Clinical significance of pretreatment prognostic nutritional index and lymphocyte-to-monocyte ratio in patients with advanced p16-negative oropharyngeal cancer—a retrospective study

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ABSTRACT

Background: Systemic inflammation and nutritional status both play roles in the survival of cancer patients. Therefore, it is important to understand the effects of prognostic nutritional index (PNI) and lymphocyte-to-monocyte ratio (LMR) on the survival of patients with advanced p16-negative oropharyngeal cancer.

Methods: A total of 142 patients diagnosed with advanced p16-negative oropharyngeal cancer between 2008 and 2015 were enrolled in this study. All patients received primary treatment with definite concurrent chemoradiotherapy (CCRT). Optimal cutoff values for PNI and LMR were determined using receiver operating characteristic curves for survival prediction. Survival rates for different level of PNI and LMR were estimated and compared using Kaplan–Meier method and log-rank test to see if there were significant effects on these end points, including 5-year overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS) rates. The effects of PNI and LMR on survival were assessed using Cox regression model adjusted for other prognostic factors.

Results: The results showed the optimal cutoff values for PNI and LMR were 50.5 and 4.45, respectively. A high PNI (≧50.5) was significantly improved the 5-year OS. A low LMR (<4.45) was significantly associated with a poor 5-year DFS, DSS, and OS. In multivariate analysis, both PNI and LMR were independent prognosticators for 5-year OS.

Conclusions: Elevated pretreatment PNI and LMR are both favorable prognosticators in advanced p16-negative oropharyngeal cancer patients undergoing CCRT.

INTRODUCTION

It is estimated that head and neck cancers are the sixth most commonly diagnosed systemic malignant tumors with more than 500,000 new cases and 300,000 associated deaths annually (*McGuire, 2016*). Oropharyngeal squamous cell carcinoma (OPSCC) is an aggressive type of head and neck cancer. The average annual percentage increase of OPSCC was 6.1% between 1980 and 2014, and the trend continues to increase with numerous OPSCC cases diagnosed as advanced stages in Taiwan (*Hsu et al., 2017*). Though investigations of OPSCC have been carried out for decades worldwide, its etiologic and clinical characteristics differ substantially among populations. For instance, human papillomavirus (HPV) infection may lead to a more favorable prognosis in patients than that without HPV infection. (*Mehanna et al., 2013*) Besides, the relatively low HPV infection rate, approximately 20–30%, in Taiwan along with the high prevalence of betel nut chewing both may deteriorate the prognosis, as investigated in our previous study (*Al-Swiahb et al., 2010*) makes the evaluation of treatments and possible prognostic factors in advanced stage HPV-negative OPSCC an urgent need.

In addition to HPV status, inflammatory biomarkers are thought to be a representation of the interaction between the tumor microenvironment and host immune system (*O'Callaghan et al., 2010*; *Aggarwal, Vijayalekshmi & Sung, 2009*). Recent studies have shown a negative prognostic value of higher neutrophil-to-lymphocyte ratio and lower lymphocyte-to-monocyte ratio (LMR) among patients with head and neck cancer (*Perisanidis et al., 2013*; *Haddad et al., 2015*; *Rassouli et al., 2015*; *Tham et al., 2018*). *Takahashi et al. (2019)* reported that a low LMR was an independent adverse prognostic factor for survival in patients with OPSCC.

Nutritional impairment has also been shown to have a negative impact on clinical outcomes (*Moon et al., 2016*). Patients with advanced stage OPSCC are often vulnerable to malnutrition at the time of diagnosis because of poor food intake due to cancer-related pain, mechanical obstruction by the tumor, or psychological problems. The prognostic nutritional index (PNI), calculated as previously described, (*Onodera, Goseki & Kosaki, 1984*) may be especially useful because it could act as a surrogate marker for both inflammation and nutritional status. This index was originally studied to demonstrate the correlation between postoperative complications and prognosis in patients with esophageal carcinoma (*Nozoe et al., 2002*). With regard to head and neck cancer, a low PNI had shown as a poor survival predictor in previous study (*Bruixola et al., 2018*).

Currently, studies on the role of PNI and LMR played in patients with advanced stage HPV-negative OPSCC are still limited. Clinically, p16 expression could be regarded as a surrogate marker for HPV in the prediction of tumor behavior in oropharyngeal cancer (*Golusiński et al., 2017*). Thus, the objective of this study was to identify the significant effects of PNI and LMR on clinical prognosis in patients with advanced stage (stage III/IV) p16-negative OPSCC.

MATERIALS AND METHODS

Study population

Patients who were histologically confirmed by biopsy to have stage III/IV p16-negative OPSCC were evaluated in the study. The TNM stage was reclassified according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system. Patients who were treated with primary concurrent chemoradiotherapy (CCRT) were eligible for this study. The determination of p16 expression in tumor cells by immunohistochemistry was done as suggested in the 8th edition AJCC staging system manual (*Amin et al., 2017*). Patients with clinical evidence of an acute infection within 4 weeks prior to the blood tests or who were diagnosed with recurrent tumors, distant metastases, other concomitant active cancers, or chronic inflammatory disease or who had a history of malignancy in the past 5 years were excluded from the study.

