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Scientific Article

18F-FDG PET Predicts Hematologic Toxicity in Patients with Locally Advanced Anal Cancer Treated With Chemoradiation

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Abstract

Purpose: Hematologic toxicity (HT) during chemoradiation therapy (CRT) for anal cancer can lead to treatment breaks that compromise efficacy. We hypothesized that CRT-induced HT correlates with changes in active bone marrow (ABM) characterized by pre-/post-CRT positron emission tomography (PET)/computed tomography.

Methods and materials: Data from 36 patients with anal cancer who were treated with 18F-fluorodeoxyglucose PET/computed tomography scans 2 weeks before and 6 to 16 weeks after CRT were analyzed. Complete blood counts with differential within 2 weeks from, weekly during, and 2 week after treatment were obtained. HT was defined as baseline complete blood count change to nadir and posttreatment recovery. Total bone marrow was segmented into 2 subregions: lumbosacral (LS) pelvis (L5 vertebrae, sacrum, and coccyx) and lower pelvis (LP) (ilium, femoral head/neck, and greater and lesser trochanter). PET ABM was characterized as the volume having standard uptake value (SUV) greater than the mean uptake of unirradiated extrapelvic bone marrow. PET variables of pre-/post-CRT and HT predictors were analyzed by linear regression.

Results: Average pelvic ABM was significantly reduced from 52% to 41% in pre- to post-CRT PET scans for all patients ($P = .0012$). Regional analysis indicated significant post-CRT reduction of LS-ABM ($P < .0001$) and LP-ABM ($P = .006$). Linear regression analysis identified post-CRT SUVmean, differential ΔSUVmean, and ΔABM as correlating significantly with pre- and posttreatment HT. ΔWBC linearly correlated with ΔABM of LS and LP pelvis ($P = .033$ and $P = .028$, respectively). Dosimetrically, ABM was sensitive to higher radiation doses (>50 Gy) in terms of acute hematologic ΔWBC ($P = .021$) and ΔANC($P = .028$). HT increased with increasing volume of ABM receiving 40 Gy. The results also suggest that ABM V40 Gy $\leq$ 20% to 25% may significantly reduce the risk of HT.

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Conclusions: HT was significantly associated with ΔABM in patients with anal cancer who were treated with CRT. LS-ABM was a robust surrogate for evaluating CRT-induced HT. Our results suggest implementation of ABM dosimetric constraints, V40 Gy $\leq$ 20-25%, may significantly reduce HT and lead to decreased treatment delays associated with clinical outcomes.

Introduction

Anal cancer comprises approximately 2.5% of gastrointestinal malignancies, with an increasing incidence over the last 30 years.[1] Despite this, treatment with standard-of-care chemoradiation therapy (CRT) results in excellent rates of long-term disease-free survival and sphincter preservation.[2] However, associated treatment-related side effects, such as hematologic toxicity (HT), can be significant.

Improvements in radiation therapy (RT) delivery techniques, such as intensity modulated RT (IMRT), have led to fewer gastrointestinal toxicities and HTs compared with 3-dimensional conformal radiation therapy.[3] IMRT allows a more conformal dose distribution to spare critical normal tissues, such as the pelvic bones and bone marrow. IMRT, compared with 3-dimensional conformal RT, has resulted in reduced grade 2 and 3 HT from 85% to 73% and 36% to 21%, respectively.[3,4] Treatment interruptions were also reduced, with the median duration of treatment decreasing from 49 days to 43 days.

Unplanned treatment breaks have been correlated with compromised oncologic efficacy owing to accelerated repopulation.[5] Increasing treatment breaks have also resulted in worse quality of life with increased 2-year colostomy rates.[4,6] Similarly, reducing systemic chemotherapy, particularly mitomycin-C (MMC), reduces HT to a greater degree than improvements in RT techniques.[7] Therefore, European centers have begun using single-dose MMC,[8] and investigators have attempted to elucidate dosimetric IMRT constraints to reduce HT. Although dosimetric parameters and IMRT have been helpful in sparing the pelvic bone marrow, functional imaging such as 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography (PET) imaging has been used to better characterize the heterogeneity of active bone marrow (ABM) stores in the pelvic bones.[9-12] ABM stores are detected on functional imaging by identifying areas of increased standard uptake value (SUV). Hypermetabolic bone marrow is thought to have increased rates of mitoses, requiring increased utilization of 18-flourine-tagged glucose, thus representing dominant areas of white and red blood cell production. Although reduction in the whole pelvis dose has been helpful, selectively reducing the dose to areas based on PET parameters can further prevent HT.[13] Currently, PET scans are used in conjunction with standard-of-care computed tomography (CT) imaging for the initial and posttreatment staging of patients with locally advanced anal cancer.

The objectives of this study were to characterize bone marrow regions within pelvic bone marrow using PET, investigate changes in ABM before and after CRT in patients with anal cancer, and correlate these changes with HT. We hypothesized that the ABM compartment of the pelvic bones was accurately represented by an increased SUV on PET, that treatment-related changes in ABM using PET were highly associated with HT and clinical outcomes, and that the resulting dose to ABM can be used to identify a clinically relevant dosimetric parameter of HT.

