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FULL PAPER

Anal cancer chemoradiotherapy outcome prediction using ^{18}F-fluorodeoxyglucose positron emission tomography and clinicopathological factors

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Objective: To assess the role of [^{18}F]fluorodeoxyglucose (FDG) positron emission tomography (PET), obtained before and during chemoradiotherapy, in predicting locoregional failure relative to clinicopathological factors for patients with anal cancer.

Methods: 93 patients with anal squamous cell carcinoma treated with chemoradiotherapy were included in a prospective observational study (NCT01937780). FDG-PET/CT was performed for all patients before treatment, and for a subgroup (n = 39) also 2 weeks into treatment. FDG-PET was evaluated with standardized uptake values ($SUV_{max/peak/mean}$), metabolic tumor volume (MTV), total lesion glycolysis (TLG), and a proposed Z-normalized combination of MTV and SUV_{peak} (ZMP). The objective was to predict locoregional failure using FDG-PET, tumor and lymph node stage, gross tumor volume (GTV) and human papilloma virus (HPV) status in univariate and bivariate Cox regression analysis.

Results: N3 lymph node stage, HPV negative tumor, GTV, MTV, TLG and ZMP were in univariate analysis significant predictors of locoregional failure ($p < 0.01$), while $SUV_{max/peak/mean}$ were not ($p > 0.2$). In bivariate analysis HPV status was the most independent predictor in combinations with N3 stage, ZMP, TLG, and MTV ($p < 0.02$). The FDG-PET parameters at 2 weeks into radiotherapy decreased by 30–40 % of the initial values, but neither absolute nor relative decrease improved the prediction models.

Conclusion: Pre-treatment PET parameters are predictive of chemoradiotherapy outcome in anal cancer, although HPV negativity and N3 stage are the strongest single predictors. Predictions can be improved by combining HPV with PET parameters such as MTV, TLG or ZMP. PET 2 weeks into treatment does not provide added predictive value.

Advances in knowledge: Pre-treatment PET parameters of anal cancer showed a predictive role independent of clinicopathological factors. Although the PET parameters show substantial reduction from pre- to mid-treatment, the changes were not predictive of chemoradiotherapy outcome.

INTRODUCTION

Anal cancer is a rare disease with increasing incidence[1,2] and chemoradiotherapy is the recommended curative treatment.[3] State-of-the-art radiotherapy is delivered as intensity modulated radiation therapy (IMRT) with concomitant mitomycin (MMC) and 5-fluorouracil (5-FU) chemotherapy.[4,5] Different treatment regimens are used based on tumor and lymph node stage.[3] Complete tumor remission may be achieved in up to 90% of the patients and the 3 year progression-free survival is around 70%.[6] Recurrences are mostly locoregional[7,8] and curatively intended salvage surgery usually involves extensive pelvic surgery.[9]

The majority of anal squamous cell carcinomas are HPV positive, and HPV status is both a prognostic factor and predictive of treatment outcome.[10,11] Other clinicopathological features such as gender, tumor and lymph node stage, radiotherapy dose, and tumor-infiltrating lymphocytes have shown prognostic value,[11–13] but there is currently no

consensus on the best combination of factors to predict treatment outcome of anal carcinomas.

Pre-treatment FDG-PET is used to detect and characterize glucose-avid malignancies, *e.g.* squamous cell carcinomas of lungs, cervix, head and neck and the anal canal, and is for anal cancer currently recommended for detection of lymph node metastases (N stage) and distant metastases.[14] For these cancers, both the FDG-accumulating tumor volume and maximal tumor uptake values have shown associations with treatment outcome and disease-free survival.[15–21]

Previous studies on FDG-PET of anal cancer have addressed the predictive role of PET-derived markers assessed by pre-or post-therapy imaging.[22–26] First, these studies did not evaluate HPV status in the same patient cohort. Second, only a limited panel of PET markers has previously been evaluated for anal cancers. Third, no studies have investigated the role of FDG-PET during the course of chemoradiotherapy.

