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**Gout is a chronic inflammatory disease where high levels of interleukin 8 (CXCL8), MRP8/14 and an altered proteome associate with diabetes and cardiovascular disease****Original research – Full length article****Authors**

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Accepted Article

**Abstract (250 words)**

**Objectives:** The frequent association with the metabolic syndrome and cardiovascular disease (CVD) suggest a systemic component in gout. Our objective was to study whether circulating pro-inflammatory cytokines are associated with comorbidities in gout patients.

**Methods:** We studied 330 gout patients from three independent cohorts and compared those with 144 healthy individuals and 276 disease controls. Circulating levels of IL-8 (CXCL8), IL-1b, IL-6, IL-10, IL-12, TNF-a were measured, after which proteome-wide analysis was performed in a selection of samples to determine possible prognostic proteins for the development of comorbidities. Replication analysis was performed specifically for MRP8/14.

**Results:** Compared to healthy and disease controls, patients with gouty arthritis (n=48) had significantly higher mean levels of CXCL8 ( $P < 0.001$ ) whereas other cytokines were almost undetectable. Similarly, patients with intercritical gout also showed high levels of CXCL8. CXCL8 was independently associated ( $P < 0.0001$ ) with diabetes in intercritical gout patients. Proteome-wide analysis in gouty arthritis (n=18) and intercritical gout (n=39) revealed MRP8/14 as the protein with the greatest differential expression and correlation with CXCL8 ( $R^2 0.49$ ,  $P < 0.001$ ), which was replicated in an independent cohort. The proteome of gout patients with high CXCL8 was associated with diabetes (OR, 95% CI; 16.5, 2.8-96.6) and CVD (OR, 95% CI; 3.9, 1.0-15.3).

**Conclusions:** Circulating levels of CXCL8 levels are increased during both the arthritis and intercritical phases of gout, and coincides with a specific circulating proteome that is associated with diabetes and CVD risk. Further research focussed on the role CXCL8 and MRP8/14 in patients with gout is warranted.

**Key words**

Gout; CXCL8; proteomics; cardiovascular diseases, diabetes

## Introduction

Gouty arthritis affects 1-4% of the adult population in developed countries where it is the most common arthritis in men.(1, 2) Gout is a disorder of the purine metabolism that culminates in hyperuricaemia and deposition of monosodium urate (MSU) crystals. Accumulating evidence suggests a worldwide increasing prevalence of gout, potentially attributable to factors including alcohol and sugar-sweetened beverage consumption, a purine-rich diet and increased longevity.(3) The association with widespread urate deposition (tophi),(4, 5) renal disease,(6) the metabolic syndrome,(7) and an increased risk of cardiovascular disease (CVD)(7-13) suggests that gout is a systemic condition rather than a localized joint problem. An unmet need in the treatment of gout is the availability of biomarkers that would identify those gout patients at risk to develop these comorbidities.

Although various studies describe the presence of local inflammatory mediators in the synovial fluid during gout attacks, similar attempts to investigate the presence of systemic inflammatory markers, either during gouty arthritis or in the intercritical phase, are to our knowledge lacking.(14-16) We studied the presence of these markers by analysing a selected group of pro-inflammatory cytokines and the circulating proteome in blood from patients with gouty arthritis and those with intercritical gout. In addition, we analysed the possible association between circulating proteins with frequent coexistent CVD and/or diabetes.

We observed that circulating levels of interleukin 8 (IL-8, CXCL8) are increased in a substantial portion of patients with gout. Using a non-hypothesis driven OMICS approach we demonstrated that high levels of CXCL8 coincide with a circulating proteome that associates with increased risk for CVD and diabetes.

## Patients and Methods

In total, 330 gout patients from three independent gout patient cohorts, 144 healthy individuals - 129 individuals originating from the Nijmegen Biomedical Study and 15 from the University Medical Center Utrecht (19) – and disease controls comprising patients with rheumatoid arthritis (RA) (n=45), systemic sclerosis (SSc) (n=31) and dermatitis (n=200) were included in this study. The first cohort consisted out of patients with gouty arthritis (n=48, **NL-ARTHRITIS** cohort) (**Figure 1**). A second cohort comprising patients with intercritical gout from New Zealand (n=169, **NZ-INTERCRITICAL** cohort) was used for replication. For replication of the results on MRP8/14, a third cohort from the Netherlands was used (**NL-INTERCRITICAL** cohort) comprising 113 patients with intercritical gout. All individuals were selected for age and gender and local ethical approval was present. All data collection and analyses were performed from 2005 until 2013

### *Detection of systemic inflammation markers in gout patients*

Pro-inflammatory cytokine levels of CXCL8, IL-1b, IL-6, IL-10, IL-12 and TNF-a were measured in the circulation of gout patients. These levels were first investigated in patients with gouty arthritis since cytokine levels are thought to be highest then. 48 patients with gouty arthritis were recruited by general practitioners in the Netherlands (n=48) from 2005 until 2006 (**NL-ARTHRITIS** cohort). After informed consent patients were referred to the rheumatology department of the regional hospital to be included in a previously performed study.<sup>(17)</sup> Patient characteristics were collected. The diagnosis gout was confirmed by MSU crystal identification in synovial fluid. Cytokine levels were measured in the remainder of frozen blood samples taken for regular blood testing.

