotherapy for the entire population was 29%. The expressions of HIF-1 and CA 9 were correlated inversely with the rate of response to radiotherapy (univariate analysis: HIF-1 +: odds ratio 0.21, 95% CI [0.06, 0.71], p = 0.012; CA 9 +: odds ratio 0.15, 95% CI [0.04, 0.59], p = 0.0063). The multivariate analysis showed that HIF-1 + (odds ratio 0.13, 95% CI [0.03, 0.65], p = 0.013), CA 9 +++ (odds ratio 0.21, 95% CI [0.04, 0.98], p = 0.047) and age (odds ratio 0.91, 95% CI [0.82, 0.99], p = 0.046) were independent predictors of response to radiotherapy.

**Conclusions:** Glioblastomas with a hypoxic profile (expression of HIF-1 and/or CA 9) were associated with a significantly worse response to radiotherapy, independently of known prognostic factors.

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#### P 970

##### **MOLECULAR PROFILING IN ADULT TYPE OF GLIOBLASTOMA MULTIFORME IN POLISH PATIENTS (LOH 9, 10, 13,17, P53 MUTATIONS AND EGFR AMPLIFICATION).**

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According to the classical model of carcinogenesis in glioblastoma multiforme (GM) the primary (de novo) GM is related mainly to the amplification or overexpression of EGFR and MDM2, LOH 10p and 10q, p16 deletion, PTEN mutation. The secondary GM, occurring after the progression of low-grade astrocytomas is related to the p53 mutation and loss of heterozygosity (LOH) on chromosome 17.

The aim of our study was identification of molecular markers in GM in 32 tumor tissue with GM and peripheral blood from patients. We examined the LOH on chromosome 9, 10, 13, 17, EGFR amplification. Mutations of p53 were examined in patients with EGFR amplification or/and LOH 10. Additionally, the immunohistochemical study was performed using antibodies against p53, Rb, EGFR, p21 and p27, Ki67. LOH 10 was detected in 25% (8/32) cases, in one case a deletion on chromosome 10 was detected; LOH 9 in 15,5% (5/32); LOH 17 in 12,5% (4/32); LOH 13 in 9,3% (3/32); EGFR amplification was identified in 21,8% (7/32) cases.

In one of cases, p53 mutation was detected together with LOH 9,10, 13. In other unusual case, the genetic alterations were more complex, consisted in p53 mutation, LOH 10, LOH 17, and EGFR amplification.

Our study reveals, that molecular changes in GM may be complex and indicates overlapping of two main genetic pathways of primary and secondary GM.

#### P 971

##### **PROGNOSTIC SIGNIFICANCE OF CELL CYCLE PROTEINS P53, P21, P27, P14, P16 AND MDM2 IN GLIOMAS.**

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**Background:** Gliomas are among the most aggressive and treatment refractory of all human tumors and molecular pathogenesis has been under intense investigation as a part of the effort to develop more effective therapeutic strategies for these tumors. Cell cycle regulatory genes and especially CDK inhibitors have been found commonly affected in gliomas. This study investigates the immunohistochemical expression of 6 essential cell cycle proteins (p53, p21, p27, p14, p16 and mdm2) in astrocytomas and their correlation with tumor grade, proliferating index (Ki67 positivity) and patients' survival.

**Material and Methods:** Paraffin-embedded sections of 63 brain biopsies from equal number of patients with astrocytomas of the central nervous system divided in low grade (I and II, N=15) and high grade (III and IV, N=48) tumors, were analyzed immunohistochemically using polyclonal and monoclonal antibodies. Follow up data were available for all patients (follow-up time: 2-70 months, mean 22.2). Cases containing >5% immunostained tumor cells were

considered as positive. Tumors with high proliferating index were considered those with >10% immunostained tumor cells. Results: The number of cases in which the expression of p53, p14, p16, p21 p27 and mdm2 were detected was: 54%(34/63), 76%(48/63), 63%(40/63), 45%(28/63), 87%(55/63) and 100%(63/63) respectively. The mean index for Ki67 was 14.58. A highly significant relationship was observed between tumor grade and survival ( $p<0.001$ ) as well as between proliferating index and survival ( $p<0.001$ ). P53 and p21 were more frequently expressed in high grade tumors ( $p<0.05$  respectively). P53 expression was associated with lower survival only in p21(+) tumors ( $p<0.05$ ). Neither p14 nor p16 expression were associated with grade, Ki67 index or overall survival. Cox regression analysis revealed that only tumor grade and Ki67 index were independent prognostic factors (CI: 0.032-0.502,  $p=0.03$  and CI:1.167-5.408,  $p=0.019$ ). Conclusions: The study confirms that in cases of gliomas, tumor grade and proliferating index, predict the outcome of the patients. P53 was associated with poor prognosis only in tumors overexpressing p21; that means high levels of p21 in tumor cells associated with aberrant p53 protein expression, may result in a state refractory to therapy.

#### P 972

##### ANTI-INVASIVE AND ANTI-ANGIOGENIC EFFECTS OF MMI166 ON GLIOMA CELLS

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Constitutive overexpression of matrix metallo-proteinases (MMPs) is frequently observed in malignant tumors. In particular, MMP-2 and MMP-9 are reported to be closely associated with invasion and angiogenesis in malignant gliomas. The aim of our study was to evaluate the anti-tumor effects of MMI-166, a new MMP inhibitor in three human glioma cell lines (T98G, U87MG, KNS2). The gene expression of MMP-2 and MMP-9 were analyzed by semi-quantitative RT-PCR. The anti-invasive effect of MMI-166 was analyzed by in vitro invasion assay. The cytotoxicity of MMI-166 was determined by MTT assay. The gelatinase activity was analyzed by gelatin zymography. In vitro angiogenesis assay was also performed. Glioma cell lines, which produced both MMP-2 and MMP-9, showed invasive capacity in invasion assay. The invasion of glioma cells was suppressed by MMI-166. No remarkable suppression of the proliferation by MMI-166 was observed in the MTT assay. Gelatin zymography revealed complete suppression of MMP-2 and MMP-9 activity by MMI-166. Angiogenesis assay showed the suppressive effect of MMI-166 on angiogenesis induced by glioma cells. These results suggest that MMI-166 has potential suppressive effects on invasion and angiogenesis of glioma.

#### P 973

##### ESTROGEN RECEPTOR BETA (ER $\beta$ ) PROTEIN EXPRESSION CORRELATES WITH BAG-1 AND PROGNOSIS IN BRAIN GLIAL TUMOURS

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Introduction: Estrogen receptor  $\beta$  (ER $\beta$ ) is an important mediator of estrogen function in a variety of tissues. Its expression declines in breast, ovarian, prostatic and colon carcinomas as well as in astrocytic tumours. BAG-1 is a multifunctional protein with anti-apoptotic action that may have an important role in neoplasia and is possibly regulated

by estrogen receptors. One of the direct targets of BAG-1 is HSP70.