Methods and Materials

Patients

We retrospectively identified treatment-naïve patients with localized nonmetastatic squamous cell cancer of the anus who were treated definitively with concurrent 5-flurouracil (5-FU), MMC, and RT between 2011 and 2016. Patient data were collected independently under institutional review board–approved protocols from 2 institutions. Patients with baseline pre- and post-CRT (range, -6 to 16 weeks; median, 12 weeks; interquartile range, 10-12.5 weeks) 18F-FDG PET/CT scans were included in the study. The same 18F-FDG PET/CT imaging protocol was used at both institutions. Patients fasted 6 hours before intravenous administration of 200 to 400 MBq of 18F-FDG 60 minutes before being scanned. The images were collected and analyzed using the same procedures. White blood cell (WBC) counts, absolute neutrophil count (ANC), lymphocytes (LC), platelets (PLT), and hemoglobin (Hg) were assessed within 8 weeks of beginning treatment, weekly during treatment, and 2 weeks after treatment.

Chemoradiation technique

Treatment-naïve patients were treated with definitive concurrent CRT. Chemotherapy included 5-FU (1000 mg/

m^2 on days 1-4 and days 29-32) and MMC (10 mg/m^2 on days 1 and 29). RT doses were 50.4 to 59.4 Gy in 28 to 33 fractions (55.8 ± 2.23 Gy). Target volumes and normal critical organs were contoured by 2 board-certified radiation oncologists using the Radiation Therapy Oncology Group (RTOG) contouring guidelines.[14] Primary tumor and clinically involved nodes were identified as gross tumor volume and expanded to clinical target and planning target volumes using expansions of 2.5 cm and 0.7 to 1 cm, respectively. Whole pelvic and inguinal nodal basins were treated to 45 Gy in 25 fractions. Clinically involved lymph nodes and primary tumors were treated with additional boost doses (5.4-14.4 Gy). In addition to other normal tissue constraints, bone marrow related constraints were specially considered: V50 Gy < 5%, V40 Gy < 35%, and V30 Gy < 50%. All patients were treated with static field–or volumetric arc–based IMRT.

Pelvic contours and segmentation

Whole-body CT scans obtained as part of PET/CT were used to segment the bone marrow. The whole pelvic bone marrow was outlined following outer contours using a bone window. Total bone marrow (TBM) of the pelvis, including bilateral L5 vertebral body, sacrum, coccyx, ilium, femoral heads, femoral necks, and greater and lesser trochanters, was contoured. Two subregions of pelvic TBM were identified, as shown in Figure 1: (a) lumbosacral bone marrow (LSBM), comprising the L5 vertebral body, entire sacrum, and coccyx; and (b) the remainder of the pelvis, referred to as the lower part of the pelvis (LPBM) and comprising the femoral head, femoral neck, greater trochanter, and lesser trochanter. The extrapelvic bone marrow was also contoured, including the C4-L4 vertebrae, scapulae/proximal humeri, clavicles/sternum, and ribs.

The aforementioned subregion segmentation is built upon the results of our preliminary study on 21 patients,[15] where the pelvis bone was divided into 3 subregions: iliac bone (ilium, iliac crest), lower pelvis (pubic, ischium, femoral head, and femoral neck), and lumbosacrum. We found that the metabolic uptake of the iliac and lower pelvic bones was similar (P = .589) yet significantly different from the lumbosacral regions (P = .034 and .011, respectively). To simplify the analysis in this study, we integrated the lower pelvic and iliac regions into lower-pelvis iliac regions as described.

Metabolic activity of the pelvic bone

The bone marrow metabolic activity represented by SUV of PET images was fused on the CT images. The average uptake of the pelvic regions, SUV_{mean}, was measured in the whole pelvic bone marrow, as well as LSBM and LPBM. To reduce intrapatient image variations, pelvic SUV_{mean} was normalized by subtracting the mean uptake of unirradiated extrapelvic bone marrow. Changes in metabolic activity before and after CRT were calculated as $\Delta SUV_{mean} = SUV_{mean\_post\text{-}CRT} - SUV_{mean\_pre\text{-}CRT}$ and correlated with treatment response.

Active bone marrow

Pelvic ABM was characterized in all PET images as the volume having an SUV greater than the threshold, where the threshold is defined as the mean uptake in the extrapelvic bone marrow. Subregional ABM was also measured for LSBM and LPBM, referred to LS-ABM and LP-ABM, respectively. We calculated ABM volumes and determined the proportion of ABM within each structure: ABM ratio = Volume of ABM/Volume of BM. The ABM volumes and their ratios to bone marrow volume were calculated before and after CRT, respectively. The CRT-induced ABM changes were calculated as the changes of ABM ratio between pre- and post-CRT, such as $\Delta ABM = ABM_{post\text{-}CRT}/BM_{post\text{-}CRT} - ABM_{pre\text{-}CRT}/BM_{pre\text{-}CRT}$. Correspondingly, ΔLS-ABM and ΔLP-ABM represent the change of ABM ratio between pre- and post-CRT in the lumbosacral and iliac-pubic regions, respectively.

