Original Article

Prognostic impact of lymphopenia and neutrophil-lymphocyte ratio for patients with anal squamous cell carcinoma

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Background: Outcomes after definitive chemoradiation for squamous cell carcinoma are generally favorable. However, biomarkers to further yield prognostic information are desired. Treatment-related lymphopenia as well as an elevated baseline neutrophil-lymphocyte ratio have been associated with worse survival in several cancer types. We evaluated absolute lymphocyte count and neutrophil-lymphocyte ratio at baseline and at treatment-related nadir in patients with anal cancer for associations with oncologic endpoints.

Methods: We conducted a retrospective analysis of 428 consecutive patients with non-metastatic anal cancer treated with definitive, intensity-modulated radiation therapy-based chemoradiation. We analyzed absolute neutrophil and lymphocyte counts at several timepoints: pretreatment, weekly during treatment, and in the six weeks following treatment completion. Neutrophil-lymphocyte ratio was calculated at baseline and treatment-related nadir. We estimated oncologic endpoints using life tables and compared them using the log-rank test. We conducted univariate and multivariable time-to-event analyses using Cox proportional hazards.

Results: Median absolute lymphocyte count at baseline and nadir were 1.80 [interquartile range (IQR), 1.45–2.32] k/μL and 0.26 (IQR, 0.18–0.36) k/μL, respectively, and 31% developed treatment-related grade 4 lymphopenia. Median neutrophil-lymphocyte ratio at baseline and nadir were 2.34 (IQR, 1.68–3.30) and 8.80 (IQR, 5.86–12.68), respectively. Estimates of overall survival, local failure-free survival, distant metastasis-free survival (DMFS), and freedom from colostomy at 5 years were 87%, 86%, 82%, and 88%, respectively. Baseline and nadir absolute lymphocyte count were not associated with selected outcomes on univariate analysis. On multivariable analysis, factors independently associated with death included T3-T4 disease, HIV-positive status, treatment break, and baseline neutrophil-lymphocyte ratio >3. Baseline neutrophil-lymphocyte ratio showed a trend toward association with distant progression or death (P=0.07). The 5-year overall survival estimates for patients with baseline neutrophil-lymphocyte ratios ≤3 and >3 were 92.3% and 80.6%, respectively.

Conclusions: Lymphopenia during and after chemoradiation for anal cancer is common but does not appear to be associated with worse survival, recurrence, or metastases. However, elevated baseline neutrophil-lymphocyte ratio was independently associated with overall survival, local recurrence-free survival, and DMFS. Further studies are needed to determine the clinical utility of baseline neutrophil-lymphocyte ratio to guide treatment and follow-up.

Keywords: Radiation; anal cancer; neutrophil-to-lymphocyte ratio (NLR); neutrophil; lymphopenia

Submitted Jun 21, 2021. Accepted for publication Aug 23, 2021.
doi: 10.21037/jgo-21-323
View this article at: https://dx.doi.org/10.21037/jgo-21-323

Introduction

The current standard of care for squamous cell carcinoma of the anal canal (SCCA) was established by the Radiation Therapy Oncology Group (RTOG) 9811 trial and includes definitive chemoradiation (CRT) with 5-fluorouracil (5-FU) and mitomycin-C (MMC) (1). However, studies have shown equivalent oncologic outcomes with concurrent 5-FU and cisplatin (2), which are also commonly used (3). While landmark studies utilized three dimensional conformal radiotherapy treatment techniques, intensity-modulated radiotherapy (IMRT) emerged as the standard of care in the treatment of SCCA after RTOG 0529 demonstrated that IMRT reduced grade 2 or greater (G2+) hematologic and G3+ dermatologic toxicity (4). While SCCA has excellent cure rates overall, patients with T3/T4 and/or node positive disease have 5-year overall survival (OS) rates ranging from 42–74% (5).