Aim: The purpose of this study was to analyse the expression pattern of ER $\beta$ , BAG-1 and HSP70 proteins in two distinct types of glial neoplasms (astrocytic and oligodendroglial), to investigate their possible correlation and probe their impact in prognosis.

Patients-Methods: ER $\beta$ , BAG-1 and HSP70 protein expression was monitored immunohistochemically in 66 cases of astrocytomas of all Grades (I-IV) and 20 oligodendrogliomas. Grading was performed according to the WHO grading system. Protein expression was detected by immunohistochemistry using the EnVision System (DAKO, Carpinteria, CA, USA) and the following antibodies: monospecific polyclonal antibody to Estrogen Receptor  $\beta$  protein (Ab No. 385P, Biogenex, San Ramon, CA, USA/MENARINI diagnostics, Greece), monoclonal mouse antibody to BAG-1 (Mob 335, DBS, Pleasanton, CA, USA/MENARINI diagnostics, Greece) and monoclonal mouse antibody to HSP70 (clone 8B11, NOVOCASTRA Lab Ltd, Newcastle Upon Tyne, UK).

Results: In astrocytic tumours low ER $\beta$  expression correlated significantly with high grade ( $p<0.001$ ), higher expression of cytoplasmic BAG-1 ( $p<0.001$ ) and worse survival (log rank  $p=0.02$ ). ER $\beta$  expression had an independent prognostic value for overall survival in this cohort of patients (Cox  $p=0.03$ ). There was also statistically significant association of BAG-1 nuclear expression with HSP70 cytoplasmic expression. This last association was noted in oligodendrogliomas as well.

Conclusion: Our results strengthen the hypothesis that ER $\beta$ , BAG-1 and HSP70 play an important role in the pathogenesis and progression of glial neoplasms, particularly those of astrocytic origin. Moreover, ER $\beta$  expression in astrocytic tumors appears to be an important, independent prognostic factor for survival, and a possible target in therapeutic strategies.

#### P 974

##### EXPRESSION OF HISTONE GENES IN ASTROCYTIC BRAIN TUMOURS AND ITS PROGNOSTIC SIGNIFICANCE IN PATIENTS AFTER SURGICAL TREATMENT

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##### Introduction:

Expression of histone genes is known to reflect proliferative activity of normal and neoplastic cells. It is hypothesised that expression of histone genes also characterizes biological aggressiveness of astrocytic tumours (AT) and can be useful in the prediction of clinical course in patients after surgical treatment.

Purpose of the study: 1) To assess the correlation between expression of histone genes H1, H2A, H2B, H3 and H4 and histological grade as well as other proliferative indices in AT. 2) To validate the usefulness of histone genes expression in prognostication in patients with AT.

##### Methods:

Eighty four patients (52 men and 32 women, mean age: 50.5 years), with supratentorial astrocytic tumour grade II (n = 13), III (n = 19) and grade IV (n = 52) were studied. The expression of histone genes was analyzed semi-quantitatively by in situ hybridization (ISH) in paraffin sections and defined as histone labeling index (HLI). In 52 patients the histone genes expression was analyzed with quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR). The

RNA extract served as a matrix in the QRT-PCR reaction for quantitative analysis of histone H1, H2A, H2B, H3 and H4 mRNAs, with application of sequence detector ABI Prism 7700 (TaqMan). The results of QRT-PCR reaction were calculated as a target gene mRNA copy number to the reference gene (beta-actin) mRNA copy number ratio. For ISH study a cocktail of fluorescent-labeled oligonucleotide probes, labeling histone H2B, H3 and H4 mRNAs was used. The Ki-67 labeling index was studied after immunostaining performed in paraffin sections with use of avidin-biotin complex.

Results:

The global expression of histone genes increased significantly with increasing grade of the tumour. Expression of histone H3 gene showed the highest correlation with histological grade (R=0.55), HLI (R=0.33) and Ki-67 (R=0.37) proliferative indices. Multivariate analysis using the Cox proportional hazard model showed that patient age, tumour grade, postoperative performance score and the expression of histone H3 gene are independent prognostic factors. Significantly shorter survival times were observed in patients with histone H3 mRNA levels higher than 1.26.

Conclusion:

Our results support value of the histone genes expression as a marker of biological malignancy, tumour progression and cellular proliferative activity in AT. The level of H3 mRNA is an independent molecular prognostic factor in patients

**P 975**

**MOLECULAR INVESTIGATIONS OF GLIOMAS IN STEREOTACTIC BIOPSY MATERIAL**

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The difficulties connected with diagnosing and treating of gliomas are a reason to develop new methods of precise determination of these tumours biology. The determination of the gliomas malignancy was supplemented with molecular analysis of transcriptional activity of some proliferation and apoptosis markers in stereotactic biopsy samples of these tumours.

The objective of our study was to determine the expression profiles of genes taking part in proliferation and apoptosis in diffuse brain astrocytomas and to search for correlation between mRNA concentration profiles of these genes, the findings of the morphological examinations, the course of the disease and the response to the implemented treatment.

The biopsy samples of 232 patients who had undergone stereotactic biopsy in the last 6 years in the Department of Neurosurgery in Sosnowiec, Poland, were investigated. In most patients, radiotherapy was the only treatment or it was adjuvant to surgery. The morphological and molecular analyses were carried out on 85 gliomas (30 cases of G2 WHO diffuse astrocytomas of low malignancy and 55 cases of G3 WHO anaplastic astrocytomas), 62 of these patients underwent radiotherapy.

An analysis of full clinical data, cytological examination, routine histopathological examination, determination of malignancy grade according to the WHO criteria and immunohistochemical proliferative responses (Ki-67) and investigation of apoptosis markers (p53, BCL2, BAX,

FAS, FAS-L) were performed. A pattern of material processing for the molecular analysis was developed - the markers were determined by the RT-QPCR method (TaqMan) with application of an ABI PRISM 7700 sequence detector. The response to brachytherapy was assessed with imaging techniques (CT, MRI).

We found that fixed in pure alcohol, dry and not-stained cytological smears are enough to obtain the amounts of mRNA sufficient to carry out molecular analyses. Analysing clinical data, histological findings, and number of the mRNA molecules coding H3, p53, BCL2, BAX and FAS proteins in the investigated material, we found that molecular investigations are better correlated with the course of the disease and the response to implemented treatment than morphological examinations. Our findings enable us to claim that assessment of the genes' expression may be considered as a valuable additional marker in the diagnosis and prognosis of diffused astrocytomas.

