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Title	Pretreatment lymphocyte-to-monocyte ratio as an independent prognostic factor for head and neck cancer
Author(s)	Kano, Satoshi; Homma, Akihiro; Hatakeyama, Hiromitsu et al.
Citation	Head & neck, 39(2), 247-253 https://doi.org/10.1002/hed.24576
Issue Date	2017-02
Doc URL	https://hdl.handle.net/2115/68256
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Type	journal article
File Information	HeadNeck39_247.pdf

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Pretreatment lymphocyte-to-monocyte ratio as an independent prognostic factor for head and neck cancer

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Running title: lymphocyte-to-monocyte ratio in head and neck cancer

Financial support: None.

Conflict of interest: None.

Keywords: NLR, PLR, LMR, head and neck cancer, chemoradiotherapy

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Abstract

Background. We analyzed the relationship between pretreatment inflammatory markers and the prognosis of patients with oropharyngeal, hypopharyngeal and laryngeal cancers.

Methods. The data for 285 patients treated with curative intent by concurrent chemoradiotherapy (CCRT) were obtained and their pretreatment inflammatory markers, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR), were calculated.

Results. Significant relationships were observed between a high NLR and oropharyngeal or hypopharyngeal cancer, T3-4, N2b-3 and clinical stage III-IV, while significant relationships were observed between a high LMR and laryngeal cancer, T1-2 and clinical stage I-II. With regard to survival outcomes, a high NLR, a high PLR and a low LMR were all significantly associated with decreases in overall survival and disease-free survival. Furthermore, multivariate analysis showed that LMR was an independent prognostic factor.

Conclusions. Pretreatment LMR was found to be an independent prognostic factor for patients with head and neck cancers treated by CCRT.

Head and neck cancer is one of the six most common cancers. More than 90% of these tumors are squamous cell carcinomas (SCC) and most of them are located in the oral cavity, nasopharynx, oropharynx, hypopharynx or larynx. The standard therapy for early stage cancer is surgical excision or radiotherapy, while that for advanced stage cancer is surgery, including reconstructive surgery or concurrent chemoradiotherapy (CCRT). The known prognostic factors for patients with head and neck cancers include performance status, number of pack years of tobacco, primary tumor size, lymph node involvement, distant metastasis and human papilloma virus (HPV) infection. [1,2] However, these parameters cannot always accurately predict the risk of patient mortality. Therefore, other useful biomarkers should be identified for the selection of appropriate treatment.

Since Virchow noted the presence of leucocytes in neoplastic tissues and made a connection between inflammation and cancer in 1863, the association between oncogenesis and a systemic inflammatory response has been gradually clarified. [3] Recently, it has been reported that inflammatory cells in the peripheral blood are associated with the prognosis in many cancer sites, with the results suggesting that inflammatory cells in the tumor microenvironment play a significant role in tumor development. [4-9] The peripheral blood neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) have

been widely investigated as useful predictors of prognosis for various cancers, including head and neck cancers. However, most of studies on inflammatory markers in head and neck cancers have targeted cancers of the nasopharynx or oral cavity, with few studies conducted on other head and neck cancers, including oropharyngeal, hypopharyngeal and laryngeal cancers. [10-22] In addition, there have been no reports on direct comparisons among NLR, PLR and LMR in head and neck cancers. Therefore, the aims of this study are to clarify the relationship between pretreatment inflammatory markers in the peripheral blood and the prognosis of patients with oropharyngeal, hypopharyngeal and laryngeal cancers, and evaluate the inflammatory marker most useful as prognostic factor.

MATERIALS AND METHODS

Patients

This is a retrospective study using the medical records of patients with head and neck cancers treated at Hokkaido University Hospital between January 2003 and December 2012. The inclusion criteria for this study were as follows: (1) previously untreated laryngeal, oropharyngeal or hypopharyngeal cancer, (2) histologically proven SCC, (3) performance status of 0 or 1, and (4) curative-intent CCRT. The exclusion criteria for

this study were as follows: (1) unavailable pretreatment hematologic parameters, (2) distant metastasis at the initial visit, (3) treatment for other cancers within 4 weeks prior to pretreatment peripheral blood examination, (4) complications with an active infection or any hematologic disease, and (5) medication with any immunosuppressive agent. A total of 306 patients met the inclusion criteria, among which 21 patients were later excluded from this study, so that the data for 285 patients were obtained and analyzed in this study. Of the 285 patients, 252 were male and 33 were female. The median patient age was 61 years (range 37-80 years). The primary site was the larynx in 67, oropharynx in 116 and hypopharynx in 102 patients. The median follow-up period for the survivors was 5.1 years (range 0.3-12 years).

Treatment

The irradiation dose was within 65-70 Gy (median, 70Gy). All patients received external radiotherapy, in the form of 4 or 6 MV photons produced by a linear accelerator, to the primary sites and regional lymphatic area. The treatment was planned using a CT simulator and a three-dimensional dose-calculation computer. Although the concomitant chemotherapy consisted of various regimens, approx. 78.2% of all patients received cisplatin, 17.5% received docetaxel and 4.2% received carboplatin. The dose, schedule and route of injection of cisplatin were as follow: 30 – 40 mg/m^2 of cisplatin was

administered intravenously to 130 patients once a week, 80 mg/m^2 of cisplatin was administered intravenously to 14 patients once every 3 weeks and the superselective intra-arterial infusion of high-dose cisplatin (100 – 120 mg/m^2) was performed for 79 patients once a week (3 – 5 cycles). On the other hand, docetaxel was administered intravenously at a dose of 10 mg/m^2 once a week and carboplatin was administered intravenously at a dose of 1.5 area under the curve (AUC) once a week.

