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LETTER TO THE EDITOR

Treatment-related leukopenia in anal cancer patients associated with worse outcome: results of a retrospective cohort study

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Introduction

Anal cancer is a rare disease, albeit with an increasing incidence [1,2]. Standard treatment consists of radiotherapy with concurrent chemotherapy. The treatment outcome is affected by a number of prognostic factors, such as age, gender, tumor stage, human papillomavirus (HPV) status, radiation and chemotherapy dosing, and treatment time [3–7]. In recent years, it has also been suggested that the immune response is important for optimal effects of anticancer treatment in various cancer types [8,9]. The impact of factors related to the immune system in anal cancer has been less explored [10–13].

This study reports on predictive factors for recurrence and survival in a well-characterized cohort of anal cancer patients, with special focus on white blood cell (WBC) toxicity and immunosuppressive disorders.

Material and methods

Study population

The study population has been described previously [14]. Briefly, consecutive patients with non-metastatic squamous cell carcinoma of the anus (anal cancer), who received intensity-modulated radiotherapy (IMRT) with curative intent at Skåne University Hospital, Sweden, during the time period August 2009–December 2017 were included ($n = 170$). Of special note, for the patients included in this study, hematologic toxicity was not a reason for radiotherapy treatment interruptions, and pelvic bone marrow was not delineated as an organ at risk for radiotherapy planning purposes. The mean radiation dose to the primary tumor and elective lymph nodes were 59.0 and 44.7 Gy, respectively (Supplementary Table S1).

Data collection

Data were extracted from medical records by a radiation oncologist (MPN). Somatic comorbidity was coded according to Charlson comorbidity index, excluding age [15]. Based on inherent immunosuppression of a disorder, or long-term use of immunosuppressive medications, the following disorders were counted as 'immunosuppressive disorders': connective tissue disorders with >1 year of systemic immunosuppressive treatment (rheumatoid arthritis, $n = 6$; systemic lupus erythematosus, $n = 2$; polymyalgia rheumatica, $n = 1$; psoriatic arthritis, $n = 2$; sarcoidosis, $n = 1$; unspecified connective tissue disorder, $n = 1$), inflammatory bowel disease (ulcerative colitis, $n = 4$; Crohn's disease, $n = 2$), organ transplant (heart, $n = 1$), and chronic leukemia (CLL, $n = 3$). Two patients with HIV and a normal CD4 count were not counted [16].

Since various radiotherapy schedules were used, crude radiation treatment time (RTT) was not a valid measure of interruptions. Instead, the 'optimal' number of days was calculated for each schedule, presuming that one fraction was given per day, Monday through Friday, and that the treatment started on a Monday. RTT ≥5 d longer than optimal was chosen as cutoff [17].

Acute (<90 d from the end of radiotherapy) toxicity was graded retrospectively according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. Hemoglobin, total WBC, neutrophils, and platelets were measured routinely before commencement of chemoradiotherapy (CRT), weekly during treatment, and post-treatment when clinically indicated. Lymphocytes and other subtypes of immune cells were not routinely measured. For this study, data on pretreatment and nadir values of WBC, neutrophils, and hemoglobin were collected. As expected, WBC and neutrophils were highly correlated, and the sample size was not deemed large enough to include both variables in multivariate models. Due to higher and more significant hazard ratios in univariate analysis (data not

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shown), WBC and not neutrophils were used in the subsequent analyses. For 'gastrointestinal toxicity', the following CTCAE terms were included: colitis, small intestinal mucositis, diarrhea, and ileus.

Endpoints and statistical analysis

The following time to event endpoints was analyzed: local recurrence (LR), distant recurrence (DR), overall survival (OS), anal cancer-specific survival (ACSS), and non-anal cancer death (NACD) [14]. Follow-up and time to endpoint were defined from the date of diagnosis, except for LR, where it was defined from the end of radiotherapy. Five-year survival outcomes were estimated by the Kaplan–Meier method. Cox proportional hazard regression was used to assess univariate predictors for all endpoints; the variables listed in Table 1 were tested. For survival endpoints, age at diagnosis, dichotomized at 65 years, was also included. Variables with a significance of $p < 0.10$ in the univariate analysis for a certain endpoint were entered into a multivariate Cox model for that endpoint. Factors associated with an increased risk of WBC G3+ toxicity were investigated using logistic regression.

All significance tests were 2-sided, and p values <0.05 were considered statistically significant. Statistical analysis was conducted using SPSS version 24 (SPSS Inc., Chicago, IL USA). The study was approved by the Regional Ethical Review Board in Lund (Dnr 2013/742, Dnr 2019/02669). We followed the STROBE guidelines [18] (checklist in Supplementary Material).

Results

One-hundred and seventy consecutive patients with non-metastatic anal cancer were treated with curative intent IMRT (concurrent chemotherapy; 89.4%). Patient, tumor, and treatment characteristics are listed in Supplementary Table S1. With a median follow-up of 50 months (range 14−117 months), the number of events were: LR ($n = 20$), DR ($n = 34$), OS ($n = 36$), ACSS ($n = 23$), and NACD ($n = 13$). As previously reported, 5-year OS and ACSS were 79.9% and 86.1%, respectively [14].