While standard clinical prognostic factors such as T- and N-stage remain important, there has been growing interest in identifying biomarkers for oncologic outcomes for SCCA (6). Blood test-based biomarkers are attractive for their non-invasive nature and ease of serial measurement. Absolute treatment-related lymphocyte count (ALC) nadir has been associated with worse OS in several solid tumors such as liver (7), pancreatic (8), esophageal (9) and lung (10) malignancies. A recent study of patients with SCCA showed treatment-related lymphopenia was common, and severe lymphopenia may be a prognostic factor for worse OS (11). In addition to lymphopenia, an elevated neutrophil-to-lymphocyte ratio (NLR) has been associated with worse OS for many solid tumor types (12) including for SCCA (13). It is unknown which plasma biomarker yields the best prognostic information for patients with SCCA. It is also unknown whether baseline values or nadir values are more closely associated with outcomes. The purpose of this study is to evaluate a large consecutive cohort of patients with SCCA treated with IMRT-based chemoradiation and to assess the prognostic value of baseline and nadir ALC and NLR on oncologic outcomes. We present the following article in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting checklist (available at https://dx.doi.org/10.21037/jgo-21-323) (14).

Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board at MD Anderson Cancer Center (protocol 2020-0513). Because of the retrospective nature of the study, the requirement for informed consent was waived. We conducted a retrospective cohort study of all patients with SCCA treated with definitive IMRT-based CRT at our institution from 1/1/2003 until 12/31/2018. We excluded patients who received chemotherapy prior to CRT, who had metastatic disease at the time of diagnosis, or who were treated with 3D conformal radiation or proton therapy.

Treatment details

A multidisciplinary team evaluated each patient prior to initiating treatment. A pathologist at our institution confirmed squamous cell histology before initiating treatment. Patients received definitive CRT using an IMRT technique previously described (15). The treating radiation oncologist selected dose and fractionation to the primary tumor based on tumor size; T1 tumors received 50 Gy in 25 fractions, T2 tumors received 54 Gy in 27 fractions and T3 and T4 tumors received 58 Gy in 29 fractions. Elective dose to the pelvis was contingent upon the number of fractions: 43 Gy in 25 fractions, 45 Gy in 27 fractions and 47 Gy in 29 fractions. Dose to involved lymph nodes was contingent upon size: 50 Gy for nodes <2 cm, 54 Gy for nodes 2–5 cm and 58 Gy for nodes >5 cm. Concurrent chemotherapy consisted of either weekly cisplatin (20 mg/m^2 intravenously once weekly) and daily 5-FU (300 mg/m^2/day infused continuously on days of radiation) (3) or MMC (10 mg/m^2 on day 1 and day 28) and 5-FU (1,000 mg/m^2/day infused continuously days 1–4 and 29–32). Some patients received capecitabine (825 mg/m^2 twice daily orally on days of radiation) in lieu of 5-FU at the treating medical oncologist's discretion.

Patients underwent laboratory studies including a complete blood count with differential before starting

treatment and weekly during treatment. The treating physicians evaluated patients every three to six months for five years.

Data collection

We analyzed all consecutive patients meeting inclusion criteria. We collected patient demographic, tumor and treatment characteristics from patient medical records. We collected white blood cell count, absolute neutrophil count (ANC) and ALC at baseline, weekly during treatment, and up to six weeks post completion of CRT. NLR was calculated. We recorded the nadir as the lowest value during the period from treatment initiation to six weeks following the conclusion of CRT. We recorded oncologic endpoints including locoregional failure (LRF), distant metastases (DM) and OS. We defined LRF as either recurrence of disease in the anal canal and/or regional lymph nodes after complete clinical response (cCR) or biopsy-proven persistence of disease at least six months after completion of CRT.

Statistical analysis

We summarized patient baseline characteristics and compared them using *t*-tests for continuous variables and χ^2 tests for categorical variables. The median follow-up with associated confidence interval was calculated using the reverse Kaplan-Meier method. We defined OS as the latency between the end of CRT and death of any cause or last follow up. We defined composite endpoints locoregional failure-free survival (LFFS) and distant metastasis-free survival (DMFS) as the latencies between the end of CRT and recurrence/death or last follow-up. We defined freedom from colostomy as the latency from the end of CRT to placement of permanent colostomy or last follow-up. We estimated time-to-event endpoints at various time points using life tables and compared using the log-rank test. We conducted univariate and multivariable time-to-event analyses using Cox proportional hazards. We assessed proportional hazards assumptions using χ^2 tests of Schoenfeld residuals. We used a threshold P value of 0.05 on univariate analysis to select variables for inclusion in each multivariable model. For multivariable time-to-event analysis, patients with missing values in chosen variables were omitted from the respective analysis. We performed statistical analysis using Stata Version 16.0 (StataCorp, College Station, TX, USA).