In this retrospective study, 142 patients with stage III/IV p16-negative OPSCC who underwent primary CCRT at the Kaohsiung Chang Gung Memorial Hospital in Taiwan between January 2008 and April 2015 were recruited. Treatment was primarily based on the American NCCN guidelines. The chemotherapy agent was cisplatin-based and the radiation technique for all patients was intensity-modulated radiation therapy (IMRT). The primary radiation dose for all of our patients was between 70 and 74 Gy with conventional fractionated daily dose of 1.8 or 2 Gy. All included patients completed the treatment programs formulated by the multidisciplinary team.

Variables and outcomes

Pretreatment clinical variables of interest were collected, including age, sex, Adult Comorbidity Evaluation-27 (ACE-27) score and clinical TNM stage of the tumor. Information collection of pretreatment complete blood count (including absolute lymphocyte and monocyte counts) and biochemical (including albumin) tests using the peripheral blood sample were also conducted within one week before treatment.

The LMR was calculated by dividing the baseline absolute peripheral lymphocyte count (cells/mm^3) by the absolute monocyte peripheral count (cells/mm^3).

The PNI was calculated as follows: $10 \times$ baseline serum albumin (g/dL) + $0.005 \times$ baseline absolute lymphocyte count (cells/mm^3).

Statistical analysis

The endpoints in our study were the 5-year overall survival (OS), 5-year disease-specific survival (DSS), and 5-year disease-free survival (DFS) rates. OS calculated the time frame from the date of the first treatment to the date of death or last follow-up; disease specific survival calculated from the date of the first treatment to the date of death because of tumor or last follow-up. Disease free survival calculated the time from the date of the first treatment to the date of recurrence, metastasis, or last follow-up. Follow-up was continued through May 2020. Receiver operating characteristic curves for survival were plotted, and Youden's index, which calculated as J = sensitivity + specificity − 1, was applied to verify the optimum cutoff value of LMR and PNI for OS. Survival rates of certain prognostic factors were estimated using the Kaplan–Meier method, and the log-rank test

was used to determine the heterogeneity of each specific factor. Sex and smoking status variables were excluded from the analysis because of the extremely imbalanced distribution. The Cox proportional hazards model was built with independent primary factors and other significant prognostic factors that were identified in prior univariate survival analyses. The variance inflation factors (VIF) were assessed to avoid multicollinearity among independent variables in the Cox model. Both VIF values for continuous PNI to continuous LMR or dichotomous PNI to dichotomous LMR were below 3 (1.004 and 1.002, respectively), which indicated that there was a low correlation between PNI and LMR. All statistics tests were two-sided, with 0.05 significant level. All statistical analyses were performed using the Social Science Software, version 20.0 package (SPSS, Chicago, IL, USA). This study was approved by the Medical Ethics and Human Clinical Trial Committees at Chang Gung Memorial Hospital (Ethical Application Reference number: 202000471B0). Patients' consent to review their medical records was not required by this hospital's committees because the patient data remained anonymous in this study.

RESULTS

Of the 142 p16-negative OPSCC patients, 99.3% (141) were men and 0.7% (1) were women. The mean age at diagnosis for all participants was 53.8 years (range: 36–85 years). The mean follow-up period was 40.7 months (3.6–111.8 months). Nine patients (6.3%) had stage III disease, 34 (23.9%) had stage IVA and 99 (69.7%) had stage IVB. This cohort included patients with clinical T classifications of T1 ($n = 4$, 2.8%), T2 ($n = 24$, 16.9%), T3 ($n = 24$, 16.9%), T4a ($n = 34$, 23.9%) and T4b ($n = 56$, 39.4%). Clinical nodal metastasis was present in 117 patients (82.4%) and 65 (45.8 %) had extranodal extensions (ENE). The clinicopathological features of the 142 cases, and their survival outcomes were listed in Table 1.

The optimal cutoff value for PNI was 50.5, and 4.45 for LMR (Fig. 1). Patients with PNI $\geq$ 50.5 or LMR $\geq$ 4.45 did not have significant correlations with age, T classification, N classification, or other clinicopathologic factors (all $p > 0.05$; Table S1).

The OS rate for patients with PNI $\geq$ 50.5 was significantly higher than that for patients with PNI < 50.5 (48.1% vs. 24.7%, $p = 0.004$). Similarly, the DSS for patients with PNI $\geq$ 50.5 was significantly higher than that for patients with PNI < 50.5 (57.2% vs. 42%, $p = 0.043$). Moreover, DFS had a similar trend by PNI difference in our cohort (44.3% vs. 34.2%), although p value did not reach statistical significance ($p = 0.108$, Fig. 2). Regarding the LMR, the 5-year OS (55.5% vs. 26.6%), DSS (66.8% vs. 41.4%) and DFS (51.4% vs. 35.0%) were all significantly increased (both $p < 0.05$, Fig. 3) among patients with LMR $\geq$ 4.45, compared with those with LMR < 4.45. Clinically positive ENE status was another significant predictor of poor outcome for 5-year OS, DSS and DFS in univariate analysis (Table 2).

Multiple regression analysis was applied to analyze the relationship between survival outcome and significant factors which were revealed in prior univariate analyses. PNI was an independent factor of OS in this cohort (hazard ratio (HR): 1.778, 95% confidence

Table 1 Clinical characteristics of 142 patients who were diagnosed with advanced p16-negative OPSCC.