Data collection

The following pretreatment hematological parameters were collected within 2 weeks prior to the initial treatment: neutrophil count, lymphocyte count, monocyte count and platelet count. NLR, PLR and LMR were calculated by division of the absolute values of the corresponding hematological parameters.

Statistical analysis

A receiver operating characteristics (ROC) curve for overall survival (OS) after 2 years from the start of treatment was plotted to verify the optimal cutoff values of the continuous NLR, PLR and LMR. The relationships between clinical characteristics and NLR, PLR as well as LMR were examined by chi-square test. Overall survival (OS) and disease-free survival (DFS) curves were calculated using the Kaplan-Meier method and differences were assessed by the log-rank test. A Cox proportional hazard regression

model was used to assess the effect of each variable on OS. Pearson correlation analysis was used to determine the correlations among NLR, PLR and LMR. A 2-tailed *p* value <0.05 was considered statistically significant. Statistical analyses were performed using XLSTAT 2011 (Addinsoft, NY, USA).

RESULTS

Optimal cutoff values of the continuous neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and lymphocyte-to-monocyte ratio

ROC curves for OS were plotted to verify the optimal cutoff values for continuous NLR, PLR and LMR (Figure 1). As shown in Figure 1, the AUC for NLR, PLR and LMR was 0.715, 0.752 and 0.752, respectively. The optimal cutoff value was 1.92 for NLR, 125 for PLR and 3.22 for LMR, with the highest specificity and sensitivity being 0.504 and 0.818 for NLR, 0.574 and 0.818 for PLR, and 0.655 and 0.791 for LMR. Based on each cutoff value, patients were divided into two groups for further analysis. As a result, there were 125 (43.9%) patients with NLR <1.92 and 160 (56.1%) patients with NLR ≥1.92, 135 (47.4%) patients with PLR <125 and 150 (52.6%) patients with PLR ≥125, and 83 (29.1%) patients with LMR <3.22 and 202 (70.9%) patients with LMR ≥3.22.

Patient characteristics and inflammatory markers

To examine the correlations among NLR, PLR and LMR and clinical parameters, the patient characteristics between the high and low NLR, PLR and LMR groups were compared (Table 1). We found that there was a significant relationship between a high NLR and oropharyngeal or hypopharyngeal cancer (P = 0.004), T3-4 (P = 0.005), N2b-3 (P < 0.001) and clinical stage III-IV (P = 0.003). In contrast to NLR, a high LMR was found to be significantly related to laryngeal cancer (P = 0.049), T1-2 (P = 0.038) and clinical stage I-II (P = 0.046). However, no significant associations were observed between PLR and any of the parameters examined.

Survival outcomes

Figure 2 shows the OS and DFS curves based on pretreatment NLR, PLR and LMR. Our results indicated that a high NLR, high PLR and low LMR were significantly associated with decreases in OS and DFS. Furthermore, multivariate Cox proportional hazard regression model analysis for OS was performed to identify the prognostic factors for patients treated with CRT. The results showed that primary site, clinical stage, chemotherapy regimen and LMR were considered to be independent prognostic factors for OS, while NLR and PLR were not independently associated with OS (Table 2). In addition, to assess the prognostic vale of LMR in the unfavorable groups, the OS curves were drawn for patients with hypopharyngeal cancer or stage III-IV disease (Figure 3).

Our results indicated that a high LMR was significantly associated with a decreased survival rate in the unfavorable subgroups as well as in all patients.

Correlations among the inflammatory markers

As shown in Figure 2, univariate analysis showed that a high NLR, high PLR and low LMR were all individually associated with an unfavorable prognosis; however, multivariate analysis showed that only a high LMR was independently associated with OS. Therefore, the correlations between LMR and NLR and between LMR and PLR were examined using Pearson correlation analysis (Figure 4). Every marker was calculated based on the lymphocyte count; nevertheless, there were weak correlations between LMR and NLR and between LMR and PLR (correlation coefficient $R^2 = 0.295$ and 0.231, respectively). Furthermore, OS was examined in 4 groups stratified according to LMR and NLR or PLR level (Figure 5). The OS rate in the high NLR/low LMR group was noticeably worse than that in the other groups, while that in the high PLR/low LMR group was slightly worse than that in the low PLR/low LMR group.

DISCUSSION

This study indicated that the NLR, PLR and LMR were all associated with the prognosis of patients with oropharyngeal, hypopharyngeal and laryngeal cancers in

terms of both OS and DFS. Although all patients enrolled in this study were treated with CCRT, the patients with a high NLR, high PLR and low LMR did not necessarily have tumors with low radiosensitivity. Table 3 shows a summary of published studies on inflammatory markers in head and neck cancer. [10-22] Most studies indicated a statistically significant difference in the survival rates stratified according to NLR, PLR and LMR level regardless of the treatment method. On the other hand, only 2 researches found no significant difference in any survival rate. [11, 22] The reason for this discrepancy is thought to be that these 2 researches used a fixed cutoff value regardless of the individually collected data. Most studies showing a significant difference in the survival rate used a cutoff value calculated from the median, tertiles or ROC curve based on the actual data. However these retrospectively calculated cutoff values cannot be used in a prospective study and are unsuitable for future clinical application to the prediction of treatment outcomes. Further large-scale studies are needed to establish specific cutoff values for the various cancers.