The aim of this study was to evaluate the predictive role of FDG uptake parameters in a large prospective anal cancer patient cohort with PET/CT performed both prior to (n = 93) and 2 weeks into chemoradiotherapy (n = 39). PET metrics at baseline and early during treatment, alongside clinicopathological factors such as tumor volume and lymph node status, gender, and HPV status, were evaluated in univariate and multivariate analysis. Moreover, a novel PET metric was proposed, the z-normalized MTV-Peak (ZMP). This metric was constructed *a priori* based on a combination of the metabolic tumor volume (MTV) and local hot spot tumor intensity (standardized uptake value, SUV_{peak}) in the FDG-PET image.

METHODS AND MATERIALS

Patients, treatment and imaging

Consecutive patients presenting with squamous cell carcinoma of the anal canal were prospectively included in the single-arm observational Anal Cancer Radiotherapy study (ANCARAD, NCT01937780). Patients were eligible if they had biopsy-proven anal squamous cell carcinoma, and were scheduled for chemoradiotherapy with curative intent at Oslo University Hospital in the period 2013–2016. The study was approved by the Regional Ethical Committee, and all patients gave written informed consent.

Tumor was staged according to the AJCC seventh edition,[27] based on clinical information, MRI, and FDG-PET/CT, and determined in a multidisciplinary team meeting. In a few cases biopsy was taken to determine lymph node status. Gross tumor volume (GTV) and clinical target volume (CTV) was delineated according to guidelines.[28] 88 patients received sequential radiotherapy with 2 Gy fractions, giving 46 Gy to the CTV and 54 or 58 Gy to the tumor (and pathologic lymph nodes if present) for patients with T1-2N0 or T3-4 or *N*+ disease, respectively. Five patients with T3-4 or *N*+ disease was treated with simultaneous integrated boost in 27 fractions, giving a total dose of 48.6 Gy to the CTV and 57.5 Gy to the GTV. Radiotherapy was delivered with three-dimensional (3D) conformal radiotherapy (n = 31), IMRT (n = 10), or volumetric modulated arc therapy (n = 52). Most patients received concomitant chemotherapy with MMC 10 mg/m^2 Day 1 (and Day 29 if T3-4 or N+) and 5-FU 1000 mg/m^2 days 1–4 (and days 29–32 if T3-4 or N+). The mean treatment duration was 40 days. Evaluation of treatment response with clinical and radiological examinations (MRI, and either CT or PET/CT) was performed 3–6 months after completed chemoradiotherapy.[29] Locoregional treatment failure was defined by incomplete tumor remission after 3–6 months or locoregional pelvic recurrence. Patients were followed every 3 months the first 2 years, thereafter every 6 months until 5 years. Follow-up included digital rectal examination, and at defined time points also anorectoscopy, MRI, and CT scans. By March 2018 the median treatment follow-up time after start of chemoradiotherapy was 31 months (range 3–53).

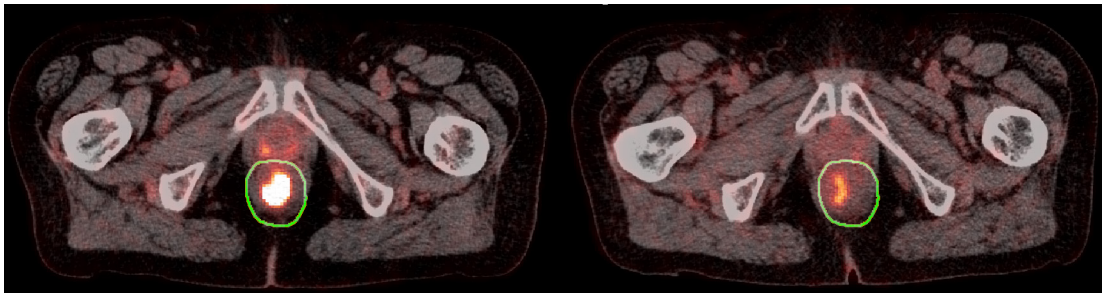
Table 1. Patient demographics for cohorts evaluated prior to CRT and 2 weeks into CRT