**P 976**

**RELATIONS OF CLINICAL AND PATHOLOGICAL CHARACTERISTICS WITH LOSS OF HETEROZYGOSITY ON CHROMOSOME 10 IN GLIOBLASTOMA.**

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**P 977**

**PROGNOSTIC SIGNIFICANCE OF STEROID HORMONE RECEPTORS AND DNA CONTENT IN MENINGIOMAS**

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The possibility that meningioma growth may be related to female sex hormone levels is suggested by several lines of evidence. Meningiomas are twice as common in women as in men, have been observed to wax and wane with pregnancy, and are positively associated with breast cancer. However, unlike breast cancer, meningiomas are much more commonly positive for progesterone receptor than for estrogen receptors. Overall, 70% of meningioma specimens have been found positive for progesterone receptor while only 30% were positive for estrogen receptor. Therefore, clinically and biologically more interesting are the unexpectedly aggressive clinical course of some meningioma, is a diagnostic dilemma for routine histopathology. Comparison between hormone levels was mostly significant. Also the relationship between expression of progesterone with the ploidy determined by image cytometric measurement (Leica Quin 500 DNA Cytometry Software) has been interpreted as signs of aggressive in the tumor tissue support the possibility of their application as a test for grading and for adequate treatment choice.

Key words: Meningioma-Steroid receptors-DNA ploidy.

**P 978**

**MULTIPLE SPINAL MENINGIOMAS WITH POSITIVE PR – CASE REPORT**

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#### Short introduction

Multiple spinal meningiomas are relatively rare neoplasms and accounting only about 3% of all spinal meningiomas. They are defined as at least two spatially separated meningiomas occurring at the same time or more than two meningiomas arising sequentially from two clearly distinct regions. We report a case of the 52-year-old woman who was admitted to Department of Neurosurgery because of a progressive motor weakness with spastic paraparesis and sensation disturbance which gradually developed in the in both legs for last 3 years. Computed tomography (CT) scan revealed clearly defined three intradural extramedullary lesions at the Th6, Th10 and Th12. After operation in which the total resected tumors were performed histopathological examination showed two psammomatous meningiomas and one meningotheial meningiomas which was progesterone receptors (PR) positive. Proliferative potential which were measured by Ki67 antibodies were lower than 1%.

#### Purpose of the study:

In the present case rare multiple spinal meningiomas were investigated by the presence and distribution of the PR and proliferative potential with Ki 67 antibody with aim to predict eventually recurrence.

#### Methods used

Tumor samples after the third operation was analyzed by standard pathohistological technics, histochemical staining and immunohistochemical methods for PR, Vimentin, EMA and Ki 67 antibodies.

#### Summary of the results

The two tumors specimens from the Th10 and Th 12 showed a psammomatous meningiomas with large numbers of psammoma bodies (round calcified bodies). One tumor specimen from the Th 6 showed classic meningotheial meningiomas with cellular whorls and pseudonuclear inclusion.

Samples of the meningotheial meningiomas showed exclusively nuclear PR stain in a 15% of the tumor cells. In contrast, PR was negative in other two psammomatous meningiomas. Proliferative index were measured by Ki67 antibodies and in all tumors samples was lower than 1%.

#### Conclusion reached

To our knowledge, this is the first report of an association between multiple (three) spinal meningiomas (three) and PR.

#### P 979

##### **CASPASE-3 EXPRESSION IS AN INDEPENDENT PREDICTOR OF RECURRENCE IN PATIENTS WITH INTRACRANIAL MENINGIOMAS**

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Caspase-3 is the ultimate executioner caspase that is essential for the nuclear changes associated with apoptosis. In this study, we investigated caspase-3 expression in relation to baseline apoptosis, in order to assess the prognostic impact of an apoptotic mode of cell death in meningiomas' recurrence.

The expression of caspase-3 was evaluated immunohistochemically in a consecutive series of 56 patients with primary intracranial, totally resected meningiomas. Caspase-3 expression was investigated in relation to baseline apoptosis -as illustrated by the expression of the ssDNA-, the

apoptosis suppressor protein bcl-2, proliferation indices (Ki-67, PCNA, Topoisomerase IIa and Mitosin C), and hormonal status as expressed by estrogen, progesterone and androgen receptors. Furthermore, caspase-3 expression was correlated to standard clinicopathological parameters and patients' disease-free survival.

Immunostaining for caspase-3 was observed in 60.7% of cases demonstrating a cytoplasmic granular pattern of expression. Caspase-3 labeling index was significantly associated with ss-DNA expression ( $p=0.03$ ). Moreover, significant positive correlations emerged between caspase-3 labeling index and mitotic index ( $p=0.002$ ), PCNA labeling index ( $p=0.004$ ), while a suggestive association was observed between caspase-3 and the proliferation associated molecule mitocinC. As far as the conventional parameters are concerned, a significant correlation was established between caspase-3 expression and histological grade of meningiomas, in that grade II and III meningiomas (atypical and malignant respectively) demonstrated a significantly increased caspase-3 labeling index ( $p<0.0001$ ). In univariate and multivariate survival analyses caspase-3 predicted meningiomas' recurrence affecting independently disease-free survival ( $p=0.0297$  and  $p=0.047$  respectively).

In conclusion, caspase-3 may prove to be a useful marker of recurrence in a group of neoplasms characterized by the frequent discordance between histology and clinical behavior.

#### P 980

##### **INTRACRANIAL LESIONS MIMICKING MENINGIOMAS: REPORT OF 18 CASES.**

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#### OBJECTIVE:

To analyze the neurosurgical cases in which both the clinical and neuroimaging features suggested intracranial meningioma but resulted in different entities from a neuropathological point of view.

#### PATIENTS AND METHODS:

The clinical history and histopathological reports of patients with intracranial mass lesions mimicking meningioma and operated in our hospital, were reviewed.

#### RESULTS:

Our analysis revealed 18 cases with different neuropathological diagnosis. They were distributed as follows: 8 cases of hemangiopericytoma, 2 cases of Rosai-Dorfman's disease, and one case of each of the following entities: solitary fibrous tumor, medulloblastoma, cerebral lymphoma, histiocytosis X, metastasis of adenocarcinoma, oligodendroglioma, pleomorphic xanthoastrocytoma and fibrous pseudotumor.

#### CONCLUSIONS:

The clinical and neuroimaging features of meningiomas are mimicked by different neoplastic and nonneoplastic entities. Because these lesions can resemble a meningioma during intraoperative biopsy, they must be considered in the differential diagnosis and kept in mind by the pathologist at this moment.

#### P 981

##### **PARTICULAR FORMS OF MENINGIOMA: ABOUT FOUR CASES**

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Meningothelial tumors or meningioma represent between 13 and 26 % of all nervous tumors in adult. The intracerebral meningotheiomatous form is the most frequent. We report

four cases which have a particular clinical presentation and particular microscopic patterns.

The first case occurred in 39-years-old women who developed a multifocal cystic tumour. The CT scan proposed the diagnosis of glioma. Microscopic pattern and immunochemistry made the diagnosis of multifocal meningioma. The second case is about another parietal cystic tumour occurring in a 60 years old woman. The diagnosis was a microcystic meningioma.

The third case is about a sixty years old woman who developing an intraventricular atypical meningioma.

The last case is about a 40 years old woman who developing an intramedullary papillary meningioma.