Results

A total of 428 patients were included in this study. Patient, disease, and treatment characteristics are summarized in *Table 1*. The median age at diagnosis was 60 (range, 31–88) years. The majority of the cohort was female (74%) and white (92%). The median tumor size in greatest dimension was 3.5 [interquartile range (IQR), 2.0–5.0] cm. Among all patients, 64% had American Joint Committee on Cancer (AJCC) 8th Edition T1 or T2 disease, 36% had T3 or T4 disease, and 51% had N1 disease. Median ALC prior to CRT and at nadir were 1.80 (IQR, 1.45–2.32) k/μL and 0.26 (IQR, 0.18–0.36) k/μL, respectively, and 31% developed treatment-related G4 lymphopenia. Median NLR prior to CRT and at nadir were 2.34 (IQR, 1.68–3.30) and 8.80 (IQR, 5.86–12.68), respectively.

cCR was achieved in 93% of patients at a median 2.8 (range, 0.1–24.5) months following the completion of CRT. At a median follow-up of 5.3 [95% confidence interval (CI), 4.8–5.8] years, 89% were alive at last follow-up. Colostomy was required in 11%. Recurrence was seen in 18% of patients at a median of 10.5 (range, 5.3–17.9) months following the completion of CRT. Time-to-event estimates are shown in *Table 2*. Estimates of OS, LFFS, DMFS, and freedom from colostomy at 5 years were 87%, 86%, 82%, and 88%, respectively.

Factors on univariate analyses significantly associated with death and/or progression are summarized in *Table 3*. Factors associated with worse OS, LFFS, and DMFS included T3-T4 disease, human immunodeficiency virus (HIV)-positive status, current smoking, treatment break, higher baseline ANC, and baseline NLR >3. Additionally, older age at diagnosis was associated with a higher risk of death. ANC nadir was associated with a higher risk of locoregional progression. A greater latency from diagnosis to RT start was associated with a higher risk of distant progression. Finally, node-positive disease was associated with a higher risk of locoregional and distant progression. Baseline ALC, ALC nadir, and lymphopenia grade were not associated with selected outcomes on univariate analysis. Kaplan-Meier curves of OS stratified by baseline NLR is shown in *Figure 1*.

Variable selection for multivariable models considered the variables shown in *Table 3*. Given significant collinearity between hematologic parameters, a single hematologic parameter with the largest significant effect size, baseline NLR ≤3 or >3, was chosen for all three multivariable

Table 1 Baseline patient, tumor and treatment characteristics

Parameter	Value
Median age at diagnosis (range)	60 [31–88]
Sex	
Male	110 (26%)
Female	318 (74%)
Race	
White	392 (92%)
Black	28 (7%)
Asian	5 (1%)
Other	3 (1%)
Smoking status	
Never	213 (50%)
Former	148 (35%)
Current	66 (15%)
HIV-positive patients	20 (5%)
Tumor size (IQR), cm	3.5 (2.0–5.0)
T stage	
1	80 (19%)
2	192 (45%)
3	105 (25%)
4	51 (12%)
N stage	
0	210 (49%)
1	218 (51%)
Chemotherapy regimen	
Cisplatin-containing	334 (78%)
MMC-containing	73 (17%)
Other	21 (5%)
Median PTVp dose [IQR], Gy	54 [54–58]
Median # of fractions [range]	27 [24–36]
Median time from diagnosis to RT [IQR], days	47 [34–62]
Duration of RT [IQR], days	38 [35–40]
Median baseline ANC in k/μL (IQR)	4.15 (3.19–5.57)
Nadir ANC (IQR)	2.28 (1.62–2.94)
Neutropenia grade (n=401)	
0	284 (71%)
1	32 (8%)
2	57 (14%)
3	22 (5%)
4	6 (1%)
Median baseline ALC in k/μL (IQR)	1.80 (1.45–2.32)
Median nadir ALC (IQR)	0.26 (0.18–0.36)
Lymphopenia grade (n=401)	
0	1 (0%)
1	1 (0%)
2	33 (8%)
3	240 (60%)
4	126 (31%)
Median baseline NLR (IQR)	2.34 (1.68–3.30)
Median nadir NLR (IQR)	8.80 (5.86–12.68)
Treatment break required	49 (11%)
Hospitalization required	63 (15%)
Achieved cCR	396 (93%)
Median time to cCR (range), months	2.8 (0.1–24.5)
Any failure	76 (18%)
Median time to any failure (range), months	10.5 (5.3–17.9)
Locoregional failure	57 (13%)
Regional failure	17 (4%)
Distant failure	35 (8%)
Colostomy required	47 (11%)
Deceased at last follow up	47 (11%)
Median follow-up time (95% CI) (reverse Kaplan-Meier method), years	5.3 (4.8–5.8)