Characteristics		Value	%
Mean age (range), year		53.8 (36, 85)	
Mean follow up time (range), months		40.7 (3.7, 111.8)	
Sex	Male	141	99.3
	Female	1	0.7
Clinical TNM Stage	III	9	6.3
	IVA	34	23.9
	IVB	99	69.7
Clinical T classification	T1	4	2.8
	T2	24	16.9
	T3	24	16.9
	T4a	34	23.9
	T4b	56	39.4
Clinical N classification	N0	25	17.6
	N1	15	10.6
	N2b	17	12
	N2c	20	14.1
	N3b	65	45.8
Clinical ENE	Negative	77	54.2
	Positive	65	45.8
PNI	Unknown	7	4.9
	<50.5	79	55.6
	≥50.5	56	39.4
LMR	<4.45	99	69.7
	≥4.45	43	30.3
Recurrence	No	59	41.5
	Yes	83	58.5
Last status	NED	35	24.6
	Alive with disease	12	8.5
	DOD	68	47.9
	DWOD	27	19.0

Note:
OPSCC, oropharyngeal squamous cell carcinoma; PNI, prognostic nutritional index = $10 \times$ serum albumin (g/dl) + 0.005 $\times$ total lymphocyte count (/mm^3); ENE, extranodal extension; LMR, lymphocyte to monocyte ratio; NED, no evidence of disease; DOD, died of disease; DWOD, die without disease.

interval (CI) [1.145–2.761]) and simultaneously adjusted by other independent factors, LMR and ENE (Table 3). In another model, the status of LMR showed a significant prognosticator in OS (HR of 2.408, 95% CI [1.439–4.029]), DSS (HR: 2.33, 95% CI [1.255–4.323]) and DFS (HR: 1.765, 95% CI [1.067–2.892]) after being adjusted by other factors (Tables 3–5). The status of clinical ENE was a significant prognosticator of OS (HR: 1.592, 95% CI [1.054–2.405]), DSS (HR: 2.159, 95% CI [1.319–3.533]) and DFS (HR: 1.86, 95% CI [1.202–2.878]) (Tables 3–5).
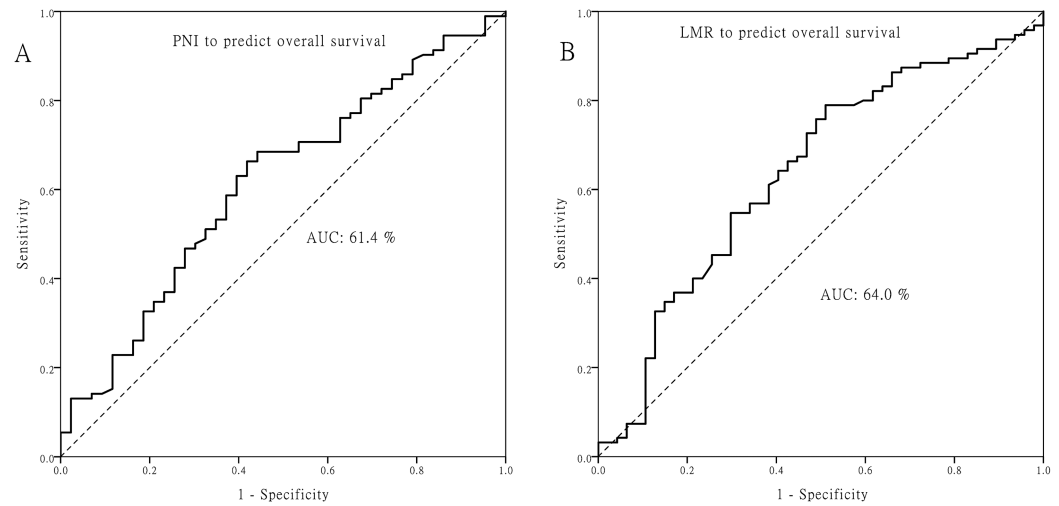
Figure 1 Receiver operating characteristic curves. Receiver operating characteristic curves for predicting the survival outcome. (A) Pretreatment prognostic nutritional index (PNI). (B) Pretreatment lymphocyte to monocyte ratio (LMR). Full-size ⬛ DOI: 10.7717/peerj.10465/fig-1
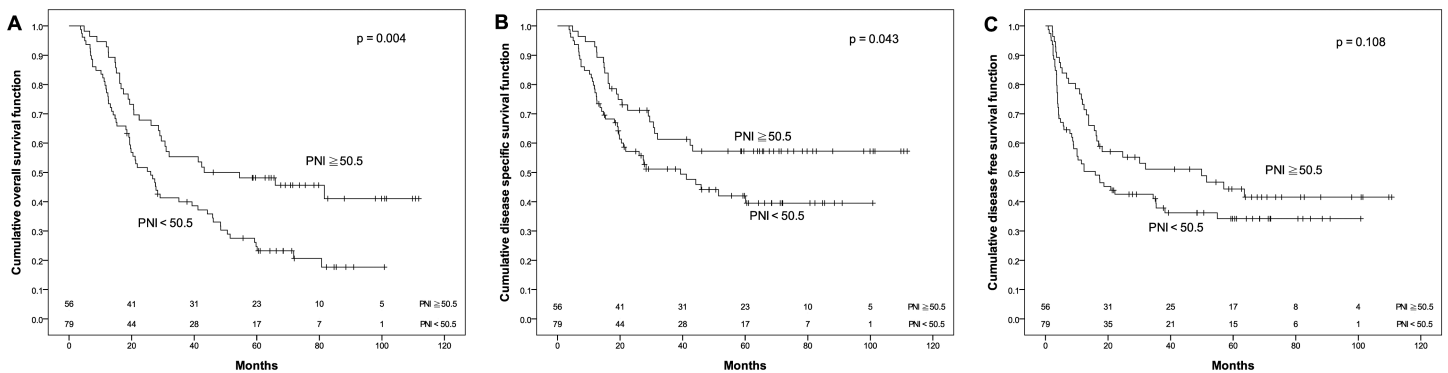