Consequences of Particular clinical and histological forms of meningiomas can be their misdiagnosis. The knowledge of this entities is important for a successful therapy

#### P 982

### CORRELATION BETWEEN VITAMIN E AND FOLIC ACID LEVELS AND THE DEVELOPMENT OF ALZHEIMERS DISEASE IN A MOUSE MODEL OF AD NEGAR OMIDI;PARICHEHR PASBAKSH;

Alzheimers disease(ad)is the most common type of dementia(75%),manifesting as a severe deterioration of mental functions.the brains of people with alzheimers disease exhibit deposition of amyloid beta peptid and progressive degeneration of nerve cells.scientist are studying ways that may help decrease or prevent neurodegeneration and may help injured neurons to regrow.we determined whether the antioxidant vitamin e+folic acid affected the presence of amyloid plaques in brain of aged pdapp transgenic mice.beta-amyloid precursor protein (app).is important for the pathogenesis of alzheimers disease(ad),which is characterized by progressive decline of cognitive functions,formation of a beta plaques,and neurofibrillary tangles,and loss of neurons.in the present study we have examined that treatment by vitamin e+folic acid in pdapp aged mice compare with control groups is able to decrease 20% in ab plaques levels of cerebral amyloidosis in neocortex.more over ,our results suggest that treatment by vitamins is able to prevent the disruption of basal cholinergic forebrain system and prevent of loss of cholinergic basal forebrain neurons(ms+hdb,vdb)

#### P 983

### AN AMINO ACID MOTIF COMMON TO BETA AMYLOID PROTEIN AND TO NEURODEGENERATIVE VIRUSES MAY TRIGGER NEURONAL DESTRUCTION.

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Research in the field of neuroscience has provided a better understanding of the cascade of biochemical events in neurodegenerative diseases. Most neurodegenerative conditions are marked by the presence of protein aggregations, and, in many cases, increased levels of oxidative damage in post-mortem tissues. The proteins associated with the neurodegenerative conditions may cause the over-production of free radicals in the neuronal tissues of the patients. We discovered that a 5-amino-acid sequence, Gly-Ala-Ile-Ile-Gly, residues 29-to-33 of the b-amyloid protein from Alzheimer's disease, is also found in proteins from three neurodegenerative viruses: HIV-1, Newcastle disease virus, and Japanese encephalitis virus. We used PC12 cells and SH-SY5Y cells to study the toxicity of this pentapeptide, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay and the measurement of caspase-3 activity. b-Amyloid 29-to-33 produces a concentration-dependent cytotoxicity to PC12 cells and to differentiate SH-SY5Y cells, but the reverse sequence (b-amyloid 33-to-29) is not toxic. Also, b-amyloid

29-to-33, but not b-amyloid 33-to-29, increases the caspase-3 activity in PC12 cells in a dose-dependent manner, which is blocked by U-83836E, U-101033E and vitamin E. These results emphasize role of free radicals in the pathogenesis of neurodegeneration.

#### P 984

### 1H MRS COMPARATIVE STUDY OF CEREBRAL METABOLISM IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT (MCI), VASCULAR DEMENTIA (VD) AND IN NORMAL ELDERLY SUBJECTS (N)

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Purpose: The aim of our study is to characterize the metabolic changes in the brain of patients with MCI, VD, and normal elderly subjects on the basis of analysis of 1H MRS data.

Materials and Methods: Three groups of patients are studied by MRI and 1H MRS with 1.5T Magnetom Vision (SIEMENS). The 1st group includes 25 patients (56-84y) with MCI. The 2nd group consists of 9 patients with VD (52-73 y). The 3rd group (N) consists of 30 healthy volunteers (60-75 y). 1H spectra are recorded in hippocampus (H), amygdala (A), frontal lobes (FL) and in entothinal cortex (EC) with the SVS STEAM method: TR/TE=1500/135,30 ms, VOI = 8cm3, NS = 128.

RESULTS: In the 1H spectra the following signals are identified: N-acetylaspartic acid (NAA)-2.02 ppm, total creatine (Cr)-3.03 ppm, choline (Cho)-3.24 ppm, and myo-Inositol (mIns)-3.56 ppm. From the spectra in all sampled regions of the brain the peak areas of main metabolites: NAA, Cr, Cho and mIns, and also the metabolite ratios are obtained. For the patients of the 1st verse the 3rd group the significant decrease of NAA and Cr and the increase of Cho peak areas in all regions and also significant decrease of NAA/Cr, and Cho/Cr in H and FL are observed. In the 1st group (NAA/Cr=1.01; mIns/Cr=0.82; Cho/NAA=0.96), in the 2nd group (NAA/Cr=1.14; mIns/Cr=0.58; Cho/NAA=0.86), and in the 3rd group (NAA/Cr=1.25; mIns/Cr=0.62; Cho/NAA=0.53) are obtained. There is the significant increasing in mIns/Cr (0.69) ratios in the patients of the 1st group compared with the patients of the 2nd group (0.52). Comparison 1H data in the right and left FL and EC for the patients of the all groups a significant reduction of NAA/Cr in the left hemisphere and non-significant reduction of NAA/Cr in the right hemisphere are found. The ratios of Cho/NAA, mIns/NAA in H, FL and EC are the most accurate values for differentiation MCI, VD and N.

CONCLUSION: 1H MRS data are useful in predicting the progression of MCI and VD and also for identification of cognitively normal subjects at risk for developing MCI.

#### P 985

### DECREASED THYMIDINE PHOSPHORYLASE EXPRESSION IN BRAINS AFFECTED BY ALZHEIMER DISEASE

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INTRODUCTION: Whether the alteration of blood-brain permeability plays a role in the pathophysiology of Alzheimer disease (AD) has long been debated and recently white matter

disturbance was implicated in AD's pathogenesis. We have recently shown, that mutations in TP present in patients with MNGIE result in loss of expression of thymidine phosphorylase in the white matter, which correlates with white matter hypointensity on MRI and albumin positive cells in the white matter suggesting increased BBB permeability. Furthermore its embryological expression reveals a maturation pattern, which coincides with the temporal and spatial maturation of the BBB.

**PURPOSE:** The aim of the pilot study is to test whether TP expression is altered in AD and this alteration correlate with increased BBB permeability

**METHODS:** We completed a pilot study on post mortem brain tissue from patients with definite AD and age and sex matched controls. Immunohistochemical stains for TP and intracellular albumin were performed and quantified by counting positive cells per 10 high-power fields.

**RESULTS:** The number of TP positive cells was 5.36 and 14.15 ( $p < 0.01$ ) in the parietal lobe, 5.97 and 16.22 ( $p < 0.01$ ) in the frontal lobe of Alzheimer and control brains, respectively and inversely correlated with the number of albumin positive cells (29.99 and 16.08 ( $p < 0.01$ ) in the parietal lobe, 25.94 and 12.06 ( $p < 0.01$ ) in the frontal lobe of Alzheimer and control brains, respectively). The number of TP positive cells was independent of age. Although patients with MNGIE do not develop AD, they succumb at a young age usually. Formal neuropsychological testing has not been performed on patients with MNGIE or their heterozygote family members to detect subtle cognitive impairment.