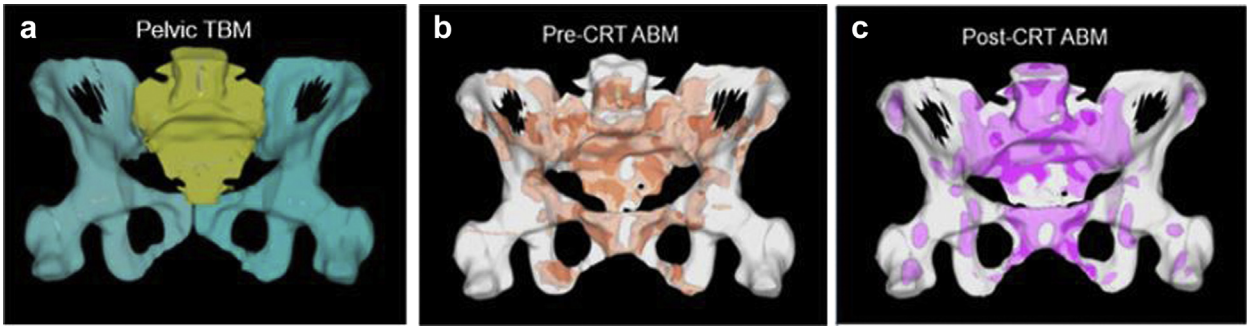

Figure 1 (a) Pelvic total bone marrow with 2 subregions identified: lumbosacral region (yellow) and lower pelvic region (cyan). (b) Pre-CRT PET-defined active bone marrow (orange). (c) Post-CRT active bone marrow (pink). *Abbreviations:* CRT = chemoradiation therapy; PET = positron emission tomography.

Dose evaluations

Radiation dose was defined as the cumulative dose to bone marrow during the course of RT treatment. We used dose-volume histograms to generate multiple dose-volume point metrics at dose levels of 10, 20, 30, 40, and 50 Gy. This analysis was performed on multiple volumes, including pelvic TBM, LSBM, LPBM, ABM, and individual structure LS-ABM and LP-ABM.

Hematologic toxicity and blood cell count nadirs

All patients were monitored weekly during and after CRT for acute toxicities, including but not limited to fatigue, erythema, bloating, urinary urgency, urinary frequency, fecal incontinence, bleeding, dermatitis, nausea, vomiting, constipation, diarrhea, and proctitis. HT was graded using Common Terminology Criteria for Adverse Events, version 4.

Acute HT is represented by the difference in blood cell counts between nadir and baseline: Acute HT = Cell counts at nadir−Cell counts before CRT, where cell counts can be WBC, ANC, PLT, LC, and Hg. We also used the post-CRT HT as an alternative endpoint, where posttreatment HT = Cell counts after CRT−Cell counts before CRT.

Statistical analysis

An independent-sample t test was used to analyze baseline differences in age, clinical stages, and Karnofsky Performance Scale scores among patients from both institutions. The Fisher exact test was used to compare categorical variables. We used the Shapiro−Wilk statistic to test for normality of variables. The primary image-based response was defined as the change of ratio of the ABM% from before to after treatment. Generalized linear modeling was used to investigate the eventual correlation between dosimetric variables and blood cells nadirs.

Posttreatment HT was used as the clinical endpoint for evaluating the dosimetric results. Significant covariates on univariable linear regression analysis were included in the multivariable linear regression model. The Pearson coefficient and multicollinearities index variance inflation factor were used to evaluate the effectiveness of input data in the multivariable analysis. The standard error of the fit and Hosmer-Lemeshow (HL) test were used to evaluate the goodness of model fit. The threshold of dosimetric parameter was validated by area under the curve of the receiver operating characteristic analysis. MATLAB Statistical Toolbox Software (version 11.1) and GraphPad Prism (version 7) were used for the analysis.

Results

Patients

Of the 36 patients, 18 were male (Table 1). Median age at diagnosis was 61.5 years (range, 39-78 years). Mean IMRT dose was 54.8 ± 2.9 Gy. The clinical stage and Karnofsky Performance Scale scores of before and after CRT are shown in Table 1. The t test results indicate there was no significant difference between the patient cohorts of both institutions.

Pelvic total bone marrow

After segmentation of both the pre and post-CRT pelvic TBM, the mean volumes of pre- and post-CRT TBM were 1230 ± 159 mm^3 and 1289 ± 168 mm^3, respectively. Figure 1 shows the TBM defined before CRT (Fig 1b) and after CRT (Fig 1c). The results of the pairwise t test show no significant difference between TBM as a function of CRT ($P = .234$).