Figure 2 Kaplan–Meier survival curves. Kaplan–Meier survival curves by different level of pretreatment prognostic nutritional index (PNI). (A) Overall survival. (B) Disease-specific survival. (C) Disease-free survival. Full-size ⬛ DOI: 10.7717/peerj.10465/fig-2
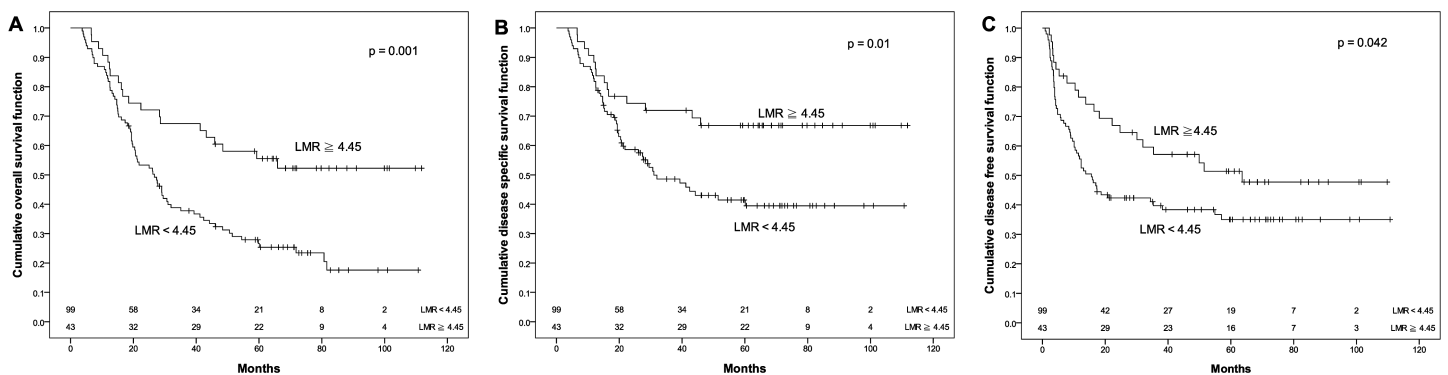

Figure 3 Kaplan–Meier survival curves. Kaplan–Meier survival curves by different level of pretreatment lymphocyte to monocyte ratio (LMR). (A) Overall survival. (B) Disease-specific survival. (C) Disease-free survival. Full-size ⬛ DOI: 10.7717/peerj.10465/fig-3

Table 2 Univariate analysis of factors impacting survival ($n = 142$).

Variable		Number	Event	5 year OS (%)	p	Event	5 year DSS (%)	p	Event	5 year DFS (%)	p
Age	<53	70	45	38.1	0.489	34	49.5	0.836	44	35.3	0.743
	≧53	72	50	33.2		34	50.1		39	44.3	
	0	93	60	37.9		44	50.4		56	39.1	
ACE-27	1	42	30	31.4	0.602	21	47.7	0.948	24	39.9	0.893
	2	7	5	28.6		3	57.1		3	57.1	
Betel nut chewing	No	36	26	36.1	0.841	16	55.7	0.672	18	48.7	0.416
	Yes	106	69	35.5		52	47.7		65	36.9	
Alcohol drinking	No	24	13	49.0	0.226	9	61.6	0.327	11	52.1	0.221
	Yes	118	82	32.8		59	46.9		72	37.3	
Clinical T classification	T1/2/3	52	34	33.5	0.693	24	50.5	0.638	29	43.6	0.407
	T4a/b	90	61	36.7		44	49.4		54	37.7	
Clinical N classification	N0	25	18	35.6	0.684	11	50.7	0.801	15	36.3	0.884
	N1–N3b	117	77	35.5		57	49.6		68	40.6	
Clinical ENE	Negative	77	48	41.0	0.037*	29	59.4	0.003*	38	49.7	0.008*
	Positive	65	47	29.1		39	38.6		45	28.2	
PNI	<50.5	79	61	24.7	0.004*	43	42.0	0.043*	50	34.2	0.108
	≧50.5	56	31	48.1		23	57.2		31	44.3	
LMR	<4.45	99	75	26.6	0.001*	54	41.4	0.01*	62	35.0	0.042*
	≧4.45	43	20	55.5		14	66.8		21	51.4	

Notes:
* Statistically significant ($p < 0.05$).
OS, overall survival; DSS, disease specific survival; DFS, disease free survival; ACE-27, adult comorbidity evaluation-27; ENE, extranodal extension; PNI, prognostic nutritional index; LMR, lymphocyte to monocyte ratio.

Table 3 Multivariate analysis of prognostic factors associated to overall survival.

Factor	Hazard ratio	95% CI	p-Value
PNI			0.01*
≧50.5	1		
<50.5	1.778	[1.145–2.761]	
LMR			0.001*
≧4.45	1		
<4.45	2.408	[1.439–4.029]	
ENE			0.027*
Negative	1		
Positive	1.592	[1.054–2.405]	

Notes:
* Statistically significant ($p < 0.05$).
ENE, extranodal extension; PNI, prognostic nutritional index; LMR, lymphocyte to monocyte ratio.