Recent reports have shown that inflammatory markers, such as NLR, PLR and LMR, can be used to predict mortality and recurrence for various cancers. Regardless of the site of the cancer, a high NLR, high PLR and low LMR trend to be associated with increased mortality and recurrence rates, which in agreement with our results.

Neutrophils are thought to produce several cytokines and angiogenic factors that participate in different steps in tumor development. [23] It has been reported that SCC tissue in the head and neck exhibits considerable polymorphonuclear granulocyte infiltration, with high levels of infiltration associated with poorer survival in advanced disease. [24] On the other hand, lymphocytes are responsible for immune surveillance resulting in the elimination of cancer cells. Previous studies have demonstrated an association between a low peripheral lymphocyte count and short survival in different types of cancer. [25, 26] Balermpas et al. reported that patients with abundant tumor-infiltrating lymphocytes had a significantly increased survival rate in head and neck cancers. [27] In addition, Partlova et al. reported that HPV-positive tumors showed a significantly higher number of infiltrating CD8+ T lymphocytes in contrast to HPV-negative tumors, indicating that the presence of high levels of CD8+ T lymphocytes might play a role in a better response to standard treatment and subsequent favorable clinical outcome. [28] Platelets have also been known to mediate tumor cell growth, dissemination and angiogenesis. Activated platelets can interact with cancer cells through paracrine signaling or direct contact, thereby promoting tumor cell growth and survival. [29, 30] Rschidi et al. concluded that a high platelet count was associated with a poor prognosis in patients with head and neck cancers, whereas treatment with

antiplatelet agents was associated with a better prognosis. [31] Monocytes, which differentiate into tissue macrophages and dendritic cells, have been reported to secrete various proinflammatory cytokines and promote tumorigenesis, angiogenesis and distant metastasis. [32, 33] As a consequence, a high monocyte count is associated with short survival. In head and neck cancers, chronic inflammation due to tobacco or chronic infection with HPV or Epstein-Barr Virus (EBV) is known to induce carcinogenesis; therefore, inflammatory cells might be associated with tumor development more strongly than in cancers located in other sites.

We performed multivariate analysis for OS to identify the prognostic factors among several clinical factors and three inflammatory markers. The results indicated that LMR, along with primary site, clinical stage and chemotherapy regimen, was an independent prognostic factor. Furthermore, in the low LMR group, the OS was significantly decreased in the unfavorable subgroups including those with hypopharyngeal cancer or stage III-IV disease. Similar to our study, comparisons among NLR, PLR and LMR have been reported for a number of other cancer sites. NLR was considered to be an independent prognostic factor in patients with gastric cancer, colorectal cancer and colorectal liver metastasis, while LMR was considered to be an independent prognostic factor in patients with bladder cancer, esophageal cancer and

malignant pleural mesothelioma. [4-9] Meta-analysis is required to clarify which inflammatory marker is the most valuable for each cancer site. Furthermore, as shown in Figure 5, a combination of inflammatory markers might reflect treatment outcomes in a more sensitive manner.

In recent years, remarkable progress in research on immune checkpoints in tumor immunity has allowed the elucidation of the molecular mechanism underlying immunological tolerance to tumor development. It was reported that overexpression of programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) in the tumor microenvironment, which suppress the activation of T cells, is associated with an unfavorable prognosis. [34-38] Based on this research, molecular targeted drugs against PD-1 or PD-L1 have been developed and applied clinically. In the case of lung cancer, the anti-PD-1 antibody was reported to be more effective in tumors with gene alterations or in the patients with a history of tobacco use; therefore, head and neck cancers, which possess a number of gene alterations and is caused by tobacco use, are also expected to be good targets for the anti-PD-1 antibody. [39] The association between peripheral inflammatory markers such as LMR and treatment outcomes for immunotherapy remains unclear; however, LMR might afford a useful predictor for immunotherapy in the treatment of head and neck cancers in the future.

In conclusion, this study indicated that pretreatment LMR can be considered an independent prognostic factor for patients with laryngeal, oropharyngeal and hypopharyngeal cancers treated by CCRT. Further large-scale analyses are needed to establish the specific cutoff value with the aim of future clinical application.

ACKNOWLEDGEMENT

This work was supported by MEXT KAKENHI Grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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FIGURE LEGENDS

Figure 1. Optimal cutoff values of the continuous neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR).

Receiver operating characteristics curves for overall survival were plotted to verify the optimal cutoff values for continuous NLR (A), PLR (B) and LMR (C). The area under the curve for NLR, PLR and LMR was 0.715, 0.752 and 0.752, respectively.

Figure 2. Kaplan-Meier curves for overall survival (OS) and disease-free survival (DFS) stratified according to neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) level.

(A) OS curves stratified according to NLR level (the 5-year OS for low NLR 79.2%, high NLR 55.7%). (B) DFS curves stratified according to NLR level (the 5-year DFS for low NLR 69.6%, high NLR 47.8%). (C) OS curves stratified according to PLR level (the 5-year OS for low PLR 75.8%, high NLR 58.0%). (D) DFS curves stratified according to PLR level (the 5-year DFS for low PLR 66.0%, high NLR 50.0%). (E) OS curves stratified according to LMR level (the 5-year OS for low LMR 44.2%, high

LMR 75.5%). (F) DFS curves stratified according to LMR level (the 5-year DFS for low LMR 38.4%, high LMR 65.3%).

