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Original Research Article

Prognostic impact of leukocyte counts before and during radiotherapy for oropharyngeal cancer

Garrett L. Jensen [a], Pierre Blanchard [a,b], G. Brandon Gunn [a], Adam S. Garden [a], C. David Fuller [a], Erich M. Sturgis [c], Maura L. Gillison [d], Jack Phan [a], William H. Morrison [a], David I. Rosenthal [a], Steven J. Frank [a,*]

[a] Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
[b] Department of Radiation Oncology, Gustave Roussy Cancer Campus, Villejuif, France
[c] Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
[d] Department of Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

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ABSTRACT

Introduction: Peripheral blood count components are accessible and evidently predictive in other cancers but have not been explored in oropharyngeal carcinoma. We examine if there is an association between the use of intensity-modulated radiotherapy (IMRT) or intensity-modulated proton therapy (IMPT) and lymphopenia, as well as if there is an association between baseline neutrophilia, baseline leukocytosis and lymphocyte nadir in oropharyngeal cancer.

Materials and Methods: Analysis started with 150 patients from a previous case to case study design, which retrospectively identified adults with oropharyngeal carcinoma, 100 treated with IMRT in 2010-2012 and 50 treated with IMPT in 2011–2014. Pretreatment leukocyte, neutrophil, lymphocyte, and hemoglobin levels were extracted, as were neutrophil and lymphocyte nadir levels during radiotherapy. We retained 137 patients with recorded pre-treatment leukocyte and neutrophil levels for associated analysis and 114 patients with recorded lymphocyte levels during radiation and associated analysis. Multivariate survival analyses were done with Cox regression.

Results: The radiotherapy type (IMRT vs. IMPT) was not associated with lymphopenia (grade 3 $P > .99$; grade 4 $P = .55$). In univariate analyses, poor overall survival was associated with pretreatment neutrophilia (hazard ratio [HR] 5.58, 95% confidence interval [CI] 1.99–15.7, $P = .001$), pretreatment leukocytosis (HR 4.85, 95% CI 1.73–13.6, $P = .003$), grade 4 lymphopenia during radiotherapy (HR 3.28, 95% CI 1.14–9.44, $P = .03$), and possibly smoking status >10 pack-years (HR 2.88, 95% CI 1.01–8.18, $P = .05$), but only T status was possibly significant in multivariate analysis (HR 2.64, 95% CI 0.99–7.00, $P = .05$). Poor progression-free survival was associated with pretreatment leukocytosis and T status in univariate analysis, and pretreatment neutrophilia and advanced age on multivariate analysis.

Conclusions: Treatment modality did not affect blood counts during radiotherapy. Pretreatment neutrophilia, pretreatment leukocytosis, and grade 4 lymphopenia during radiotherapy were associated with worse outcomes after, but establishing causality will require additional work with increased statistical power.

Introduction

Radiotherapy, with or without chemotherapy, is the treatment of choice for most patients with early [1,2] or advanced [3–5] oropharyngeal carcinoma (OPC). Five-year survival rates remain less than optimal for patients with localized disease (83%), regional disease (59%), and distant disease (36%) [6], although the discovery of human papillomavirus (HPV) as a causal factor in OPC has led to the identification of subgroups of patients with improved prognosis [7]. Although other biomarkers of survival have been examined, none other than HPV status have affected clinical care or are used routinely [8–14].

Both leukocytosis and neutrophilia at diagnosis and leukopenia during treatment have been previously associated with survival.

* Corresponding author at: Department of Radiation Oncology, Unit 1422, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.
E-mail address: sjfrank@mdanderson.org (S.J. Frank).

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Pretreatment leukocytosis is a marker of heightened inflammation and is associated with poor survival in many types of cancer [15–29]. Tumor-related leukocytosis has been associated with resistance to radiotherapy, immune suppression, and promotion of metastasis [28,29]. Like leukocytosis, neutrophilia may be a marker of late or aggressive disease [25,30]. Increased neutrophil to lymphocyte ratio and neutrophilia itself have been associated with survival in multiple cancers [17,25,27].