		Prior to CRT (N = 93)		2 weeks into CRT (N = 39)	
		#N	%	#N	%
Gender	Female	71	76	29	74
	Male	22	24	10	26
HPV	Positive	67	72	29	74
	Negative	23	25	8	21
	Missing	3	3	2	5
TNM*	T0**	1	1	0	0
	T1	5	4	2	5
	T2	43	46	20	51
	T3	18	19	8	21
	T4	26	30	9	23
	N0	42	45	17	44
	N1	13	14	6	15
	N2	24	26	10	26
	N3	14	15	6	15
Stage	I	1	1	1	3
	II	36	39	11	28
	IIIa	16	17	9	23
	IIIb	40	43	18	46

CRT, chemoradiotherapy; HPV, human papillomavirus.
*According to the TNM seventh edition
**One patient with no visible primary tumor but large anorectal/mesorectal squamous cell carcinoma lymph node metastases was treated as a primary anal cancer.

Patients with visible tumor on pre-treatment PET/CT (n = 93) were included in the analysis. Thus, patients with small tumors that had been surgically excised were not included. Included patients were on median (range) 63 years old (40, 88), weighted 70 kg (47, 117) and 76% were female (Table 1). FDG-PET/CT was performed median 15 days (0, 43) prior to start of

Figure 1. Example of FDG-PET/CT images in one anal cancer patient with identical window levels; at baseline (left) and mid-chemoradiotherapy (right). The green delineation is the gross tumor volume. FDG, fluodeoxyglucose; PET, positron emission tomography.



chemoradiotherapy. In addition, for patients who consented to an extra study-specific procedure and when it was logistically feasible, patients underwent an additional mid-therapy pelvic PET/CT (n = 39). This scan was conducted a median (range) of 14 days (7, 19) into chemoradiotherapy (Figure 1). This time point was chosen based on a publication of sequential PET during chemoradiotherapy for locally advanced rectal cancer,[30] showing that assessment at 2 weeks was optimal for discriminating between pathological responders and non-responders. PET scanning was performed on a Siemens Biograph mCT (Siemens Medical Solutions, Erlangen, Germany) including 3D point spread function (PSF) and time of flight (TOF) reconstruction. The reconstruction matrix was 400 × 400 with a 2 mm in plane resolution and 3 mm slice spacing. Reconstruction was done with OSEM 2i21s and a 2 mm Gaussian filter. The patients fasted for 6 h prior to injection of FDG with a mean (range) activity of 253 MBq (150, 401) and images were obtained 1 hour post-injection (median 62 min, range 55–80 min). All patients included had acceptable blood glucose levels below the upper threshold of 7 mmol l^{-1}.

Tumor characteristics

The TNM stage and the largest tumor diameter were determined at the multidisciplinary team meeting based on all available diagnostic information, including clinical assessment, anorectoscopy, MRI and PET/CT. The GTV was delineated by experienced gastrointestinal oncologists in the Eclipse planning software (Varian Medical Systems, Palo Alto, CA according to recommendations[28] and imported to custom software for analysis (IDL 8.5, Harris Geospatial Solutions, Broomfield, CO). The GTV delineation included GTV and the anal canal circumference in the areas of tumor involvement. We have previously demonstrated high agreement between our oncologists in GTV delineation of anal cancer.[31]

SUV were calculated for the FDG-PET images by correcting for patient weight and injected activity. Within the GTV the maximum voxel value (SUV_{max}) and the maximum mean value of a 1 cm^3 sphere (SUV_{peak})[32] were calculated. The MTV[33] was defined as the region with an activity greater than 50% of SUV_{peak} or SUV >2.5. The segmented MTV was morphologically closed by a kernel with a radius of 16 mm and opened by a kernel with a radius of 2 mm. Total lesion glycolysis (TLG) was calculated by adding all SUV voxel values within the MTV. The novel metric ZMP was calculated by adding z-normalized values of MTV and SUV_{peak} in order to provide an equally weighted measure of these two metrics (Supplementary Material 1). This metric was thus generated *a priori* without any testing on outcome data.