IQR, interquartile range; CRT, chemoradiation; MMC, mitomycin C; IMRT, intensity modulated radiotherapy; VMAT, volumetric modulated arc therapy; PTVp, planning target volume of primary mass; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; NLR, neutrophil to lymphocyte ratio; cCR, clinical complete response.

Table 2 Time to event estimates at 1, 2, 3, and 5 years following treatment

Metric	Median (95% CI)	1-yr (95% CI)	2-yr (95% CI)	3-yr (95% CI)	5-yr (95% CI)
Overall survival	Not reached	96.7% (94.5–98.0%)	93.8% (91.0–95.7%)	90.0% (86.6–92.3%)	86.5% (82.5–89.6%)
Locoregional progression-free survival	Not reached	91.9% (88.8–94.1%)	88.3% (84.8–91.1%)	87.4% (83.8–90.3%)	86.3% (82.4–89.4%)
Distant metastasis-free survival	Not reached	92.3% (90.0–95.0%)	88.8% (85.3–91.4%)	85.3% (81.4–88.4%)	81.7% (77.3–85.3%)
Freedom from colostomy	Not reached	94.0% (91.1–95.9%)	90.1% (85.8–92.6%)	89.5% (85.1–92.2%)	88.4% (86.7–91.2%)

CI, confidence interval.

Table 3 Univariable analysis for factors associated with survival outcomes

Parameters	For death			For locoregional failure or death			For distant failure or death		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Age	1.026	1.00001–1.05208	0.050*	1.007	0.987–1.027	0.489	1.016	0.994–1.039	0.162
Sex									
Female	(Ref)			(Ref)			(Ref)		
Male	1.644	0.981–2.756	0.059	1.211	0.782–1.875	0.392	1.495	0.936–2.387	0.092
T stage									
T1-T2	(Ref)			(Ref)			(Ref)		
T3-T4	2.268	1.385–3.713	0.001*	2.357	1.586–3.504	<0.001*	2.534	1.628–3.945	<0.001*
N stage									
0	(Ref)			(Ref)			(Ref)		
1	1.544	0.932–2.558	0.092	2.022	1.335–3.062	0.001*	1.963	1.240–3.109	0.004*
HIV	3.128	1.422–6.879	0.005*	2.279	1.105–4.701	0.026*	2.701	1.299–5.616	0.008*
Smoking history									
Never	(Ref)			(Ref)			(Ref)		
Former	0.953	0.527–1.724	0.874	0.920	0.568–1.487	0.732	0.874	0.516–1.480	0.617
Current	2.098	1.148–3.833	0.016*	2.480	1.541–3.992	<0.001*	1.954	1.136–3.361	0.015*
Radiation dose									
≤54 Gy	(Ref)			(Ref)			(Ref)		
>54 Gy	2.892	1.741–4.805	<0.001*	3.070	2.047–4.605	<0.001*	3.299	2.090–5.206	<0.001*
Time from diagnosis to RT start (weeks)	1.002	0.998–1.006	0.421	1.003	0.9995–1.0073	0.081	1.005	1.001–1.008	0.010*
Concurrent chemo									
Cisplatin-containing	(Ref)			(Ref)			(Ref)		
MMC-containing	1.715	0.951–3.094	0.073	1.059	0.625–1.795	0.830	1.419	0.824–2.442	0.207
Other	1.965	0.831–4.647	0.124	1.723	0.828–3.583	0.145	1.876	0.851–4.133	0.119

Table 3 (*continued*)

Table 3 (*continued*)