To study whether these cytokine levels were similar during different disease states of gout, they were also measured in the circulation of intercritical gout patients. 169 intercritical gout patients were recruited from rheumatology outpatient clinics in New Zealand from 2008 until 2013 (**NZ-INTERCRITICAL** cohort), and had a diagnosis of gout according to the American College of Rheumatology preliminary diagnostic

criteria for acute gout.(18) In most of them MSU crystals were also identified in synovial fluid. All patients previously had gout attacks. Patient characteristics were collected, and blood samples were frozen. Recruitment of gout patients was approved by the New Zealand Multi-region Ethics Committee (MEC/05/10/130). All patients provided written informed consent. This cohort comprised patients of European Caucasian (n=106), Māori (n=25) and Pacific Island (n=38) descent. Samples from all Māori and all Pacific Island patients were measured for all cytokines. Because these samples, and the samples of the NL-ARTHRITIS cohort showed high levels of CXCL8, but normal levels of the other cytokines, only 23 of the European Caucasians samples were measured for all cytokines. To reduce costs the remainder 83 European Caucasians samples were analyzed for CXCL8 only.

Cytokines in the NL-ARTHRITIS cohort and in the NL-INTERCRITICAL cohort were measured by using BD™ Cytometric Bead Array (CBA, BDBiosciences; USA), because this method was used in the hospital (Rijnstate Hospital, Arnhem, The Netherlands) of which these patients originated from. Capturing beads of known size and fluorescence were conjugated with a cytokine specific antibody to bind cytokines in solution. A mixture of phycoerythrin (PE) conjugated antibodies, used as detector reagent, provided a fluorescent signal in proportion to the amount of bead-bound cytokine. A series of 10 recombinant cytokine standards with a known concentration of the cytokines (ranging from 20-5000 pg/ml and a blank control) were measured each experiment. Each individual standard curve for a given cytokine defines the minimum and maximum quantifiable levels using the BD CBA kit. By applying a 4-parameter curve fit option, values for sample intensities that fall outside the limits of the standard curve (<20 pg/ml or >5000 pg/ml) could be extrapolated. After the flowcytometric data acquisition, FCAP Array™ software was used to extrapolate the cytokine concentrations from the PE-fluorescence intensities. Cytokines in the NZ-INTERCRITICAL cohort, the healthy individuals and the disease controls were measured by using the well-known Luminex® Assays. The cytokine levels were shown as mean (standard deviation (SD)).

To study whether circulating CXCL8 levels were associated with the level of other cytokines the 86 of the 169 NZ-INTERCRITICAL patients in which all cytokines were measured were arbitrarily stratified in

roughly equally sized groups based on low, intermediate and high levels of CXCL8. The levels of other cytokines were displayed in these three groups to find correlations.

### *Proteomic analysis*

To get more insight in the proteins involved in the systemic inflammation, we analysed the proteome of CXCL8, i.e. whether patients with high CXCL8 levels had different proteins highly expressed than patients with low CXCL8 levels. To be able to find differences in the circulating proteome profile only patients with the highest and the lowest CXCL8 levels were used. The proteome profile was first investigated in a subgroup of the NL-ARTHRITIS cohort with the lowest (n=9) and the highest (n=9) CXCL8 levels. To validate the proteomics results the proteome profile was also investigated in a selected subgroup of the NZ-INTERCRITICAL cohort with the lowest (n=20) and the highest (n=19) CXCL8 levels. Proteomic analysis was performed using the SELDI-TOF-MS technology (Ciphergen Biosystems, Fremont, USA). For the details of this method we refer to an earlier published paper.(20)

### *Statistical analysis*

#### *1) The association of systemic inflammation markers (CXCL8 levels) with comorbidities*

The association of CXCL8 levels with clinical characteristics (age at onset of the disease, number of gout attacks, body mass index (BMI), systolic and diastolic blood pressure, serum creatinine levels, and serum uric acid levels) and comorbidities (diabetes, CVD, hypercholesterolemia, tophi, and chronic kidney disease) was tested in the NZ-INTERCRITICAL cohort. CVD was defined as transient ischemic attack, cerebrovascular accident, myocardial infarction, peripheral vascular disease, heart rhythm disorder, angina pectoris, and/or heart failure. As shown in the Results section, CXCL8 levels turned out to associate only with the comorbidity diabetes. Therefore it was further analyzed whether high CXCL8 levels were an independent risk factor for diabetes in gout patients. First, possible confounders for CXCL8 in the relationship with diabetes were evaluated. Variables which could be possible confounders for this association were selected based on the literature. After univariate linear regression variables with beta-

