***Title Page (with author names and affiliations)**

Humidification mitigates acute mucosal toxicity during radiotherapy when factoring volumetric parameters. Trans Tasman Radiation Oncology Group (TROG) RadioHUM 07.03 substudy.

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Introduction

The rationale for domiciliary humidification during head and neck radiotherapy to ameliorate symptoms associated with mucositis using a high flow rate humidifier with nasal prong interface is based on principles of moist wound care, or in other words to reduce the impact of wound desiccation on tissue injury. This may help mitigate the intense pro-inflammatory environment characteristic of mucositis pathobiology [i] [ii]. Moisturisation aids nutrient delivery and facilitates migration of cells across epithelial surfaces during wound healing. A moist environment provides a cushioning effect to nerve endings, which can attenuate pain. Changes to the oral microbiome composition during radiotherapy which allow micro-organism species with virulence characteristics to become more dominant may be exacerbated by the desiccated environment [iii]. Moisturisation may contribute to more favourable host-microbiome interactions that influence mucositis [iv]. Patients with bulky pharyngeal tumours may also experience mass effect symptoms similar to sleep apnoea syndrome, and overnight high flow rate humidification may provide some of the symptomatic benefits associated with continuous positive airways pressure (CPAP) therapy. [v].

The TROG 07.03 RadioHUM study was a phase 3 multi centre trial evaluating the role of domiciliary humidification using the Fisher & Paykel Healthcare MR880 humidifier. The primary analysis has been previously reported [vi]. Although the results were negative for the primary endpoint involving CTCAE version 3.0 *clinical mucositis* scores, there were several positive secondary endpoints suggestive of a benefit from humidification in reducing symptom burden. In compliant (per protocol; PP) patients, the area under the curve for CTCAE v3.0 functional mucositis was significantly reduced (p 0.009). Mean total inpatient hospital days (p 0.006) and proportion of patients requiring acute hospital admissions (p 0.013) were also reduced in the PP population. Estimates of McMaster University Head & Neck questionnaire patient reported symptom burden in the PP population were also in the direction favouring humidification with less symptom severity, although differences at most time points did not reach significance. The influence of humidification compliance on the results moderated recommendations regarding its practical utility.

There is evidence that mucositis symptom burden for individual patients correlates with dosimetric and volumetric radiotherapy parameters derived from tumour and normal tissue regions of interest (ROI) [vii]. In this report we have modelled the association between humidification use and mucositis symptom burden when factoring in radiotherapy parameters, using a subset of patients from TROG 07.03 managed at a single institution. Radiotherapy parameters used include the Planning Target Volume 70Gy (PTV70) as a surrogate of tumour volume, and two normal tissue regions of interest (ROI) centred on the oral cavity (MOIST), and oropharynx and supraglottic larynx (TSV). The primary objective of this study is to use an ordinal regression model to investigate the effect of humidification on CTCAE v3.0 functional mucositis score when factoring in these tumour and normal tissue dosimetric variables. Secondary objectives are to investigate the effect of humidification on nutritional mode and hospitalisation events when factoring in dosimetric variables.

Methods

TROG 07.03 RadioHUM study was a phase 3 multi centre trial in which patients receiving radiotherapy (RT) for head and neck cancer were randomised 1:1 to receive either their institutional standard of care for managing mucositis, or the standard of care plus domicile based humidification (clinical trials. gov; NCT01917942). Eligibility criteria were the presence of pathologically confirmed cancer involving the nasopharynx, oropharynx, oral cavity, larynx, hypopharynx, or squamous cell carcinoma unknown primary provided eligible mucosal sites were irradiated. This subset analysis involves patients enrolled on TROG 07.03 recruited from Auckland City Hospital and treated with a prescribed dose of $\geq$ 70 Gy. This substudy was approved by the local hospital and university ethics committees.

