

Research Paper

Low Preoperative Lymphocyte to Monocyte Ratio Serves as a Worse Prognostic Marker in Patients with Esophageal Squamous Cell Carcinoma Undergoing Curative Tumor Resection

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Abstract

Preoperative lymphocyte to monocyte ratio (LMR) has been considered a prognostic factor in various cancers. However, the application of LMR in the assessment of patients with esophageal squamous cell carcinoma (ESCC) remains controversial. This study aimed to investigate whether preoperative LMR could serve as a prognostic marker in patients with ESCC undergoing curative tumor resection. Medical records of 680 patients of ESCC after curative surgery without preoperative adjuvant therapy were obtained. The median of LMR was determined as the optimal cut off value. The association of LMR with clinical features of ESCC was analyzed using chi-square tests. Spearman's correlation coefficient was used to calculate the correlation. Disease-free survival (DFS) and overall survival (OS) stratified by LMR were evaluated using Kaplan–Meier method and log-rank test. The LMR was negatively correlated with sex ($r=-0.245$, $P<0.001$). Low LMR ($LMR<3.17$) predicted a shorter DFS and OS in patients with ESCC. Multivariate analyses revealed that LMR was independently correlated with DFS (hazard ratios 0.854; 95% confidence interval 0.768-0.949; $P=0.003$) and OS (hazard ratios 0.864; 95% confidence interval 0.779-0.958; $P=0.006$). Our study indicated that low LMR could serve as an independent worse prognostic marker in patients with ESCC.

Key words: lymphocyte to monocyte, esophageal squamous cell carcinoma, prognosis

Introduction

Esophageal cancer is identified as the sixth leading cause of cancer-related mortality in the world [1]. In China, esophageal cancer is one of the most frequent malignant carcinomas and the fourth most common cause of cancer-related death [2]. Meanwhile, 90 percent of cases are esophageal squamous cell carcinomas (ESCC) [3]. Although significant advances achieved in multimodality therapies including surgery, chemotherapy and radiotherapy, patients with ESCC still face with unfavorable prognosis that the 5-year survival rate is

only around 30% [4-6]. Several factors are associated with the prognosis of ESCC, including clinicopathological characteristics such as TNM stage and tumor differentiation. Nevertheless, patients with the same TNM stage have disparate prognosis [7]. Therefore, it is imperative to identify new and accurate prognosis biomarkers of ESCC.

A growing number of studies have showed that cancer-related inflammation results in worse prognosis. Moreover, systemic inflammation plays an outstanding role in carcinoma development,

progression and metastasis [8]. A variety of inflammatory biomarkers including neutrophil to lymphocyte ratio (NLR), C-reactive protein to albumin ratio (CAR) have been extensively studied in various cancers [9-11]. The lymphocyte to monocyte ratio (LMR) has been associated with worse prognosis in various cancers such as hepatocellular carcinoma, ovarian cancer and breast cancer [12-14]. However, because of the inconsistent results, whether LMR are associated with the prognosis in ESCC remains controversial [15-18]. Therefore, this retrospective study aimed to investigate whether preoperative LMR plays a key role in survival of patients with ESCC.

Materials and Methods

Patient selection

A total of 731 patients newly diagnosed with ESCC from Feb 2008 to Jan 2015 at the Zhejiang Cancer Hospital were selected for the research. The histological grades were determined based on the World Health Organization classification criteria. The inclusion standard as follows: (1) ESCC was pathologically confirmed; (2) received esophagectomy after diagnosis without preoperative chemotherapy or radiotherapy; (3) sufficient clinical information and follow-up data. 51 patients were excluded: 43 patients with preoperative chemotherapy, 7 patients with preoperative radiotherapy and 1 patient without preoperative LMR available. As result, 680 patients with ESCC were enrolled in the study.

The preoperative peripheral blood cell count was examined within 1 week prior to surgery. The LMR was calculated by dividing the absolute lymphocyte count with the absolute monocyte count. The conduct of this study was approved by the Ethics Committee of Zhejiang Cancer Hospital. All individual participants completed written informed consent.

Blood-routine markers

Patients were requested to be fasted after midnight prior to draw blood sample. On the morning, venous blood sample (2 mL) was drawn into the EDTA-K2 anticoagulative tubes. The lymphocyte and monocyte were examined by the SYSMEX XE-2100 (Sysmex, Kobe, Japan) Automatic Blood Cell Analyzer.

Statistical analysis

Categorical data are expressed as percentage and continuous data without a normal distribution as median and interquartile range. The lymphocyte, monocyte and LMR were analyzed as continuous variables and all clinicopathologic characteristics were counted as categorical variables.