coefficient  $\leq 0.2$  were considered as possible confounders. Logistic regression was performed by adding possible confounders by forward selection. When the beta-coefficient changed more than 10% the variable was considered as a confounder. Then the confounder was kept in the multivariate logistic regression model. If the beta-coefficient was significant for CXCL8 ( $P < 0.05$ ) in the multivariate model after correction for confounders, then CXCL8 was considered independently associated with diabetes. Next we tested CXCL8 as an independent predictor for diabetes using CXCL8 as a continuous variable.

## 2) Analysis of proteome and association with comorbidities

The measured proteins were hierarchically clustered based on Pearson correlation coefficients. Hierarchical clustering and statistical analysis was performed using the software GenePattern.(21) For comparisons a T test was used and adjusted  $P$  values were calculated using exhaustive permutation, when possible, or 100.000 fold permutation when software limits were reached. The program TagIdent was used for the identification of candidate proteins corresponding to the M/Z scores from the heatmaps by performing a query on the molecular weight ( $\pm 1\%$ ) of each protein.(22) The scan in UniProtKB/Swiss-Prot was restricted by organism name “homo sapiens”, and the use of the keyword “secreted”, referring to the cellular component of the protein. Gene Ontology was performed on the predicted protein lists using the Functional Annotation tool of the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7.(23)

The protein with the greatest differential expression between patients with low and high CXCL8, MRP8/14 as shown in the Results section, was further investigated. To validate whether this protein was increased in gout, we wanted to specifically measure MRP8/14 in the NZ-INTERCRITICAL cohort by using ELISA.(24-26) Unfortunately, these samples had become haemolytic. Therefore MRP8/14 was measured in a different cohort of 113 patients with MSU crystal-proven intercritical gout (NL-INTERCRITICAL cohort). These patients were included from 2011 until 2012 at the rheumatology department of a regional hospital in the Netherlands. All patients provided written informed consent for

the freezing of blood samples. Both MRP8/14 and CXCL8 levels were measured in these samples. The correlation between MRP8/14 and CXCL8 levels was calculated.

The association of comorbidities with the proteome of low and high circulating CXCL8 was studied to identify proteins, which could be used as predictors to detect gout patients at risk for the development of comorbidities. The association of comorbidities with the proteome profile associated with low and high CXCL8 levels in gout patients was tested in the subgroup of the lowest (n=20) and the highest (n=19) CXCL8 levels from the NZ-INTERCRITICAL cohort. To analyze whether clustering on the basis of the proteome of low and high CXCL8 levels showed an association between CXCL8 levels and the co-occurrence of diabetes and CVD odds ratios (ORs) and 95% confidence intervals (CI) were calculated. The OR for CVD was calculated after stratification for hypercholesterolemia and diabetes.

## Results

**Figure 1** and **table 1** provide an overview of the clinical characteristics of the patient cohorts.

### *Detection of systemic inflammation markers in gout patients*

Compared to healthy donors (47 pg/ml  $\pm$  13 (mean  $\pm$  SD) and patients with rheumatoid arthritis (n = 45, 38  $\pm$  28), systemic sclerosis (n = 31, 44  $\pm$  51) and dermatitis (n = 200, 44  $\pm$  34) using the NL-ARTHRITIS cohort a remarkably high mean level of circulating CXCL8 (226 pg/ml  $\pm$  574,  $P < 0.001$ ) was measured. If we consider the upper cut-off value for normal as 2-times SD above mean ( $< 73$  pg/ml) then 27% of patients of the NL-ARTHRITIS cohort and 51% of the NZ-INTERCRITICAL cohort exceeded normal circulating CXCL8 levels. In contrast to CXCL8 IL-1b, IL-6, IL-10, IL-12 and TNF-a levels were almost undetectable (**figure 2a**). No association was present between circulating CXCL8 levels and inflammatory markers such as C-reactive protein or erythrocyte sedimentation rate. In the NZ-INTERCRITICAL cohort similar high levels of CXCL8 were observed compared to the other inflammatory mediators thus replicating previous observations (**figure 2b**). Again, no differences in CXCL8 levels, nor in IL-1b, IL-6, IL-10, IL-12 or TNF-a levels were observed between the three different ethnic groups.