Tissue samples for HPV classification were prepared from formalin-fixed paraffin-embedded material as one thin-section microtome and analyzed using INFORM HPV probes *In Situ* Hybridization (Ventana Medical Systems, Tucson, AZ) by one pathologist (DK). Biopsies were classified according to manufactory protocols as high risk HPV (types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66), low risk HPV (types: 6, 11) and HPV negative.

Statistical analysis

The clinical primary endpoint in the current study was locoregional treatment failure. Typical locoregional failure rates of anal cancer with state-of-the-art chemoradiotherapy were expected to be 10–20%.[6–8] Thus, it was decided to divide the patients into a potential non-responder group (20% of the patients) and a responder group (80% of the patients) for all parameters where a cut-off value could be defined. Thus, cut-offs were obtained from the 80th percentile for these parameters. Group compositions based on several parameters were attempted and analyzed with log-rank test and both univariate and bivariate Cox proportional hazard regression models. p-values less than 0.05 were considered statistically significant. In bivariate Cox regression, imaging values were combined with age, gender, weight, T/N stage, and HPV status, and if both risk factors retained their statistical significance they were considered complementary. Further multivariate analysis was not undertaken due to limited sample size. Kaplan–Meier plots, Log Rank tests, Student's t-tests and linear regression were performed in R (v3.3.3, The R Project for Statistical Computing, Vienna, Austria). Correlation analysis and Cox regression were performed in Matlab (R2015a, Mathworks).

RESULTS

Of the 93 patients, 49% had T3/T4 tumor, 55% node-positive disease and 72% HPV positive tumor (Table 1). In median (range), tumor diameter was 4.1 cm (1.3, 14), GTV 94 cm^3 (23, 534), MTV 8 cm^3 (1, 252), SUV_{max} 18 (3, 46), SUV_{peak} 12 (2, 24), SUV_{mean} 10 (1, 21) and TLG 62 (2, 2020). All patients completed chemoradiotherapy, and 11 (12 %) had locoregional failure at a median follow-up of 31 months. Of these 11 patients, 9 had local failures only and two had both local and nodal failures. Moreover, seven showed residual disease after treatment while four had recurrent disease during follow-up. Five patients had

Table 2. Results from univariate cox regression analysis of whether one group had a significantly ($p < 0.05$) higher chance of treatment failure than another group with an associated HR

	Group	HR	P
T stage	T4	0.9	0.9
	T3-4	1.3	0.7
N stage	N3	8.0	0.001
	N1-3	8.2	0.05
Gender	male	2.0	0.3
Weight*	>86 kg	0.9	0.8
Age*	>70 years	1.0	1.0
HPV	negative	5.9	0.005
SUVmax*	>13	2.2	0.2
SUVpeak*	>8	1.4	0.6
SUVmean*	>6	0.8	0.8
Tumor ø*	>6 cm	3.9	0.02
GTV*	>178	5.0	0.008
MTV*	>23	5.2	0.007
TLG*	>259	5.2	0.007
ZMP*	>0.54	5.3	0.006