The association of LMR with clinicopathological features of ESCC was analyzed using chi-square tests. The median of LMR was used to determine the cutoff value. Overall survival (OS) and disease-free survival (DFS) were calculated by the Kaplan-Meier method. The log-rank test was used to evaluate the effect of clinicopathological features on survival. Hazard ratio and 95% confidence interval were calculated using multivariate COX regression analyses. All statistical tests were two-sided, and $P < 0.05$ was considered to be statistically significant. The SPSS software (version 19.0) was utilized for statistical analysis.

Table 1. Difference in LMR according to clinical characteristics in ESCC patients

Variables	Cases		
	N	%	
Sex	Male	582	85.6
	Female	98	14.4
Age at therapy initiation (years)	Median	61	
	Interquartile range	(56-67)	
Pathology grade	Well differentiated	49	7.2
	middle differentiated	449	66.0
	Poorly differentiated	166	24.4
	Undifferentiated	2	0.3
	Missing	14	2.1
Depth of tumor	T1a-1b	64	9.4
	T2	131	19.3
	T3	485	71.3
Lymph node metastasis	N0	296	43.5
	N1	213	31.3
	N2	118	17.4
	N3	53	7.8
Pathological stage	1a-1b	116	17.1
	2a-2b	229	33.7
	3a-3c	335	49.3
Vessel invasive	Yes	210	30.9
	No	470	69.1
Nerve infiltration	Yes	254	37.4
	No	426	62.6
Treatment regimen	S	461	67.8
	S plus postoperative C	155	22.8
	S plus postoperative CRT	64	9.4
Hospital time after operation (days)	Median	11	
	Interquartile range	(10-14)	
LMR	Median	3.17	
	Interquartile range	(2.33-4.33)	

Abbreviations: S: surgery; C: chemotherapy; CRT: chemoradiotherapy; LMR: lymphocyte to monocyte ratio.

Results

Patient characteristics

We enrolled 680 patients with ESCC in this study, including 582 (85.6%) males and 98 (14.4%) females. The median age at therapy initiation was 61 years (Interquartile range: 56-67 years). There were 461 (67.8%) patients received surgery without any other auxiliary treatment, 155 (22.8%) patients received chemotherapy and 64 (9.4%) had access to

radiotherapy after the operation. The clinical characteristics were shown in Table 1.

Table 2. Relationship between preoperative LMR and clinicopathological features in patients with ESCC

Characteristics	Total patients	LMR<3.17 (n=343)	LMR≥3.17 (n=337)	P value
Sex	Male	321	261	<0.001
	Female	23	75	
Age at therapy initiation(years)	≤60	148	164	0.130
	>60	196	172	
Pathology grade	Well differentiated	28	21	0.539
	middle differentiated	228	221	
	Poorly differentiated	81	85	
	Undifferentiated	0	2	
	Missing	7	7	
Depth of tumor	T1a-1b	25	39	0.151
	T2	67	64	
	T3	252	233	
Lymph node metastasis	N0	148	148	0.307
	N1	102	111	
	N2	61	57	
	N3	33	20	
Pathological stage	1a-1b	55	61	0.572
	2a-2b	113	116	
	3a-3c	176	159	
Vessel invasive	Yes	106	104	0.969
	No	238	232	
Nerve infiltration	Yes	127	127	0.813
	No	217	209	
Treatment regimen	S	234	227	0.641
	S plus postoperative C	81	74	
	S plus postoperative CRT	29	35	
Hospital time after operation(days)	≤14	264	273	0.149
	>14	80	63	

Abbreviations: S: surgery; C: chemotherapy; CRT: chemoradiotherapy; LMR: lymphocyte to monocyte ratio.

The median of the LMR was 3.17(Interquartile range: 2.33-4.33). We chose the median as the cutoff value and divided the patients into low LMR group (LMR<3.17) and high LMR group (LMR≥3.17). Thus, 343 patients with the low LMR and 337 patients were grouped into the high LMR.

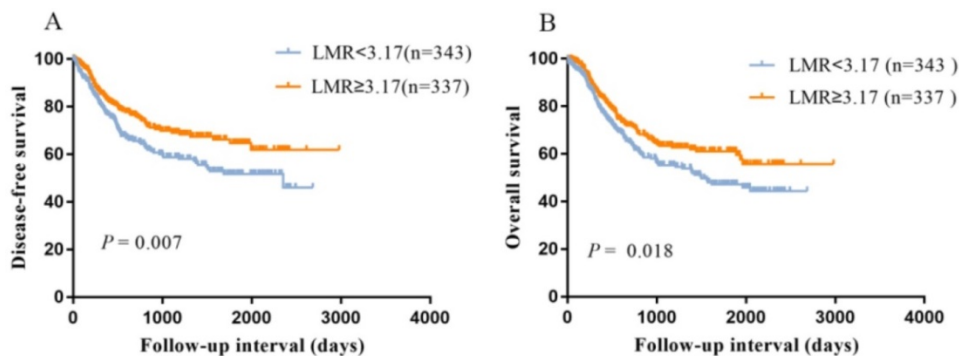


Figure 1. Kaplan-Meier survival curves showing the relationship between lymphocyte to monocyte ratio (LMR) and ESCC. A: Disease-free survival according to LMR; B: Overall survival according to LMR.