Predictors for recurrence and survival

In multivariate analysis, WBC G3+ toxicity, immunosuppressive disorders, T4 tumor stage, and RTT ≥5 d longer than optimal were predictors for LR. WBC, G3+ toxicity, and immunosuppressive disorders were predictors for DR (Table 1).

In Table 2, significant predictors in multivariate Cox analyses are listed for each endpoint. The complete univariate and multivariate Cox models for all endpoints are presented in Supplementary Table S2.

Treatment-related leukopenia

Treatment-related leukopenia, defined as WBC G3+ toxicity, showed independent associations across all endpoints except for NACD (Table 2).

Logistic regressions were performed to investigate if any factors were associated with an increased risk of WBC G3+ toxicity. Apart from gastrointestinal toxicity and other measures of hematologic toxicity, the only variable that showed a significant association with WBC G3+ toxicity was female gender (OR 2.6; CI 1.1–6.0). The odds ratio for concomitant chemotherapy was high, but the confidence interval wide, due to small numbers in the subgroup not receiving chemotherapy (odds ratio, OR 2.8; CI 0.5–14.1) (Supplementary Table S3).

Sensitivity analyses were performed by Cox analyses, restricted to patients who received two cycles of chemotherapy ($n = 103$), and to patients who received 2 cycles of chemotherapy without any dose reduction ($n = 87$), respectively. In these analyses, WBC G3+ toxicity retained its statistical significance for most endpoints (Supplementary Table S4). Furthermore, restricting the analyses to patients with an RTT <5 d longer than optimal did also not affect the statistical significance of the results (Supplementary Table S4).

Table 1. Univariate and multivariate Cox analyses for local recurrence and distant recurrence.

	Local recurrence				Distant recurrence			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	HR	*p Value*	HR	*p* Value	HR	*p* Value	HR	*p* Value
Male gender	1.98	0.16	–	–	1.51	0.29	–	–
Active smoking	0.73	0.57	–	–	0.49	0.15	–	–
Immunosuppressive disorders	2.83	0.04	3.46	**0.03**	2.53	0.02	2.62	**0.03**
T4	2.50	0.045	3.76	**0.008**	2.02	0.051	1.93	0.09
Primary tumor size ≥ 4 cm	1.64	0.38	–	–	1.97	0.13	–	–
N+	0.93	0.86	–	–	1.18	0.63	–	–
Time to treatment initiation ≥ 62 d	1.81	0.21	–	–	1.29	0.50	–	–
RTT ≥5 d longer than optimal	2.36	0.08	2.88	**0.04**	1.83	0.14	–	–
No concomitant chemotherapy	1.51	0.51	–	–	1.41	0.48	–	–
Dose reduction of chemotherapy	0.24	0.17	–	–	1.12	0.81	–	–
Pretreatment hemoglobin < 120 g/L	0.83	0.71	–	–	0.51	0.13	–	–
Pretreatment leukocyte count > 10 × 10^9/L	2.10	0.13	–	–	1.31	0.53	–	–
Fall of hemoglobin > 30 g/L during radiotherapy	2.48	0.056	1.36	0.56	2.11	0.04	1.32	0.50
Gastrointestinal G3 + toxicity	1.73	0.23	–	–	1.50	0.25	–	–
White blood cell G3 + toxicity	4.38	0.009	4.48	**0.01**	2.46	0.02	2.20	**0.045**
Charlson comorbidity index ≥1	0.87	0.77	–	–	1.43	0.30	–	–

HR: hazard ratio; RTT: radiation treatment time
Bold values represent p values <0.05.

Table 2. Significant predictors for all endpoints on multivariate Cox analyses.

Endpoints and variables	HR (95% CI)	*p* Value
Local recurrence		
Immunosuppressive disorders	3.5 (1.1–10.5)	0.03
T4	3.8 (1.4–10.0)	0.008
RTT $\geq$ 5 d longer than optimal	2.9 (1.0–8.0)	0.04
White blood cell G3+ toxicity	4.5 (1.3–14.8)	0.01
Distant recurrence		
Immunosuppressive disorders	2.6 (1.1–6.1)	0.03
White blood cell G3+ toxicity	2.2 (1.0–4.8)	0.045
Overall survival		
Male gender	4.6 (2.0–10.3)	<0.001
Time to treatment initiation $\geq$ 62 d	2.5 (1.2–5.3)	0.01
RTT $\geq$ 5 d longer than optimal	3.2 (1.3–7.8)	0.01
White blood cell G3+ toxicity	3.2 (1.4–7.1)	0.004
Anal cancer specific survival		
Male gender	3.3 (1.2–8.7)	0.02
Immunosuppressive disorders	4.1 (1.6–10.7)	0.003
No concomitant chemotherapy	4.0 (1.1–15.0)	0.04
White blood cell G3+ toxicity	3.7 (1.2–11.2)	0.02
Non- ->anal cancer death		
Male gender	4.9 (1.5–16.5)	0.01
Gastrointestinal G3+ toxicity	4.0 (1.3–12.7)	0.02

RTT: radiation treatment time

Taken together, these sensitivity analyses did not find any evidence to suggest that the inferior prognosis of patients with WBC G3+ toxicity was a consequence of reduced doses of chemotherapy or a prolonged RTT.