**CONCLUSION:** Whether loss of TP expression contributes to the pathogenesis of AD or it is a secondary phenomenon, needs further elucidation.

#### **P 986**

##### **RETROSPECTIVE STUDY OF SKIN NEUROFIBROMATOSIS (SN) OF TWENTY YEARS PERIOD (1991 -2000) IN 4 IRANIAN PATHOLOGY CENTERS**

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The present study was designed to assess the frequency and stage of Skin Neurofibroma in the samples of patients with age, gender and biopsy locations.

Data obtained from annual record files of four different pathology centers with considering factors of Diagnosis, Age, and Gender and Biopsy location, examined for statistical difference. Each diagnosis has been coded by ICD method. Statistical examination has been done in 4186 samples of skin related to 52907 cases. The results of this experiment showed that

1) SN distributed in 52.9% of male cases and 47.1% of female cases.

2) Among all cases, trunk was the most common site of biopsy with 691 cases (24.7%). 632 cases (22.6%) were in face & 438 cases (15.8%) were in head & neck. Three samples (5.3%) were taken from the eyelids, 9 (15.8%) were taken from the faces, 11(19.3%) were taken from the heads and necks, 9 (15.8%) were taken from the trunks, 9 (15.8%) were taken from the upper limbs, and 19 samples (28.1%) were taken from the lower limbs showed Neurofibroma.

3) Among all cases, the third decades of life was the most common time of SN appearance with 860 cases (21.7%). 648 cases (16.4%) were in 4th decade & 592 cases (14.9%) were in 5th decade. This results may indicated that we should work more about (SN) and its effects on our life story.

#### **P 987**

##### **INTERACTION OF VESICULAR MONOAMINE TRANSPORTER2 (VMAT2)AND NEUROMELANIN**

##### **PIGMENT AMONG THE MIDBRAIN DOPAMINERGIC NEURONS ,IN MAN**

P.PASBAKHSH(Ph.D);D.C.GERMAN(Ph.D);N.OMIDI(M) Neuromelanin(nm)pigment accumulates with age in catecholaminergic neurons in man,and the ventral substantia nigra dopaminergic neurons that are the most vulnerable to degeneration in parkinsons disease(pd)contain the greatest amount of this pigment.in vitro data indicate that nm pigment is formed from the excess cytosolic catecholamine that is not accumulated into synaptic vesicles via the vesicular monoamine transporter2(vmat2),using semi-quantitative immunohistochemical methods in human postmortem brain,we sought to examine the relationship between the cotents of vmat2 and nm pigment.the immunostaining intensity(isi)was measured for vmat2 in two regions of the midbrain dopaminergic cell complex. The isi of the cells was related to the density of nm pigment within the cells.we also measured the isi for tyrosine hydroxylase(th)and examined the noradrenergic neurons in the locus coeruleus(lc).in brains 22-65 years of age:1)ventral substantia nigra neurons had the lowest vmat2 isi of all neurons in the midbrain cell complex,whereas over 2-fold higher levels are found in most ventral tegmental area neurons :2)there was an inverse relationship between vmat2 isi and neuromelanin pigment in the midbrain dopaminergic neurons:3)neurons with the highest vmat2 isi resided in lc:4)neurons with high vmat2 isi also had high th isi:and 5)inthe newborn brain,which has not yet accumulated neuromelanin pigment in the aminergic neurons,the regional distribution of vmat2 and th-isi was similar to that found in the adult brain.these data support the hypothesis that among the midbrain dopaminergic neurons,the ventral substantia nigra dopamine neurons accumulate the highest levels of nm pigment because they have the lowest levels of vmat2,which thereby renders them especially vulnerable to degeneration in pd.

#### **P 988**

##### **DENSITIES OF PARVALBUMIN-IMMUNOREACTIVE NEURONS IN CORTICAL DYSPLASIAS AND NON-MALFORMED EPILEPTIC TEMPORAL NEOCORTEX**

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The changes in density of inhibitory parvalbumin-immunoreactive (PV-ir) interneurons were quantitatively studied by immunohistochemistry in series of human neocortical samples comprising a whole spectrum of malformations of cortical development (MCD) and the non-malformed epileptic temporal neocortex in patients with temporal lobe epilepsy (NET). The highest counts of PV-ir cells were obtained in the control samples ( $n = 21$ ). The number of PV-ir neurons was significantly decreased in NET ( $n = 73$ , 80.5 % of control values). In a proportion of patients with NET as well as in two control samples we observed patchy regions of absence of PV staining. The total count of PV-ir cells in categories of MCD was further decreased, representing 72.4%, 55.0% and 12.2% of control values in groups of 'mild MCD' ( $n = 25$ ), focal cortical dysplasia (FCD) type I ( $n = 16$ ) and type II ( $n = 15$ ), respectively. Significantly different PV-ir cell densities were demonstrated between the FCD types IIA and IIB. In MCD, we observed the decrease of

PV-ir cells to be more pronounced in infragranular layers. No significant differences were revealed between the temporal and extratemporal examples of MCD. This study provides evidence for reduction of inhibitory PV-ir interneurons in the epileptic neocortex affected by MCD as well as in morphologically unaffected temporal neocortex representing thus a possible mechanism for their epileptogenicity. Supported by Grants: GACR No. 309/02/D076, IGA NF 7411-3 and Research Project VZ FNM 0000064203.

#### P 989

##### 1H MRS STUDY OF METABOLIC ALTERATIONS IN PATIENTS AFTER ISCHEMIC STROKE IN ACUTE AND REHABILITATION PERIODS

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**Purpose:** We propose the quantitative indicators for the characteristics of regional multivoxel distributions of the brain metabolites (Cho, Cr, NAA) in patients after ischemic stroke in acute and rehabilitation periods.

**Materials and Methods:** Two groups of subjects are examined by 1H MRS using 1.5T Magnetom Vision (SIEMENS). The 1st group (VG) consists of 75 volunteers (in the age from 18 to 73 years). The 2nd group (SG) includes 80 (60 females and 20 males) patients (in the age from 46 to 87 years) in the acute (<12 hours), subacute (<10 days) and chronic (from 10 days to 12 months) stages of ischemic stroke. In all patients 12x12 spectral matrixes in supraventricular region parallel to the canthomeatal line are obtained. 2DCSI 1H spectra are recorded with: TR/TE=1500/135 ms, VOI=8x8x2cm.