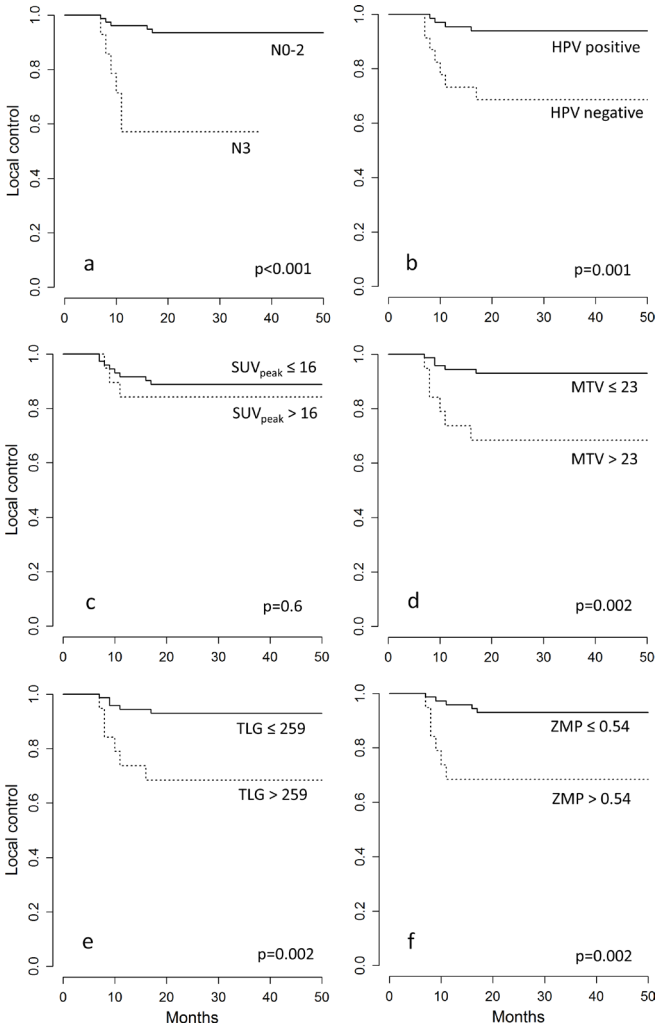
GTV, gross tumor volume; HPV, human papilloma virus; HR, hazard ratio; MTV, metabolic tumor volume; SUV, standardized uptake value; TLG, total lesion glycolysis.
*Group divisions were created by separating the 20% highest values from the 80% lowest values.

metastases, but these distant failures were not included in the primary endpoint.

Locoregional failures occurred in all T stages, and T stage was not a significant predictor for outcome in univariate analysis [T4: $p = 0.9$, hazard ratio (HR) = 0.9 | T3-4: $p = 0.7$, HR = 1.3, Table 2]. However, patients with a tumor diameter greater than 6 cm had a significantly worse outcome ($p = 0.02$, HR = 3.9). N1-3 stage was a borderline significant predictor for outcome in univariate analysis ($p = 0.05$, HR = 8.2), while N3 disease was significant ($p = 0.001$, HR = 8.0, Figure 2a). HPV negative tumor was reported in 25% of the patients and was predictive of outcome ($p = 0.005$, HR = 5.9, Figure 2b). One-third of the patients ($n = 31$) were HPV positive with N0 disease and none of these had treatment failure. Gender, age, radiotherapy treatment dose, duration, and treatment technique (IMRT/VMAT *vs* 3DCRT) were not predictive of outcome ($p = 0.3$, 1.0, 0.1, 0.6, 0.7, respectively). GTV greater than 178 cm^3 was a significant predictor ($p = 0.008$) and patients with N3 disease had significantly larger GTVs compared to those with N0-2 ($p = 0.03$). HPV status on the other hand did not correlate with the size of GTVs ($p = 0.15$).

Although MTVs were considerably smaller than GTVs, these values were highly correlated ($r = 0.86$) and MTV greater than 23 cm^3 was a significant predictor of locoregional failure ($p = 0.007$, Figure 2d). While SUV_{max}, SUV_{peak} and SUV_{mean} were not

Figure 2. Kaplan–Meier plots of locoregional control for N3 status (a), tumor HPV status (b), SUV_{peak} (c), MTV (d), TLG (e), and ZMP (f). All PET metrics are based on pre-treatment scans. Log-rank *p*-values are given. HPV, human papilloma virus; MTV, metabolic tumor volume; SUV, standardized uptake value; TLG, total lesion glycolysis.



significant ($p = 0.2$, 0.6, and 0.8, respectively, Figure 2c) a TLG value greater than 259 was significant ($p = 0.007$, Figure 2e). Finally, the proposed ZMP value was a significant predictor ($p = 0.006$, Figure 2f). As intended the ZMP was only moderately correlated to other PET derived values ($r = 0.52$–0.68).