A humidifier (model MR880 and HC211 flow source; Fisher & Paykel Healthcare, Auckland, New Zealand), set at 37^{o}C and 100% relative humidity, delivered 44 mg of water suspended in a vapour state per litre of air via nasal prongs. Humidification commenced at flow rates of 25L/min, increasing if tolerated to 30L/min. The aim was to just exceed inspiratory demand, thus avoiding entrainment of non-humidified air and promoting some mouth leakage to moisturise the oral cavity.

Humidifiers were provided for home use with instructions to start on day 1 of RT; the recommendation was to use the humidifier overnight with additional usage throughout the day. Humidification continued until week 12 after RT commencement. In those patients with CTCAE version 3.0 *clinical mucositis* $\geq$ 2 at week 12 (persistent ulceration), humidification continued until the resolution of ulcerative component of mucositis or week 16, whichever occurred earlier.

Study Endpoints

Acute toxicity was evaluated weekly using CTCAE version 3.0, from the commencement of radiotherapy until week 12. Both *clinical mucositis* and *functional mucositis* endpoints were recorded. As previously reported in the TROG 07.03 primary analysis, there was significant variability and range in *clinical mucositis* scoring across individuals and institutions. The low intra-class correlation reported in several studies for clinician scoring of the presence and severity of mucosal ulceration has resulted in the *clinical mucositis* score being removed from CTCAE version 4.0 [viii]. The mucositis endpoint in CTCAE version 4.0 is classified by mucosal site but otherwise resembles *functional mucositis* score in CTCAE version 3.0. This subset analysis has used CTCAE version 3.0 *functional mucositis* scores only as the clinician assessment of mucosal toxicity.

Nutritional mode is a clinician reported assessment of nutritional status including feeding tube use employing a 6 point scale (supplemental online table). Nutritional mode was assessed weekly until week 8, and at weeks 10, 12, 16 and 20.

Hospitalisation events up to 20 weeks beyond the commencement of radiotherapy were recorded.

Structure Definitions

Two normal tissue ROIs were used in the analysis.

1. MOIST volume (minor oral including sublingual salivary tissue) has been proposed as an ROI to encompass minor salivary glands within the mucosa of the oral cavity and anterior oropharynx (MOIST volume includes the floor of mouth, tongue, base of tongue, hard palate, soft palate, uvula, buccal mucosa, inner lips, retromolar trigone, lateral alveolar margin and anterior faucial pillars) [ix]. MOIST was used as a surrogate for *total mucosal volume* of the oral cavity and anterior oropharynx.
2. *Total swallowing volume* (TSV) was defined as a composite ROI including the superior pharyngeal constrictors, middle pharyngeal constrictors, inferior pharyngeal constrictors, cricopharyngeus muscle, supraglottic larynx and glottic larynx.

Structure definition was performed by a head and neck Radiation Oncologist (AM) using RayStation version 4.0 treatment planning system (RaySearch Laboratories AB, Stockholm, Sweden). Atlas based segmentation was used within RayStation 4.0 to develop a template for these volumes from the first 20 patients. Subsequent patients thereafter had the template initiated to automatically generate ROI volumes which were then manually adjusted. A second head and neck Radiation Oncologist (GS) audited the normal tissue ROI volumes in 5 randomly chosen cases (figure 1).

Planning Target Volume 70 Gy (PTV70) was generated by a 3mm expansion of the 70 Gy clinical target volume (the expansion was limited peripherally by a contour reduced by 3 - 5mm from the external contour). In 2 patients treated to a total of 72 Gy, the PTV 72 was generated.

Statistical Methods

Three regression models were established to explore the association of humidification use with dosimetric and volumetric parameters, and the influence of other control variables described below and in table 1. The coefficient variance estimates were adjusted to account for additional variation from the imputation.

1. *Functional mucositis* was analysed using an ordinal regression model (also known as cumulative link model) [x].
1. Given the unreliability of the categorisation of the ordinal regression model with nutritional mode data, the 6 levels within the scale were combined into 2 categories (levels 1-3 representing more unfavourable nutritional mode involving feeding tube use, and levels 4-6 without feeding tube use). This reduced the data to a binary variable which could then be analysed using a logit regression model [xi], and improved the stability of the resulting model fit
2. Hospitalisation days were analysed using a Hurdle regression model. This model consists of a component modelling the probability of getting into hospital, i.e. admission (zero model), and a second component fitting the number of hospitalisation days if hospitalisation was necessary (count model).

