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HEAD AND NECK

Systemic immune response in squamous cell carcinoma of the head and neck: a comparative concordance index analysis

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Abstract

Purpose The objective of this study was to investigate the prognostic role of three inflammatory markers: the neutrophil to lymphocyte ratio (NLR), the lymphocyte to monocyte ratio (LMR), and the platelet to lymphocyte ratio (PLR) as prognostic indicators in squamous cell carcinoma of the head and neck (HNSCC).

Methods Patients with HNSCC treated with primary surgery, with or without adjuvant radiochemotherapy were enrolled. The preoperative NLR, LMR, and PLR were recorded. Confounding variables were also recorded: age, sex, BMI, comorbidities, performance status, AJCC T and N stage and HPV status. Endpoints were overall survival (OS) and event-free survival (EFS). Survival analysis was performed using Kaplan–Meier analysis, and multivariable analysis was performed using Cox proportional hazards regression. Survival models were evaluated using Harrell's concordance index (c-index).

Results NLR ($p=0.2413$), PLR ($p=0.1593$), and LMR ($p=0.0552$) were not significantly associated with OS in the multivariable analysis. With regard to EFS, low LMR (HR = 2.95, 95% CI 1.54–5.65, $p=0.001$), high PLR (HR = 2.68, 95% CI 1.42–5.09, $p=0.003$), and high NLR (HR = 3.37, 95% CI 1.7–6.69, $p<0.001$) were associated with EFS. The multivariable c-index was highest for LMR (0.762), followed by NLR (0.761) and PLR (0.739).

Conclusion The LMR, PLR, and NLR were not associated with OS, but were associated with EFS in HNSCC. These markers are easily obtainable, and in the age of individualized patient care and precision medicine, they might represent further risk stratification tools for HNSCC patients.

Keywords Head and neck cancer · Prognosis · Neutrophil lymphocyte ratio · Platelet lymphocyte ratio · Lymphocyte monocyte ratio · Inflammation

Introduction

A complex set of interactions exist between the tumor microenvironment and the immune system [1], and it is generally acknowledged that lymphocytes, particularly T cells, are critical agents in the immune surveillance of cancer cells. It was previously shown that the presence of $CD8^+$ cytotoxic T lymphocytes in cancer cell nests confers better survival outcomes [2, 3]. These 'infiltrating' cytotoxic T cells have also been shown to express cytoplasmic granzyme B^+, a marker of an activated cytotoxic phenotype [2]. The current mechanistic theory is that MHC Class I bearing tumor cells would be susceptible to cytotoxic T cell-mediated cell death through the caspase cascade and subsequent apoptosis [2, 4].

Recent data have shown that a relative lymphopenic state might exist in a subgroup of patients who have head and neck cancer (HNC) [5]. The exact mechanism of this

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lymphopenic state is unknown at time of this writing [6]. However, this state of relative lymphopenia has also been linked to poorer survival outcomes [7]. There have been attempts to measure this state of relative lymphopenia using easily obtained serum markers—the ratio of lymphocyte count to monocyte count (LMR), the ratio of neutrophil to lymphocyte count (NLR), and the ratio of the platelet to lymphocyte count (PLR) [8–14]. In previous meta-analyses, the NLR and LMR were found to be significant prognostic indicators in HNC [6, 15]. A meta-analysis by another group also found that the PLR was a prognostic factor for survival in HNC [16].

To date, there is a lack of data regarding which of these three inflammatory markers—the NLR, LMR, and PLR—are superior, and in which specific cohorts they would be predictive of survival [6, 15]. Based on current reviews of the literature [6, 15, 16], there have been no published data on the direct comparisons across the different markers in squamous cell carcinoma of the head and neck (HNSCC). Since all three markers involve the lymphocyte count, there will be strong collinearity (a linear statistical relationship) between them. For any comparisons to be made, separate multivariable regression models reporting Harrell's concordance index (c-index) [17–19] would thus be the ideal type of analysis [20].

In this context, the primary aim of our study was to compare the prognostic performance of all three inflammatory markers (NLR, LMR, and PLR) in patients with HNSCC treated with primary surgery. This is the first study reporting a comparative analysis of this type.

Materials and methods

Sample size calculation and power analysis

Sample size calculations with power analyses were performed prior to data collection. Previous survival estimates represented in published Kaplan–Meier (KM) curves that reported the survival difference in HNC between high and low inflammatory markers were used for this calculation. The group ratio was set at 0.5, power at 80% and α at 0.05. The log-rank test was chosen as the hypothesis test for the sample size calculation. For the NLR, a survival difference of 90% and 55% would require at least 84 patients [21]; for the LMR, a survival difference of 76% and 44% would require 108 patients [22]; and for the PLR, a survival difference of 85% and 55% would require at least 111 patients [23]. In our study, we were able to enroll 123 patients with HNSCC.