An unintended consequence of chemotherapy and radiation is suppression of the immune system, sometimes reflected by lymphopenia. Treatment-related lymphocytopenia, both during treatment and for up to 1 month afterwards, has been associated with shorter survival in a variety of cancer types [31,32–38]. Lymphocytes are known to be extremely radiosensitive [39], and there is a concern that radiotherapy-related lymphopenia may affect responses to immunotherapy [40,41].

Radiotherapy for OPC delivers high radiation doses to the cervical lymph nodes, which are located near the carotid arteries and jugular vein, and to the large amounts of blood circulating through these vessels. Use of intensity-modulated proton therapy (IMPT) for OPC has been shown to reduce the radiation dose to normal structures relative to intensity-modulated radiation therapy (IMRT) by an average of 25 Gy [42–46]. We hypothesized here that IMPT would be associated with lower rates of treatment-related lymphocytopenia in a cohort of 2:1 case-matched patients given IMRT or IMPT with curative intent. We analyzed the predictive significance of pretreatment leukocytosis, neutrophilia, and lymphopenia along with nadir levels of lymphocytes and neutrophils during radiotherapy.

Materials and methods

Patients

This is an update of a previous case-matched study not conducted for this purpose. That study included 50 adult OPC patients treated with IMPT from 2011 through 2014 as part of a prospective observational study of clinical outcomes, as well as 100 adult OPC patients treated with IMRT, selected from an institutional database of 512 consecutive adult patients treated with IMRT from 2010 through 2012 [43]. Out of the 150 patients, we retained 137 patients with recorded pre-treatment leukocyte and neutrophil levels for associated analysis and 114 patients with recorded lymphocyte levels during radiation for associated analysis. Because we found no difference between treatment modalities regarding blood counts or prognosis, both modalities were combined for analysis. The two groups were matched based on treatment laterality (unilateral vs. bilateral), disease site (tonsil vs. base of tongue), p16/HPV status (positive vs. negative, with missing data considered as "any category"), T status (T1–T2 vs. T3–T4), N status (N0–N1 vs. N2–N3), receipt of concurrent chemotherapy, and smoking status. Patients were not matched by age to ensure inclusion of sufficient numbers of patients. This case-matched study was approved by the appropriate institutional review board.

Treatment

The standard processes and sequence of treatment for patients with OPC at MD Anderson Cancer Center have been reported elsewhere [47–49]. At least two radiation oncologists examined all patients and target volumes were peer-reviewed for quality assurance purposes. Gross tumor plus margins were prescribed a dose of 66 Gy for small-volume disease and 70 Gy for more advanced disease, and elective regions received 54–63 Gy. For IMPT patients, a relative biological effectiveness (RBE) value of 1.1 was used. Planning for IMPT was done with an Eclipse proton therapy treatment planning system (version 8.9, Varian Medical Systems, Palo Alto, CA, USA). Planning for IMRT was done with a Pinnacle planning system (Philips Medical Systems, Andover, MA, USA). Treatment was delivered with a static gantry approach. IMRT was delivered with a Varian Medical Systems (Palo Alto CA) linear accelerator as 6-MV photons with daily image guidance [50].

Data collection and endpoint definition

Baseline patient and tumor characteristics, including smoking status (as number of pack-years [PY]) and comorbid conditions according to the Charlson Comorbidity Index [51] (CCI) were collected from the medical record. All data were prospectively recorded for the IMPT cohort and retrospectively collected for the IMRT cohort. For the current study, pretreatment leukocyte, lymphocyte, and hemoglobin levels were extracted from the electronic medical record along with nadir levels of lymphocytes and neutrophils during radiotherapy, which were measured weekly when concurrent chemotherapy was administered and sporadically if it was not. For patients who received induction chemotherapy, pretreatment levels had been measured in the blood sample drawn soonest before induction was begun. For patients who did not receive induction chemotherapy, pretreatment levels had been measured in the blood draw soonest before radiotherapy was begun. Lymphopenia was graded using the Common Terminology Criteria for Adverse Events (CTCAE), and neutrophilia and leukocytosis were defined when patient values exceeded upper normal limits.

Vital status and the dates of local and/or distant failure were updated using the electronic medical record. Survival times were updated and calculated from the end of radiotherapy to the date of the first event of interest. Events were defined as follows: death from any cause for overall survival (OS); death from any cause or disease recurrence for progression-free survival (PFS); and locoregional recurrence or distant recurrence for locoregional control and distant control. Patients were censored at their last follow-up date.