For the functional mucositis and nutritional mode models, linear and quadratic temporal effects **(time and time^2)** were used to capture the complex non-linear effects of time.
For each of the models, the following predictors were also used.

1. Treatment group (control/humidification) to test the effect of humidification **(humidification vs control).**
3. Control variables for demographic characteristics **(gender - male vs female, age - continuous, ethnicity - Caucasian vs other)**.
4. Control variables from radiation treatment parameters.
   - Equivalent uniform dose (EUD) to the MOIST ROI (a value = 1) in Gy **(EUD MOIST)**.
   - Equivalent uniform dose (EUD) to the TSV ROI (a value =1) in Gy **(EUD TSV)**.
   - Volume of the PTV 70 in cm^3 **(PTV70)** (variable multiplied by factor of 1/10)**.**
5. Group indicator for type of radiotherapy treatment plan **(Intensity Modulated Radiotherapy (IMRT) vs 3D conformal)**.

None of the patients were current smokers during radiotherapy. Smoking history data was insufficient to include in the models. A logit model examining chemotherapy utilisation based on age indicated that age was highly significant in explaining whether a patient received chemotherapy ($p < 0.01$). Chemotherapy was therefore not included as a variable to avoid introducing highly correlated variables into the analysis. Similarly there was a strong association between T stage and dosimetric variables (PTV70, EUD MOIST and EUD TSV; see figure 4). For reasons of model stability, T stage was therefore not included in the models. HPV status using the surrogate of p16 staining was not routinely performed in New Zealand and Australia when TROG 07.03 RadioHUM was developed and p16 status was not prospectively recorded.

All analysis were conducted using the R software [xii]. For ordinal regression we used the ordinal package[xiii], and for the Hurdle model we used the pscl package[xiv]. Furthermore, the Amelia II software package was used to perform multiple imputations of the functional mucositis and nutritional mode data to correct for missing values and capture the time series/cross sectional structure of the data [xv]. Imputation diagnostics were then run to ensure the imputed values aligned with these structures [xvi].

Results

The analysis included 39 patients (humidification 20; control 19). Patient characteristics are outlined in table 1. Thirteen of 20 or 65% of the humidification patients met the predefined humidification compliance standard based on 4 hours daily use (compliance definition see reference 1). This is higher than in the overall TROG 07.03 study where 44% met the compliance standard [7].

A descriptive analysis of the CTCAE version 3.0 *functional mucositis* scores over time differentiated by treatment arm is shown in figure 2. Figure 3 provides a descriptive representation of the volumetric and dosimetric data. The values for PTV 70, EUD MOIST, and EUD TSV are bracketed by functional mucositis score and treatment arm: control, humidification. The figure includes the incidence for all patients within each bracketed category. This means that any given patient may be represented several times depending on which grades of *functional mucositis* they were scored at during the 0 to 12 week time period. The baseline median values of volume of the PTV 70 are very similar between the control and humidification arms as evidenced by the functional mucositis scores of 0. There is some indication that humidification patients that reach a functional mucositis score of 3 will be more likely to have larger PTV 70 volumes. There are similar trends that humidification patients that reach grade 3 *functional mucositis* will be more likely to have higher EUD MOIST values.

The results using the ordinal regression model for CTCAE version 3.0 *functional mucositis* scores are displayed in table 2. There is a signification odds reduction of 0.29 associated with the use of humidification ($p < 0.001$). Within the parameters of the model therefore, at any given time point, the risk of a humidification patient being scored as experiencing a one-step increase in *functional mucositis* is 3.45 times lower (1 / 0.29) than for control patients. The risk of *functional mucositis* score increasing by one grade is 1.05 higher when a patient's PTV 70 volume increases by 10 cm^3 (p 0.001). There is a similar significant association between EUD MOIST (*total mucosal volume*) (p 0.018) and EUD TSV (*total swallowing volume*) (p 0.002), and *functional mucositis* scores, where the odds ratios are 1.03 and 1.04, respectively.