Study design

In this retrospective cohort study, 123 eligible patients were selected consecutively, and were treated at the New York Head and Neck Institute from 2008 to 2016. This study was approved by the Institutional Review Board of the Feinstein Institute of Medical Research (FIMR) and Northwell Health. The inclusion criteria for the study were the following: (a) tumors of the oral cavity, oropharynx, or larynx managed with curative intent treatment with primary surgery, with or without adjuvant chemoradiation; (b) histologically confirmed squamous cell carcinoma (SCC); (c) preoperative platelet count, lymphocyte count, monocyte count, and neutrophil count available; (d) patient was followed up at our institution; (e) complete clinical data and records.

The exclusion criteria for our study were the following: (a) evidence of comorbidities such as HIV, immunodeficiency, or acute infection, or autoimmune disease, which could potentially bias the peripheral immune cell counts; (b) non-SCC histology; (c) benign disease; (d) palliative procedure performed; (e) no preoperative immune cell counts available; (f) incomplete medical records; (g) neoadjuvant chemotherapy or radiation; (h) unknown primary tumors. Patients were identified through billing records using predefined ICD-9/10 codes.

Variable selection and data collection

Established prognostic factors for HNSCC were included in this cohort study: age, sex, ethnicity, BMI, alcohol history, smoking history, ECOG score, and Karnofsky performance status (KPS). Comorbidities were retrospectively graded according to the Adult Comorbidity Evaluation 27 (ACE-27) score [24]. The tumor and treatment-related variables recorded were tumor differentiation, T stage, N stage, type of adjuvant treatment, and surgical margins. All tumors, including HPV-positive tumors, were staged according to the 8th Edition of the American Joint Committee on Cancer (AJCC) [25].

At our institution, HPV status is routinely obtained for all oropharyngeal tumors, but not for other HNSCC sites. Information on HPV testing was available for 40/43 (93%) of the oropharyngeal tumors in the cohort. A patient was defined as being HPV positive if there was either strong staining on p16 immunohistochemistry (IHC), or HPV 16/18 DNA detection via in situ hybridization (ISH).

Lastly, preoperative inflammatory markers of interest were recorded: the platelet count, the lymphocyte count, the monocyte count, and the neutrophil count. These were used to calculate the PLR, LMR, and NLR, respectively. They were calculated as the platelet count (10^3/μL) divided lymphocyte count (10^3/μL); lymphocyte count

Table 1 Cohort characteristics stratified by inflammatory markers

	Total (*n*)/mean	LMR			PLR			NLR		
		> 2.8	≤ 2.8	*p*	≤ 164.8	> 164.8	*p*	≤ 2.87	> 2.87	*p*
Total (*n*)	123	72	51		83	40		68	55	
Age	62.2 (11.7)	60.8 (11.6)	64.2 (11.6)	0.972	60.7 (12.6)	65.3 (8.8)	0.014	60.7 (11.6)	64.1 (11.6)	0.98
Sex (*n*)				0.068			0.492			< 0.001
Male	88	47	41		61	27		37	51	
Female	35	25	10		22	13		30	5	
BMI	27.0 (5.7)	26.3 (5.8)	27.6 (5.6)	0.213	27.1 (6.6)	26.9 (4.9)	0.793	25.9 (5.8)	27.6 (5.6)	0.133
Alcohol	61	37	24	0.638	42	19	0.748	36	25	0.002
Smoking	68	37	31	0.304	44	24	0.467	35	33	0.346
ECOG score (*n*)				0.032			0.092			0.003
0	88	57	31		64	24		57	31	
1	31	12	19		16	15		9	22	
2	4	3	1		3	1		2	2	
KPS	1.5 (0.7)	1.5 (0.8)	1.5 (0.6)	0.895	1.5 (0.7)	1.6 (0.8)	0.571	1.4 (0.8)	1.6 (0.6)	0.189
ACE-27 score (*n*)				0.008			0.059			< 0.001
0	29	22	7		24	5		23	6	
1	62	39	23		41	21		35	27	
2	26	9	17		13	13		6	20	
3	6	2	4		5	1		4	2	
Subsite (*n*)				0.63			0.038			0.046
Oropharynx	43	26	17		32	10		26	17	
Oral cavity	67	40	27		46	21		39	28	
Larynx	13	6	7		5	8		3	10	
Differentiation[a] (*n*)				0.851			0.737			0.44
Well	23	14	9		16	7		15	8	
Moderate	74	42	32		47	27		37	37	
Poor	21	11	10		15	6		11	10	
pT stage (*n*)				0.263			0.003			0.002
pT1	54	36	18		39	15		36	18	
pT2	39	21	18		30	9		22	17	
pT3	13	5	8		3	10		1	12	
pT4	17	10	7		11	6		9	8	
pN Stage (*n*)				0.068			0.385			0.19
pN0	62	41	21		42	20		36	26	
pN1	33	20	13		25	8		19	14	
pN2	14	7	7		9	5		9	5	
pN3	14	4	10		7	7		4	10	
HPV (*n*)				0.011			0.182			0.006
Yes	36	28	8		28	8		26	10	
No	9	3	6		5	4		2	7	
RT (*n*)	65	38	27	0.986	44	11	0.126	30	35	0.032
CT (*n*)	27	11	16	0.034	14	13	0.051	11	16	0.087
Positive margins (*n*)	10	9	1	0.036	8	2	0.38	6	4	0.756