**Results:** In each voxel of the spectral matrix we introduce two indicators: the metabolite content AM as the peak area and the metabolite concentration CM as the ratio of the peak area to the sum of all the peak areas S:  $CM=AM/S$ . We describe the metabolic state in each voxel by the triad  $T^* = \{A_{Cho}, A_{Cr}, A_{NAA}\}$ , where  $A_{Cho}$ ,  $A_{Cr}$ , and  $A_{NAA}$  are the peak areas of the signals from Cho, Cr and NAA. For each of the areas we assign three values: 1, 2 and 3, to obtain six symbolic spectral configurations:  $1^* = \{1,2,3\}$ ,  $2^* = \{2,1,3\}$ ,  $3^* = \{1,3,2\}$ ,  $4^* = \{3,2,1\}$ ,  $5^* = \{3,1,2\}$  and  $6^* = \{2,3,1\}$ . We analyzed the temporal alterations of the triad distributions after ischemic stroke. In the VG triads  $1^*$  and  $2^*$  dominate in all voxels of VOI. The most frequent configurations in the acute period are  $5^*$  and  $6^*$ . The triad regional variability decreased during rehabilitation period. Triads  $3^*$  and  $4^*$  are more often in elderly subjects.

**Conclusion:** The triad distributions give us a quantitative way for the estimation of efficiency of stroke therapy.

#### P 990

##### REGIONAL ALTERATIONS OF CONTENT AND VALUES OF T2 RELAXATION TIME OF THE MAIN CEREBRAL METABOLITES IN PATIENTS WITH PARKINSON'S DISEASE (PD): IN VIVO 1H MRS STUDY

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**PURPOSE:** From the analysis of  $^1H$  MRS data and their echo-time dependence we propose the quantitative indicators for the characteristics of the brain state in patients with PD.

**MATERIALS AND METHODS:** Two groups of patients are studied by  $^1H$  MRS with 1.5T Magnetom Vision (SIEMENS). The 1st group (PG) includes 40 patients with PD (48-70 y). The 2nd group (VG) consists of 15 healthy

volunteers (50-73 y). Spectra are recorded in the temporoparietal cortex (TC), lentiform nucleus (LN), putamen (P) and substantia nigra (SN) with STEAM method: TR/TE=1500/135,155,175,200,270 ms.

**RESULTS:** We introduce two indicators: the metabolite content  $A_{M</sup>M</sup>}$  as the peak area and the concentration  $C_{M</sup>M</sup>}=A_{M</sup>M</sup>}/S. We describe the metabolic state in VOI by the triad  $T^* = \{A_{Cho</sup>Cho</sup>}, A_{Cr</sup>Cr</sup>}, A_{NAA</sup>NAA</sup>}$ , where  $A_{Cho</sup>Cho</sup>}$ ,  $A_{Cr</sup>Cr</sup>}$ , and  $A_{NAA</sup>NAA</sup>}$  are the peak areas of the signals of metabolites. For each of the areas we assign three values: 1,2,3, to obtain six symbolic spectral configurations:  $1^* = \{1,2,3\}$ ,  $2^* = \{2,1,3\}$ ,  $3^* = \{1,3,2\}$ ,  $4^* = \{3,2,1\}$ ,  $5^* = \{3,1,2\}$ ,  $6^* = \{2,3,1\}$ . In the VG triads  $1^*$  and  $2^*$  dominate in all VOI,  $3^*$  and  $4^*$  are often in elderly subjects. In the PG the most frequent configurations in TC are  $2^*$  and  $5^*$  and in LN, P and SN are  $5^*$  and  $6^*$ . From comparison of  $T_{2</sup>2</sup>}</sup>M</sup>M</sup>}$  values in P, LN and SN in the both groups the shortening of  $T_{2</sup>2</sup>}</sup>M</sup>M</sup>}$  in the PG are obtained. In the TC the  $T_{2</sup>2</sup>}</sup>M</sup>M</sup>}$  values are similar in PG and VG.$

**CONCLUSION:** This study gives a new insight into brain biochemistry in patients with PD.

#### P 991

##### PERONEAL NERVE REGENERATION THROUGH A HEALTHY TIBIAL NERVE: A DETAILED MORPHOMETRIC ANALYSIS

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**INTRODUCTION:** End-to-end nerve reconstruction is considered to be the treatment of choice, in the repair of peripheral nerve injuries. However, in cases of long nerve defects, the optimal surgical procedure has not yet been established, but the gold standard continues to be the use of autologous nerve grafts. For short nerve defects, a lot of different conduits have been studied, as well as the end-to-side nerve suture.

**PURPOSE:** The purpose of this study was to evaluate the regeneration of the transected peroneal nerve utilizing the tibial nerve as a conduit, with the use of proximal and distal neurography.

**METHODS:** Forty male Wistar rats, weighing 230-300g, were included in this study. The peroneal nerve was transected and the animals were divided in five groups. Group I: End-to-end neurography. Group II: The proximal stump of the peroneal nerve was sutured in an end-to-side fashion to the tibial nerve. The distal stump was sutured end-to-side 1.0 cm distally, along the tibial nerve. All coaptations were performed with epineurotomy of the tibial nerve. Group III: The proximal and distal stumps were left in situ. Group IV: Double end-to-side neurography with intraperitoneal injection of valproic acid. Group V: Double end-to-side neurography, plus subcutaneous injection of erythropoietin. During the waiting period, walking track analysis was performed in all animals. After 16 weeks, all rats were sacrificed. Specimens including sciatic, tibial and peroneal nerves from both sides were dissected and fixed in 2.5% Glutaraldehyde in 0.1M Cacodylate. Representative samples were taken, embedded in a resin mixture and semi-thin sections (1µm) were stained with a modified solution of Toluidin blue and evaluated. A semiquantitative morphometric analysis using an image analyzer, was performed. The tibialis anterior muscle weight was recorded. **RESULTS:** Axonal counting, the mean axonal diameter and area and the mean myelin thickness were estimated in all

groups, and compared to the contralateral unoperated side. The morphometric parameters of the Group II were comparable to Group I, while the preliminary data regarding Group V, suggest a further slight improvement.

**CONCLUSIONS:** The morphometric data presented in this study support the use of the tibial nerve as a conduit for the regeneration of the peroneal nerve, when the immediate repair is not possible

#### P 992

##### **COMPARATIVE STUDY OF THE ABNORMALITIES OF CENTRAL NERVOUS SYSTEM IN CHILDREN AND ADULTS AUTOPSIED AFTER BONE MARROW TRANSPLANTATION**

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**Background:** We compare the neuropathological abnormalities found in children and adults after bone marrow transplantation (BMT) who were autopsied in the Department of Pathology, University of Paraná, Brazil.

**Methods:** The autopsy reports of 180 patients were reviewed. They were divided in two groups: patients under 15 years old and those with 15 or more. Age, gender, clinical diagnosis at time of BMT, survival time, neuropathological abnormalities and cause of death were analyzed.

**Results:** In children (26,6% of total) and in the adult group (73.4% of total), the main clinical diagnosis prior to BMT were respectively: severe aplastic anemia (31.2%) and chronic myeloid leukemia (36.3%). The mean survival time for children was 102.6 days and for adults 185.9 days after BMT. The brain lesions were considered cause of death in 20.8% of paediatric cases and 11.3% of the adult group. The neuropathological abnormalities were morphologically similar in children and adults with the following respectively prevalence: cerebrovascular diseases in 58.3 and 56% (p=0.8655), neurotoxoplasmosis in 6.2% and 3% (p=0.3856) and infections in 27 and 25.7% (p=0.8489).