Statistical analysis

Follow-up was calculated by the reverse Kaplan–Meier method [52]. The distribution of categorical variables between patients, regardless of radiotherapy modality, with and without neutrophilia, leukocytosis, or lymphopenia were compared with chi-square or Fisher's exact tests. Survival distributions were compared with log-rank tests. Survival curves and estimates of survival at specific time points were computed with the Kaplan–Meier method. Multivariate survival analyses were done with Cox regression and included variables with $P < .25$ in univariate analysis, as well as neutrophilia or lymphopenia status, selected through an ascending stepwise selection procedure. The statistical analysis plan was predefined before the statistical analysis. All P values were 2-sided and $P < .05$ was considered to indicate a statistically significant difference. Statistical analyses were done with SAS software (Release 9.3; SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Patient, tumor, and treatment characteristics according to the presence or absence of baseline pretreatment neutrophilia and grade 4 lymphopenia during treatment are presented in Tables 1 and 2. Patients with and without baseline neutrophilia differed

Table 1
Patient, tumor, and treatment characteristics according to the presence or absence of pretreatment neutrophilia (n = 137).

Characteristics		All Patients, No. (%)	Patients with Baseline Neutrophilia, No. (%)	Patients Without Baseline Neutrophilia, No. (%)	*P* Value
Age	≤60 years	81 (59)	5 (50)	76 (60)	0.74
	>60 years	56 (41)	5 (50)	51 (40)	
Sex	Female	17 (12)	1 (10)	16 (13)	>.099
	Male	120 (88)	9 (90)	111 (87)	
Smoking status	0 PY	61 (44)	3 (30)	58 (46)	0.13
	1–10 PY	19 (14)	0 (0)	19 (15)	
	>10 PY	57 (42)	7 (70)	50 (39)	
CCI	0–1	123 (90)	7 (70)	116 (91)	0.03
	≥2	14 (10)	3 (30)	11 (9)	
Tumor site	Tonsil	71 (52)	3 (30)	68 (54)	0.20
	Base of Tongue	66 (48)	7 (70)	59 (46)	
P16 status	Positive	119 (87)	9 (90)	110 (87)	0.88
	Negative	3 (2)	0 (0)	3 (2)	
	Unknown	15 (11)	1 (10)	14 (11)	
T status	T1-T2	106 (77)	5 (50)	101 (80)	0.05
	T3-T4	31 (23)	5 (50)	26 (20)	
N status	N0-N1	22 (16)	1 (10)	21 (16)	>0.99
	N2-N3	115 (84)	9 (90)	106 (84)	
Induction CT	Yes	64 (47)	6 (60)	58 (46)	0.51
	No	73 (53)	4 (40)	69 (54)	
RT Laterality	Bilateral	117 (85)	10 (100)	107 (84)	0.36
	Unilateral	20 (15)	0 (0)	20 (18)	
Concurrent CT	Yes	94 (69)	8 (80)	86 (68)	0.72
	No	43 (31)	2 (20)	41 (32)	
Neck Dissection	Not done	105 (77)	7 (70)	98 (77)	0.25
	Before RT	12 (9)	0 (0)	12 (9)	
	After RT	20 (14)	3 (30)	17 (13)	

Abbreviations: PY, pack-years; CCI, Charlson comorbidity index; CT, chemotherapy; RT, radiotherapy.

Table 2
Patient, tumor, and treatment characteristics according to the presence or absence of grade 4 lymphopenia during treatment (n = 114).