NLR neutrophil to lymphocyte ratio, *PLR* platelet to lymphocyte ratio, *LMR* lymphocyte to monocyte ratio, *ACE-27* comorbidity score, *RT* radiation therapy, *CT* chemotherapy, *KPS* Karnofsky performance status, *BMI* body mass index

[a]Missing = 5

Table 2 Univariable Cox proportional hazards regression of overall survival (OS) and event-free survival (EFS)

Variable	Univariable model (OS)		Univariable model (EFS)	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
BMI	0.92 (0.84–1.02)	0.104	0.95 (0.9–1.01)	0.073
KPS	1.74 (1.17–2.58)	0.006	1.54 (1.17–2.03)	0.002
Age > 65	1.3 (0.5–3.39)	0.587	1.3 (0.73–2.34)	0.375
Sex (Male)	3.82 (0.87–16.88)	0.077	1.52 (0.77–3.02)	0.229
Alcohol	1.2 (0.46–3.12)	0.704	0.93 (0.52–1.66)	0.792
Smoking	1.78 (0.65–4.87)	0.258	1.15 (0.64–2.06)	0.639
Subsite		0.631		0.135
Oropharynx	1 (–)	Reference	1 (–)	Reference
Oral cavity	1.05 (0.35–3.14)	0.929	1.79 (0.87–3.7)	0.116
Larynx	1.9 (0.45–7.96)	0.381	2.49 (0.98–6.33)	0.056
Differentiation		0.712		0.719
Well	1 (–)	Reference	1 (–)	Reference
Moderate	0.64 (0.21–1.9)	0.416	1.1 (0.54–2.27)	0.789
Poor	0.82 (0.19–3.43)	0.782	0.77 (0.28–2.11)	0.606
ECOG score		0.034		0.005
0	1 (–)	Reference	1 (–)	Reference
1	2.07 (0.69–6.22)	0.197	2.38 (1.27–4.45)	0.007
2	7.2 (1.55–33.56)	0.012	4.13 (1.24–13.75)	0.021
ACE-27 score		0.055		0.038
0	1 (–)	Reference	1 (–)	Reference
1	1.35 (0.27–6.71)	0.712	2.59 (0.99–6.77)	0.053
2	4.03 (0.84–19.45)	0.082	4.43 (1.6–12.31)	0.004
3	6.98 (0.98–49.89)	0.053	3.16 (0.61–16.4)	0.171
Staging pT		0.004		0.004
pT1	1 (–)	Reference	1 (–)	Reference
pT2	1.84 (0.41–8.24)	0.424	1.32 (0.63–2.78)	0.467
pT3	7.12 (1.57–32.25)	0.011	2.5 (0.96–6.55)	0.062
pT4	9.17 (2.27–37.11)	0.002	3.86 (1.78–8.39)	0.001
Staging pN		< 0.001		< 0.001
pN0	1 (–)	Reference	1 (–)	Reference
pN1	0.52 (0.06–4.51)	0.554	0.75 (0.32–1.77)	0.51
pN2	3.99 (1.06–15.07)	0.041	1.75 (0.74–4.13)	0.199
pN3	11.51 (3.45–38.47)	Reference	4.79 (2.22–10.34)	< 0.001
HPV	–	0.991	0.26 (0.1–0.65)	0.004
RT	0.96 (0.37–2.5)	0.932	0.72 (0.4–1.28)	0.26
CT	2.02 (0.74–5.48)	0.17	1.77 (0.94–3.32)	0.075
Positive margins	0.76 (0.1–5.76)	0.793	1.25 (0.45–3.51)	0.667
NLR	3.46 (1.12–10.68)	0.031	3.14 (1.7–5.79)	< 0.001
PLR	3.86 (1.48–10.03)	0.006	2.51 (1.41–4.48)	0.002
LMR	3.07 (1.13–8.35)	0.028	2.96 (1.63–5.38)	< 0.001

NLR neutrophil to lymphocyte ratio, *PLR* platelet to lymphocyte ratio, *LMR* lymphocyte to monocyte ratio, *ACE-27* comorbidity score, *RT* radiation therapy, *CT* chemotherapy, *KPS* Karnofsky performance status, *BMI* body mass index

(10^3/μL) divided by monocyte count (10^3/μL); neutrophil count (10^3/μL) divided by lymphocyte count, respectively (10^3/μL). All preoperative markers were obtained from the same blood sample that was performed as part of the patient's routine preoperative lab work.