**Conclusions:** The paediatric patients had a shorter survival than adults with increasing prevalence of neurotoxoplasmosis and the brain lesions were considered cause of death in twice as many as compared to adult patients.

#### P 993

##### **PATHOGENESIS OF HETEROTOPIC ECTOPIC GRAY MATTER IN THE BRAIN OF MOUSE PRENATALLY EXPOSED TO IRRADIATION**

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The developing brain is one of the fetal structures most susceptible to irradiation. Studies of survivors exposed to atomic bombing in Hiroshima and Nagasaki or to x-rays during medical procedures indicated that mental retardation and microcephaly with heterotopic gray matter are prominent findings after in utero radiation exposure. The heterotopic gray matter could be caused by cell death and abnormal neuronal migration during a critical gestational stage of sensitivity to irradiation. However, the possible cellular and molecular mechanisms involved in the abnormal neuronal migration caused by irradiation are unknown. We have examined the pattern of brain malformations in 8-week-old mice prenatally exposed to doses of radiation ranging from

0.5 to 2.0 Gy at different embryonic days. Brain malformations, identical to radiation-related small-sized heads with heterotopic gray matter, were reproduced in mice exposed to 1.5 Gy on embryonic day 13 (E13). In the present experiment, pathogenesis of such brain malformation was studied with immunohistochemical detection methods. Pregnant females were exposed to irradiation at a dose of 1.5 Gy on E13. Brains from fetuses and pups were obtained every day from 6 hrs after exposure to different postnatal days. Six hrs after exposure extensive dead cells appeared throughout the brain mantle. On E16 some cells came together to form rosettes. On E18 a high proportion of BrdU-labeled cells reached the superficial layers of the cortical plate with the remaining cells located in the heterotopic neuronal masses. The quantitative study showed that labeled-cells in layers II-III were fewer and those in layers IV-VI more numerous in the adult mice prenatally irradiated than controls. These results indicated the disruption of normal cell migration in the irradiated brain. The anti-GFAP immunostaining revealed that radial glial fibers were markedly straight and perpendicular to the pial surface in the controls, but disorganized after the irradiation exposure. Migration of young neurons along radial glial fibers is the prevalent mode of neuronal cell movement in the developing mammalian brain. The crumpled fibers might have presented unpassable, only partially passable, or disoriented pathway for neuronal migration. These findings suggested that the heterotopic gray matter in the brain of mice prenatally exposed to irradiation resulted from interrupted neuronal cell migration and migratory pathway.

#### P 994

##### **EXPRESSION AND CELLULAR DISTRIBUTION OF MULTIDRUG RESISTANCE PROTEINS (MRP-1 AND MDR-1) IN FOCAL CORTICAL DYSPLASIA CASES**

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##### **PURPOSE:**

Focal cortical dysplasia (FCD) is a congenital cortical developmental abnormality, which is generally characterized by drug resistant epilepsy. We aimed to investigate, multidrug resistance-associated protein 1 (MRP-1) and multidrug resistance gene-1 P glycoprotein (MDR-1) in the resection material of patients with FCD who underwent surgery.

##### **METHODS:**

Formalin-fixed paraffin-embedded human brain tissues obtained from 20 patients and 10 age-match autopsy controls were studied. Sections of 5 µm were cut and the sections were stained with HE, Bielschowsky and Luxol-Fast-Blue. The resection materials were evaluated according to Palmieri's Focal Cortical Dysplasia classification. Different type neurons, reactive astrocytes, endothelial cells within the dysplastic cortex were examined for the expression MDR-1 and MRP-1 by immunocytochemistry. Immunoreactivity in the cells was scored as -, -/+, + and ++.

##### **RESULTS:**

The Distribution of cases were 2 type IA, 4 Type IB, 8 Type IIA (40%) and Type IIB (30%) FCD.

##### **Evaluation of immunoreactivity:**

Neuron: MDR-1 and/or MRP1 was strongly positive in 16 cases (80%) of patients whereas in control cases MDR-1 was none or MRP-1 weakly positive in 50% of cases. Astrocytes: 10 % (2 cases) with MDR-1, and 55% (11 cases) with MRP-1 positivity were observed in FCD patients whereas MDR-1 was none and MRP-1 was 22% weakly positive for controls. Capillary endothelial cell: MDR-1 in 90% of controls, 50% of patients, whereas MRP-1 50% of patients, 10% of control were positive. In type IIA and type IIB FCD cases, MRP-1 positivity was detected 70 % (10/14 cases) in dysmorphic neurons and 20% (3/14 cases) giant neurons whereas MDR-1 positivity were seen only 15 % in dysmorphic neurons (2/14

cases) and 7% in giant neurons (1/14 cases). Immunoreactivity for MDR-1 and MRP-1 in the balloon cells of Type IIB FCD cases was observed in 4 cases (33%).

#### CONCLUSIONS:

The distribution of MRP-1 was more strong than MDR-1 in FCD cases.

MDR-1 and MRP-1 expression were prominent in neuronal cell were than the other cells. The overt expression of MDR proteins within the dysplastic tissue may be an evidence for the drug resistant seizures in patients with FCD comparing the control cases.

#### P 995

##### NEUROCYSTICERCOSIS: BIOPSY ANALYSIS OF FIVE YEARS PERIOD

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**Introduction.** Neurocysticercosis (NC) is caused by larval stage of the pork tapeworm, *Taenia solium*. It is the most common parasitic cerebral infection encountered in worldwide neurosurgical practice. In some geographic regions it is the leading cause of space-occupying intracranial lesions.

**Purpose** of this study was neuropathological analyses of NC in surgical material of patients with space-occupying intracranial lesions, operated on during five years period (2000-2004).

**Methods.** Surgical biopsy specimens of NC were examined using standard histological and histochemical staining procedures.

**Results.** Of 4987 patients with intracranial lesions, NC was diagnosed in 18 cases (0,37%). The most frequent clinical manifestations included: hydrocephalus, epileptic seizures and chronic psychoorganic syndrome. The mean age of the patients was 51,66 (ranged from 40 to 68) years. Female to male ratio was 1:1. The great majority (16/18) of cases had solitary cystic lesion. In other two cases there were two parenchymal cystic lesions associated in one of them with third, solid, partly calcified suprasellar nodus. Cysticercus was located more frequently in the ventricular system (13 cases - 72%) than in brain parenchyma (5 cases - 28%). Inside of ventricular system, left lateral ventricle was the most frequently involved (7 cases).