### *The association of systemic inflammation markers (CXCL8 levels) with comorbidities*

In the NZ-INTERCRITICAL cohort CXCL8 levels were significantly associated with a higher number of gout attacks ( $P < 0.001$ , data not shown) and with serum uric acid levels ( $P < 0.001$ , **figure 3a**) whereas other clinical variables such as age of onset of the disease, gender, BMI, presence of tophi, systolic and diastolic blood pressure, and serum creatinine levels were not (data not shown). The frequency of diabetes, but not CVD, hypercholesterolemia or chronic kidney disease, was clearly associated with a high CXCL8 level ( $P < 0.0001$ ) (**figure 3b**). To investigate whether CXCL8 is an independent risk factor for diabetes in gout we performed multivariate modelling in which possible confounders for CXCL8 in the relationship with diabetes were first evaluated (**suppl. table 1**). Next we tested CXCL8 as an independent

predictor for diabetes using CXCL8 levels as a continuous variable (**suppl. table 2**). In these models CXCL8 independently predicted the co-occurrence of diabetes in gout patients.

#### *Proteomic analysis with SELDI-TOF*

We have previously successfully used SELDI-TOF to identify proteins associated with diseases in a non-hypothesis driven fashion.(27-28) Given that increased circulating CXCL8 levels did not correlate with high levels of the expected cytokines like IL-1 and TNF- $\alpha$  we postulated that a non-hypothesis driven OMICS approach – by measuring the proteome - would provide most efficient insights into the possible components coinciding with highest circulating CXCL8 levels. SELDI-TOF provides such an OMICS tool measuring a wide spectrum of the proteome, which we chose to perform in two “extreme” subgroups, those patients with lowest and highest CXCL8 levels, an approach which is often applied in OMICS research. SELDI-TOF expression profiles indeed revealed clearly distinct subsets of candidate proteins differentially expressed in the NL-ARTHRITIS patients with low (<10 pg/mL; n=9) and high (>100 pg/mL; n=9) CXCL8 levels (**figure 4a**). 574 and 1028 candidate proteins had high expression in patients with low and high CXCL8 levels, respectively, with most having a role in the regulation of innate and adaptive immune responses (**suppl. table 3**). The candidate protein with the greatest differential expression between low and high CXCL8 was MRP8/14 ( $P < 0.001$  and  $P < 0.0001$  for MRP8 and MRP14, respectively) (**figure 4b**). Measurement of MRP8/14 in the NL-INTERCRITICAL cohort demonstrated a positive correlation of MRP8/14 with CXCL8 ( $R^2 0.49$ ,  $P < 0.001$ ) (**figure 4c**). Consistent with the NL-ARTHRITIS cohort, the expression profiles from proteomic analysis in the NZ-INTERCRITICAL cohort also revealed distinct profiles in patients with low (<100 pg/ml; n=20) and high (>500 pg/ml; n=19) CXCL8 levels ( $P < 0.002$ ) (data not shown).

#### *The co-occurrence of diabetes is associated with a specific proteome in patients with high CXCL8*

In the subgroup of NZ-INTERCRITICAL patients with low (n=20) and high (n=19) CXCL8 levels we investigated whether the proteome associated with low and high circulating CXCL8 levels was related

with co-occurrence of diabetes. After stratification for CXCL8 there was a clear association (16.5; 2.8-96.6 (OR; 95% CI);  $P < 0.0001$ ). The association was predicted by low expression of proteins between 4565 - 4585 Daltons (vaso-intestinal peptide and liver expressed antimicrobial peptide 2) and 58995 - 60392 Daltons (elastin) and high expression of chemokines (CCL18, CCL20, CXCL4, and other inflammatory mediators) (**figure 5**). Next we investigated in the same group of patients whether the CXCL8-associated proteome could also be used to identify those gout patients that have CVD after stratification for diabetes and hypercholesterolemia. Although less clear compared to the presence of diabetes, the co-occurrence of CVD could be linked to high CXCL8 levels and its associated proteome (3.9; 1.0-15.3 (OR; 95% CI);  $P < 0.001$ ). Candidate proteins that displayed lowest expression in gout patients with high CXCL8 levels and CVD were chromogranin A, natriuretic peptide B and oncostatin M, whereas proteins with highest expression comprised thrombopoietin, CXCL7, apelin, CXCL4, renin, IL-32 and complement factors (**suppl. table 4**).

## Discussion

We demonstrate, for the first time, that circulating CXCL8 levels are increased in patients both with the gouty as well as the intercritical phase of the disease, both compared to healthy donors as well as to patients with other inflammatory conditions like RA, SSc and dermatitis. High circulating CXCL8 levels were associated with the co-occurrence of diabetes. Further investigation of the circulating proteome of patients with low and high CXCL8 levels revealed distinct proteomes that were clearly associated with the presence of diabetes and CVD, both recently featured as clinically significant complications of gout (29-30). The protein MRP8/14 was found to be most closely associated with circulating CXCL8 levels.