DISCUSSION

In the current study of patients with advanced stage (stage III/IV) p16-negative OPSCC, the 5-year DFS, DSS, and OS rates were 39.9%, 49.8% and 35.6%, respectively. PNI, LMR and clinical ENE status were all significant independent factors of OS in our multivariate cox regression analysis.

Table 4 Multivariate analysis of prognostic factors associated to disease-specific survival.

Factor	Hazard ratio	95% CI	p-Value
PNI			0.066
≧50.5	1		
<50.5	1.624	[0.968–2.723]	
LMR			0.007*
≧4.45	1		
<4.45	2.33	[1.255–4.323]	
ENE			0.002*
Negative			
Positive	2.159	[1.319–3.533]	

Notes:
* Statistically significant ($p < 0.05$).
ENE, extranodal extension; PNI, prognostic nutritional index; LMR, lymphocyte to monocyte ratio.

Table 5 Multivariate analysis of prognostic factors associated to disease-free survival.

Factor	Hazard ratio	95% CI	p-Value
LMR			0.027*
≧4.45	1		
<4.45	1.765	[1.067–2.892]	
ENE			0.005*
Negative	1		
Positive	1.86	[1.202–2.878]	

Notes:
* Statistically significant ($p < 0.05$).
ENE, extranodal extension; LMR, lymphocyte to monocyte ratio.

Clinical ENE is the extension of metastatic lymph node through an affected lymph node capsule. It has always been considered a marker of poor prognosis as tumor recurrence and oncological survival in head and neck cancer; thus, it was proposed to be incorporated into the newest edition of the AJCC staging system manual (*Amin et al., 2017*). Our cohort also revealed similar results, showing that the presence of ENE was associated with poor oncologic outcomes.

A low PNI indicated a decrease in the serum albumin and/or a low absolute lymphocyte count. Serum albumin is an important factor of the host inflammatory response and nutritional status (*Gupta & Lis, 2010*). The absolute lymphocyte count is also believed to be an important participant in the inhibition of cancer growth by initiating a cytotoxic immune response (*Mantovani et al., 2008*). Taken together, this existing evidence showed that malnutrition and lymphocytopenia may be factors affecting a chronically impaired immune system. The cutoff value for PNI reported in previous studies in other type of cancer was 40–60 (*Feng & Chen, 2014*; *Lee et al., 2017*; *Jian-Hui et al., 2016*; *Shibutani et al., 2015*; *Yang et al., 2016*; *Zhang et al., 2019*). With regard to head and neck cancer, several studies found that lower PNI predicted poor oncologic outcomes in head and neck squamous cell carcinoma (HNSCC) (Table 6) (*Bruixola et al., 2018*;

Table 6 Different studies about PNI in HNSCC.

Reference	Site	Case number	Cut off for PNI	Primary treatment strategy	Statically significant Outcome measurement
Bruixola et al. (2018)	Locoregionally advanced HNSCC	145	45	ICT followed by CCRT	OS
Kono et al. (2017)	HNSCC	101	40	Radiotherapy	Toxicity of radiotherapy
Chang et al. (2018)	Advanced oral cavity, oropharynx, hypopharyngeal cancer	143	36	CCRT	Treatment tolerance and toxicity of CCRT
Fu et al. (2016)	Laryngeal squamous cell carcinoma	975	48.65	Radical surgery	DSS and OS
Our current study	Advanced stage p16 negative OPSCC	142	50.5	CCRT	OS

Note:
PNI, prognostic nutritional index; HNSCC, head and neck squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma; ICT, induction chemotherapy; CCRT, concurrent chemoradiotherapy; OS, overall survival; DSS, disease-specific survival.

Table 7 Different studies about LMR in HNSCC.

Reference	Site	Case number	Cut off for LMR	Primary treatment strategy	Statically significant Outcome measurement
Takahashi et al. (2019)	Oropharyngeal carcinoma	75	4.97	Heterogeneity (76% of population were CRT)	OS
Tham et al. (2019)	HNSCC	123	2.8	Radical surgery	Event free survival
Furukawa et al. (2019)	Tongue cancer	103	4.29	Radical surgery	OS
Yang et al. (2018)	Hypopharyngeal carcinoma	197	2.98	Not well documented	OS, DSS and DFS
Kano et al. (2017)	Oropharyngeal, hypopharyngeal, and laryngeal cancers	285	3.22	Concurrent CRT	OS and DFS
Our current study	Advanced stage p16 negative OPSCC	142	4.45	Concurrent CRT	OS, DSS and DFS

Note:
LMR, lymphocyte to monocyte ratio; HNSCC, head and neck squamous cell carcinoma; OPSCC, oropharyngeal squamous cell carcinoma; CRT, chemoradiotherapy; OS, overall survival; DSS, disease-specific survival; DFS, disease-free survival.

Kono et al., 2017; *Chang et al., 2018*; *Fu et al., 2016*). *Bruixola et al. (2018)* demonstrated that low PNI (cutoff value: 45) was an independent prognostic biomarker in locoregional advanced HNSCC. *Fu et al. (2016)* studied 975 patients with laryngeal squamous cell carcinoma treated with curative laryngectomy, and found that patients with PNI < 48.65 had a low probability of cancer-specific survival and OS. Our results are comparable with these findings, showing that a low PNI is an indicator of poor prognosis in patients with advanced stage (stage III/IV) p16-negative OPSCC undergoing primary CCRT, with a cutoff value similar to previous studies (*Bruixola et al., 2018*; *Kono et al., 2017*; *Chang et al., 2018*; *Fu et al., 2016*). In our study, patients with PNI < 50.5 had significantly reduced survival with adjusted for other prognostic factors in the multivariate analysis.