Table 2 also displays the results using the logit regression model with nutritional mode as response. The odds reduction associated with the use of humidification is 0.24. In other words, a control patient is 4.17 times (1 / 0.24) more likely to receive a more unfavourable nutritional mode involving a feeding tube (nutritional mode score 0, 1 or 2) than a humidification patient ($p < 0.001$). The PTV 70 volume odds ratio is 1.11 ($p < 0.001$); thus, the risk of receiving a nutritional mode score of 0, 1 or 2 is 1.11 times higher when a patient's PTV 70 volume increases by 10 cm^3. Interestingly, there is a non-significant trend seen with increasing EUD TSV and unfavourable nutritional mode (p 0.059) which is not present with MOIST. If PTV70 is removed from the model, both EUD MOIST and EUD TSV become significantly associated with nutritional mode ($p < 0.001$; data not shown).

The risk of being admitted to hospital decreased by a factor of 11.11 (1 / 0.09) for humidification patients (p 0.013) as shown in table 3. There were no other significantly associated variables for hospital admission. The duration of hospital admissions for humidification patients in the count model tended to be 1.49 times longer (p 0.018) than for control patients. So humidification patients were much less likely to need hospital admission, but if admission was required, tended to stay longer. Multiplicative effects for EUD MOIST and TSV on the admission duration are close to 1. There is a strong effect of PTV70 on admission duration. When a patient's PTV 70 volume increases by 10 cm^3, the duration of hospital admission increased 1.11 times on average ($p < 0.001$).

Discussion

These regression models have demonstrated significant benefit from humidification in this subset analysis in reducing clinician reported symptom burden, limiting functional nutritional status decline during radiotherapy, and reducing hospitalisation.

CTCAE version 3.0 *functional mucositis* represents a clinician assessment of symptom burden. Previous studies examining symptom clusters during radiotherapy in head and neck cancer have highlighted the importance of symptoms such as difficulties with mouth/throat mucus, mouth/throat pain, and difficulties swallowing [xvii]. These symptoms associate with structures in the oral cavity, oropharynx and larynx rather than just the oral cavity. This becomes increasingly important with a patient cohort as in this study where there are no oral cavity malignancies and oropharynx is the dominant site.

Two summary ROIs were identified; MOIST as a surrogate for *total mucosal volume* of the oral cavity and anterior oropharynx, and TSV as a surrogate of total swallowing volume. EUD TSV was significantly associated with functional mucositis decline, with a strong trend for association with nutritional mode decline. The association between functional mucositis and EUD MOIST was also significant, but there was no association between EUD MOIST and nutritional mode decline. One of the advantages of the humidifiers in TROG 07.03 RadioHUM is that given their high flow rate characteristics, they moisturise the larynx, oropharynx and supraglottic larynx as well as the oral cavity. These findings support the hypothesis that inflammation in the oropharynx and larynx (in addition to oral cavity) contributed to symptoms associated with mucositis symptom burden in these patients (all of whom were treated for non oral cavity malignancies), and humidification helped ameliorate the symptoms. The associations between EUD MOIST, EUD TSV and outcome in an oral cavity malignancy population may well be different.

Different anatomical definitions for normal tissue regions of interest can result in inconsistent dosimetric correlations with toxicity [xviii]. This study used summary regions of interest, but a future analysis will apply machine learning techniques for data-driven variable selection which is able to analyse a much larger set of treatment plan parameters (including many more individual OARs, and physical parameters such as overlap between OARs and target, and respective geometric distances between OARs and targets). A future analysis will also explore out to 2 years beyond radiotherapy, clinician reported nutritional mode and CTCAE version 3.0 dysphagia scores, patient reported symptom burden using the McMaster Head and Neck questionnaire, and feeding tube insertion and duration parameters.