In gout patients we showed that there is an association between CXCL8 levels and diabetes, however we did not detect such an association between CXCL8 levels and CVD. Although many reasons could underlie this observation, we believe that is due to the low number of gout patients having CVD without

diabetes in our cohort. CXCL8 is a prototypical chemokine of the CXC subfamily.(31) Although all nucleated cells are potential sources of CXCL8, typically monocytes and macrophages are considered as the main producers. However, accumulating evidence suggests the role of activated endothelial cells to circulating CXCL8 levels,(31-32) and a pivotal role for CXCL8 in CVD. For instance, high CXCL8 levels were observed in human arterial atherosclerotic wall but also in fibrous plaques.(33) Especially macrophages, but practically every cell of the vascular wall, were demonstrated to be the source of CXCL8 in atherosclerotic plaques.(34) In addition, many non-gout-focussed studies showed a role for CXCL8 in predicting CVD. CXCL8 functions as an independent predictor for clinical outcome in patients with coronary artery disease,(35) and for CVD in patients with end-stage renal disease.(36) Finally, CXCL8 was found to be increased in diabetes and suggested to be a marker linking obesity with metabolic complications such as atherosclerosis and diabetes.(11) Altogether, CXCL8 is clearly implicated in inflammatory processes that drive atherosclerosis and diabetes.

Proteome wide analysis identified MRP8/14, also known as S100A8/A9, as a potential marker to be associated with CVD and diabetes risk in gout. MRP8/14 was identified as a toll-like receptor (TLR) 4 agonist.(37) The increased levels of MRP8/14 both during gouty attacks as well as during its intercritical phase might provide a possible link between chronic inflammation and the risk of CVD and/or diabetes in gout patients. This is in line with recent observations where MRP8/14 was identified as a biomarker for mortality prediction in patients with heart failure,(38) associated with systemic inflammation in stable coronary atherosclerosis,(39) and involved with the aberrant TLR-mediated inflammatory responses in humans with acute coronary syndrome.(40) Similar to CVD, MRP8/14 is associated with the hallmark features of diabetes: diabetic retino- and nephropathy.(41) In addition to CXCL8 and MRP8/14, we showed that the overall proteome profiles stratified patients with CVD and/or diabetes from those who were free of these comorbidities. Interestingly, markers that were lowly expressed in patients with high CXCL8 and increased CVD prevalence constitute a group of proteins normally designed as negative modulators of the neuroendocrine and/or immune responses. For instance, chromogranin A is a precursor

to vasostatin, pancreastatin, and parastatin. Reduced levels of vasostatin were recently associated with the presence and severity of coronary artery disease,(42) and chromogranin A knock-out mice displayed hypertension, higher adipokine levels and altered glucose homeostasis further substantiating a crucial role for homeostatic control of inflammation and diabetes.(43) Another example is oncostatin M, which has a proven major role in cardiac protection and repair.(44) In contrast, various inflammatory mediators (IL-32, CXCL7, CXCL4, thrombopoietin, apelin) previously shown to have a direct effect role on immune activation and/or CVD were associated with CXCL8 levels.(45) These further substantiate the validity of our observations for potential clinical use to predict which gout patients should be more intensively managed for the presence of CVD and/or diabetes.

Recently several conceptual frameworks have been proposed trying to explain the pathogenesis of gout. Although the role for MSU crystals has long been unclear, it is generally well accepted that MSU crystals exert a direct pro-inflammatory effect including the production of cytokines such as IL-1b via engagement of the TLR family and the inflammasome.(46-47) More recently, neutrophil extracellular traps (NETs)(48) which trap and kill microbes to avoid spreading of potential pathogens, are also linked to mediators causing sterile inflammation such as CXCL8 and tumor necrosis factor.(49-50) In contrast to the knowledge on the mechanisms causing gouty arthritis, the relationship with associated chronic conditions is poorly understood. For instance, hyperuricemia itself has been associated to chronic renal dysfunction,(10) hyperinsulinaemia,(10) hypertension,(10) CVD,(11) obesity and diabetes.(7) However, the mechanisms underpinning these associations are unknown and uncertainty as to whether or not circulating urate *per se* is detrimental is prevailing.

Although high levels of IL-1b initiated by inflammasome activation might play an important role in gout attacks, the high levels of CXCL8 do not support a direct role for the inflammasome in intercritical gout nor in relation with comorbidity coinciding with gout. In fact, our observed increased levels of CXCL8 did not correlate with that of IL-1b and therefore by itself cannot be explained by inflammasome