Studies of investigating the clinical effects of LMR on HNSCC prognosis have increased in recent years. White blood cell differential could be divided into myeloid lineage and lymphoid lineage. It is believed that lymphoid lineage preponderance of white blood cell was related to better survival based on previous HNSCC study (*Wu et al., 2017*). Several studies found that lower LMR predicted reduced DSS and OS in HNSCC (Table 7) (*Takahashi et al., 2019*; *Tham et al., 2019*; *Furukawa et al., 2019*; *Yang et al., 2018*;

Kano et al., 2017). In addition, the relationship between LMR and advanced stage OPSCC was not thoroughly evaluated. Our results are comparable with these findings, showing that a low LMR is an indicator of poor prognosis in advanced stage (stage III/IV) p16-negative OPSCC. Patients with LMR < 4.45 have significantly reduced OS, DSS and DFS according to the multivariate analysis.

The mechanism between an increased systemic inflammatory response and promotion of tumor cell invasion is not clearly understood. A possible explanation might lie in the antitumoral roles that lymphocyte plays by inhibiting tumor cell proliferation and migration, and reinforcing human's immune response to cancer (*De Giorgi et al., 2012*). Fewer infiltrating lymphocytes have been correlated to poor prognosis (*Gooden et al., 2011*). In contrast, higher levels of monocyte-derived macrophages have been associated with greater tumor aggressiveness and poorer survival outcomes (*Pollard, 2004*). This is postulated to happen through tumor microenvironment mediators such as TNF-α, vascular endothelial growth factor and epidermal growth factor (*Pollard, 2004*; *Xiong et al., 1998*). A low LMR implies a relative decrease in lymphocytes and/or increase monocytes. Perhaps, the prognostic ability of LMR is owing to its action as a crude marker for the pro-tumor versus anti-tumor dynamic in the immune system (*Lin, Chien & Chuang, 2017*).

PNI, which calculated as $10 \times$ baseline serum albumin (g/dL) + $0.005 \times$ baseline absolute lymphocyte count (cells/mm^3), is used to evaluate the immune-nutritional status and may influence the prognosis of cancer patients (*Yao et al., 2013*). Poor immune-nutritional status has been reported as its' association with an immunosuppressed condition, which provides a favorable microenvironment for tumor relapse (*Colotta et al., 2009*). That may be the reason why this immunosuppressed condition in low-PNI patients may cause the poor outcomes. Recently, remarkable progress in research on immune checkpoints in tumor immunity has allowed the elucidation of the molecular mechanism underlying immunological tolerance to tumor development. The association between peripheral inflammatory biomarkers and treatment outcomes for immunotherapy remains unclear. These biomarkers might serve as a useful predictor for immunotherapy in the treatment of head and neck cancer in the future.

In our study, we have identified the clinical significance of PNI and LMR on survival in patients with p16 negative oropharyngeal cancer treated by CCRT. Moreover, we had control cancer stage and HPV status these two well-known prognostic factors in oropharyngeal cancer, making this cohort homogenous for our analysis findings. However, the drawback of our study is that it is retrospective, and selective bias may exist. A prospective study or large series study from multiple institutes is necessary to confirm our findings.

CONCLUSION

In summary, our current study showed that patients with higher pretreatment LMR (≧4.45) showed significantly better survival than those with lower LMR (<4.45); Patients with higher PNI (≧50.5) revealed significantly better 5-year OS and 5-year DSS than those with lower PNI (<50.5). According to Cox regression analysis from this

cohort, pretreatment LMR and PNI were also an independent prognostic factor that predicts OS. Interestingly, it may be possible to incorporate pretreatment LMR and PNI into the treatment strategy for patients with advanced stage p16-negative OPSCC undergoing CRT/RTO in the future.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Ming-Hsien Tsai conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Tai-Lin Huang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Hui-Ching Chuang conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Yu-Tsai Lin conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Fu-Min Fang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Hui Lu analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Chih-Yen Chien conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):
   This study was approved by the Medical Ethics and Human Clinical Trial Committees at Chang Gung Memorial Hospital (Ethical Application Reference number:202000471B0).

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.10465#supplemental-information.

REFERENCES

Aggarwal BB, Vijayalekshmi RV, Sung B. 2009. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clinical Cancer Research* **15(2)**:425–430 DOI 10.1158/1078-0432.CCR-08-0149.

Al-Swiahb JN, Huang CC, Fang FM, Chuang HC, Huang HY, Luo SD, Chen CH, Chen CM, Chien CY. 2010. Prognostic impact of p16, p53, epidermal growth factor receptor, and human papillomavirus in oropharyngeal cancer in a betel nut-chewing area. *Archives of Otolaryngology—Head & Neck Surgery* **136(5)**:502–508 DOI 10.1001/archoto.2010.47.

Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP. 2017. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA: A Cancer Journal for Clinicians* **67(2)**:93–99 DOI 10.3322/caac.21388.

Bruixola G, Caballero J, Papaccio F, Petrillo A, Iranzo A, Civera M, Moriana M, Bosch N, Maroñas M, González I, Pastor M, Cervantes A. 2018. Prognostic nutritional index as an independent prognostic factor in locoregionally advanced squamous cell head and neck cancer. *ESMO Open* **3(6)**:e000425 DOI 10.1136/esmoopen-2018-000425.