Characteristics		All Patients, No. (%)	Patients with Grade 4 Lymphopenia, No. (%)	Patients without Grade 4 Lymphopenia,No. (%)	*P* Value
Age	≤60 years	70 (61)	10 (62)	60 (61)	>0.99
	>60 years	44 (39)	6 (38)	38 (39)	
Sex	Female	16 (14)	3 (19)	13 (13)	0.70
	Male	98 (86)	13 (81)	85 (87)	
Smoking status	0 PY	51 (45)	7 (44)	44 (45)	0.68
	1–10 PY	14 (12)	1 (6)	13 (13)	
	>10 PY	49 (43)	8 (50)	41 (42)	
CCI	0–1	104 (91)	13 (81)	91 (97)	0.12
	≥2	10 (9)	3 (19)	7 (7)	
Tumor site	Tonsil	48 (49)	8 (50)	48 (49)	>0.99
	Base of Tongue	85 (51)	8 (50)	50 (51)	
P16 status	Positive	98 (86)	13 (81)	85 (87)	0.60
	Negative	2 (2)	0 (0)	2 (2)	
	Unknown	14 (12)	3 (19)	11 (11)	
T status	T1-T2	86 (75)	12 (75.0)	74 (76)	>0.99
	T3-T4	28 (25)	4 (25.0)	24 (24)	
N status	N0-N1	18 (16)	1 (6)	17 (17)	0.46
	N2-N3	96 (84)	15 (94)	81 (83)	
Induction CT	Yes	54 (47)	8 (50)	46 (47)	>0.99
	No	60 (53)	8 (50)	52 (53)	
RT Laterality	Bilateral	100 (88)	15 (94)	85 (87)	0.69
	Unilateral	14 (12)	1 (6)	13 (13)	
Concurrent CT	Yes	96 (84)	15 (93.7)	81 (83)	0.46
	No	18 (16)	1 (6.3)	17 (17)	
Neck Dissection	Not done	86 (75)	13 (81)	73 (75)	0.77
	Before RT	13 (11)	1 (6)	12 (12)	
	After RT	15 (13)	2 (13)	13 (13)	

Abbreviations: PY, pack-years; CCI, Charlson comorbidity index; CT, chemotherapy; RT, radiotherapy.

only with respect to comorbidities (CCI ≥ 2) (n = 3 [30%] with vs. n = 11 [9%] without, *P* = .03) and T status ≥ 3 (n = 5 [50%] with vs. n = 26 [20%] without, *P* = .05). Patients with and without grade 4 lymphopenia seemed to have differences in comorbidities (CCI ≥ 2: n = 3 [19%] with vs. n = 7 [7%] without, *P* = .12) but not in T status. Neutrophilia was found to be associated with lymphopenia; grade 4 lymphopenia during radiotherapy occurred in 11 of 101 patients with normal baseline neutrophil numbers (11%) and in 5 of 9 patients with baseline neutrophilia (56%) (*P* = .0003).

Thirteen patients were excluded from the neutrophilia analysis owing to missing data on pretreatment neutrophil counts. All 13 of

those patients had received induction chemotherapy; most had unilateral radiation (n = 10 [77%]) without concurrent chemotherapy (n = 11 [85%]). Thirty-six patients were excluded from lymphopenia analysis because of missing data for lymphocyte nadir during treatment; none of those 36 patients had received concurrent chemotherapy, and most had received bilateral radiation (n = 20 [56%]) without induction chemotherapy (n = 26 [72%]).

The type of radiotherapy (IMPT vs. IMRT) was not associated with pretreatment neutrophil level ($P > .99$). The mean pretreatment neutrophil level was $4.84 \times 10^3/\mu L$ (SD = 2.37), with 10 patients (7%) having neutrophil counts above the upper limit of normal. The type of radiotherapy was not associated with nadir neutrophil number ($P > .99$) or grade 4 neutropenia during treatment ($P = .55$). The mean neutrophil nadir during treatment was $2.99 \times 10^3/\mu L$ (SD = 1.7), and 11 patients (10%) had grade 3 neutropenia.

The type of radiotherapy (IMPT vs. IMRT) was not associated with pretreatment leukocyte level ($P = .33$). The mean pretreatment leukocyte level was $7.47 \times 10^3/\mu L$ (SD = 2.55). Eleven patients (8%) had leukocyte levels above the upper limit of normal.

The type of radiotherapy was also not associated with grade 3 ($P > .99$) or grade 4 lymphopenia ($P = .26$) during treatment. The mean pretreatment lymphocyte level was $1.72 \times 10^3/\mu L$ (SD = 0.56), and the mean lymphocyte nadir during radiotherapy was $0.49 \times 10^3/\mu L$ (SD = 0.50). Grade 3 lymphopenia was present in 88 patients (77%), and grade 4 lymphopenia in 16 patients (14%). The mean pretreatment hemoglobin level was 13.1 g/dL (SD = 1.9).