Figure 3. Overall survival (OS) curves stratified according to lymphocyte-to-monocyte ratio (LMR) level in the unfavorable groups.

(A) OS curves stratified according to LMR level in patients with hypopharyngeal cancer (the 5-year OS for low LMR 27.1%, high LMR 69.7%). (B) OS curves stratified according to LMR level in patients with stage III-IV disease (the 5-year OS for low LMR 36.1%, high LMR 74.3%).

Figure 4. Correlations among the inflammatory markers.

(A) Correlation chart between lymphocyte-to-monocyte ratio (LMR) and neutrophil-to-lymphocyte ratio (NLR) (regression line: $Y = -0.476 \times X + 4.562$, correlation coefficient: $R^2 = 0.295$). (B) Correlation chart between LMR and platelet-to-lymphocyte ratio (PLR). (regression line: $Y = -22.137 \times X + 245.488$, correlation coefficient: $R^2 = 0.231$).

Figure 5. Overall survival (OS) curves in 4 groups stratified according to lymphocyte-to-monocyte ratio (LMR) and neutrophil-to-lymphocyte ratio (NLR), or LMR and platelet-to-lymphocyte ratio (PLR) level.

(A) OS curves stratified according to LMR and NLR (the 5-year OS for low NLR/high LMR 81.1%, high NLR/high LMR 68.2%, low NLR/low LMR 64.3%, high NLR/low LMR 37.4%). (B) OS curves stratified according to LMR and PLR (the 5-year OS for low PLR/high LMR 79.9%, high PLR/high LMR 70.8%, low PLR/low LMR 51.0%, high PLR/low LMR 40.9%).