Endpoints

The two primary endpoints in this study were overall survival (OS) and event-free survival (EFS). EFS has been previously shown to be a good surrogate for OS over other

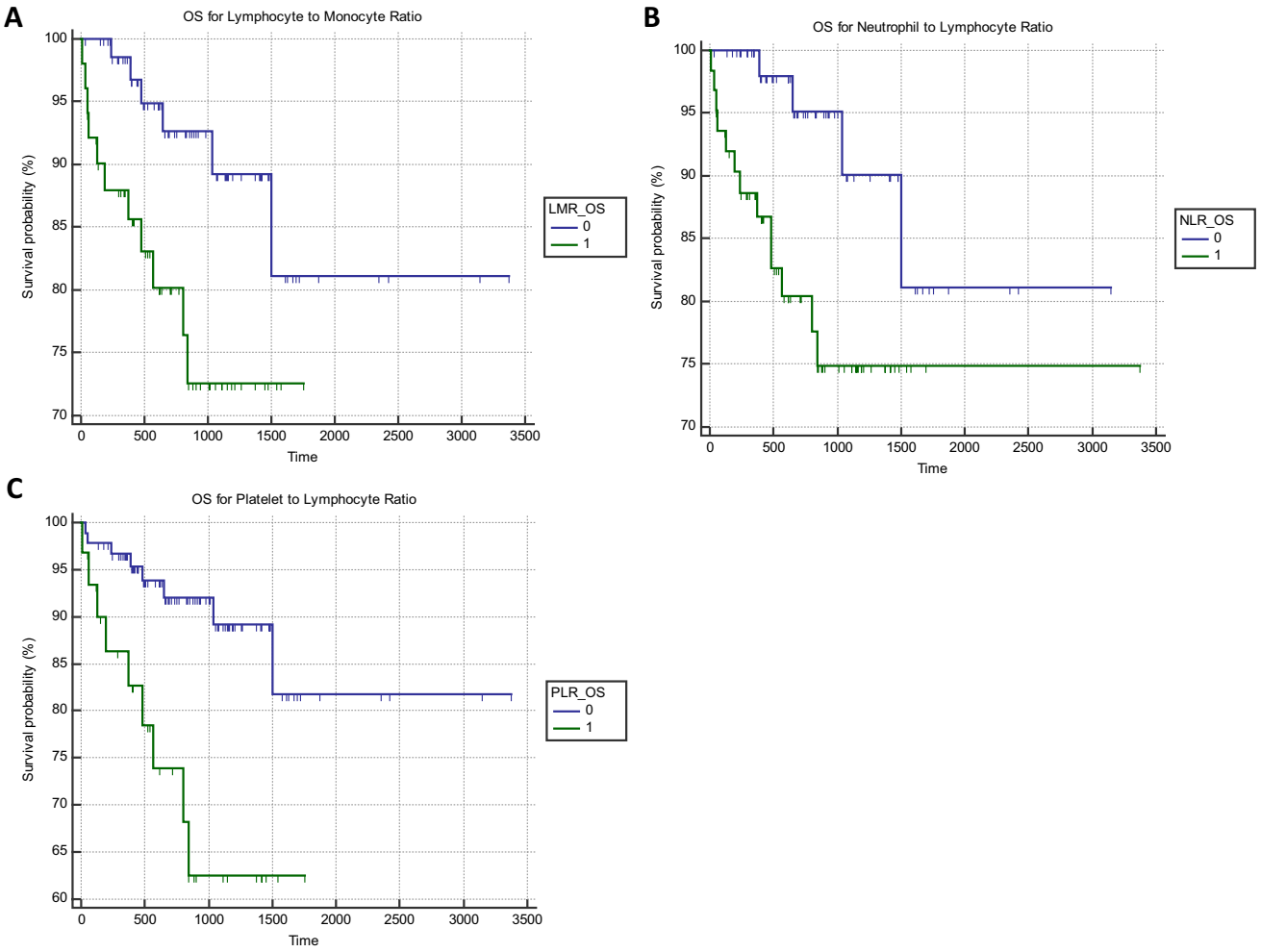

Fig. 1 Overall survival (OS) stratified by inflammatory markers. Kaplan–Meier curves of overall survival (OS) stratified by inflammatory marker status. Patients with lower LMR (**a**, log-rank test, $p = 0.0208$), higher NLR (**b**, log-rank test, $p = 0.0218$), and higher PLR (**c**, log-rank test, $p = 0.0028$) had worse OS

endpoints in HNC [26]. OS was calculated as time from surgery till date of last follow-up or death. Patients who were alive at time of last follow-up were censored for the survival analysis. EFS was defined as date of surgery to last follow-up or event. 'Event' was defined as any progression, local or distant recurrence, or death from any cause [26]. Patients who did not experience any 'event' were censored.

Statistical analysis

The appropriate parametric and non-parametric tests were used to evaluate the differences in the relationship between the inflammatory markers and clinicopathological characteristics of the cohort (Table 1). The optimal cutoff points for the NLR, LMR, and PLR have not yet been determined [6, 15, 16]. Therefore, we obtained cutoff values for these markers using receiver-operating characteristic (ROC) curve analysis. The Youden index [27] was used in the ROC curve to find the optimal discriminatory threshold that would maximize sensitivity and specificity of the inflammatory marker. A bootstrap of 1000 iterations was performed to obtain credible ROC curve parameters. Using this method, separate cutoff values were used for the two endpoints OS and EFS, for the three inflammatory markers NLR, LMR, and PLR. The optimal cutoff values obtained through ROC curve analysis were 2.8 (LMR: OS and EFS), 2.87 and 2.69 (NLR: OS and EFS, respectively), and 194 and 164.8 (PLR: OS and EFS, respectively).