Differences of pelvis metabolic activity before and after chemoradiation therapy

The change in metabolic activity because of CRT treatment was compared with respect to SUV_{mean} for TBM, LSBM, and LPBM (Fig 2). Patients' post-CRT SUV_{mean} was significantly lower than that before CRT, and the SUV_{mean} of the LSBM ($P < .0001$) decreased more significantly than that of the LPBM ($P = .011$).

Difference of pelvic active bone marrow before and after chemoradiation therapy

The ABM was segmented using the SUV of bone marrow, which was higher than the mean SUV of the extrapelvic bone marrow. We compared the difference between normalized ABM before and after CRT treatment (Fig 1b, 1c). The ABM in the whole pelvic region was significantly reduced from 50% before CRT to 40% after CRT ($P = .0012$; Fig EB, available online at https://doi.org/10.1016/j.adro.2019.06.005). Regional analysis also indicated that the lumbosacral ABM was more significantly ($P < .0001$) reduced than the LP pelvis region ($P = .006$).

Acute and posttreatment hematologic toxicity

Different cell types reached their nadir at different time points: week 5 or 6 for WBC, ANC, Hg, and LC versus the week 3 for PLT nadir (Fig EC; available online at https://doi.org/10.1016/j.adro.2019.06.005). Consistently across all cell types, the greatest reduction toward nadir

Table 1 Patient characteristics

Variables (N = 36)	All (36 pts)	Institution I (21 pts)	Institution II (15 pts)	*P*-value
Age, median (range), y	61.5 (39-78)	62 (39-78)	60 (45-77)	.395
Sex				.326
Male	18 (50%)	9 (42.8%)	9 (60%)	
Female	18 (50%)	12 (57.2%)	6 (40%)	
Clinical stage				.663
I	3 (8.3%)	2 (9.5%)	1 (7%)	
II	20 (55.7%)	12 (57.2%)	8 (53%)	
III	13 (36.0%)	7 (33.3%)	6 (40%)	
IV	0 (0%)	0 (0%)	0 (0%)	
KPS score, before CRT				.0982
80-90	26 (72%)	13 (62%)	13 (86%)	
60-70	7 (20%)	6 (29%)	1 (7%)	
40-50	3 (8%)	2 (9%)	1 (7%)	
N/A	0 (0%)	0 (0%)	0 (0%)	
KPS score, after CRT				.249
80-90	18 (50%)	8 (38%)	10 (67%)	
60-70	11 (31%)	11 (53%)	0 (0%)	
50-40	4 (11%)	2 (9%)	2 (13%)	
N/A	3 (8%)	0 (0%)	3 (20%)	
Treatment				-
Chemo 5-flurouracil	36 (100%)	21 (100%)	15 (100%)	
Chemo mitomycin-C	36 (100%)	21 (100%)	15 (100%)	
RT Dose mean (std)	54.8 ± 2.9 Gy	55.8 ± 2.1 Gy	53.5 ± 3.4 Gy	

Abbreviations: CRT = chemoradiation therapy; KPS = Karnofsky performance status; N/A = not available; RT = radiation therapy.

occurred between weeks 1 and 3, followed by a persistent negative slope through the course of treatment and recovery in all cases 2 weeks after CRT. The exception was PLT counts, which recovered shortly after the week 3 nadir during treatment. Average WBC count significantly decreased from pre-CRT (7.8 ± 2.4 k/μL) to week 4 (4.5 ± 1.9 k/μL) and then remained low through weeks 5, 6, and 7. WBC counts after treatment increased (5.1 ± 1.5 k/μL). A similar trend was observed in ANC, LC, and Hg levels.

Predictors of hematologic toxicity

We further examined the correlation between HT and image-defined variables (SUV_{mean}, ABM) and dosimetric parameters. Acute and post-CRT HT in relation to PET-defined variables and dosimetric parameters are represented in Table 2. WBC, ANC, LC, and Hg significantly correlated with multiple variables (indicated in Table 2). No significant correlations were found for PLT. The parameters most significantly correlated with ΔWBC and ΔANC were ΔSUV_{mean} and ΔABM for LSBM. In terms of subregional HT, the LS pelvic region had more significant associations (eg, ΔSUV_{mean} and ΔABM) than the LP pelvic region. To better interpret the relationship between HT and clinical imaging and dosimetric variables, we performed linear regression modeling for ABM and HT, as well as radiation dose distribution and HT.