Parameters	For death			For locoregional failure or death			For distant failure or death		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Treatment break	2.244	1.337–4.394	0.004*	1.735	1.016–2.963	0.044*	2.268	1.310–3.927	0.003*
Baseline ANC	1.1190	1.079–1.312	0.001*	1.159	1.070–1.256	<0.001*	1.157	1.056–1.268	0.002*
Baseline ALC	0.881	0.574–1.353	0.562	0.929	0.669–1.288	0.658	0.891	0.614–1.293	0.544
Baseline NLR (continuous)	1.118	1.017–1.228	0.021*	1.104	0.023–1.192	0.011	1.143	1.045–1.251	0.004
Baseline NLR (dichotomized)									
≤3	(Ref)			(Ref)			(Ref)		
>3	2.399	1.383–4.161	0.002*	1.696	1.105–2.604	0.016*	2.034	1.259–3.286	0.004*
ANC nadir	1.137	0.849–1.522	0.389	1.244	1.0003–1.5468	0.050*	0.958	0.741–1.238	0.741
ALC nadir	0.980	0.284–3.385	0.975	0.745	0.229–2.422	0.625	0.744	0.197–2.817	0.664
ALC nadir (dichotomized)									
≤G3 lymphopenia	(Ref)			(Ref)			(Ref)		
≥G4 lymphopenia	0.918	0.507–1.660	0.776	0.963	0.610–1.521	0.872	1.098	0.663–1.819	0.717
NLR nadir (continuous)	0.977	0.931–1.025	0.342	0.995	0.963–1.027	0.743	0.982	0.944–1.022	0.378
NLR nadir (dichotomized)									
≤3	(Ref)			(Ref)			(Ref)		
>3	0.560	0.201–1.557	0.266	0.984	0.361–2.687	0.975	0.468	0.202–1.083	0.076

*, significant values (P<0.05). A hazard ratio >1 indicates an increased likelihood of progression or death, whereas a hazard ratio <1 indicates a decreased likelihood. HR, hazard ratio; CI, confidence interval; HIV, human immunodeficiency virus; RT, radiotherapy; MMC, mitomycin C; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; NLR, neutrophil to lymphocyte ratio; G3, grade 3; G4, grade 4.

analyses. The final models for death, locoregional progression or death, and distant progression or death each included 400 patients to account for one or more missing values across included variables. A test of the proportional hazards assumptions for death, death, locoregional progression or death, and distant progression or death using Schoenfeld residuals yielded P values of 0.882, 0.190, and 0.476 and thus we failed to reject the null hypotheses that the hazards were proportional. Results of the multivariable analyses are summarized in *Table 4*. Factors independently associated with death included T3-T4 disease, HIV-positive status, treatment break, and baseline NLR >3. Factors independently associated with locoregional progression or death included node-positive disease, current smoking history, and treatment break. Finally, factors significantly associated with distant progression or death included T3-T4 disease, node-positive disease, increased latency between diagnosis and RT, and treatment break. In addition to independent association with OS (P=0.01), baseline NLR showed a trend toward association with distant progression or death (P=0.07). The 2- and 5-year OS estimates for patients with baseline NLR ≤3 were 96.6% (95% CI, 93.5–98.2%) and 92.3% (95% CI, 87.8–95.1%), respectively, whereas the 2- and 5-year OS estimates for patients with baseline NLR >3 were 90.5% (95% CI, 83.8–94.5%) and 80.6% (95% CI, 71.8–86.9%), respectively.

Discussion

In this retrospective review of 428 patients with non-metastatic SCCA treated with definitive IMRT-based CRT, we found no significant relationship between baseline or

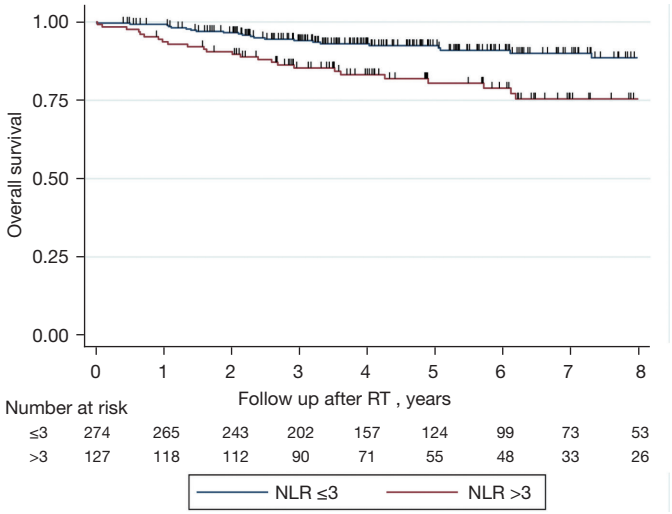

Figure 1 Overall survival for all patients stratified by baseline neutrophil to lymphocyte ratio. A significant difference was noted on log-rank test (P=0.001).