In bivariate regression, HPV status was an independent risk factor that provided complementary information to N3 stage, GTV, MTV, TLG and ZMP (Table 3). N3 stage was found to be a significant risk factor, but tumor diameter, GTV, MTV, TLG or ZMP were no longer significant when combined with N3 stage. A combination of HPV and N3 stage was the best predictive model of treatment outcome, while a combination of HPV and ZMP, MTV, or TLG performed almost equally well.

Comparing pre- and mid-FDG-PET, median (range) changes of −40 (−78, 13) % for SUV_{mean}, −23 (−77, 52) % for SUV_{max}, −30 (−74, 10) % for SUV_{peak}, −37 (−97, 85) % for TLG, and 2

Table 3. Hazard ratios and *p*-values from bivariate Cox regression analyses

	T3-4	N3	Diam	Gender	Peak	GTV	MTV	TLG	ZMP	HPV	
T3-4	-	<0.001	0.05	0.3	0.6	0.006	0.005	0.005	0.005	0.005	T3-4
	-	8.8	9	2	1.4	10	10.2	10.2	7.3	5.9	
N3	0.6	-	0.1	0.04	0.4	0.1	0.1	0.1	0.2	0.007	N3
	0.7	-	2.7	4.1	0.6	2.9	2.7	2.7	2.6	5.4	
Diam	0.3	0.003	-	0.4	0.7	0.1	0.06	0.06	0.04	0.01	Diam
	0.3	6.6	-	1.7	1.3	3.7	3.7	3.7	3.9	4.9	
Gender	0.7	<0.001	0.03	-	0.6	0.007	0.004	0.006	0.004	0.008	Gender
	1.3	12.2	3.7	-	1.5	5.1	5.9	5.3	5.7	5.8	
Peak	0.8	<0.001	0.03	0.3	-	0.008	0.008	0.008	0.003	0.005	Peak
	1.2	9.9	3.9	2	-	5	5.1	5.1	7.4	5.8	
GTV	0.2	0.009	0.5	0.2	0.7	-	0.4	0.3	0.1	0.02	GTV
	0.3	5.5	1.6	2.1	1.3	-	2.6	2.6	3	4.5	
MTV	0.2	0.01	0.3	0.1	0.8	0.5	-	0.6	0.1	0.01	MTV
	0.3	5.2	2	2.6	1.2	2.2	-	2.3	3	5	
TLG	0.2	0.01	0.3	0.2	0.9	0.4	0.6	-	0.2	0.01	TLG
	0.3	5.2	2	2.2	1.1	2.4	2.3	-	2.9	5	
ZMP	0.4	0.02	0.2	0.2	0.3	0.2	0.2	0.2	-	0.01	ZMP
	0.5	5.1	2.3	2.3	0.5	2.7	2.9	2.7	-	5	
HPV	0.7	0.001	0.1	0.9	0.7	0.05	0.02	0.02	0.01	-	HPV
	1.2	7.1	2.7	1	1.3	3.4	4	4	4.6	-	
	T3-4	N3	Diam	Gender	Peak	GTV	MTV	TLG	ZMP	HPV	

GTV, gross tumor volume; HPV, human papilloma virus; MTV, metabolic tumor volume; SUV, standardized uptake value; TLG, total lesion glycolysis. The abscissa signifies the *p*-value and hazard ratio of a parameter (column) when paired with a variable on the ordinate (row). That is; *e.g.* the bivariate model of N3 combined with T3–T4 is significant ($p < 0.001$) for N3 stage and non-significant ($p = 0.6$) for T stage. Factors that are significant in univariate and bivariate analysis are coded in blue. Yellow signifies pairs of values where both are significant in univariate analysis but only one value is significant in bivariate analysis.