Correlation between active bone marrow and hematologic toxicity

The graphs in Figure 3 are linear regression models that were fit to correlate the ΔABM within LSBM with ΔWBC and ΔANC. The ΔWBC was linearly correlated

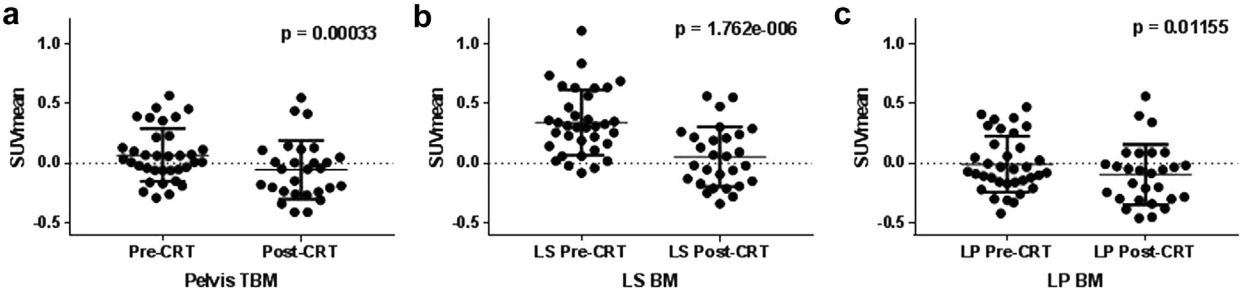

Figure 2 Pelvic metabolic uptakes (SUV_{mean}) comparison between pre- and post-CRT PET. (a) whole pelvis ($P = .0003$); (b) LS-pelvis: the lumbosacral region ($P < .001$); and (c) LP-pelvis: ilium and lower pelvis region ($P = .011$).

Table 2 Correlation between CRT-induced hematologic toxicity measurements and subregional pelvic and dosimetric parameters

Parameter	Δ WBC		Δ ANC		Δ lymphocyte		Δ platelet		Δ Hgb	
	Acute	*After CRT*	*Acute*	*After CRT*	*Acute*	*After CRT*	*Acute*	*After CRT*	*Acute*	*After CRT*
Whole pelvis										
SUV_{mean}, before	0.143	0.261	0.213	0.437	0.556	0.250	0.048	0.647	**0.019***	0.892
SUV_{mean}, after	**0.055**	0.248	**0.041***	0.143	0.462	0.482	0.237	0.683	0.123	0.653
Δ SUV_{mean}	0.980	0.220	0.986	**0.033***	0.586	0.837	0.693	0.355	0.716	0.179
ABM before	0.252	0.226	0.105	0.477	0.320	0.162	0.094	0.996	0.059	0.698
ABM after	0.078	0.201	**0.038***	0.152	0.338	0.447	0.273	0.559	0.115	0.884
Δ ABM	0.463	0.259	0.590	**0.043***	0.862	0.935	0.944	0.315	0.486	0.355
LSBM										
SUV_{mean}, before	0.099	0.786	0.935	0.744	0.504	0.382	0.225	0.630	**0.036***	0.446
SUV_{mean}, after	0.147	0.183	**0.043***	0.210	0.626	0.389	0.352	0.472	0.117	0.887
Δ SUV_{mean}	0.521	**0.002***	0.199	**0.015***	0.427	0.453	0.975	0.322	0.825	0.914
ABM before	0.255	0.406	0.123	0.649	0.580	0.183	0.051	0.714	**0.025***	0.957
ABM after	0.283	0.124	0.065	0.214	0.398	0.344	0.347	0.404	0.158	0.832
Δ ABM	0.758	**0.033***	0.388	**0.029***	0.743	0.576	0.870	0.085	0.390	0.790
LPBM										
SUV_{mean}, before	0.125	0.488	0.743	0.332	0.846	0.226	0.100	0.632	**0.032***	0.559
SUV_{mean}, after	**0.049***	0.303	**0.054***	0.148	0.383	0.563	0.203	0.811	0.136	0.617
Δ SUV_{mean}	0.847	**0.057***	0.336	**0.051***	0.505	0.995	0.880	0.509	0.619	0.542
ABM before	0.286	0.176	0.217	0.415	0.398	0.170	0.119	0.818	0.094	0.601
ABM after	**0.053***	0.270	**0.044***	0.142	0.299	0.548	0.251	0.662	0.114	0.827
Δ ABM	0.369	0.452	0.474	0.062	0.997	0.888	0.926	0.611	0.436	0.269
Dose										
Mean dose	0.464	0.720	0.430	0.427	0.323	0.393	0.391	0.260	0.073	0.722
TBM V10 Gy	0.560	0.945	0.396	0.922	0.490	0.981	0.778	0.344	0.562	0.167
TBM V20 Gy	0.928	0.838	0.309	0.483	0.249	0.562	0.756	0.256	0.890	0.189
TBM V30 Gy	0.295	0.372	0.177	0.394	0.542	0.321	0.681	0.607	0.324	0.766
TBM V40 Gy	0.058	0.262	0.235	0.330	0.843	0.807	0.759	0.196	0.321	0.094
TBM V50 Gy	0.073	0.605	**0.027***	0.438	**0.033***	0.422	0.448	0.893	0.747	0.468
ABM V10 Gy	0.992	0.430	0.864	0.368	0.656	0.315	0.649	0.102	0.335	0.064
ABM V20 Gy	0.411	0.686	0.665	0.521	0.967	0.390	0.901	0.125	0.625	0.116
ABM V30 Gy	0.907	0.752	0.932	0.828	0.982	1.000	0.913	0.895	0.363	0.509
ABM V40 Gy	**0.014***	0.904	0.077	0.934	0.529	0.922	0.783	0.416	0.370	0.070
ABM V50 Gy	**0.021***	0.088	**0.028***	0.102	0.100	0.206	0.134	0.294	0.226	0.388

Abbreviations: ABM = active bone marrow; ANC = absolute neutrophil count; CRT = chemoradiation therapy; Hgb = hemoglobin; LSBM = lumbosacral bone marrow; LPBM = lower pelvis bone marrow; SUV = standard uptake value; TBM = total bone marrow; WBC = white blood cell count.