Overall survival

The median follow-up time was 50 months for all patients (41 months for the IMPT group and 56 months for the IMRT group). Nine patients were censored before 2 years of follow-up after treatment. Nineteen patient deaths were recorded, 5 in the IMPT group and 14 in the IMRT group. The OS rates at 4 years were 93.6% in the IMPT group and 85.1% in the IMRT group, corresponding to an overall hazard ratio (HR) of 0.808 (95% confidence interval [CI] 0.29–2.27, $P = .69$). Twenty-seven PFS events (recurrence or death) were observed, 9 in the IMPT group and 18 in the IMRT group, leading to 4-year PFS rates of 78% in the IMPT group and 82% in the IMRT group, corresponding to an overall HR of 1.03 (95% CI 0.46–2.30, $P = .94$).

Findings from the analyses of OS are presented in Table 3 and Fig. 1. In univariate analyses, pretreatment neutrophilia, pretreatment leukocytosis, grade 4 lymphopenia during treatment, and smoking status of >10 PY were associated with poorer OS (hazard ratios [HRs]: 5.58 for pretreatment neutrophilia [95% CI 1.99–15.7, $P = .001$], 4.85 for pretreatment leukocytosis [95% CI 1.73–13.6, $P = .003$], 3.28 for grade 4 lymphopenia during treatment [95% CI 1.14–9.44, $P = .03$], and 2.88 for smoking >10 PY [95% CI 1.01–8.18, $P = .05$]). In multivariate analyses, T status was the only possibly significant factor affecting OS (HR 2.64 [95% CI 0.99–7.00], $P = .05$). Apparent differences were noted in CCI and grade 4 lymphopenia during treatment, but the P values for these comparisons were not significant (CCI: HR 3.05 [95% CI 0.93–10.0, $P = .06$]; grade 4 lymphopenia: HR 2.34 [95% CI 0.77–7.06, $P = .13$]). Grade 3 lymphopenia was not associated with OS. Findings from the multivariate analysis of leukocytosis are not presented because leukocytosis is strongly correlated with neutrophilia, is not particularly specific, and was not associated with any other variable on multivariate analysis.

Associations between blood counts and progression-free survival

Results from the PFS analysis are presented in Table 4. In univariate and multivariate analyses, pretreatment neutrophilia (HR 3.7,

Table 3
Univariate and multivariate analyses of associations with overall survival.

Characteristics		Univariate		Multivariate	
		HR (95% CI)	P	HR (95% CI)	P
RT type	IMRT	1		–	
	IMPT	0.81 (0.29–2.27)	0.69	–	
Pre-RT neutrophilia	No	1			
	Yes	5.58 (1.99–15.7)	0.001		
Pre-RT leukocytosis	No	1		–	
	Yes	4.85 (1.73–13.6)	0.003	–	
Grade 4 lymphopenia during RT	No	1		1	
	Yes	3.28 (1.14–9.44)	0.03	2.34 (0.77–7.06)	0.13
Age	≤60 years	1		–	
	>60 years	2.45 (0.96–6.24)	0.06	–	
Sex	Female	1		–	
	Male	2.77 (0.37–20.7)	0.32	–	
Smoking status	0 PY	1		–	
	0–10 PY	1.30 (0.25–6.72)	0.75	–	
	>10 PY	2.88 (1.01–8.18)	0.05	–	
Charlson Comorbidity Index	0–1	1		1	
	≥2	2.62 (0.86–7.97)	0.09	3.05 (0.93–10.0)	0.06
Tumor site	Tonsil	1		–	
	Base of Tongue	1.37 (0.55–3.38)	0.50	–	
T status	T1-T2	1		1	
	T3-T4	3.9 (1.59–9.70)	0.003	2.64 (0.99–7.00)	0.05
N status	N0-N1	1		–	
	N2-N3	2.42 (0.56–10.5)	0.24	–	
Induction CT	No	1		–	
	Yes	1.78 (0.71–4.42)	0.21	–	
Concurrent CT	No	1		–	
	Yes	3.18 (0.93–10.9)	0.07	–	
Neck dissection	No	1		–	
	Yes	1.56 (0.59–4.10)	0.37	–	

Abbreviations: HR, hazard ratio; CI, confidence interval; IMRT, intensity-modulated radiotherapy; IMPT, intensity-modulated proton therapy; PY, pack-years; CCI, Charlson comorbidity index; CT, chemotherapy; RT, radiotherapy. HRs were not estimated for HPV-negative or unilateral RT patients, owing to the small numbers of patients/events in these groups.