This subset analysis included a cohort of patients from TROG 07.03 managed at a single institution with higher (although still suboptimal) humidification compliance than reported overall in the TROG 07.03 primary analysis. As mentioned in the original TROG 07.03 published report, compliance issues with humidification are similar to those reported with CPAP therapy. This report contributes to evidence supporting the hypothesis that humidification can help mitigate the effects of mucositis. Reducing hospitalisation is an important outcome in itself, but additionally may figure in discussions on how toxicity interventions can enhance the value of healthcare expenditure. The findings are also consistent with the hypothesis that a certain level of humidification compliance is needed to provide benefit. Future research needs to focus on improving humidification compliance.

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Table

Characteristic	No. of control patients (19)	No. of HUM patients (20)
Age (mean: range)	58 (29-74)	56 (45-75)
Sex Male Female	 15 4	 14 6
Primary Site Nasopharynx Oropharynx Larynx Hypopharynx	 4 13 1 1	 0 19 1 0
Ethnicity Caucasian Maori/Pacifc Islander/Asian	 15 4	 18 2
ECOG 0 1 2 3	 13 4 2 0	 14 3 2 1
Technique IMRT 3D Conformal	 12 7	 11 9
Radiotherapy total dose 70 Gy 72 Gy	 18 1	 19 1

Table 1: Patient Characteristics

	Functional Mucositis		Nutritional Mode	
	odds	significance	odds	significance
Humidification	0.29	<0.001	0.24	<0.001
time	9.98	<0.001	2.42	<0.001
time^2	0.86	<0.001	0.96	<0.001
Age	1.01	0.548	0.97	0.053
Ethnicity	0.96	0.902	0.25	0.005
Gender	1.15	0.596	4.02	<0.001
IMRT	0.61	0.084	0.29	0.015
EUD MOIST	1.03	0.018	1.02	0.373
EUD TSV	1.04	0.002	1.05	0.059
PTV 70 volume	1.05	0.001	1.11	<0.001

Table 2: Regression Models: Ordinal model for CTCAE version 3.0 *functional mucositis* scores; logit model for nutritional mode.

	Zero Model (Admission)		Count Model (Total Days)	
	odds	significance	odds	significance
Humidification	0.09	0.013	1.49	0.018
Age	0.49	0.619	1.07	<0.001
Ethnicity	0.83	0.318	0.18	<0.001
Gender	0.71	0.405	2.36	<0.001
IMRT	0.68	0.501	0.51	0.001
EUD MOIST	0.51	0.686	0.98	0.003
EUD TSV	0.52	0.097	0.97	0.008
PTV 70 volume	0.49	0.274	1.12	<0.001

Table 3: Hurdle regression model for hospitalisation consisting of zero model (admission) and count model (total hospitalisation days).