* $P \leq .05$.

with LSBM ΔABM ($P = .033$; $\beta = 2.557$; $R^2 = 0.175$; 95% confidence interval, 0.22-4.894; Table E1 , available online at https://doi.org/10.1016/j.adro.2019.06.005). Similarly, we found that the ΔANC linearly correlated with LSBM ΔABM ($P = .029$; $\beta = 2.041$; $R^2 = 0.2$; 95% confidence interval, 0.234-3.847).

Correlation between radiation dose and hematologic toxicity

TBM and ABM within increasing dosimetric subregions (ie, V10 Gy, V20 Gy, V30 Gy, V40 Gy, and V50 Gy) were correlated with HT (Table 2). The pre-CRT ABM and TBM were used for dose-volume histograms. The V50 Gy of ABM was significantly associated with acute HT for ΔWBC ($P = .021$) and ΔANC ($P = .028$). The V50 of TBM was significantly associated with acute HT for ΔANC ($P = .027$) and ΔLC ($P = .033$). These results, in conjunction with the RTOG 0529 bone marrow constraints, resulted in a linear regression analysis of ABM V40 Gy (Fig 4). A negative linear regression can be observed for ΔWBC and ΔANC related to ABM V40 Gy. With linear regression, the ΔWBC can be modeled as a function of ABM V40, expressed as ΔWBC = −0.052 × V40 to 1.53, with $P = .014$ and standard error of the fit Sy.x = 1.967. The HL test was used to test the goodness of fit, with $P = .0122$.

Similarly, ΔANC can be fitted as a linear function of ABM V40 Gy, expressed as ΔANC = -0.039 × V40 to

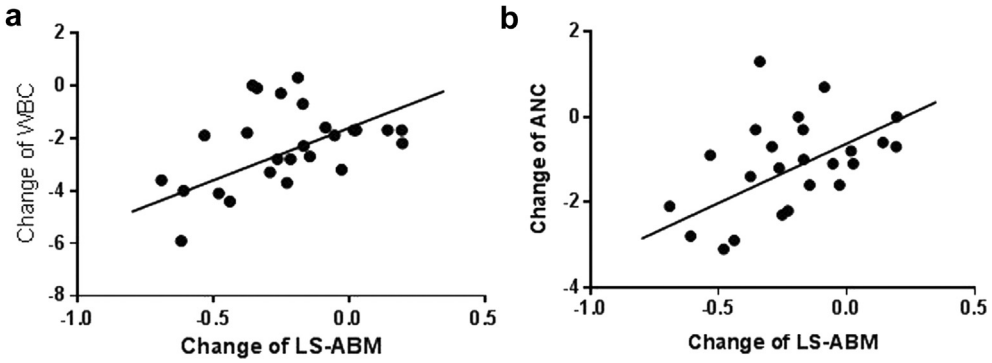

Figure 3 Hematologic toxicity as a function of changes of lumbosacral pelvic active bone marrow. (a) Post-chemoradiation therapy change in white blood cell count is linearly correlated to lumbosacral pelvic change in active bone marrow ($P = .033$); and (b) post-chemoradiation therapy change in absolute neutrophil count as a function of lumbosacral pelvic change in active bone marrow ($P = .029$)

0.542, with $P = .077$ and standard error of the fit Sy.x = 1.618. The goodness of fit of the HL test achieves a $P = .077$.

We further identified the threshold of ABM V40 corresponding to the median post-CRT HT in our data. Relying on the linear regression function derived as described, the median value of ΔWBC −2.79 corresponded to ABM V40 = 24.2%, whereas the median value of ΔANC (-1.31) indicated ABM V40 = 20%. Our results suggest that ABM V40 Gy $\leq$ 20% to 25% correlates to low post-CRT HT. We used multivariable linear regression to identify the correlation between dose ABM V40 and 2 HT variables (ΔWBC and ΔANC). The results of the regression show that the area under the curve of the receiver operating characteristic analysis is 0.625 for V40 $>$ 20%, 0.571 for V40 $>$ 25%, and 0.569 for V40 $>$ 18%. For V40 $>$ 20%, the sensitivity of the classification is 0.565, specificity is 0.692, positive predictive value is 0.764, and negative predictive value is 0.474 (true positive = 13; false negative = 10; false positive = 4; and true negative = 9). The performance of the classification is also interpreted by the Matthews correlation coefficient of 0.248. We determined 2 variable correlations (ΔWBC and ΔANC) to have a Pearson coefficient of 0.877 and a multicollinearities index variance inflation factor of 4.334, which is less than the ad hoc data range of 5 to 10.