The Kaplan–Meier (KM) product limit method was used to estimate survival endpoints, and the log-rank test was used to find survival differences in the KM curves. Cox proportional hazards regression (CPH) was used to calculate the hazard ratio (HR) of the variables in a univariable and subsequently multivariable analysis. Variables that were statistically significant in the univariable analysis were selected for inclusion in the multivariable model. Final variable selection

in the multivariable model was performed using backward selection. Variables were removed from the multivariable model with a p value threshold of 0.05. Due to the expected and subsequently observed strong collinearity between the NLR, PLR, and LMR values (Spearman correlation coefficient: $r > 0.6$, $p < 0.001$, Supplementary Figure S1), we performed the analysis in three separate multivariable models.

Additionally, to ascertain which marker had the strongest predictive association with the endpoints, Harrell's concordance index (c-index) was calculated for each model [17–20]. The c-index is widely used to evaluate the prediction performance of a model with a continuous outcome that can be censored. According to Harrell, the c-index is a probability of concordance between predicted and observed survival, ranging from 0.5 (no discrimination) to 1 (perfect discrimination). The c-index is also relatively unaffected by the amount of censoring [28].

HRs with corresponding 95% confidence intervals (95% CI) are presented, with two-sided p values. The α level was set to 0.05, and p values less than 0.05 were considered statistically significant. The Tukey-adjusted pairwise multiple comparisons were performed within categorical variables. All statistical analyses were performed using MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium), SAS version 9.4 (SAS Institute Inc., Cary, NC), and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Cohort characteristics and survival analysis

The cohort characteristics of the 123 patients are presented in full in Table 1. A majority were male (71%), with a cohort mean age of 62 years. The most common subsite was the oral cavity (54%), followed by the oropharynx (35%) and larynx (11%). A majority of tumors were early T stage (pT1–2, 76%), and the presence of nodal disease was evenly distributed (pN0, 50%; pN1–3, 50%). HPV status was available in 40 (93%) oropharyngeal tumors. Of these, 31 were positive for HPV (78%).

Seventeen patients died during this follow-up period, and 47 had a recurrence, progression, or death. The 3- and 5-year OS rates were 82% and 76%, and the 3- and 5-year EFS rates were 58% and 42%, respectively.

Overall survival (OS)

In the univariable analysis, the following prognostic variables were found to be associated with worse OS: higher ECOG, higher KPS, higher T stage, and higher N stage (Table 2). With regard to the inflammatory markers, the following were associated with worse OS in the univariable analysis: an elevated NLR > 2.87 (HR = 3.46, 95% CI 1.12–10.68, $p = 0.031$); elevated PLR > 194 (HR = 3.86, 95% CI 1.48–10.03, $p = 0.005$); a decreased LMR < 2.8 (HR = 3.07, 95% CI 1.13–8.35, $p = 0.028$) (Fig. 1).

In the multivariable model, T stage and N stage were significantly associated with OS. NLR ($p = 0.2413$), PLR ($p = 0.1593$), and LMR ($p = 0.0552$) were not significantly associated with OS in the multivariable analysis (Table 3).

Table 3 Multivariable Cox proportional hazards regression of overall survival (OS)

Variable	Multivariable model (OS)	
	HR (95% CI)	p value[a]
Staging pT		0.037
pT1	1 (–)	Reference
pT2	1.28 (0.17–9.48)	0.7486
pT3	4.45 (0.58–34.13)	0.0598
pT4	6.09 (0.89–41.81)	0.0159
Staging pN		0.003
pN0	1 (–)	Reference
pN1	0.79 (0.04–14.11)	0.8359
pN2	4.14 (0.7–24.65)	0.0405
pN3	9.54 (1.83–49.78)	0.0005
NLR > 2.87	–	0.2413
LMR < 2.8	–	0.0552
PLR > 194	–	0.1593

[a]The Tukey-adjusted pairwise multiple comparisons were performed within categorical variables

Event-free survival (EFS)

In the univariable analysis, the following variables were associated with worse EFS: higher ECOG score, higher KPS, higher ACE-27 comorbidity score, higher T and N stage (Table 2). HPV-positive status was associated with better EFS (HR = 0.26, 95% CI 0.10–0.65, $p = 0.004$). With regards to the inflammatory markers, the following were associated with worse EFS: an elevated NLR > 2.69 (HR = 3.14, 95% CI 1.70–5.79, $p < 0.001$); an elevated PLR > 164.8 (HR = 2.51, 95% CI 1.41–4.48, $p = 0.002$); a decreased LMR < 2.8 (HR = 2.96, 95% CI 1.63–5.38, $p < 0.001$) (Fig. 2).