Chang PH, Hsieh JC, Yeh KY, Chen EY, Yang SW, Huang JS, Lai CH, Wu TH, Huang YM, Chang YS, Chou WC, Wang CH. 2018. Prognostic nutritional index relevance in chemoradiotherapy for advanced oral cavity, oropharyngeal and hypopharyngeal cancer. *Asia Pacific Journal of Clinical Nutrition* **27**:996–1001 DOI 10.6133/apjcn.032018.04.

Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. 2009. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* **30(7)**:1073–1081 DOI 10.1093/carcin/bgp127.

De Giorgi U, Mego M, Scarpi E, Giuliano M, Giordano A, Reuben JM, Valero V, Ueno NT, Hortobagyi GN, Cristofanilli M. 2012. Relationship between lymphocytopenia and circulating tumor cells as prognostic factors for overall survival in metastatic breast cancer. *Clinical Breast Cancer* **12(4)**:264–269 DOI 10.1016/j.clbc.2012.04.004.

Feng JF, Chen QX. 2014. Significance of the prognostic nutritional index in patients with esophageal squamous cell carcinoma. *Therapeutics and Clinical Risk Management* **10**:1–7 DOI 10.2147/TCRM.S56159.

Fu Y, Chen SW, Chen SQ, Ou-Yang D, Liu WW, Song M, Yang AK, Zhang Q. 2016. A preoperative nutritional index for predicting cancer-specific and overall survival in Chinese patients with laryngeal cancer: a retrospective study. *Medicine* **95(11)**:e2962 DOI 10.1097/MD.0000000000002962.

Furukawa K, Kawasaki G, Naruse T, Umeda M. 2019. Prognostic significance of pretreatment lymphocyte–to–monocyte ratio in patients with tongue cancer. *Anticancer research* **39(1)**:405–412 DOI 10.21873/anticanres.13126.

Golusiński P, Pazdrowski J, Szewczyk M, Misiołek M, Pietruszewska W, Klatka J, Okła S, Kaźmierczak H, Marszałek A, Filas V, Schneider A, Masternak MM, Stęplewska K, Miśkiewicz-Orczyk K, Golusiński W. 2017. Is immunohistochemical evaluation of p16 in oropharyngeal cancer enough to predict the HPV positivity? *Reports of Practical Oncology & Radiotherapy* **22(3)**:237–242 DOI 10.1016/j.rpor.2017.01.003.

Gooden MJ, De Bock GH, Leffers N, Daemen T, Nijman HW. 2011. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *British Journal of Cancer* **105(1)**:93–103 DOI 10.1038/bjc.2011.189.

Gupta D, Lis CG. 2010. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutrition Journal* **9(1)**:69 DOI 10.1186/1475-2891-9-69.

Haddad CR, Guo L, Clarke S, Guminski A, Back M, Eade T. 2015. Neutrophil-to-lymphocyte ratio in head and neck cancer. *Journal of Medical Imaging and Radiation Oncology* **59(4)**:514–519 DOI 10.1111/1754-9485.12305.

Hsu WL, Yu KJ, Chiang CJ, Chen TC, Wang CP. 2017. Head and neck cancer incidence trends in Taiwan, 1980–2014. *International Journal of Head and Neck Science* **1**:180–189 DOI 10.6696/IJHNS.2017.0103.05.

Jian-Hui C, Iskandar EA, ShI Cai, Chen CQ, Wu H, Xu JB, He YL. 2016. Significance of Onodera's prognostic nutritional index in patients with colorectal cancer: a large cohort study in a single Chinese institution. *Tumour Biology* **37(3)**:3277–3283 DOI 10.1007/s13277-015-4008-8.

Kano S, Homma A, Hatakeyama H, Mizumachi T, Sakashita T, Kakizaki T, Fukuda S. 2017. Pretreatment lymphocyte-to-monocyte ratio as an independent prognostic factor for head and neck cancer. *Head & Neck* **39(2)**:247–253 DOI 10.1002/hed.24576.

Kono T, Sakamoto K, Shinden S, Ogawa K. 2017. Pre-therapeutic nutritional assessment for predicting severe adverse events in patients with head and neck cancer treated by radiotherapy. *Clinical Nutrition* **36(6)**:1681–1685 DOI 10.1016/j.clnu.2016.10.021.

Lee SH, Chung MJ, Kim B, Lee HS, Lee HJ, Heo JY, Kim YJ, Park JY, Bang S, Park SW, Song SY, Chung JB. 2017. The significance of the prognostic nutritional index for all stages of pancreatic cancer. *Nutrition and Cancer* **69(3)**:512–519 DOI 10.1080/01635581.2016.1250921.

Lin CN, Chien CY, Chuang HC. 2017. Are friends or foes? New strategy for head and neck squamous cell carcinoma treatment via immune regulation. *International Journal of Head and Neck Science* **1**:105–113 DOI 10.6696/IJHNS.2017.0102.03.

Mantovani A, Allavena P, Sica A, Balkwill F. 2008. Cancer-related inflammation. *Nature* **454(7203)**:436–444 DOI 10.1038/nature07205.

McGuire S. 2016. World cancer report 2014—Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. *Advances in Nutrition* **7**:418–419 DOI 10.3945/an.116.012211.

Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, Roberts S. 2013. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. *Head & Neck* **35(5)**:747–755 DOI 10.1002/hed.22015.