Correlation between LMR and clinical features

The correlation between LMR and clinical characteristics is listed in Table 2. None of the clinical features was significantly associated with the LMR including age at therapy initiation, pathology grade, depth of tumor, lymph node metastasis, pathological stage, vessel invasive, nerve infiltration, treatment regimen and hospital time after operation. Nevertheless, a decreased LMR was notably correlated with sex (P<0.001). The LMR was negatively correlated with sex (r = -0.245, P<0.001) using Spearman analysis (Table 3).

Table 3. Spearman analysis of correlation between preoperative LMR and clinicopathological features

Variables	LMR	
	spearman correlation	P value
Sex	-0.245	<0.001
Age at therapy initiation(years)	-0.061	0.110
Depth of tumor	-0.054	0.161
Lymph node metastasis	-0.026	0.506
Pathological stage	-0.037	0.335
Vessel invasive	0.008	0.825
Nerve infiltration	0.004	0.927
Treatment regimen	0.001	0.977
Hospital time after operation(days)	0.013	0.742

Prognostic variables for DFS and OS

As shown in Figure 1, the Kaplan-Meier curves indicated that patients with low LMR had a worse DFS (P=0.007, Figure 1A) and OS (P=0.018, Figure 1B) compared with high LMR group.

Univariate analysis revealed that in addition to LMR (P=0.020), lymph node metastasis (P<0.001), pathological stage (P<0.001), vessel invasive (P=0.027), nerve infiltration (P<0.001) and treatment regimen (P<0.001) were also significantly correlated with DFS. After adjustment for confounders, lymph node metastasis (P<0.001), nerve infiltration (P=0.003), treatment regimen (P<0.001) and LMR (P=0.003) were significantly associated with DFS (Table 4).

Table 4. Disease-free survival analyses according to preoperative LMR in 680 patients with ESCC.

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Sex (male vs. female)	1.246	0.826-1.879	0.295			
Age (>60 vs. ≤60)	0.995	0.977-1.012	0.559			
Depth of tumor (3 vs. 1+2)	1.143	0.909-1.439	0.253			
Lymph node metastasis (+ vs. -)	1.667	1.453-1.910	<0.001	1.648	1.314-2.068	<0.001
Pathological stage (3 vs. 1+2)	1.64	1.332-2.021	<0.001	0.84	0.611-1.157	0.286
Vessel invasive (+ vs. -)	1.382	1.037-1.842	0.027	0.81	0.578-1.136	0.222
Nerve infiltration (+ vs. -)	1.643	1.250-2.159	<0.001	1.57	1.160-2.126	0.003
Treatment regimen (S vs. S+CRT/C)	1.306	1.170-1.458	<0.001	1.299	1.147-1.471	<0.001
Hospital time after operation(days) (>14 vs. ≤14)	1.003	0.989-1.017	0.716			
LMR (≥3.17 vs. <3.17)	0.892	0.810-0.982	0.020	0.854	0.768-0.949	0.003

Abbreviations: S: surgery; C: chemotherapy; CRT: chemoradiotherapy; LMR: lymphocyte to monocyte ratio.

Table 5. Overall survival analyses according to preoperative LMR in 680 patients with ESCC.

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Sex (male vs. female)	1.242	0.836-1.844	0.283			
Age (>60 vs. ≤60)	1.001	0.984-1.018	0.926			
Depth of tumor (3 vs. 1+2)	1.513	1.178-1.943	0.001	0.987	0.714-1.364	0.935
Lymph node metastasis (+ vs. -)	1.755	1.544-1.995	<0.001	1.692	1.317-2.173	<0.001
Pathological stage (3 vs. 1+2)	1.929	1.563-2.382	<0.001	0.909	0.605-1.365	0.644
Vessel invasive (+ vs. -)	1.783	1.369-2.324	<0.001	0.885	0.634-1.237	0.475
Nerve infiltration (+ vs. -)	1.829	1.411-2.371	<0.001	1.524	1.12-2.075	0.007
Treatment regimen (S vs. S+CRT/C)	1.006	0.896-1.13	0.916			
Hospital time after operation(days) (>14 vs. ≤14)	1.007	0.995-1.019	0.234			
LMR(≥3.17 vs. <3.17)	0.895	0.816-0.981	0.018	0.864	0.779-0.958	0.006

Abbreviations: S: surgery; C: chemotherapy; CRT: chemoradiotherapy; LMR: lymphocyte to monocyte ratio.