Grossly, majority of cystic lesions were approximately 2 cm in diameter with thin, translucent membrane and water-clear fluid. Three ventricular cysts had a grapelike appearance (racemose form of cysticerci). Occasionally, single small (2-4 mm) whitish nodule was attached to the luminal surface of the wall. Microscopically, typical three-layered vesicular membrane was consisted of external wavy eosinophilic lamina, cellular layer and interior to this loose connective tissue. Rudimentary parasitic body and scolex showed different stages of involution. In some sections of viable scolex there were muscular suckers and hooklets. Around degenerated and dead parasite there was so called reactive membrane, composed of histiocytes, multinuclear giant cells, collagen fibers, prominent inflammatory cells (lymphocytes, plasmocytes and eosinophils) and layer of reactive astrogliosis.

**Conclusions.** According to our series of neurosurgical biopsy material, NC accounts for only 0,37% of surgically treated space-occupying intracranial lesions. This percent is significantly less than in series of NC from well known endemic regions in the world

#### P 996

##### A NEUROPATHOLOGICAL STUDY OF AUTISM

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Autism is a severe developmental disorder characterized by impairments in reciprocal social interaction and communication, restricted and stereotyped patterns of behaviour and interests, and an onset before 3 years of age (World Health Organization, 1992). Many regions of the brain have been implicated in the genesis of autism, but the neurobiological basis of the disorder remains unknown. Autism is a rare disorder which was described relatively recently and there are only a few post-mortem studies. Few data are available about molecular mechanisms of this pathology. A neuropathological study of autism was established and post-mortem brain tissue examined from one male individual.

It is known that RhoA, a small GTPase, inhibits axonal growth during early neuronal differentiation and axonal regeneration, while promotes the axonal growth during late neuronal differentiation. Infact RhoA controls a wide variety of cellular processes such as actin cytoskeleton rearrangement, microtubule dynamics, cell adhesion and polarity.

The cerebellar cortex was fixed in 10% formaldehyde, embedded in paraffin and sectioned.

Immunohistochemistry for glial fibrillary acid protein (GFAP), neurofilaments and RhoA was performed using the avidin-biotin complex method with diaminobenzidine as the chromogen.

The sections were acquired on digital support. Studied antigens distribution and expression analysis were evaluated by image analysis software Quantimet 500 plus.

Our results showed a reduced Purkinje cells number in the autistic cerebellum compared to the control one. RhoA expression was greater in Purkinje cells than other cells. GFAP and RhoA expression were reduced, while neurofilaments production was increased in the autistic cerebellum compared to the control one.

GFAP expression is mainly localized in granular layer cells both in control and autistic specimens. These data, together with the literature, suggest that an abnormal behaviour of cerebellar cells can occur, as shown by GFAP and neurofilament expression, also because of an alteration of RhoA expression.

#### P 997

##### STRUCTURAL CHANGES OF THE HUMAN PINEAL GLAND IN RELATION TO HYPOXY DURATION.

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**Introduction:** Morphological data of the relation of hypoxia to the structure of the human pineal gland (PG) are very few.

The aim of this study was to investigate the morphological changes of PG in relation to acute and chronic hypoxia.

**Methods:** Seventy three PG's were observed - 56 male and 17 female. Length, width, weight and density of the glands were measured. To determine the duration of hypoxia, the cases were separated in three groups: 1st - 26 died of mechanical asfixia of hanging (MAH), mean age  $57.24 \pm 11.34$ ; 2nd - 28 died of acute respiratory insufficiency (ARI), mean age  $51.5 \pm 10.93$ ; 3rd - 19 died of chronic respiratory insufficiency with exacerbation (CRIE), mean age  $52.33 \pm 13.06$ . Paraffin slides were stained with hematoxylin-eosine and Azan.

**Results:** PG's of the 1st group were significantly ( $t=7.3$ ,  $P<0,05$ ) heavier ( $168.15 \pm 42.15$  mg) than 2nd ( $111.67 \pm 42.94$  mg) and 3rd ( $115.61 \pm 50.58$  mg). In the cases of ARI the density is significantly higher than those of MAH ( $2.6 \pm 1.77$

kg/m<sup>3</sup>) and CRIE (3.45±1.97 kg/m<sup>3</sup>). In the cases of CRIE the stroma increases and the pseudolobes are atrophic. The frequency of well shaped pseudolobes is significantly higher in the 3rd (58.92%) than in the 1st (26.92%) and 2nd group (25%). The frequency of glial proliferation in MAH (46.15%) and ARI (64.29%) is significantly lower ( $t=2,39$ ,  $P<0,05$ ) than CRIE(76.47%).

Conclusions: Continuous hypoxia in the cases of CRIE develops gliosis and increases the relative share of well shaped pseudolobes of PG and proliferation of stroma and different stages of pseudolobar atrophy. The acute hypoxia of MAH and ARI does not develop structural changes of the PG.

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#### **PATHOGENESIS OF HETEROTOPIC ECTOPIC GRAY MATTER IN THE BRAIN OF MOUSE PRENATALLY EXPOSED TO IRRADIATION**

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The developing brain is one of the fetal structures most susceptible to irradiation. Studies of survivors exposed to atomic bombing in Hiroshima and Nagasaki or to x-rays during medical procedures indicated that mental retardation and microcephaly with heterotopic gray matter are prominent findings after in utero radiation exposure. The heterotopic gray matter could be caused by cell death and abnormal neuronal migration during a critical gestational stage of sensitivity to irradiation. However, the possible cellular and molecular mechanisms involved in the abnormal neuronal migration caused by irradiation are unknown. We have examined the pattern of brain malformations in 8-week-old mice prenatally exposed to doses of radiation ranging from 0.5 to 2.0 Gy at different embryonic days. Brain malformations, identical to radiation-related small-sized heads with heterotopic gray matter, were reproduced in mice exposed to 1.5 Gy on embryonic day 13 (E13). In the present experiment, pathogenesis of such brain malformation was studied with immunohistochemical detection methods. Pregnant females were exposed to irradiation at a dose of 1.5 Gy on E13. Brains from fetuses and pups were obtained every day from 6 hrs after exposure to different postnatal days. Six hrs after exposure extensive dead cells appeared throughout the brain mantle. On E16 some cells came together to form rosettes. On E18 a high proportion of BrdU-labeled cells reached the superficial layers of the cortical plate with the remaining cells located in the heterotopic neuronal masses. The quantitative study showed that labeled-cells in layers II-III were fewer and those in layers IV-VI more numerous in the adult mice prenatally irradiated than controls. These results indicated the disruption of normal cell migration in the irradiated brain. The anti-GFAP immunostaining revealed that radial glial fibers were markedly straight and perpendicular to the pial surface in the controls, but disorganized after the irradiation exposure. Migration of young neurons along radial glial fibers is the prevalent mode of neuronal cell movement in the developing mammalian brain. The crumpled fibers might have presented unpassable, only partially passable, or disoriented pathway for neuronal migration. These findings suggested that the heterotopic gray matter in the brain of mice prenatally exposed to irradiation resulted from interrupted neuronal cell migration and migratory pathway.