Due to strong collinearity among the inflammatory markers (Supplementary Figure S1), we performed multivariable analysis for EFS in separate models (Table 4). In the LMR model, T stage ($p = 0.011$), N stage ($p = 0.004$), HPV status ($p = 0.013$), and low LMR (HR = 2.95, 95% CI 1.54–5.65, $p = 0.001$) were significantly associated with EFS. In the PLR model, T stage ($p = 0.026$), N stage ($p = 0.002$), HPV

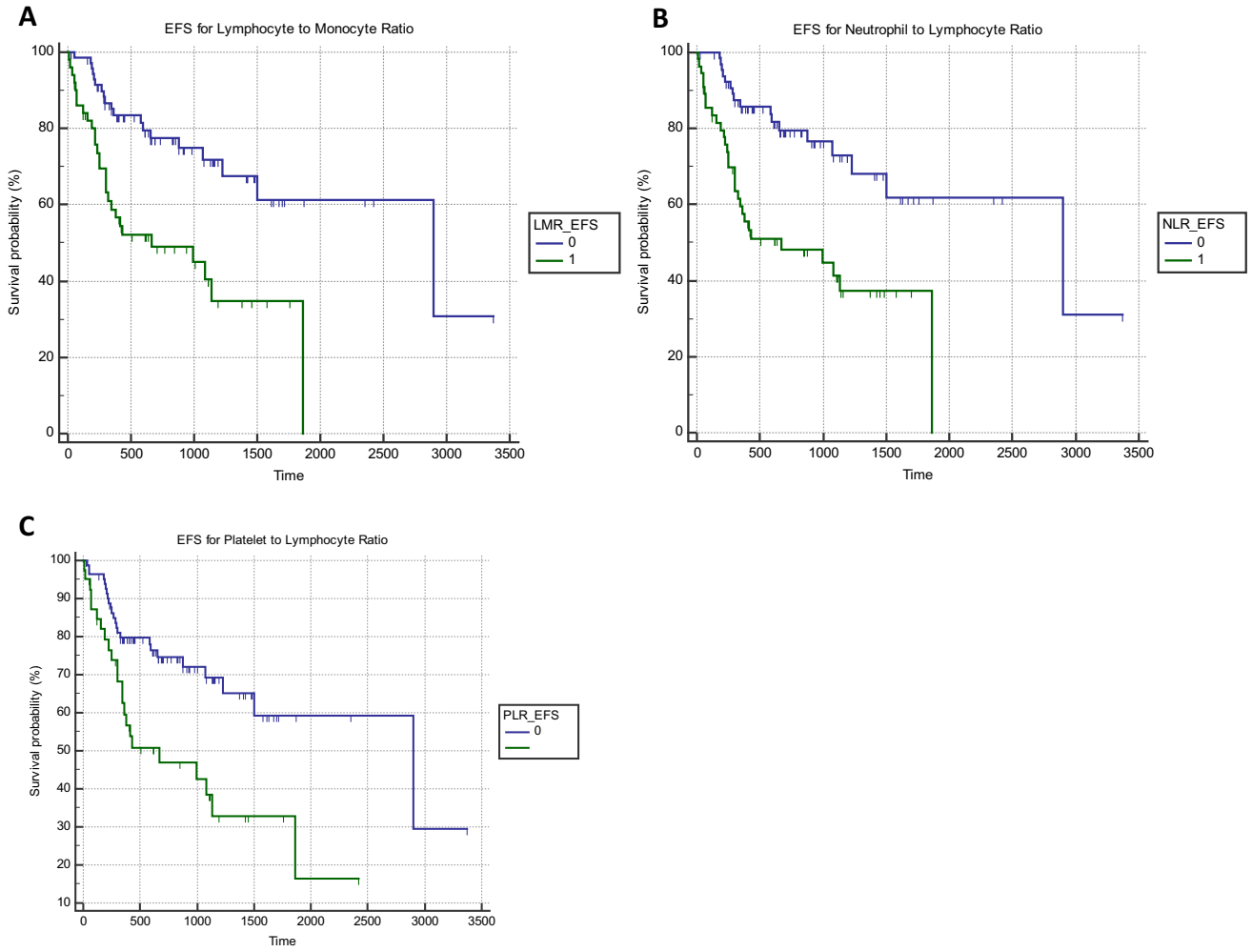

Fig. 2 Event-free survival (EFS) stratified by inflammatory markers. Kaplan–Meier curves of event-free survival (EFS) stratified by inflammatory marker status. Patients with lower LMR (**a**, log-rank test, $p = 0.0002$), higher NLR (**b**, log-rank test, $p = 0.0018$), and higher PLR (**c**, log-rank test, $p = 0.0012$) had worse EFS

status ($p = 0.007$), and high PLR (HR = 2.68, 95% CI 1.42–5.09, $p = 0.003$) were associated with EFS. Lastly, in the NLR model, T stage ($p = 0.007$), N stage ($p = 0.001$), HPV status ($p = 0.0026$), and high NLR (HR = 3.37, 95% CI 1.7–6.69, $p < 0.001$) were associated with EFS.

Comparison of inflammatory markers—NLR, LMR, and PLR

Harrell's concordance index (c-index) was measured for all inflammatory markers (Table 5). In the univariable analysis for OS, NLR had the highest c-index (0.681), followed by LMR (0.670) and PLR (0.666). The multivariable c-index was obtained for EFS, since all three markers were found to be associated with EFS in the multivariable analysis. The multivariable c-index was highest for LMR (0.762), followed by NLR (0.761) and PLR (0.739).