activation. An alternative explanation might be that the activation of macrophages due to TLR stimulation by MSU crystals forms the initiating event. Subsequently, these macrophages transubstantiate inflammasome activation but also produce high levels of CXCL8, which results in chemo-attraction and activation of neutrophils in the affected joint, clinically reflected by a gouty arthritis. These activated neutrophils form NETs that in turn lead to activation of plasmacytoid dendritic cells recently shown to be able to produce high quantities of CXCL8.<sup>(20)</sup> In addition, the main constituent of activated neutrophils is MRP8/14, which is released in high quantities when these neutrophils perish, as is likely in gouty arthritis. Upon elimination of MSU crystals, inflammasome activation might resolve after a few days, which is reflected by improved clinical signs and symptoms. However, consistent high CXCL8 and MRP8/14 levels might directly activate synovial macrophages via TLR-dependent pathways, leading to endothelial dysfunction and possibly other yet unidentified pathways that culminate in low-grade subclinical chronic inflammatory responses and promoting CVD and/or diabetes. This concept would explain the clinical responses observed by therapies targeting IL-1b in gout, and it does also link MSU crystals with chronic low-grade inflammation and risk for CVD, diabetes and other comorbidities so often observed. Why such a chronic self-perpetuation loop pertains however is unexplained.

Taken together, we show that gout can be considered a chronic systemic inflammatory disease in which circulating CXCL8 levels are high irrespective of disease activity. Increased levels of CXCL8 in patients with gout associated with diabetes and the CXCL8-associated proteome associated with co-occurrence of diabetes and CVD. Although the true clinical value of these biomarkers needs validation in a large prognostic cohort, our results suggest a potential role in the identification of those gout patients at risk for diabetes and CVD and requiring early intervention.

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**Table 1. Phenotypic characteristics of gout patients.**

<b>Cohort Type</b>	<b>Gouty arthritis The Netherlands (n=48) (NL-ARHTRITIS)</b>	<b>Intercritical gout New Zealand (n=169) (NZ- INTERCRITICAL)</b>	<b>Intercritical gout The Netherlands (n=113) (NL- INTERCRITICAL)</b>
Age (years) (mean (SD))	57.3 ± (15.2)	51.3 (14.8)	65.1 (12.8)
Disease duration (years)	n.a.	16.0 (12.8)	n.a.
Male (%)	87.5	87.3	
Body mass index (kg/m <sup>2</sup> ) (mean (SD))	28.9 ± 4.7	33.5 ± 8.1	29.3 (4.8)
Presence of tophi (%)	14.6	42.2	34.5
Serum uric acid (mmol/l) (mean (SD))	0.50 (0.1)	0.57 (0.1)	0.36 (0.14)
C-reactive protein (mg/l) (mean (SD))	33.8 (56.2)	n.a.	11.2 (18.2)
Hypertension (%)	56.3	56.4	70.8
Diabetes (%)	8.3	22.1	17.7
Cardiovascular disease* (%)	33.3	43.6	53.1
Hypercholesterolemia (%)	29.2	46.6	43.4
Chronic kidney disease** (%)	25.0	37.4	37.2
Medication used			
Diuretics (%)	33.3	18.4	40.7
Steroids (%)	4.2	59.6	4.4
NSAIDs (%)	22.9	82.9	13.3
Allopurinol (%)	2.1	87.7	64.6
Probenecid (%)	0	10.0	0
Benzbromarone (%)	0	n.a.	16.8
Febuxostat (%)	0	n.a.	2.7
Colchicine (%)	8.3	73.1	55.8

n.a. not applicable

\*Cardiovascular disease is defined as transient ischemic attack, cerebrovascular accident, myocardial infarction, peripheral vascular disease, heart rhythm disorder, angina pectoris, and/or heart failure.

\*\*Chronic kidney disease is defined as glomerular filtration rate <60 ml/min.

## Figure legends

### **Figure 1. Patient cohorts and performed analyses on these groups.**

NL= The Netherlands; NZ= New Zealand

### **Figure 2. CXCL8 is highly increased in patients during gouty attacks and in the intercritical phase.**

Circulating levels of CXCL8, IL-1, IL-6, IL-10, TNF- $\alpha$  and IL-12 in patients with recent onset gouty attack (n=48) (NL-ARTHRITIS) (**panel A**). In patients with intercritical gout from New Zealand consisting of European Caucasians (n=106 for CXCL8, and n=23 for the other cytokines), Māori (n=25) and those from Pacific Islands (n=38) extremely increased levels of CXCL8 were observed (**panel B**). The lower panel displays the levels of circulating cytokines IL-1, IL-6, IL-10, IL-12 and TNF- $\alpha$  after stratification for low CXCL8 (<100 pg/ml), intermediate CXCL8 (100–500 pg/ml) and high CXCL8 (>500 pg/ml) (**panel C**).