Discussion

These results build on a growing body of literature suggesting PET imaging is an effective approach for evaluating changes in ABM due to CRT and the impact on HT in patients with anal cancer. Our technique for identifying areas of ABM was similar to that of Noticewala et al,[12] such that irradiated pelvic regions were normalized to unirradiated extrapelvic bone marrow. We used both pre- and posttreatment PET images and identified significant changes in mean SUV and ABM as a function of CRT. This approach was similar to the one used by Lee et al[16] and Freese et al,[17] but our novelty of using both pre- and post-PET images allowed for identification of changes in the ABM regions. Specifically, the lumbosacral ΔABM was the area of the strongest correlation. Next, we derived a novel formula for estimating

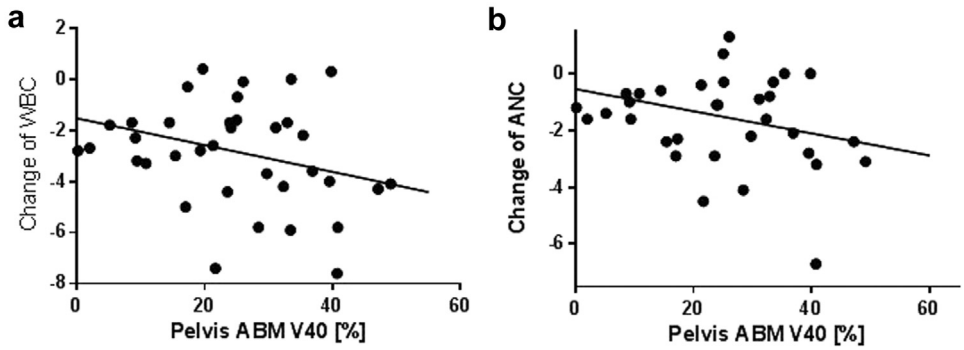

Figure 4 Hematologic toxicity as a function of V40 of active bone marrow. V40 Gy of change in active bone marrow negative linear regression with post-chemoradiation therapy. (a) Change in white blood cell count ($P = .014$); and (b) change in absolute neutrophil count ($P = .077$).

the ΔWBC and ΔANC by using a V40 Gy dose distribution. Lastly, to our knowledge, this is the first study to suggest that patients whose ABM V40 Gy ≤ 20% to 25% had a significant reduction in HT, which correlated with pre- and post-PET scans.

Our study suggests that changes in pelvic ABM during CRT, as identified using PET/CT scans, correlates well with HT, including neutropenia, lymphopenia, and thrombocytopenia. The significance of these findings seems to be greater for lumbro-sacral bone marrow compared with lower pelvic bone marrow ABM. In addition, the analysis of bone marrow dosimetric parameters suggests that the severity of HT linearly correlates with increasing dose to ABM. The slope of the linear fit can be used to estimate changes in WBC counts. For example, to avoid ΔWBC ≤1, our results suggest that V40 Gy of ABM should be <20%. Meanwhile, to achieve ΔANC ≤1, ABM V40 Gy should be <25%.

Several reports suggest that low-dose irradiated pelvic bone marrow is associated with leukopenia and other HTs. Mell et al[18] reported that low-dose irradiation of whole pelvic bone marrow PBM-V10 was associated with HT, and patients with PBM-V10 ≥ 90% were more likely to develop HT. Rose et al also recommended PBM-V10 < 95% and V20 < 80% to reduce grade 3 (G3) leukopenia risk. Additional reports have indicated that high doses of specific subregional pelvic bone marrow are more important in the development of HT. For example, Franco et al showed that LSBM mean dose is significantly correlated with ≥G3 leukopenia and suggested an LSBM mean dose <32 Gy to minimize G3 HT in patients with anal cancer.[19] Another study of the same group suggested that patients with LSBM-V40 > 41% were more likely to develop ≥ G3 HT.[20] Lee et al reported that acute G3 neutropenia and leukopenia may be limited by constraining the lower pelvic bone marrow to V40 Gy ≤ 25% and a V20 Gy ≤ 90%. The latter results are in line with our findings of LS-ABM-V40 ≤ 25% based on PET and CT-based bone marrow contours.

In our patient cohort, the lumbro-sacral bone marrow most significantly correlated with posttreatment ΔANC ($P = .029$) and ΔWBC ($P = .033$). Furthermore, areas of ABM were significantly sensitive to high radiation dose. Although the volume of ABM receiving 50 Gy was relatively small (3.2% ± 4.5% for TBM and 2.8% ± 5.3% for ABM), THE V50 Gy of ABM was significantly associated with the acute HT variables ΔANC ($P = .027$ for TBM and $P = .028$ for ABM) and ΔWBC ($P = .073$ for TBM and $P = .021$ for ABM). Therefore, our results suggest that changes in ABM predict leukopenia, which can be alleviated or avoided using the appropriate constraints.