Relationship of pretreatment inflammatory markers and clinicopathological characteristics

The correlations of interest in the cohort (Table 1) were the higher ECOG status observed for low LMR ($p = 0.032$) and high NLR ($p = 0.003$), higher proportion of comorbidities in low LMR ($p = 0.008$) and high NLR ($p < 0.001$), as well as the higher T stage for high NLR ($p = 0.002$). Of interest, HPV-positive patients had a higher LMR ($p = 0.011$) and lower NLR ($p = 0.006$) than HPV-negative patients. A non-parametric test was used to confirm this finding, which showed a higher LMR (median 3.5 vs 2.1, Mann Whitney test $p = 0.0269$) and lower NLR for HPV-positive patients (median 2.1 vs 3, Mann Whitney test $p = 0.0090$) (Supplementary Table S2).

To further explore this unexpected association with HPV, an ad hoc supplementary survival analysis was performed in the small HPV-positive cohort. Decreased LMR

Table 4 Multivariable Cox proportional hazards regression of event-free survival (EFS)

Variable	Multivariable model (EFS)	
	HR (95% CI)	*p* value[a]
Lymphocyte to monocyte ratio (LMR)		
Staging pT		0.011
pT1	1 (–)	Reference
pT2	0.79 (0.28–2.23)	0.9357
pT3	0.91 (0.23–3.51)	0.9977
pT4	2.93 (1.02–8.39)	0.0433
Staging pN		0.004
pN0	1 (–)	Reference
pN1	1.11 (0.34–3.62)	0.996
pN2	3.37 (0.99–11.43)	0.052
pN3	3.54 (1.2–10.51)	0.0148
HPV	0.27 (0.09–0.76)	0.013
LMR < 2.8	2.95 (1.54–5.65)	0.001
KPS	–	0.409
ACE-27	–	0.334
ECOG	–	0.116
Platelet to lymphocyte ratio (PLR)		
Staging pT		0.026
pT1	1 (–)	Reference
pT2	0.91 (0.28–2.23)	0.9959
pT3	0.77 (0.23–3.51)	0.9638
pT4	2.63 (1.02–8.39)	0.0975
Staging pN		0.002
pN0	1 (–)	Reference
pN1	1.56 (0.34–3.62)	0.7816
pN2	3.76 (0.99–11.43)	0.0247
pN3	4.13 (1.2–10.51)	0.0042
HPV	0.24 (0.09–0.76)	0.007
PLR > 164.8	2.68 (1.42–5.09)	0.003
KPS	–	0.409
ACE-27	–	0.334
ECOG	–	0.116
Neutrophil to lymphocyte ratio (NLR)		
Staging pT[b]		0.007
pT1	1 (–)	Reference
pT2	0.84 (0.3–2.37)	0.971
pT3	0.76 (0.2–2.92)	0.951
pT4	3.17 (1.07–9.33)	0.031
Staging pN		0.001
pN0	1 (–)	Reference
pN1	1.25 (0.38–4.07)	0.963
pN2	4.22 (1.23–14.4)	0.014
pN3	3.93 (1.33–11.6)	0.007
HPV	0.31 (0.11–0.87)	0.026
NLR > 2.69	3.37 (1.7–6.69)	< 0.001
KPS	–	0.548
ACE-27	–	0.389
ECOG	–	0.184

[a]The Tukey-adjusted pairwise multiple comparisons were performed within categorical variables

[b]Overall, there is a staging T effect; however, the adjusted pairwise comparisons did not show any significant differences among any of the 6 pairwise comparisons

had poorer EFS (log-rank test, $p = 0.0026$); Increased NLR had poorer EFS compared to lower NLR (log-rank test, $p = 0.0001$); lastly, PLR was not associated with EFS (log-rank test, $p = 0.0998$) (Supplementary Figures S3). OS survival analysis was not performed due to low number of deaths in the HPV-positive cohort.

Discussion

In this study, the three inflammatory markers, LMR, PLR, and NLR, were not associated with OS in the multivariable analysis. For EFS, all three markers were found to be significantly associated with survival in multivariable analysis. Additionally, analysis of multivariable model performance using Harrell's c-index illustrated that LMR and NLR (c-index 0.762 and 0.761) were superior measures of prognosis compared to PLR (c-index 0.739). Using separate multivariable modeling as well as Harrell's concordance index analysis, we believe this to be the first study reporting detailed comparisons of the inflammatory markers in HNSCC.

Out of all three markers, there is a lack of data regarding the prognostic role of LMR in HNSCC [15]. In a recent meta-analysis of the LMR [15], there were only two studies reporting the LMR in the OC [29] and OP [22] sites. Additionally, none of the previously published literature reporting on LMR had controlled for HPV status [22].

There are few reports of studies reporting all three inflammatory markers [6, 15]. The study by Eltohami et al. investigated only LMR and albumin in a combined systemic inflammation score (SIS) [30]; the study by Park et al. investigated all three markers, isolated to the oral cavity [31]. In the study by Kano et al. they reported all three markers in a cohort of patients receiving concurrent chemoradiation therapy (CCRT) [22]. Building on those previous findings, this study has confirmed the findings presented by Kano et al. [22] in a cohort that has been treated surgically instead.