In the univariate analysis, we found that LMR was significantly associated with OS ($P=0.018$). Meanwhile, there was a close relationship between depth of tumor ($P=0.001$), lymph node metastasis ($P<0.001$), pathological stage ($P<0.001$), vessel invasive ($P=0.027$), nerve infiltration ($P<0.001$) and monocyte ($P=0.022$). In the multivariate analysis, lymph node metastasis ($P<0.001$), nerve infiltration ($P=0.007$) and LMR ($P=0.006$) were significantly related to OS (Table 5). Above all, LMR was an independent prognostic factor for ESCC after esophageal radical surgery.

Discussions

Up to now the association between preoperative LMR and the prognosis of patients with ESCC remains inconclusive. Therefore, we conducted this retrospective study concerning the patients without preoperative adjuvant treatment, which have an impact on the count and percentage of lymphocyte and monocyte. In the present research, low LMR was discovered to be significantly related to sex ($P<0.001$), with 70% of patients being male. There is a 2-fold to 3-fold difference in incidence and mortality rates between the gender worldwide [19]. A total of 731 patients newly diagnosed with ESCC from Feb 2008 to Jan 2015 at the Zhejiang Cancer Hospital were selected for the present study. With 51 patients excluded, 680 patients undergoing radical surgery

were enrolled. Therefore, there was a possibility that decreased LMR was significantly associated with gender. The reason for no relationship between clinical features and LMR was that the patients undergoing radical surgery were in early and middle stage but not in advanced stage. Low LMR was correlated with the unfavorable prognosis of ESCC after radical surgery. We concluded that LMR was an independent prognostic factor for ESCC by using the Cox regression model.

The system inflammation in the tumor microenvironment has an impact on the tumorigenesis and progression. There were increasing studies suggest that the inflammation-based score were associated with the outcome of various cancers [20-25]. The representative markers of system inflammation are the peripheral inflammatory cells such as neutrophils, lymphocytes and monocytes. LMR ratio is the calculated value between lymphocytes and monocytes. As result, LMR ratio that is correlated with worse prognosis of patients with ESCC is on behalf of lymphocytes and monocytes. In other words, LMR presents tumorigenesis and progression through lymphocytes and monocytes. Lymphocytes play a major role in suppressing cancer cell proliferation and migration [26]. Besides, cytotoxic lymphocytes, mainly cytotoxic T cells, are essential for eliminating residual cancer

cell and being applied in immunotherapy [27-28].

Monocytes are thought to have an impact on tumorigenesis through differentiation to tumor-associated macrophages (TAMs). TAMs are recruited to the tumor site by obtaining the signal from tumor-derived chemotactic factors [29]. Therefore, the amount and percentage of monocytes could represent for TAMs reflecting the tumor burden. Recent studies reported that increased infiltration of TAMs was associated with the outcome of various cancers [30-31]. Above all, LMR may substitute for the balance between the promotion of tumor reaction and anti-tumor immune response.

There was also a study that focused on the relationship between LMR and outcome in ESCC patients after radical surgery. The author concluded that low LMR ($LMR \leq 4.0$) was correlated with the worse cancer specific survival and overall survival. The clinical information was based on 147 cases of ESCC patients in Japan. Besides, the cut off value was determined by the Receiver operating characteristics (ROC) curve analysis [16]. In our study, we chose the median value of LMR as the optimal cut off value. Disease free survival and overall survival represented the survival of ESCC patients. Based on 680 cases patients with primary diagnosis of ESCC, we found that low LMR was an independent prognostic factor in ESCC patients in China.

There were several limitations of this study: first, it was the single-center design and retrospective analysis. Second, it was uncertain whether a different cut off value determined by other ways including ROC curve analysis and C index would serve as an optimal indicator of survival in ESCC patients. The cut off value of LMR ranged from 2.93 to 5.3 in the reported studies. We chose the median value of LMR, which minimized a false positive result, as the cut off value. Therefore, multi-center design and prospective trials are required to confirm the reproducibility of these findings.

In summary, a low preoperative LMR indicates a worse survival of patients with primary diagnosis ESCC who received radical surgery. This finding may help clinicians to assess the prognosis of ESCC patients after operation.

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Competing Interests

The authors have declared that no competing interest exists.

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