Figure

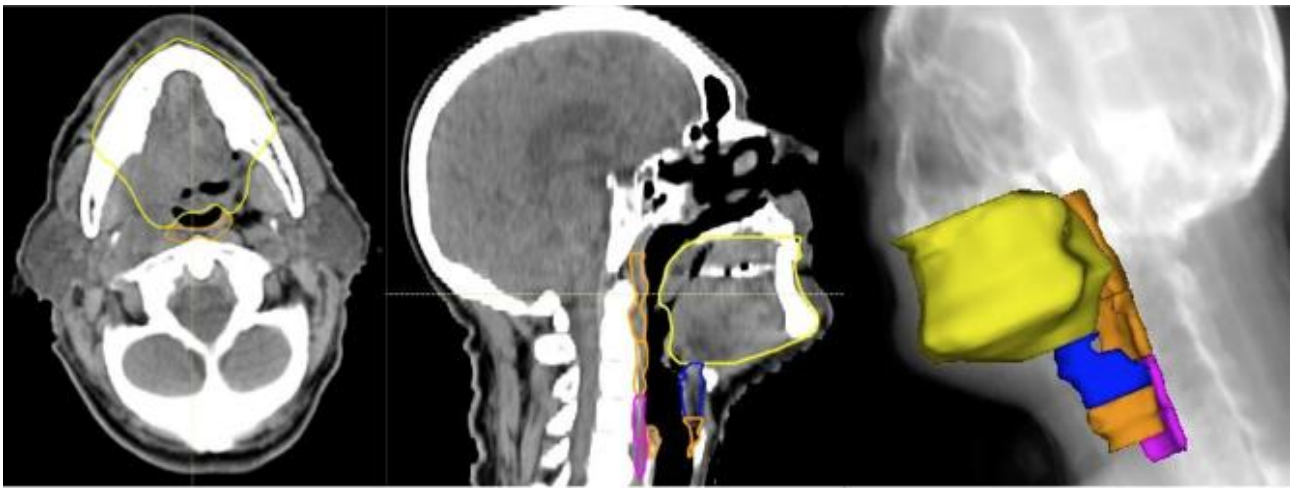

Figure 1: Structure definition of the MOIST (yellow) and TSV (aggregation of the remaining coloured parts) regions of interest using RayStation version 4.0 treatment planning system.

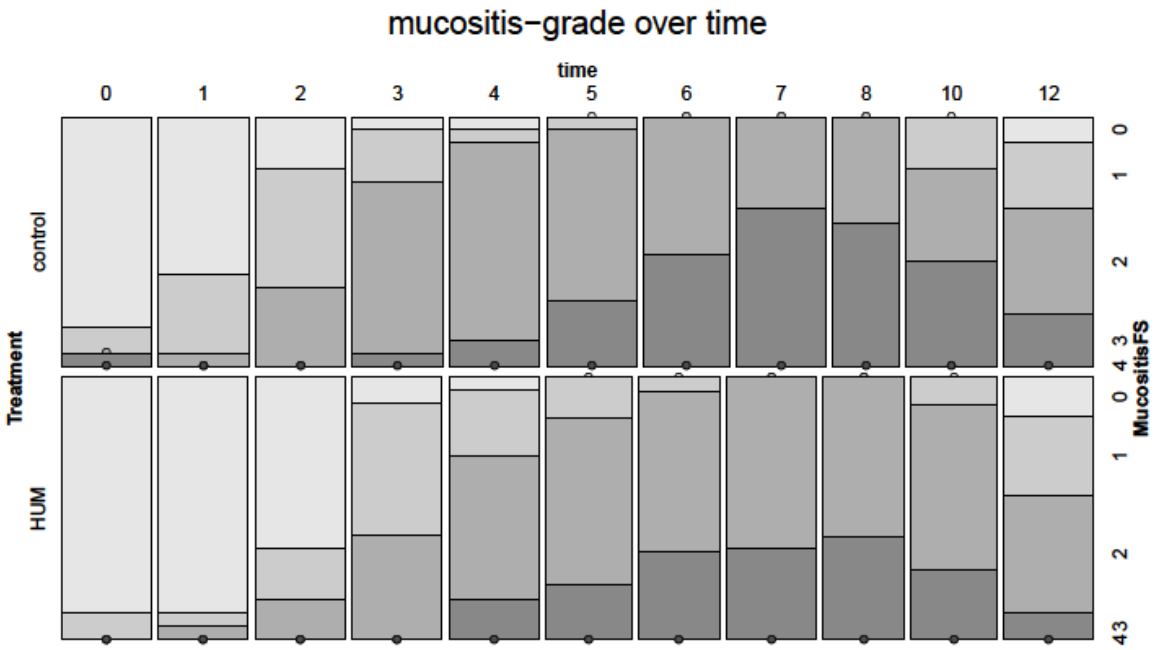

Figure 2: Descriptive analysis of CTCAE version 3.0 functional mucositis grade over time differentiated by treatment arm.