Table 4 Multivariable analysis for factors associated with survival outcomes

Parameters	For death (# of events =64)			For locoregional progression or death (# of events =99)			For distant progression or death (# of events =80)		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Age	1.029	0.999–1.060	0.060						
T stage									
T1-T2	(Ref)			(Ref)			(Ref)		
T3-T4	2.019	1.134–3.597	0.017*	1.594	0.994–2.556	0.053	1.812	1.056–3.107	0.031*
N stage									
0				(Ref)			(Ref)		
1				1.984	1.208–3.258	0.007*	1.756	1.002–3.078	0.049*
HIV	3.805	1.300–11.142	0.015*	1.502	0.643–3.509	0.347	1.801	0.759–4.269	0.182
Smoking history									
Never	(Ref)			(Ref)			(Ref)		
Former	0.869	0.446–1.695	0.681	0.794	0.471–1.340	0.389	0.696	0.384–1.262	0.233
Current	1.625	0.792–3.332	0.185	1.887	1.098–3.244	0.021*	1.489	0.791–2.803	0.218
Time from dx to RT start (weeks)							1.005	1.001–1.008	0.016*
Treatment break	2.841	1.462–5.519	0.002*	2.434	1.371–4.321	0.002*	3.003	1.652–5.459	<0.001*
Baseline NLR (dichotomized)									
≤3	(Ref)			(Ref)			(Ref)		
>3	2.056	1.167–3.623	0.013*	1.428	0.919–2.219	0.113	1.584	0.960–2.615	0.072

*, significant values (P<0.05). A hazard ratio >1 indicates an increased likelihood of progression or death, whereas a hazard ratio <1 indicates a decreased likelihood. Shaded cells indicate that variables were excluded from the final model for that particular endpoint. HR, hazard ratio; CI, confidence interval; HIV, human immunodeficiency virus; RT, radiotherapy; NLR, neutrophil to lymphocyte ratio.

nadir ALC and oncologic outcomes. We also found no significant relationship between nadir NLR and oncologic outcomes, though we did find higher NLR at baseline was independently associated with worse OS.

Our study highlighted several clinical prognostic factors that have been consistently demonstrated elsewhere, including T-stage, HIV positive status, and having an unplanned break during CRT. T-stage has often been shown to be strongly prognostic for LRF, DFS and OS (1,5,16). The correlation between HIV positive status and worse oncologic outcomes is less clear. Older studies performed before the advent of effective antiretroviral therapy suggest poorer survival for patients living with HIV (17). However, modern series have shown patients living with HIV have similar survival compared with patients who are not (18). Our data suggest worse outcomes for these patients, despite the fact that most patients in our cohort were taking antiretroviral therapy and had CD4 counts >200. However, these results should be approached with caution given that less than 5% in our series were living with HIV. Finally, large database studies have suggested prolongation of CRT with unplanned treatment breaks may be associated with worse survival (19,20).

Lymphopenia is common during CRT as circulating lymphocytes are exquisitely sensitive to radiation (21). Lymphopenia is a potential surrogate for decreased immune-mediated systemic tumor surveillance, and this may be of particular importance for HPV-associated cancers (22). Human papillomavirus (HPV) status and p16 expression are associated with excellent local response of SCCA to CRT. Conversely, increased p53 expression is associated with worse outcomes after CRT for SCCA (23).

Treatment-related ALC nadir has been associated with worse OS in several cancer types (7-10,24). The lack of significant association between treatment-related ALC nadir and oncologic outcomes in our cohort is notable. In contrast, Lee *et al.* recently found a 3.7-fold increase in death for patients with non-metastatic SCCA who developed treatment-related G4 lymphopenia compared to those who did not; 5-year OS was 32% and 86%, respectively (11). However, the number of patients who developed G4 lymphopenia in this paper was small: 11 (8%) patients. It is possible this large nominal difference in OS was driven by small subgroup sizes. Patients in the Lee *et al.* study who developed G4 lymphopenia also had a lower baseline ALC, potentially confounding the interpretation of these results. There were also some important differences between our cohort and the cohort described by Lee *et al.* In the current analysis, the majority of patients were treated with weekly cisplatin and 5-FU. Only a minority (17%) were treated with MMC, compared with 92% of patients in the Lee *et al.* cohort. Despite adjustment for chemotherapy type on multivariable analysis, however, we failed to observe a significant association between treatment-related ALC nadir and survival. The present study is not the only large study of HPV-related squamous cell carcinoma that failed to show a relationship between ALC and survival; in a cohort of 850 patients treated with CRT for oropharyngeal cancer, treatment-related ALC nadir was also not significantly associated with worse outcomes (25).