Figure 1

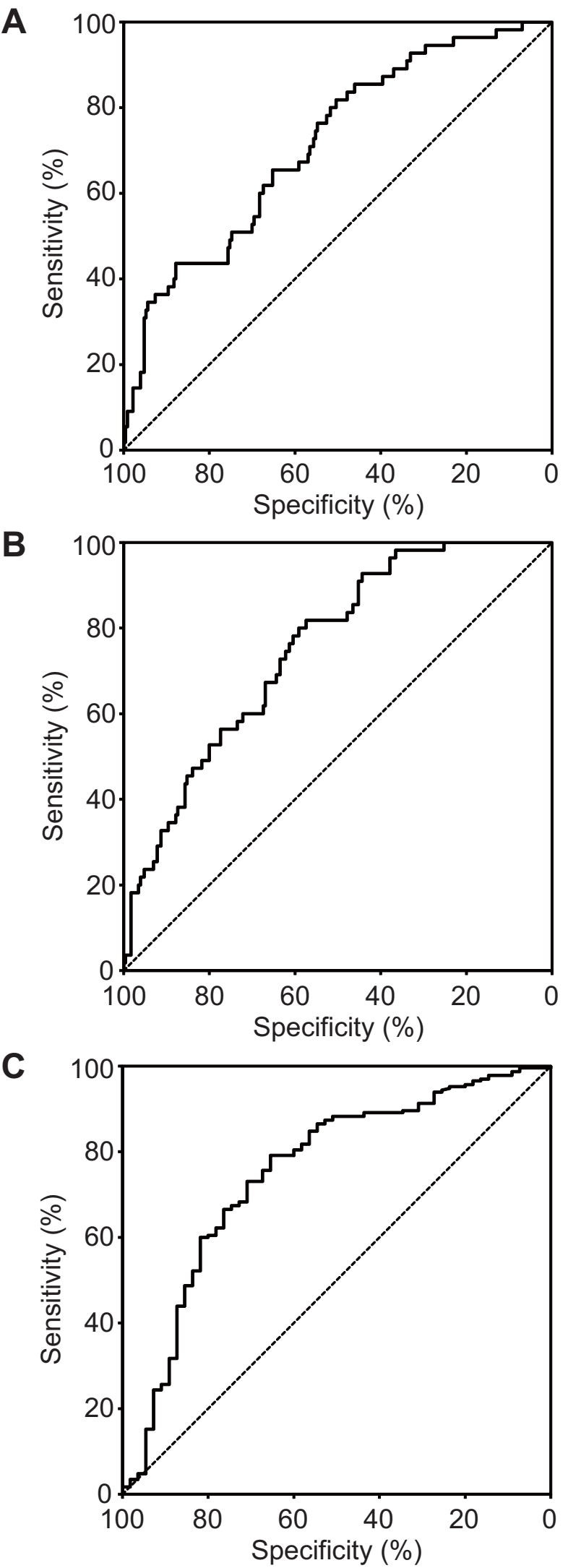


Figure 2

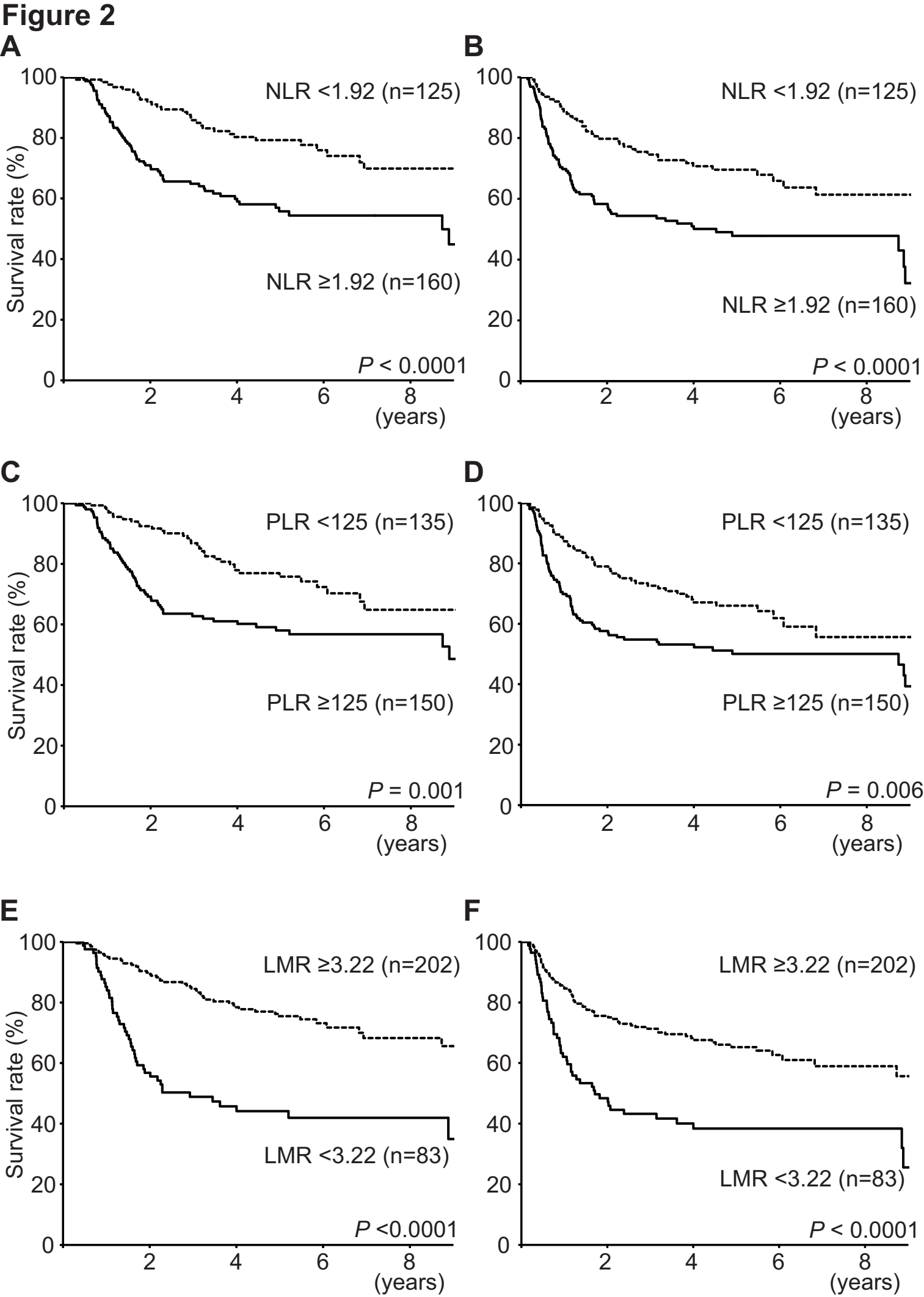

A
100
80
60
40
20
0
Survival rate (%)
NLR <1.92 (n=125)
NLR ≥1.92 (n=160)
P < 0.0001
2
4
6
8
(years)
B
NLR <1.92 (n=125)
NLR ≥1.92 (n=160)
P < 0.0001
(years)
C
PLR <125 (n=135)
PLR ≥125 (n=150)
P = 0.001
(years)
D
PLR <125 (n=135)
PLR ≥125 (n=150)
P = 0.006
(years)
E
LMR ≥3.22 (n=202)
LMR <3.22 (n=83)
P <0.0001
(years)
F
LMR ≥3.22 (n=202)
LMR <3.22 (n=83)
P < 0.0001
(years)