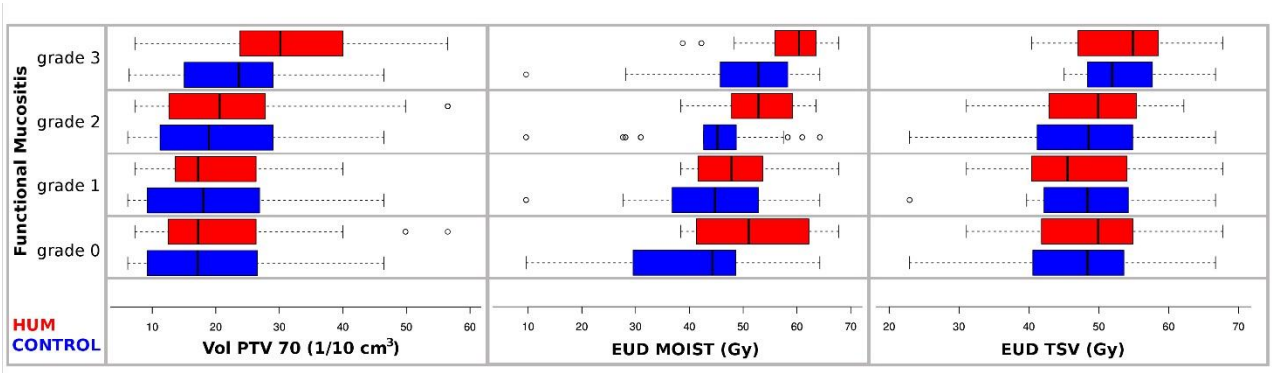

Figure 3: Descriptive analysis of the values for PTV 70, EUD MOIST, and EUD TSV bracketed by treatment arm and functional mucositis scores. The results represent incidence for all patients within each of the bracketed categories; any given patient may be represented several times depending on their functional mucositis scores. There is a non-significant trend that humidification patients that reach a functional mucositis grade of 3 will be more likely to have larger PTV 70 volumes and similar trends that humidification patients that reach grade 3 will be more likely to have higher EUD MOIST values.

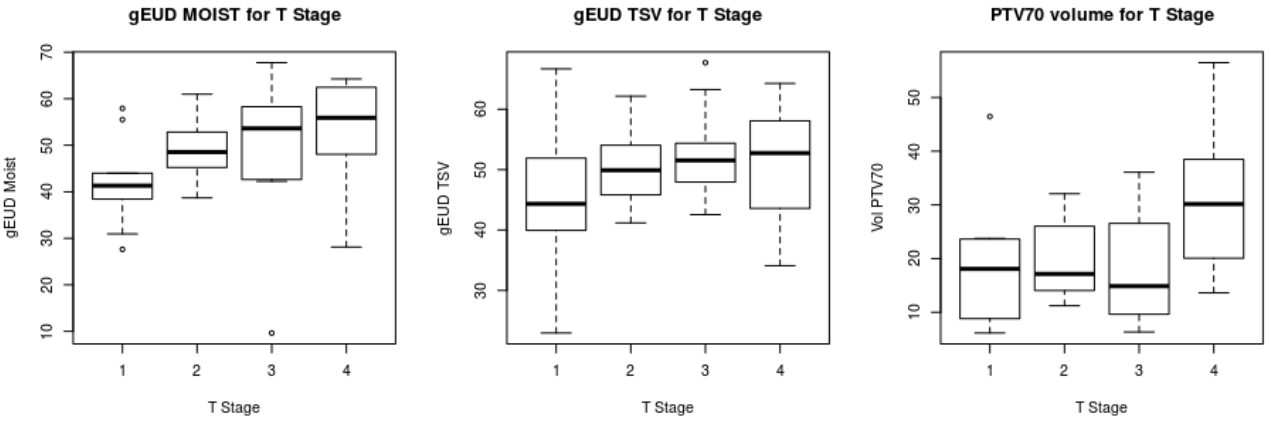

Figure 4: Descriptive analysis demonstrating the associations between T stage and the dosimetric variables PTV70, EUD MOIST and EUD TSV.