Moon H, Roh JL, Lee SW, Kim SB, Choi SH, Nam SY, Kim SY. 2016. Prognostic value of nutritional and hematologic markers in head and neck squamous cell carcinoma treated by chemoradiotherapy. *Radiotherapy and Oncology* **118(2)**:330–334 DOI 10.1016/j.radonc.2015.10.029.

Nozoe T, Kimura Y, Ishida M, Saeki H, Korenaga D, Sugimachi K. 2002. Correlation of pre-operative nutritional condition with post-operative complications in surgical treatment for oesophageal carcinoma. *European Journal of Surgical Oncology* **28(4)**:396–400 DOI 10.1053/ejso.2002.1257.

Onodera T, Goseki N, Kosaki G. 1984. Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients. *Nihon Geka Gakkai Zasshi* **85**:1001–1005.

O'Callaghan DS, O'Donnell D, O'Connell F, O'Byrne KJ. 2010. The role of inflammation in the pathogenesis of non-small cell lung cancer. *Journal of Thoracic Oncology* **5(12)**:2024–2036 DOI 10.1097/JTO.0b013e3181f387e4.

Perisanidis C, Kornek G, Pöschl PW, Holzinger D, Pirklbauer K, Schopper C, Ewers R. 2013. High neutrophil-to-lymphocyte ratio is an independent marker of poor disease-specific survival in patients with oral cancer. *Medical Oncology* **30(1)**:334 DOI 10.1007/s12032-012-0334-5.

Pollard JW. 2004. Tumour-educated macrophages promote tumour progression and metastasis. *Nature Reviews Cancer* **4(1)**:71–78 DOI 10.1038/nrc1256.

Rassouli A, Saliba J, Castano R, Hier M, Zeitouni AG. 2015. Systemic inflammatory markers as independent prognosticators of head and neck squamous cell carcinoma. *Head & Neck* **37(1)**:103–110 DOI 10.1002/hed.23567.

Shibutani M, Maeda K, Nagahara H, Ohtani H, Iseki Y, Ikeya T, Sugano K, Hirakawa K. 2015. The prognostic significance of the postoperative prognostic nutritional index in patients with colorectal cancer. *BMC Cancer* **15(1)**:521 DOI 10.1186/s12885-015-1537-x.

Takahashi H, Sakakura K, Tada H, Kaira K, Oyama T, Chikamatsu K. 2019. Prognostic significance and population dynamics of peripheral monocytes in patients with oropharyngeal squamous cell carcinoma. *Head & Neck* **41(6)**:1880–1888 DOI 10.1002/hed.25625.

Tham T, Olson C, Khaymovich J, Herman SW, Costantino PD. 2018. The lymphocyte-to-monocyte ratio as a prognostic indicator in head and neck cancer: a systematic review and meta-analysis. *European Archives of Oto-Rhino-Laryngology* **275(7)**:1663–1670 DOI 10.1007/s00405-018-4972-x.

Tham T, Wotman M, Chung C, Ahn S, Dupuis H, Gliagias V, Movsesova T, Kraus D, Costantino P. 2019. Systemic immune response in squamous cell carcinoma of the head and neck: a comparative concordance index analysis. *European Archives of Oto-Rhino-Laryngology* **276(10)**:2913–2922 DOI 10.1007/s00405-019-05554-x.

Wu CN, Chuang HC, Lin YT, Fang FM, Li SH, Chien CY. 2017. Prognosis of neutrophil-to-lymphocyte ratio in clinical early-stage tongue (cT1/T2N0) cancer. *OncoTargets and Therapy* **10**:3917–3924 DOI 10.2147/OTT.S140800.

Xiong M, Elson G, Legarda D, Leibovich SJ. 1998. Production of vascular endothelial growth factor by murine macrophages: regulation by hypoxia, lactate, and the inducible nitric oxide synthase pathway. *American Journal of Pathology* **153(2)**:587–598 DOI 10.1016/S0002-9440(10)65601-5.

Yang J, Hsueh CY, Cao W, Zhou L. 2018. Pretreatment lymphocyte-to-monocyte ratio as an independent prognostic factor for hypopharyngeal squamous cell carcinoma. *Acta Oto-Laryngologica* **138(8)**:734–740 DOI 10.1080/00016489.2018.1449965.

Yang L, Xia L, Wang Y, Hong S, Chen H, Liang S, Peng P, Chen Y. 2016. Low prognostic nutritional index (PNI) predicts unfavorable distant metastasis-free survival in nasopharyngeal carcinoma: a propensity score-matched analysis. *PLOS ONE* **11(7)**:e0158853 DOI 10.1371/journal.pone.0158853.

Yao ZH, Tian GY, Wan YY, Kang YM, Guo HS, Liu QH, Lin DJ. 2013. Prognostic nutritional index predicts outcomes of malignant pleural mesothelioma. *Journal of Cancer Research and Clinical Oncology* **139(12)**:2117–2123 DOI 10.1007/s00432-013-1523-0.

Zhang H, Shang X, Ren P, Gong L, Ahmed A, Ma Z, Ma R, Wu X, Xiao X, Jiang H, Tang P, Yu Z. 2019. The predictive value of a preoperative systemic immune-inflammation index and prognostic nutritional index in patients with esophageal squamous cell carcinoma. *Journal of Cellular Physiology* **234(2)**:1794–1802 DOI 10.1002/jcp.27052.

Tsai et al. (2020), *PeerJ*, DOI 10.7717/peerj.10465

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