Figure 3

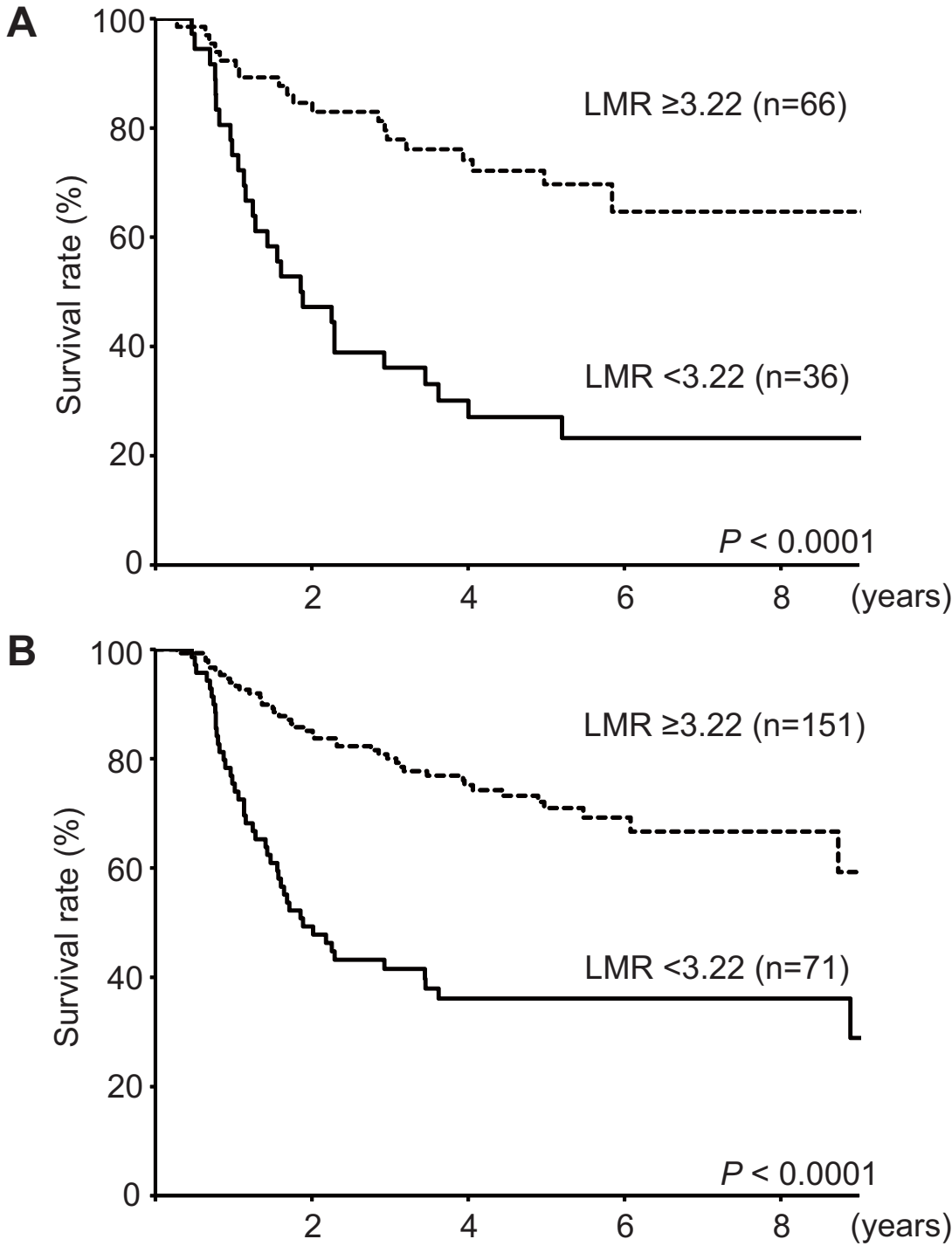

A
100
80
60
40
20
0
Survival rate (%)
LMR ≥3.22 (n=66)
LMR <3.22 (n=36)
P < 0.0001
2
4
6
8
(years)
B
100
80
60
40
20
0
Survival rate (%)
LMR ≥3.22 (n=151)
LMR <3.22 (n=71)
P < 0.0001
2
4
6
8
(years)

