Utility of Blood Biomarkers as a Predictor of Pathological Lymph Node Metastasis in Clinical Stage T1N0 Esophageal Squamous Cell Carcinoma

Manato Ohsawa  
Hiroshima University

Yoichi Hamai (✉ yyhamai@hiroshima-u.ac.jp)  
Hiroshima University

Manabu Emi  
Hiroshima University

Yuta Ibuki  
Hiroshima University

Tomoaki Kurokawa  
Hiroshima University

Toru Yoshikawa  
Hiroshima University

Ryosuke Hirohata  
Hiroshima University

Nao Kitasaki  
Hiroshima University

Morihito Okada  
Hiroshima University

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Abstract

Background

Accurate preoperative evaluation of lymph node (LN) metastasis is important for determining the treatment strategy for superficial esophageal cancer. There have been reports on the clinical significance of blood biomarkers, such as the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and lymphocyte-monocyte ratio (LMR), as predictors of LN metastasis for different cancers.

Methods

Patients (n = 185) with cT1N0M0 esophageal squamous cell carcinoma (ESCC) who underwent esophagectomy with R0 resection between April 2003 and August 2021 were enrolled. This study investigated the ability of the pretreatment NLR, PLR, and LMR to predict pathological LN metastasis.

Results

The optimal cut-off NLR, PLR, and LMR values to predict pathological LN metastasis were 2.1, 122, and 4.8, respectively. Univariate and multivariate analyses revealed that the primary tumor length, depth of invasion, and NLR were significant predictors of LN metastasis. Furthermore, similar to the five-year overall survival, the five-year relapse-free survival was significantly better in the low NLR group than in the high NLR group.

Conclusions

The NLR was the most useful predictor of pathological LN metastasis, which is difficult to predict by imaging for clinical stage T1N0M0 ESCC. Patients diagnosed with clinical stage I ESCC and with a high NLR require adequate LN dissection and may be good candidates for preoperative adjuvant therapy.

Background

Significant advances have been made in the treatment of superficial esophageal cancers, most of which can now be resected en bloc by endoscopic mucosal resection or endoscopic submucosal dissection [1]. When the tumor has invaded the mucosa or shallow submucosa and there is no lymph node (LN) metastasis, endoscopic mucosal resection or submucosal dissection is the treatment of choice, and the results are as satisfactory as those for esophagectomy [2, 3].

Although endoscopic ultrasound (EUS), computed tomography (CT), and fluorodeoxyglucose (FDG)-positron emission tomography (PET) are commonly used to diagnose LN metastasis, their diagnostic performance is unsatisfactory [4–11]. Pathological evaluation has shown that 11–56% of patients with
clinical N0 esophageal cancer develop metastasis to the regional LNs after esophagectomy [12]. Therefore, accurate preoperative assessment of regional LN metastasis and assessing the depth of tumor invasion are important to determine the appropriate treatment strategy for superficial esophageal cancer. Although the accuracy of the assessment of LN metastasis has been improved by advances in diagnostic imaging, it remains inadequate and needs to be improved by use of other diagnostic tools.

Lymphocytes act as tumor suppressors and are widely used as indicators of immunocompetence [13, 14]. Neutrophils, platelets, and monocytes are indicators of inflammation, which is associated with increased tumor growth, invasion, and angiogenesis, and can be used to evaluate tumor progression [15–17]. The neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and lymphocyte-monocyte ratio (LMR) are indicators of nutritional status and the inflammation associated with increased tumor growth, invasion, and angiogenesis and can be used to assess tumor progression and immune status. Changes in NLR, PLR, and LMR values may reflect broader changes in the tumor microenvironment and are reportedly associated with poor survival in patients with many types of solid tumors [18–22]. Furthermore, there have been reports on the usefulness of these biomarkers as predictors of LN metastasis for different cancers in recent years [23–26] but not for esophageal cancer.

Therefore, we hypothesized that these biomarkers may also be useful tools for detecting microscopic metastasis of cancer cells to the LNs for esophageal cancer with an early tumor stage, which is difficult to predict by imaging. The aim of this study was to investigate the usefulness of NLR, PLR, and LMR as predictors of pathological LN metastasis and prognosis in patients with clinical stage T1N0M0 esophageal squamous cell carcinoma (ESCC).

**Patients And Methods**

**Patients**

Patients with pretreatment clinical stage T1N0M0 ESCC who underwent esophagectomy with R0 resection at Hiroshima University Hospital between April 2003 and August 2021 were enrolled (n = 185). Table 1 shows the patient demographic and clinicopathological characteristics. The study was approved by the Institutional Review Board of Hiroshima University (E-2225).
| Variable                                           | n = 185                     |
|----------------------------------------------------|----------------------------|
| Age (mean ± SD, years)                             | 63.9 ± 7.9                 |
| Sex                                                |                            |
| Male                                               | 159 (85.9%)                |
| Female                                             | 26 (14.1%)                 |
| Performance status                                 |                            |
| 0                                                  | 150 (81.1%)                |
| 1                                                  | 35 (18.9%)                 |
| Tumor marker                                       |                            |
| SCC (mean ± SD, ng/mL)                             | 1.2 ± 0.9                  |
| CEA (mean ± SD, ng/mL)                             | 3.1 ± 1.9                  |
| Primary tumor location                              |                            |
| Upper                                              | 35 (19.0%)                 |
| Middle                                             | 96 (51.9%)                 |
| Lower                                              | 54 (29.1%)                 |
| Macroscopic tumor type                             |                            |
| IP                                                 | 21 (11.4%)                 |
| IPa                                                | 28 (15.1%)                 |
| IPb                                                | 1 (0.5%)                   |
| IPc                                                | 135 (73.0%)                |
| IP                                                  | 0 (0%)                     |
| Primary tumor length (mean ± SD, mm)               | 35.1 ± 17.7                |
| Tumor invasion depth measured with EUS             |                            |
| EP-LPM                                             | 18 (9.7%)                  |
| MM-SM1                                             | 37 (20.0%)                 |