Discussion

This study investigated predictors for recurrence and survival in a well-characterized cohort of anal cancer patients treated with CRT. The most important finding was that treatment-related WBC G3+ toxicity was an independent predictor for both recurrence and survival. Sensitivity analyses suggested that the inferior prognosis for patients with WBC G3+ toxicity was not a consequence of reduced doses of chemotherapy or prolongation of RTT.

In a *post hoc* analysis from the ACT II trial, Glynne-Jones *et al*. recently reported that reductions/delays of the second cycle of chemotherapy, and prolongation of RTT were associated with worse survival [17]. Specific causes for delays and interruptions were not reported, but one reason for dose reductions of the second cycle of chemotherapy and interruptions to radiotherapy according to the ACT II study protocol was G3+ hematological toxicity. While we strongly agree with the authors' conclusion – namely, that radiotherapy should be delivered in a timely manner in high volume facilities, avoiding interruptions – the results of our sensitivity analyses suggest that completion of per-protocol CRT does not abrogate the inferior prognosis for patients with WBC G3+ toxicity. However, the relatively small number of events and heterogeneous treatment delivery might have biased our data, and further studies are needed.

A limitation of our study was the lack of information on different subtypes of leukocytes. A majority of the patients with WBC G3+ toxicity also had G3+ neutropenia (60 of 74; 81%). In univariate analysis, the associations between G3+ neutropenia and recurrence/survival were weaker than the associations between WBC G3+ and recurrence/survival, indicating that the predictive ability of WBC was not driven mainly by neutropenia. Since no differential counts were performed we do not know for certain, but given the fact that the non-neutrophil pool of WBC mainly consists of lymphocytes, it seems reasonable to assume that the associations could be explained by lymphopenia. This is well in line with several previous studies showing that radiation-induced lymphopenia is associated with inferior outcome in other types of cancer, e.g., cervical cancer and lung cancer [8,19–21], and also in a recently published study in anal cancer [13]. Tumor-infiltrating lymphocytes have been associated with improved effect of CRT [22], and activation of CD4 T-helper cells was associated with increased efficacy of chemotherapy in metastatic anal cancer [23], suggesting that an active immune system is important for the treatment outcome.

Interestingly, WBC toxicity was associated not only with LR, but also with DR. An increased rate of DR is likely not explained by the inability of CRT to eradicate tumor cells within the treated volume, but rather, it might indicate a defective systemic immunosurveillance in these patients. Of note, the results do not prove causality between WBC toxicity and recurrence. In other words, patients who more often experience WBC toxicity once exposed to CRT might have a worse functioning immune system in the first place. If so, in statistical terms, WBC toxicity is merely a confounding factor. The alternative hypothesis is that there really exists a causal relationship between WBC toxicity and recurrence following CRT; if that is the case, it could have an impact on the treatment of cancer in the future.

In our cohort, variables related to the immune system (WBC toxicity and immunosuppressive disorders) were better and more consistent predictors for recurrence and survival, than variables associated with 'classic' radiobiology (e.g., RTT, fall of hemoglobin during radiotherapy, and tumor size), or tumor phenotype (e.g., T4 and N+). Given the limited sample size and the retrospective nature of our study, the results should not be over interpreted. The profound effect of RTT, radiation dose, etc., on tumor control probability is indisputable [24–26]. However, we do believe that future interventional studies should focus not only on ways to increase direct cell death (e.g., dose escalation, acceleration, hypoxia modification), but also on ways to modify and enhance the immunological response against tumors (e.g., immune checkpoint inhibitors or ways to decrease myelosuppression). Immune checkpoint inhibitors have already shown an impressive impact on the prognosis of lung cancer following CRT [27], and the results of ongoing and planned clinical trials in the curative setting of anal cancer are eagerly awaited [28]. Bone marrow sparing IMRT and proton therapy could probably reduce WBC toxicity, but whether a reduction in WBC toxicity leads to improved outcomes remains to be proven [29].

Conclusions

Overall, variables related to the immune system, i.e., WBC toxicity and immunosuppressive disorders were better and more consistent predictors for recurrence and survival in our retrospective analysis of anal cancer patients, than variables

associated with 'classic' radiobiology, or tumor phenotype. Further studies are needed to confirm the findings, preferably with prospective study designs.

Disclosure statement

The authors report no conflict of interest. MPN and AG conceived of the study and collected the clinical data. MPN and JS collected the radiotherapy data. MPN and EDN carried out the statistical analyses and interpreted the results. AG, OL, and AJ were involved in the interpretation of the results. MPN drafted the manuscript and all authors critically revised and approved the final manuscript.

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