Figure 4

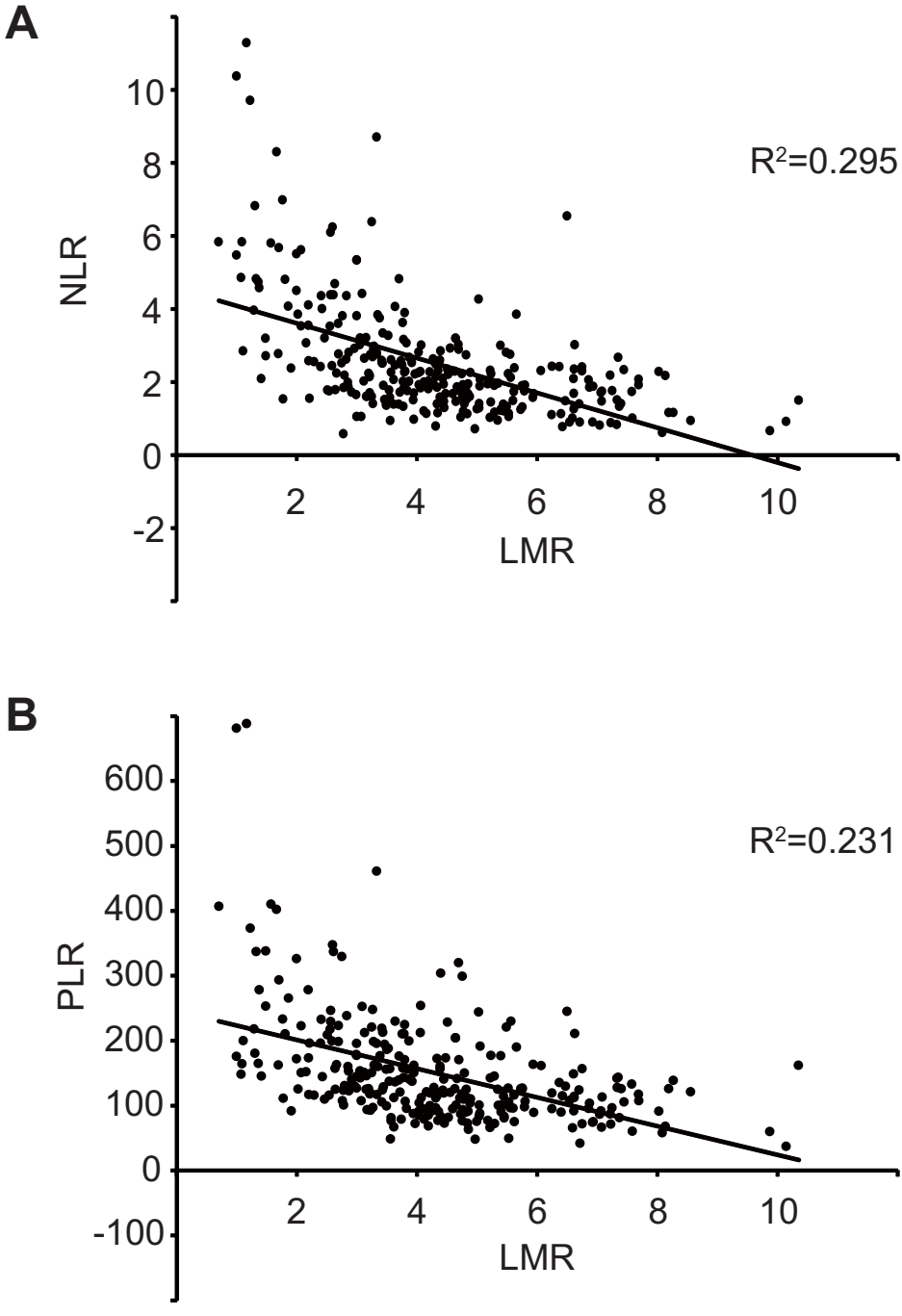

A
10
8
6
4
2
0
-2
NLR
2
4
6
8
10
LMR
R²=0.295
B
600
500
400
300
200
100
0
-100
PLR
2
4
6
8
10
LMR
R²=0.231

Figure 5

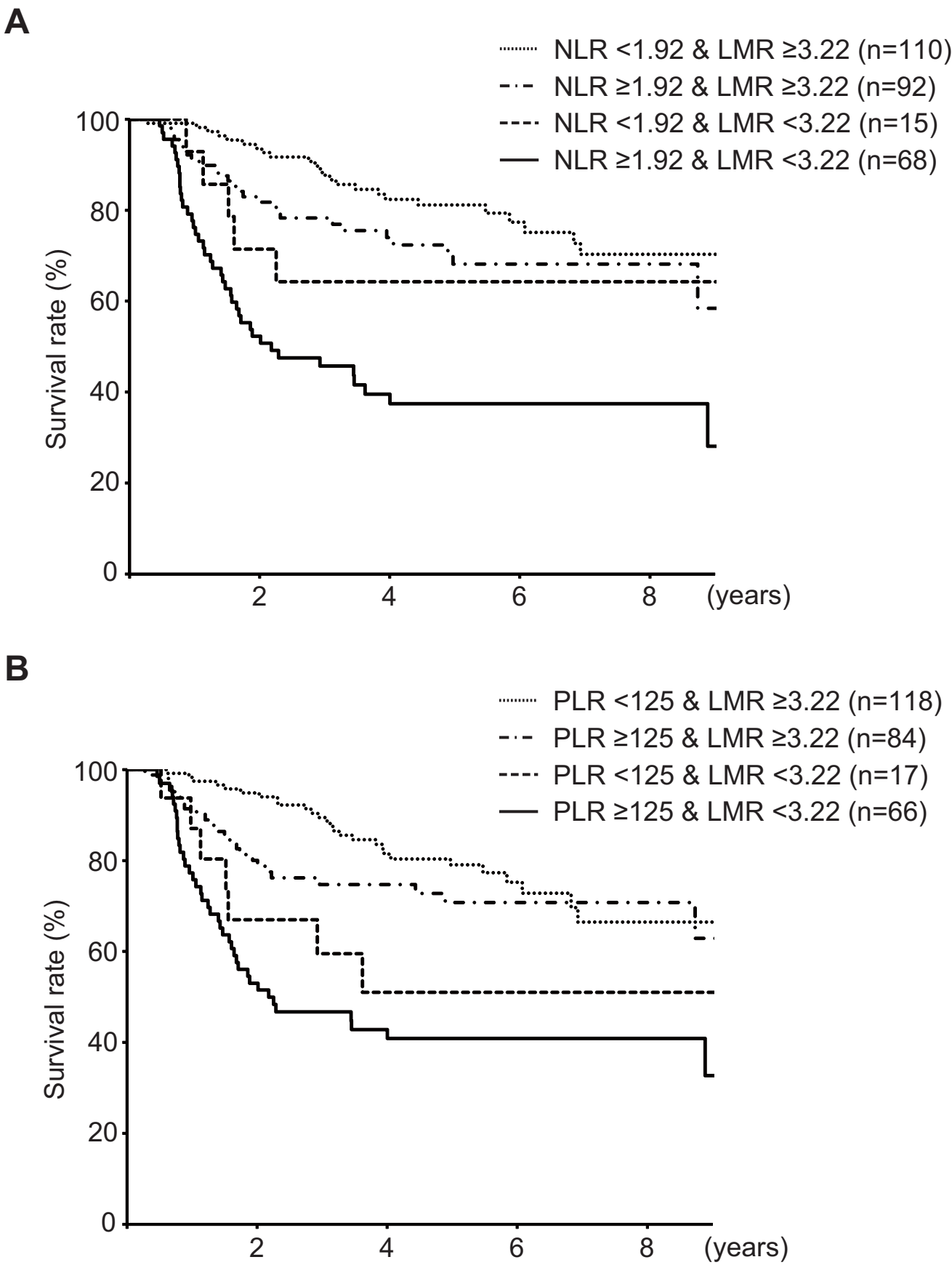

A
NLR <1.92 & LMR ≥3.22 (n=110)
NLR ≥1.92 & LMR ≥3.22 (n=92)
NLR <1.92 & LMR <3.22 (n=15)
NLR ≥1.92 & LMR <3.22 (n=68)
Survival rate (%)
100
80
60
40
20
0
2
4
6
8
(years)
B
PLR <125 & LMR ≥3.22 (n=118)
PLR ≥125 & LMR ≥3.22 (n=84)
PLR <125 & LMR <3.22 (n=17)
PLR ≥125 & LMR <3.22 (n=66)
Survival rate (%)
100
80
60
40
20
0
2
4
6
8
(years)

Table 1. Characteristics of total patients and stratified by neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and lymphocyte-to-monocyte ratio

Variable		NLR			PLR			LMR		
	Total	<1.92	≥1.92		<125	≥125		<3.22	≥3.22	
	No. of patients	No. of patients		*P*	No. of patients		*P*	No. of patients		*P*
Age (yr)										
<61	139	64	75	0.469	63	76	0.500	40	99	0.900
≥61	146	61	85		72	74		43	103	
Gender										
Male	252	111	141	0.860	123	129	0.178	76	176	0.287
Female	33	14	19		12	21		7	26	
Primary										
Larynx	67	41	26	**0.004**	38	29	0.141	12	55	**0.049**
Oropharynx	116	43	73		55	61		35	81	
Hypopharynx	102	41	61		42	60		36	66	
T classification										
1, 2	144	75	69	**0.005**	76	68	0.065	34	110	**0.038**
3, 4	141	50	91		59	82		49	92	
N classification										
0 - 2a	135	75	60	**<0.001**	69	66	0.230	33	102	0.099
2b - 3	150	50	100		66	84		50	100	
Clinical stage										
I, II	63	38	25	**0.003**	35	28	0.140	12	51	**0.046**

III, IV	222	87	135		100	122		71	151	
Chemotherapy										
Cisplatin	223	94	129	0.271	99	124	0.057	64	159	0.765
Others	62	31	31		36	26		19	43	

Abbreviations: NLR, neutrophil-to-lymphocyte ratio, PLR; platelet-to-lymphocyte ratio, LMR; lymphocyte-to-monocyte ratio.

Table 2. Cox proportional hazard regression model analysis for overall survival