(-89, 214) % for MTV were observed. Changes in ZMP are not reported due to that z-scores by definition are normalized. Values obtained mid therapy were strongly correlated with their respective baseline values for both MTV and TLG ($p < 0.0001$, Figure 3), while SUV_{peak} showed a slightly weaker correlation ($p < 0.001$, $r = 0.49$). Both MTV and TLG had a non-significant intercept while the SUV_{peak} had a significant intercept of 4.5 ($p < 0.001$). There were five patients with locoregional failure among the 39 that received the PET examination two weeks into therapy. While the MTV was still a significant predictor of locoregional failure ($p = 0.03$) neither its absolute changes nor relative changes were significant predictors (data not shown).

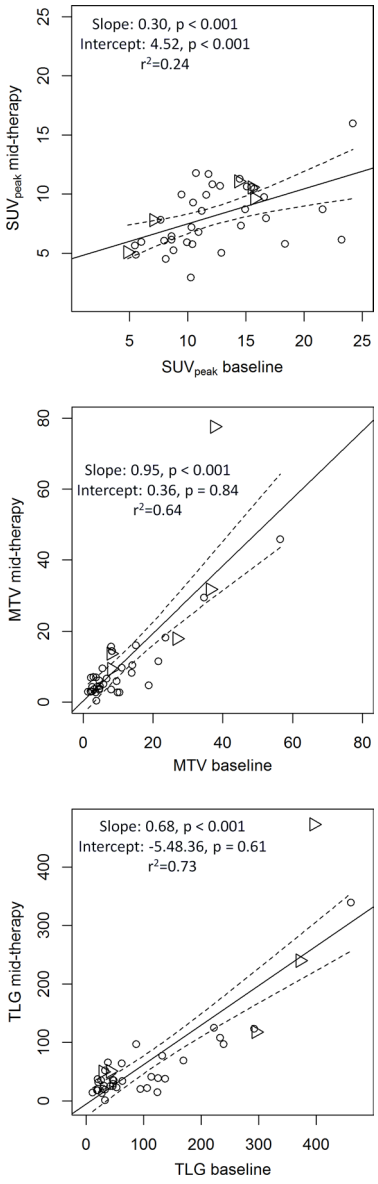
DISCUSSION

In the current study, PET uptake values at baseline and 2 weeks into chemoradiotherapy of anal cancer were evaluated together with clinicopathological factors for modelling locoregional failures. HPV status, N3 stage and the PET metrics MTV, TLG and ZMP showed clear associations with outcome, although the strongest predictors were N3 stage and HPV status. Lymph node metastases have previously been shown to be a significant prognostic factor[13,34] and HPV negative tumors tend to be more treatment resistant.[10–12] Our analysis showed that 25% of the patients had HPV negative tumors, which was higher than previously reported.[35] This could be because our HPV assay did not specifically target the presence of E6/E7 mRNA, and that *in situ* hybridization was used without cross-testing with another HPV detection tool.

Tumor diameter ≥5 cm has previously been associated with poor survival[21,36] and in the current work a tumor diameter ≥6 cm was a significant negative prognostic factor. GTV was delineated according to guidelines for anal cancer.[28,37] At Oslo University Hospital GTV delineation includes the tumor and the anorectal circumference whenever macroscopic tumor is present in the transversal plane, and may thus include rectal gas, stools and non-involved tissues. Although this makes GTV considerably larger than the macroscopic tumor volume it still had significant predictive value. In previous studies, T stage and gender have not been consistent predictors[16,21,36] and in the current work they were not found to be significant.