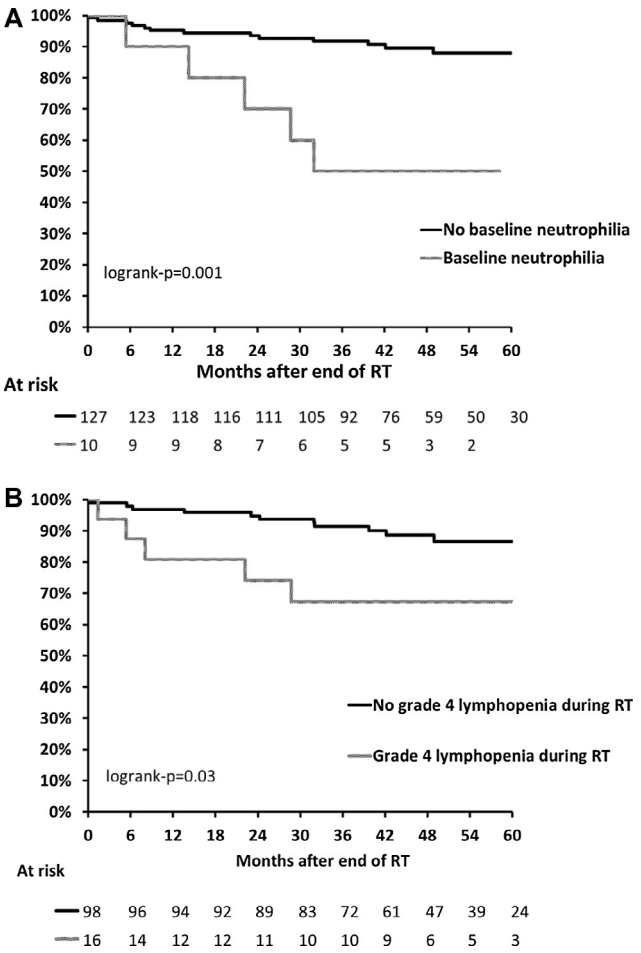

Fig. 1. Overall survival according to the presence of pretreatment neutrophilia (A) or grade 4 lymphopenia during radiotherapy (B) for oropharyngeal cancer.

95% CI 1.35–10.18, $P = .01$) and age > 60 years (HR 3.46, 95% CI 1.39–8.60, $P = .008$) were associated with poorer PFS. Pretreatment leukocytosis (HR 3.74, 95% CI 1.51–9.30, $P = .004$) and T status (HR 2.54, CI 1.16–5.54, $P = .02$) were significant only on univariate analysis.

Finally, in terms of locoregional and distant control, univariate analysis showed no association between locoregional control pretreatment neutrophilia ($P = .36$) or grade 4 lymphopenia during radiotherapy ($P = .17$), but distant control may have been associated with pretreatment neutrophilia (HR 4.37, $P = .07$) or grade 4 lymphopenia during radiotherapy (HR 3.93, $P = .05$). Multivariate analysis showed that age > 60 was associated with locoregional control (HR 2.97, 95% CI 1.10–8.05, $P = .02$), but neither age nor grade 4 lymphopenia during radiotherapy were associated with distant control (HR 3.33, 95% CI 0.83–13.34, $P = .09$; and HR 3.66, 95% CI 0.91–14.7, $P = .07$).

Discussion

Our key findings from this analysis of the implications of abnormal blood cell counts at diagnosis and during radiotherapy for OPC are as follows. High neutrophil counts before treatment were associated with high comorbidity scores and possibly with larger tumors, whereas lymphopenia during treatment was not associated with any clinical or tumor-related characteristics. No differences in lymphocyte nadir during radiotherapy were found according to use of IMPT versus IMRT. However, baseline neutrophilia and grade 4 lymphopenia during treatment were both associated with OS in multivariate analysis (Fig. 1).