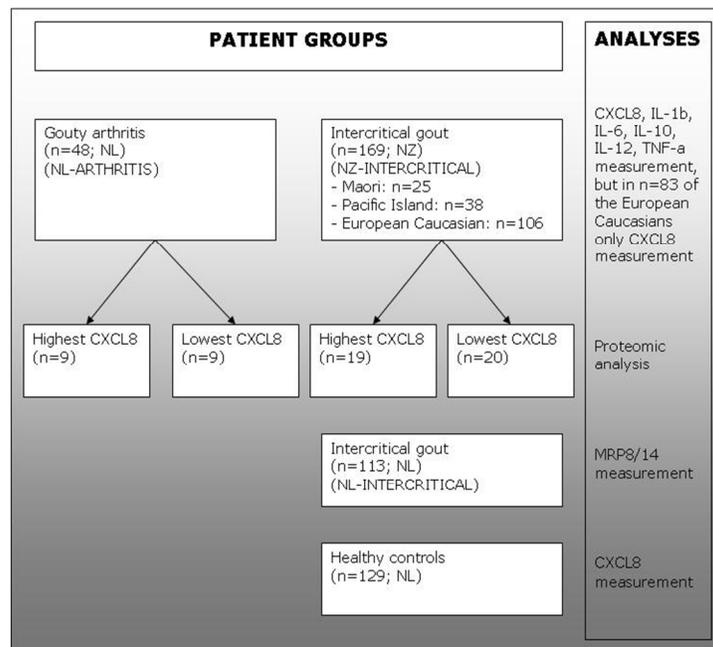
**Figure 3. High circulating CXCL8 levels associate with the co-occurrence of diabetes in patients with intercritical gout.** After stratification of intercritical gout patients for low (<100 pg/ml; n=68), intermediate (100-500 pg/ml; n=42) and high levels of CXCL8 (>500 pg/ml; n=59) high CXCL8 levels were found to be associated with the levels of circulating urate (**panel A**) and co-occurrence of diabetes (**panel B**).

**Figure 4. A serum proteome-wide analysis on gout patients during gouty attack identifies a unique protein profile associated with CXCL8.** Proteome-wide analysis exploiting SELDI-TOF of the serum was performed on patients with gout during a gouty attack (n=18). Stratification for those patients with a high CXCL8 level (>100 pg/ml; n=9) and those with low CXCL8 levels (<10 pg/ml; n=9) revealed a highly distinct predicted protein profile. Proteins that were lowly expressed are shown in green whereas those proteins present in high concentrations are presented in red (**panel A**). Myelin-related protein (MRP)

8 and MRP 14 were identified as being proteins most differentially expressed in patients with high and low CXCL8 (**panel B**). After validation in a cohort of Dutch gout patients with intercritical gout (n=113) increased levels of MRP8/14 were found to be closely correlating with increased levels of circulating CXCL8 ( $R^2$  0.49,  $P < 0.001$ ) (**panel C**).

**Figure 5. Unsupervised clustering identifies a CXCL8 dependent proteome in gout patients during intercritical phase that is associated with the co-occurrence of diabetes.** In proteome-wide analysis of serum from New Zealand patients with intercritical gout (n=39) unsupervised clustering reveals that the CXCL8-related proteome is associated with the co-occurrence of diabetes (16.5; 2.8-96.6 (OR; 95% CI). Proteins that were lowly expressed are shown in green whereas those proteins present in high concentrations are presented in red.

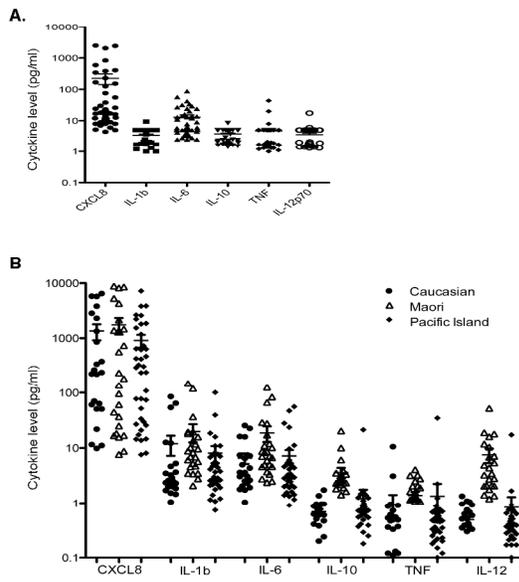
Figure 1.