NLR is considered to be a composite marker of inflammation and immune response. Though inflammation plays an important role in the progression of several cancer types, data on the prognostic value of NLR are mixed. Studies in lung cancer show a strong relationship between high pretreatment NLR and worse OS and PFS (26). Studies in esophageal cancer show elevated baseline NLR is significantly associated with worse overall survival for patients treated with CRT, but not for patients treated with surgery alone (27). Similarly, studies in colorectal cancer treated with surgery alone show worse OS for patients with a high preoperative NLR, though interestingly, no significant relationship between NLR and cancer-specific survival (28). Studies in resectable cholangiocarcinoma also failed to show a significant relationship between pre- or post-treatment NLR and survival outcomes (29). Our data did not demonstrate a significant relationship between treatment-related NLR nadir and oncologic outcomes, but did show a significant association between high baseline NLR and worse OS, local failure and distant metastases.

Limitations of this study include the retrospective nature of this study and the variable time points of blood collection for ALC and NLR analysis. While blood was collected weekly during treatment, laboratory assessment was less standardized in the six weeks after CRT completion. It is possible the true nadir was missed for some patients, potentially attenuating the odds ratios associated with these hematologic parameters. Additionally, circulating ALC and NLR are likely imperfect measures of immune response potential and generalized inflammation. Tumor infiltrating lymphocytes may be of superior prognostic value for anal cancer (30) and will be prospectively evaluated in the PLATO study (ISRCTN88455282). Additional biomarkers under investigation include circulating tumor HPV DNA (31,32) and PD-L1 (33). Only a minority of patients in this study were treated with MMC, potentially limiting the

generalizability of our results.

In conclusion, data in the present analysis demonstrate that lymphopenia during and after CRT for SCCA is common but does not appear to be associated with worse OS, local control or development of distant metastases. Elevated baseline NLR was independently associated with worse OS, LFFS and DMFS. Further studies are needed to determine the clinical utility of baseline NLR to guide treatment and follow-up.

Acknowledgments

Funding: This work was supported in part by the National Institutes of Health through Cancer Center Support Grant P30CA016672.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://dx.doi.org/10.21037/jgo-21-323

Data Sharing Statement: Available at https://dx.doi.org/10.21037/jgo-21-323

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/jgo-21-323). BD reports honoraria from Sermo, Inc. VKM reports honoraria from Projects in Knowledge; consulting/advisory relationships with Array Biopharma, Incyte, Servier, and Boehringer Ingelheim; and research funding from Bristol-Myers Squibb, Array BioPharma, EMD Serono, Boehringer Ingelheim, and Immatics. PD reports consulting/advisory relationships with Adlai Nortye and MD Anderson Cancer Center Madrid. CT reports a consulting/advisory role with Accuray. EJK reports grants from National Institutes of Health, Stand Up 2 Cancer, MD Anderson Cancer Center, Philips Healthcare, Elekta, and GE Healthcare; personal fees from RenovoRx and Taylor and Francis; and a consulting/advisory role with Augmenix. ACK reports ownership of shares in Aravive, Inc. EBH reports research funding from Merck Serono. BM serves as an unpaid editorial board member of the *Journal of Gastrointestinal Oncology* from January 2021 to December 2022. He was not involved in editorial review of the submitted work. All reported conflicts are outside of the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board at MD Anderson Cancer Center (protocol 2020-0513) Because of the retrospective nature of the study, the requirement for informed consent was waived.

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Cite this article as: De B, Ludmir EB, Messick CA, Cagley MC, Morris VK, Das P, Minsky BD, Taniguchi CM, Smith GL, Koay EJ, Koong AC, Mohan R, Holliday EB. Prognostic impact of lymphopenia and neutrophil-lymphocyte ratio for patients with anal squamous cell carcinoma. J Gastrointest Oncol 2021;12(5):2412-2422. doi: 10.21037/jgo-21-323