Patients with ABM toxicity leading to increased HT have more treatment breaks.[21] The absence of hospitalizations and treatment breaks during CRT for anal cancer results in significantly improved clinical outcomes.[5] IMRT has been instrumental in reducing HT and simultaneously allows for dose escalation,[5,22] which has been shown to improve colostomy-free survival[4,6,23] and overall survival.[5] However, guidelines on proper dose constraints and identification of active areas in the pelvic bones have not used PET/CT. Although studies have used constraints similar to those used in RTOG 0529,[3] such as TBM V50 Gy < 5%, V40 Gy < 35%, and V30 Gy < 50%, we believe that PET/CT allows more accurate identification of changes in ABM and development of more clinically relevant bone marrow dose constraints. The use of PET/CT is feasible for centers with a PET/CT scanner because it is typically incorporated into the standard workup of anal cancer patients. Our results suggest that a constraint on ABM of V40 Gy ≤ 20% to 25% may reduce HT, which can lead to a reduction in treatment breaks and thereby improve clinical outcomes.

The reduction in ABM dose should be incorporated with known pelvic dose parameters. For example, Lee et al have shown that reducing the whole pelvis BM volume to <750 cm^3 yielded no G3 leukopenia.[16] Additionally, a V40 dose constraint for CT-based lower pelvis correlated with reduced G3 leukopenia. These constraints can be achieved with lower prophylactic pelvic doses[24] for node-negative patients or for omission of inguinal nodal irradiation in select patients.[25,26] Despite compelling evidence in small, single-institution, retrospective studies suggesting omission of elective inguinal nodal irradiation, the risk of clinically detected regional nodal involvement was 29% for all patients in a Surveillance, Epidemiology, and End Results data analysis,[27] and T/N stage was shown to be strongly prognostic of disease-free survival, overall survival, and colostomy failure in RTOG 98-11.[4] Additionally, Ortholan et al showed inguinal recurrence rates of 12% and 30% in patients with cT1/2 and cT3/4 tumors, respectively, when elective inguinal nodal irradiation was omitted and inguinal failure rates of only 2% when it was included.[28] Based on these and similar data, the National Comprehensive Cancer Network recommends inclusion of inguinal nodal basins for all patients with nonmetastatic anal carcinoma treated with definitive chemoradiation.[2]

Perhaps the most significant contributor to HT is chemotherapy. Several experimental chemotherapy regimens have been prospectively compared with the current standard of 5-FU/MMC. In the ACT II trial, cisplatin with 5-FU resulted in improved grade 3/4 HT compared with 5-FU/MMC but yielded no difference in neutropenic sepsis or survival.[8] Capecitabine has also been investigated in large retrospective studies as an alternative to infusional 5-FU. Goodman et al identified a significant reduction of grade ≥3 HT and treatment interruptions of 32% and 26%, respectively, with equivalent clinical outcomes, and suggested capecitabine as a suitable alternative to 5-FU.[29] In cases where dose constraints are not achievable, some consider following a European

approach using single-dose MMC in select patients, which likely has a greater effect on HT than RT.[30,31] Additional studies will be required to assess the combined impact of varying pelvic radiation dose, elective treatment of inguinal nodes, and type of chemotherapy on both clinical outcomes and HT.

We recognize there are limitations to our study. This study is retrospective, with a limited number of patients. Therefore, it may lack generalizability. Furthermore, data were collected from 2 institutions, so there may be differences in PET/CT scanners and protocols, which may create minor differences in SUV_{max}.[32] However, intrapatient normalization of SUV_{max} to background extrapelvic bone uptake likely mitigated this issue.[12] There may also be differences in the laboratory analysis of blood samples between the institutions, although ie, unlikely to affect the delta for each individual patient throughout treatment. The threshold V40 Gy $\leq$ 20% to 25% is derived from the limited number of patients and may introduce uncertainty due to the fitting of the model. The timing of post-CRT PET had a range of 6 to 16 weeks, but there was only a 2-week interquartile range around a median of 12 weeks; there is possibility for variations in PET SUV values. Lastly, the effect of chemotherapy may vary between patients because 5-FU and MMC have a range for standard doses, which may be different for each patient. However, again one would expect that the differences in systemic effect would be mitigated when normalizing background extrapelvic bone uptake.

Conclusions

Our results are consistent with and build upon a growing body of literature indicating that ABM can be correlated with hematologic values. The use of pre- and post-18F-PET/CT scans provides a robust approach to the delineation of the ABM. Determination of changes in ABM between pre- and post-CRT PET is significantly associated with HT in patients with anal cancer undergoing CRT. LSBM ABM has the strongest correlation with HT, and a constraint on V40 Gy for ABM <20% to 25% can be used to minimize HT. Ultimately, avoidance of PET-based ABM may significantly reduce the risk of HT and can lead to decreased treatment delays, which are associated with superior clinical outcomes.

Supplementary data

Supplementary material for this article can be found at https://doi.org/10.1016/j.adro.2019.06.005.

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