Based on recent reviews of the literature [6, 15], no previous studies have accounted for multicollinearity among the three markers. Since all three markers rely on the lymphocyte count, there would be a significant and strong collinearity (linear relationship) between them, which are shown in a supplementary analysis (Supplementary Table S1). When covariates in multivariable models are not independent, collinearity/multicollinearity problems arise in the analysis, and this has been shown to lead to biased estimations [32]. In the current study, collinearity was accounted for by performing separate multivariable analyses, as well as separately analyzing the multivariable c-indexes for each marker.

Table 5 Harrell's concordance index (c-index) of the inflammatory markers

Variable	OS	EFS	
	Univariable c-index (95% CI)[a]	Univariable c-index (95% CI)[a]	Multivariable c-index (95% CI)[a]
NLR	0.681 (0.809–0.553)	0.653 (0.729–0.576)	0.761 (0.851–0.671)
PLR	0.666 (0.775–0.558)	0.606 (0.677–0.534)	0.739 (0.829–0.649)
LMR	0.67 (0.795–0.545)	0.64 (0.716–0.565)	0.762 (0.852–0.671)

The c-index is also relatively unaffected by the amount of censoring [28]

NLR neutrophil to lymphocyte ratio, *PLR* platelet to lymphocyte ratio, *LMR* lymphocyte to monocyte ratio, *95% CI* 95% confidence interval

[a]The c-index is widely used to evaluate the prediction performance of a model with a continuous outcome that can be censored. According to Harrell, the c-index is a probability of concordance between predicted and observed survival, ranging from 0.5 (no discrimination) to 1 (perfect discrimination).

With regard to the cutoff values of the markers, there is no agreement on the cutoff value. We obtained our cutoffs through ROC curve analysis. The cutoff obtained for LMR (2.8) is within the range of published values according to a previous meta-analysis (range 2.475–5.3) [15]. Cutoffs for NLR were 2.87 (OS) and 2.69 (EFS), which are similar to cutoffs found previously (interquartile range 2.38–2.79) [6]. Takenaka et al. recently published a meta-analysis of the PLR in HNC [16], and this study's cutoff value for EFS in PLR (164.8) is similar to the published range (82–150). On the other hand, this study's OS cutoff for PLR (194) was found to be much higher than the published range [16].

The other additional finding in this paper is the prognostic effect of the inflammatory markers stratified by HPV status. To date, only one other study has investigated the effect of NLR in an HPV-positive cohort [33]. The study by So et al. found that the NLR was an independent prognostic indicator in HPV-positive oropharyngeal SCC. However, they did not investigate the PLR or LMR in their cohort. This study's results for NLR were in agreement with those by So et al. (Supplementary Figure S3). Additionally, we also found the suggestion that HPV-positive cancers might have an additional benefit of having a higher baseline LMR and lower NLR versus HPV-negative cancers (Supplementary Table S2). The literature has shown that HPV + OPSCC to be a biologically different entity from HPV− OPSCC. The findings in this study further support this fact, as we observed that the inflammatory markers between HPV− and HPV + OPSCC (significantly different LMR and NLR levels) were different. Whether or not the native intrinsic factors of HPV + OPSCC or the inflammatory markers themselves are responsible for the superior prognosis of HPV + OPSCC is outside the scope of this current study.

In this study, the inflammatory markers were associated with the EFS endpoint, and not with the OS endpoint. A possible explanation for this outcome is that the follow-up time for the OS endpoint was not sufficient. In a meta-analysis of individual patient data, Michiels et al. had shown the EFS endpoint is a valid surrogate for OS in head and neck cancer [26]. Therefore, it is possible that OS would be related to these markers with longer follow-up time, which is consistent with the findings in previous meta-analyses [6, 15, 16]. Among the tumor subsites, laryngeal cancers have been known to have better prognoses. However, in the univariable Cox regression analysis, laryngeal tumors were not shown to have superior survival versus other subsites in this cohort. This might partially be due to the lower numbers of laryngeal cancers versus other subsites in this cohort.

We acknowledge that the current study was not a priori designed to investigate impact of HPV on these markers, thus there is a smaller sample size of oropharyngeal patients in this cohort. Nevertheless, these new data would assist in directing the efforts of future research. Further research is recommended to ascertain if HPV status has any bearing on the survival effect presented by inflammatory markers such as LMR and NLR. The other limitations to this study are that data were unavailable for specific lymphocyte populations ($CD8^+$) that have a relative decrease, or information about tumor-infiltrating T lymphocytes (TILs). Thus, our current results are only relative to lymphocytes in general and not for specific lymphocyte subpopulations. Additionally, this study is retrospective in nature, and thus subject to biases inherent in this study design.

In conclusion, the LMR, PLR, and NLR were not found to be independently related to OS in this cohort. However, they were found to be independently associated with EFS. The LMR and NLR were found to be superior predictors of prognosis versus the PLR. These markers are easily obtainable, and in the age of individualized patient care and precision medicine, they might represent further risk stratification tools for HNSCC patients [34, 35].

Compliance with ethical standards

Conflict of interest The authors have nothing to disclose. No potential conflicts of interest exist among the authors.

Ethical approval Research involving human participants was conducted, with IRB approval #17-0280-LHH. Informed consent was waived as per institutional and IRB approval #17-0280-LHH.

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