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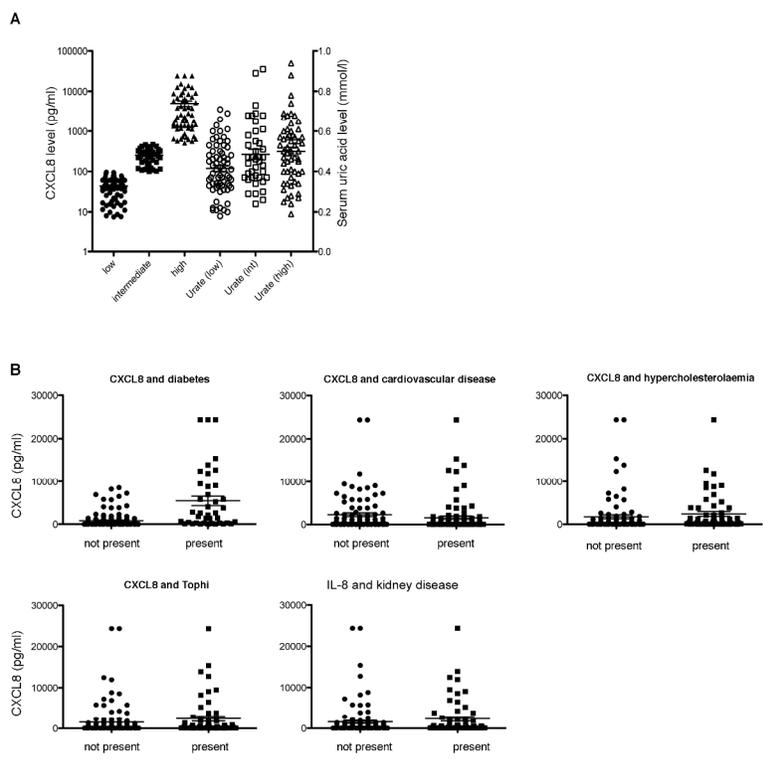
Figure 2.



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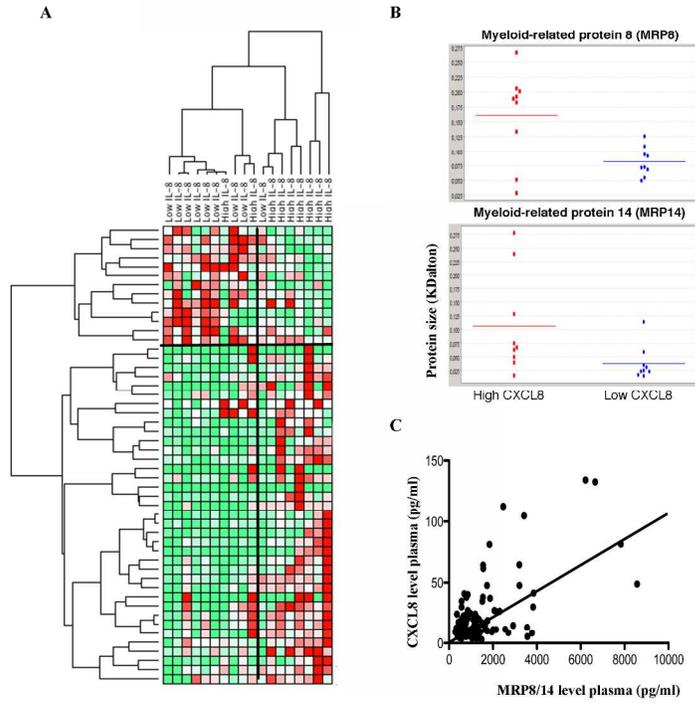
Figure 3



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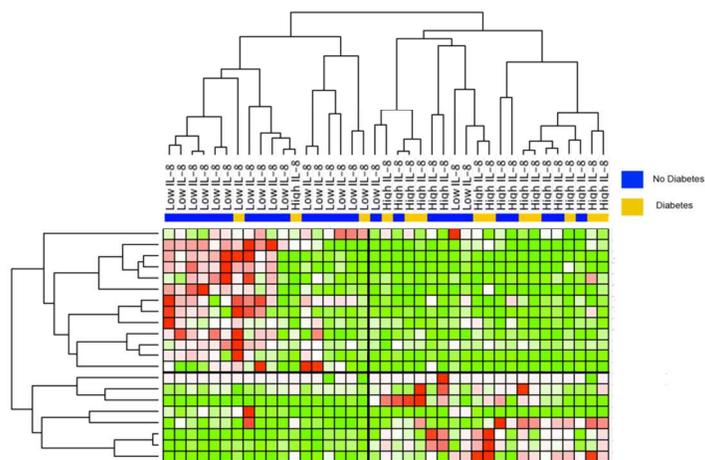
Figure 4



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Figure 5.



Common significant peak	Higher in	Possible Proteins
4565-4585	patients with low IL-8	COL, LEAP2, PENK, VIP, PPAP
58995-60392	patients with low IL-8	ELN
7650-7707	patients with high IL-8	IL8, CCL18, CCL20, CCL22, CCL3, CCL4, CCL5, CXCR4, CX1F, CL31, CXCL5, DB110, GRDA, IGF1, ISK5, CXCL4, SG1D2, TOR2A
12692-12698	patients with high IL-8	CCL26, CE064, CMGA, F19A1, FNDCS, GDF1, GHR11, IL15, INHBB, INSL4, KLK8, LYMX1, NODAL, NRG4, PS1C2, RSPRY, SAA4, SG11B, TGFb, YQ032

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