Compared with IMRT, use of IMPT was not associated with differences in blood cell counts or outcomes (OS, PFS, and locoregional and distant control) in this study. This finding contradicts our hypothesis that avoiding unnecessary radiation by using IMPT [42] would reduce the incidence of lymphopenia during radiotherapy, as was shown for esophageal carcinoma [53]. The high rate of grade 3 lymphopenia during treatment in this study (77%) also contradicts our hypothesis. However, the noted correlation between pretreatment neutrophilia and grade 4 lymphopenia during treatment suggests that susceptibility to lymphopenia during radiotherapy is increased by a heightened cancer-related inflammatory state.

Others have shown that leukocytosis and neutrophilia before treatment and treatment-related lymphocytopenia have predictive value in anal and cervical cancer [25,54]. These squamous cell cancers are similar biologically to OPC, primarily because of their association with chronic HPV infection. Schernberg and colleagues found that both neutrophilia and leukocytosis were strongly associated with OS, PFS, and local control on multivariate analysis of anal cancer, and Cho and colleagues found similar results for treatment-related lymphocytopenia in cervical cancer [25,54]. Notably, because most of our patients in both the IMRT and IMPT groups were HPV-positive, we could not assess potential differences between viral-induced and alcohol- or tobacco-induced malignancies.

Leukocytosis and neutrophilia both indicate heightened inflammation. In our study, both were found to be associated with comorbidity and tumor size (T status), but lymphopenia during treatment was not. This finding may indicate that leukocytosis and neutrophilia could reflect advanced disease, leading to poorer outcomes regardless of chemotherapy or radiation [16–18,20–22]. However, some have suggested that addressing (reversing) pretreatment neutrophilia may reverse the poor prognosis associated with this factor [55,56]. Neutrophils are known to alter the tumor microenvironment through various mechanisms that support cancer growth [56–58], as was specifically demonstrated in lung colonization of breast cancer cells [59–61]. Although the pro-tumor effects of neutrophils may have therapeutic potential [62,63], neutrophils also have antitumor effects as well [56]. These properties [55,64], and their potential interactions with immunotherapy and radiotherapy [40,65–67], are also being explored. Patients in our study who experienced grade 4 lymphopenia had normal lymphocyte levels before induction chemotherapy or concurrent chemoradiation; if lymphopenia reflects an adverse treatment reaction and immune depression, that may explain its link to poorer outcomes [31–34,36,38,54].

In this study, blood components were unaffected by the type of radiotherapy used. In a similar propensity-matched study of patients with esophageal cancer treated with IMRT or IMPT, receipt of IMRT and having a larger planning target volume both predicted grade 4 lymphopenia [53]. This discrepancy between studies may have two primarily anatomic explanations. First, high splenic doses have been shown to increase the risk of developing severe lymphopenia after concurrent chemoradiation [68]; the splenic doses associated with treating OPC were undoubtedly much lower than those for treating esophageal cancer. The second explanation may involve differences in numbers of circulating lymphocytes. Treatment of esophageal cancer with IMRT generally involves large radiation doses to the heart, superior vena cava, and aorta; these doses can all be reduced by using IMPT. Treatment of OPC involves considerably less exposure of the larger blood vessels, and the carotids are within the treatment volume regardless of the technique used.

This small, retrospective study had certain inherent limitations. The numbers of patients were small, all were treated at a single institution, and several patients included in the initial

Table 4
Univariate and multivariate analyses of associations with progression-free survival.