Variables	HR (95% CI)	*P*
Age, y (<61)	0.896 (0.595-1.349)	0.598
Gender (Female)	0.880 (0.431-1.799)	0.726
Primary (Larynx)	0.479 (0.256-0.896)	**0.021**
(Oropharynx)	0.571 (0.359-0.908)	**0.018**
Clinical stage (I, II)	0.359 (0.183-0.706)	**0.003**
Chemotherapy (Cisplatin)	0.585 (0.354-0.968)	**0.037**
NLR (<1.92)	0.742 (0.458-1.204)	0.228
PLR (<125)	0.778 (0.493-1.228)	0.281
LMR (≥3.22)	0.439 (0.282-0.685)	**<0.001**

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio.

Table 3. Published studies of the inflammatory marker in head and neck cancer

Author (year)	No. of patients	Primary site	Treatment	Cut-off value			Statistically significant difference in survival rate
				NLR	PLR	LMR	
Perisandis (2013)	97	OCC	NAC+ST	1.98	N/A	N/A	DSS (NLR)
Fang (2013)	226	OCC	ST	2.44	N/A	N/A	OS, DFS (NLR)
Li (2013)	1547	NPC	RT/CRT	N/A	N/A	5.22	OS, DFS (LMR)
Tsai (2014)	202	OCC	Any	5	N/A	N/A	None
Jin (2015)	229	Metastatic NPC	CT	3.6	N/A	N/A	OS (NLR)
Rassouli (2015)	273	Any	Any	4.27	170	N/A	DFS (NLR), OS (PLR)
Farhan-Alanie (2015)	178	OCC	ST	5	150/300	N/A	None
Zelzer (2015)	170	OCC/LC/OPC/HPC	RT/CRT/BRT	5	150/300	N/A	OS (NLR)
Song (2015)	146	HPC	ST	2.3	N/A	N/A	OS (NLR)
Jiang (2015)	672	Metastatic NPC	CT	N/A	N/A	2.475	OS (LMR)
Haddad (2015)	46	LC/OPC/HPC	CRT	5	N/A	N/A	OS (NLR)
Rachidi (2015)	543	OCC/LC/PC	Any	2.36/4.39	N/A	N/A	OS (NLR)
Sun (2015)	251	NPC	RT/CRT	2.7	167.2	N/A	OS (NLR, PLR)

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; N/A, not available; OCC, oral cavity carcinoma; NPC, nasopharyngeal carcinoma; OPC, oropharyngeal carcinoma; HPC, hypopharyngeal carcinoma; LC, laryngeal carcinoma; PC, pharyngeal carcinoma; NAC, neo-adjuvant chemotherapy; ST, surgical therapy; RT, radiotherapy; CRT, chemoradiotherapy; BRT, bio-radiotherapy; CT, chemotherapy; DSS, disease specific survival; OS, overall survival; DFS, disease free survival.