$SUV_{max/peak/mean}$ were non-significant predictors, which underlines the uncertain predictive value of SUV_{max} for anal cancers.[15,20,38,39] TLG has previously been associated with treatment outcome for other cancer types,[40,41] and was found to be

Figure 3. Comparison of SUV_{peak}, MTV and TLG at baseline (pre-therapy) with values at mid-treatment. The non-responders (triangles) were distributed above and below the linear regression line and did not separate from the responders (circles). 95% confidence interval in dashed lines. MTV, metabolic tumor volume; SUV, standardized uptake value; TLG, total lesion glycolysis.



significant also in the current study. The proposed ZMP metric, combining SUV_{peak} with MTV, gave equal predictive strength as TLG. All volume parameters were significant and it appears that tumor size is a robust predictive factor for anal cancer treatment outcome.

HPV negative status displayed the most complementary information to other significant predictors, *e.g.* N3 status or any volumetric measure, as both values retained their significance in bivariate analysis. N3 stage also retained its significance in combinations with other clinical parameters or PET values. However, in this case the volumetric parameters were rendered non-significant, indicating redundant information where the N3 stage had superior statistical properties. As such the HPV status seemed to provide less confounded information than N3.

A practical approach for identifying the most treatment resistant tumors could be to assume that patients with either N3 disease or HPV negative tumor ($n = 32$) could be potential non-responders. This produces a coarse but significant predictor. A refined approach could be to supplement this information with TLG, MTV, or ZMP, as a bivariate model with HPV status and, *e.g.* ZMP rendered both variables significant. By identifying patients at high risk of treatment failure, patients could be eligible for clinical trials aiming at intensifying treatment (see dose painting below). These findings and models need to be further investigated and validated in independent cohorts.

One reason for including local hot spots in the ZMP metric was in the context of radiotherapy. Increasing the radiation dose during primary radiotherapy may give a higher local control rate,[42,43] but may also result in unacceptable normal tissue toxicity. Local dose intensification (dose painting) guided by molecular imaging is an organ sparing approach where only the most aggressive part of the tumor is given increased radiation dose and is being explored for squamous cell carcinomas of the head and neck[44,45] and lung.[46] The *a priori* developed hypothesis that the combination of the size and intensity of the FDG uptake volume combined into the ZMP would be predictive, under the assumption that both the total tumor burden *and* local hot spots contribute to treatment resistance, was supported in the current work. However, the lack of predictive power of SUV_{peak}, and the comparable predictive strength of ZMP and TLG, indicates that the methodology did not offer significant improvements over MTV. The results also imply that targeting MTV with dose painting would be a better approach than targeting local hotspots. ZMP may be a valuable addition to the current panel of established PET metrics, but further tests and validation in other cohorts and cancer sites is required.

Janssen et al[30] found that both SUV_{max} and SUV_{mean} was reduced from baseline to 2 weeks into chemoradiotherapy by about 30% (averaged over responders and non-responders) in a cohort of 30 patients with locally advanced rectal cancer. These findings are in line with the observed decrease in SUV-based metrics in our current cohort of anal cancer patients. Still, neither absolute nor relative changes in these metrics were predictive of locoregional failure in the present cohort, again pointing to their uncertain clinical value discussed above. Pre- and mid-therapy MTV and TLG were highly correlated, suggesting that patients remained in their respective risk groups during treatment. For SUV_{peak} we observed that low intensity tumors tended to have a lower relative decrease than high intensity tumors, indicated by the significant positive intercept in the linear regression (Figure 3). This could be due to treatment-induced inflammation, which adds to the residual tumor FDG uptake. Moreover, this could have contributed to the lack of substantial MTV change during treatment. Thus, our analysis could not identify any added predictive value from the PET scans performed 2 weeks into treatment.

In conclusion, this study shows that lymph node status, HPV status and pre-treatment PET uptake volumes are predictive of locoregional failure of anal cancer. The *a priori* proposed PET-based metric ZMP showed a predictive role, but MTV and TLG performed equally well. Of the clinicopathological parameters, N3 was the strongest predictor of outcome, while HPV gave the most complementary information to the PET metrics. For the subset of patients examined by FDG-PET during treatment the additional investigation did not improve predictions.

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