Characteristics		Univariate		Multivariate	
		HR (95% CI)	P	HR (95% CI)	P
RT type	IMRT	1		–	
	IMPT	1.03 (0.46–2.30)	0.94	–	
Pre-RT neutrophilia	No	1		1	
	Yes	4.36 (1.75–10.9)	0.002	3.70 (1.35–10.18)	0.01
Pre-RT leukocytosis	No	1		–	
	Yes	3.74 (1.51–9.30)	0.0044	–	
Grade 4 lymphopenia during RT	No	1		–	
	Yes	2.34 (0.86–6.38)	0.10	–	
Age	≤60 years	1		1	
	>60 years	3.44 (1.54–7.66)	0.003	3.46 (1.39–8.60)	0.008
Sex	Female	1		–	
	Male	3.92 (0.53–28.9)	0.18	–	
Smoking status	0 PY	1		–	
	0–10 PY	0.37 (0.05–2.90)	0.34	–	
	>10 PY	2.23 (0.99–4.99)	0.05	–	
Charlson Comorbidity Index	0–1	1		–	
	≥2	1.70 (0.59–4.92)	0.33	–	
Tumor site	Tonsil	1		–	
	Base of Tongue	1.06 (0.50–2.26)	0.87	–	
T status	T1-T2	1		–	
	T3-T4	2.54 (1.16–5.54)	0.02	–	
N status	N0-N1	1		–	
	N2-N3	0.89 (0.36–2.20)	0.80	–	
Induction Chemotherapy	No	1		–	
	Yes	1.37 (0.65–2.92)	0.41	–	
Concurrent Chemotherapy	No	1		–	
	Yes	0.99 (0.45–2.15)	0.97	–	
Neck Dissection	No	1		–	
	Yes	2.03 (0.93–4.44)	0.07	–	

Abbreviations: HR, hazard ratio; CI, confidence interval; IMRT, intensity-modulated radiotherapy; IMPT, intensity-modulated proton therapy; PY, pack-years; CCI, Charlson comorbidity index; CT, chemotherapy; RT, radiotherapy. HRs were not estimated for HPV-negative or unilateral RT patients, owing to the small numbers of patients/events in these groups.

case-matched analysis were excluded owing to missing blood cell measurements. However, these patients are typical of those who seek treatment at MD Anderson. Data were prospectively recorded only for IMPT patients, which could have led to bias. We could not investigate the relationship between leukocyte counts and prognosis in HPV-negative OPC, because most of the patients in our study had HPV-positive OPC. However, the reproducibility of the present findings in non-viral–related tumors (e.g., esophageal carcinoma) suggests that this relationship may be valid in a broad range of tumors. Finally, almost all patients in this study received induction or concurrent chemotherapy; indeed, blood cell counts were obtained to monitor chemotherapy-induced toxicity. Thus, whether the observed relationships hold true for use of radiotherapy alone, or whether radiotherapy, chemotherapy, and blood cell count abnormalities interact in some way, remains unknown.

Another line of evidence underscoring the importance of immune system preservation for patients undergoing radiotherapy for cancer is the documentation of a dose-volume association between irradiated bone marrow and moderate to severe lymphopenia [69]. Because radiation has both depressive and enhancing effects on the immune response, it could reduce the effectiveness of immunotherapy [40,41]. Treatment plans could be modified to emphasize sparing of the bone marrow or blood vessels, although this could be difficult for patients with head and neck cancer owing to the proximity of blood vessels and nodal basins.

In conclusion, neutrophilia and leukocytosis before treatment, as well as grade 4 lymphopenia during treatment, were associated with worse outcomes in patients who received chemotherapy and radiation for OPC. Use of IMPT versus IMRT did not affect blood component counts during treatment, demonstrating that the tissue-sparing effects of IMPT for patients with squamous cell, HPV-positive OPC may not significantly affect lymphocyte counts. Because none of the patients for whom data on lymphocyte nadir during radiation were missing had received chemotherapy, prospective investigation of blood counts in patients receiving radiation without concurrent chemotherapy is warranted. Our own future work includes expansion of our institutional database of patients with OPC to enable us to conduct analyses with more statistical power and potentially elucidate causal relationships. A multi-institutional randomized trial comparing concurrent chemoradiation strategies involving IMPT or IMRT is underway and is expected to provide additional robust data on blood cell counts before and during treatment to validate the associations determined in the current study. Post-treatment blood counts will also be analyzed to assess potential relationships between chronic toxicity and disease outcomes, with emphasis given to investigating the relationship between vascular structure dose and lymphopenia and the relationship between blood component abnormalities and both toxicity and efficacy outcomes.

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Conflicts of interest statement

No author has relevant conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ctro.2017.09.008.

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