Values are shown as the number (percentage) or mean ± standard deviation. Pathological staging according to the TNM Classification (8th edition). CEA, carcinoembryonic antigen; EUS, endoscopic ultrasonography; EP, epithelium; LPM, lamina propria mucosa; MM, muscularis mucosa; SM, submucosa; SCC, squamous cell carcinoma-related antigen; SD, standard deviation.
| Variable                        | n = 185 |
|--------------------------------|---------|
| SM2-SM3                        | 130 (70.3%) |
| **Histology**                  |         |
| Well differentiated            | 59 (31.9%) |
| Moderately differentiated      | 88 (47.6%) |
| Poorly differentiated          | 38 (20.5%) |
| **Lymphatic invasion**         |         |
| ly0                            | 131 (70.8%) |
| ly1                            | 39 (21.1%) |
| ly2                            | 13 (7.0%)  |
| ly3                            | 2 (1.1%)   |
| **Venous invasion**            |         |
| v0                             | 152 (82.2%) |
| v1                             | 30 (16.2%) |
| v2                             | 2 (1.1%)   |
| v3                             | 1 (0.5%)   |
| **Pathological T**             |         |
| pTis                           | 2 (1.1%)   |
| pT1a                           | 55 (29.7%) |
| pT1b                           | 115 (62.2%) |
| pT2                            | 9 (4.9%)   |
| pT3                            | 4 (2.1%)   |
| **Pathological N**             |         |
| pN0                            | 140 (75.7%) |
| pN1                            | 39 (21.1%) |
| pN2                            | 6 (3.2%)   |

Values are shown as the number (percentage) or mean ± standard deviation. *Pathological staging according to the TNM Classification (8th edition). CEA, carcinoembryonic antigen; EUS, endoscopic ultrasonography; EP, epithelium; LPM, lamina propria mucosa; MM, muscularis mucosa; SM, submucosa; SCC, squamous cell carcinoma-related antigen; SD, standard deviation.
Clinicopathological diagnosis

Tumors were clinically staged using endoscopy, EUS, upper gastrointestinal radiography, and helical CT of the neck, chest, and abdomen. Pre-treatment evaluation of patients with esophageal cancer using FDG-PET/CT was started in October 2006. Clinicopathological staging was performed according to the 8th edition of the TNM classification [27]. Macroscopic superficial ESCC was graded as type I, IIa, IIb, IIc, or III according to the Japanese classification system (type I, superficial and protruding; type II, superficial and flat [type IIa, slightly elevated; type IIb, flat; type IIc, slightly depressed]; and type III, superficial and with excavated growth) [28]. Macroscopically, superficial tumors in Japan are summarized as elevated (type I) or as tumor invasion of the lamina propria mucosa (T1a-LPM), muscularis mucosa (T1a-MM), or the upper, middle, or lower third of the submucosal layer (T1b-SM1, T1b-SM2, or T1b-SM3, respectively) [29–31].

Endoscopic treatment

LN metastasis was extremely rare in mucosal lesions involving the epithelium and lamina propria, and endoscopic mucosal or submucosal dissection was performed as the initial treatment unless endoscopic resection was technically difficult. Patients whose pathological results after endoscopic treatment indicated that the lesion was deeper than the muscularis mucosa or had a positive vertical margin were eligible for additional surgical resection and were included in this study.

Surgical treatment

All patients underwent open transthoracic (n = 112) or thoracoscopic esophagectomy (n = 73) and LN dissection in at least two fields (thoracic and abdominal). Esophageal cancer in the upper or middle third of the thoracic esophagus was treated by cervical lymphadenectomy. A gastric tube was subsequently raised for cervical anastomosis with the esophagus. The reconstruction path was in the retrosternal (n = 89) or posterior mediastinal (n = 88) regions or in the region before the chest wall (n = 8). Three experts in esophageal surgery performed these procedures.

Adjuvant chemotherapy

Patients with stage II or higher disease based on postoperative pathology results received adjuvant chemotherapy, which consisted of cisplatin plus 5-fluorouracil. Cisplatin 80 mg/m² was administered via intravenous infusion on day 1 and 5-fluorouracil 800 mg/m² was administered from days 1 to 5 by intravenous injection. Two courses were administered at 3-week intervals.

NLR, PLR, and LMR data

Blood samples were collected preoperatively. In patients who underwent additional surgical resection after endoscopic treatment, blood samples were collected before the endoscopy procedure to avoid the effects of inflammation associated with invasive endoscopic treatment. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count, PLR as the platelet count divided by
the absolute lymphocyte count, and LMR as the absolute lymphocyte count divided by the absolute monocyte count.

**Statistical analysis**

The results are presented as the number (percentage) or as the mean, unless otherwise stated. Survival was analyzed using Kaplan–Meier curves, which were compared using the log-rank test. Relapse-free survival (RFS) was defined as the interval between the date of surgery and either the first event (recurrence or death from any cause) or the most recent follow-up. Overall survival (OS) was defined as the interval between the date of surgery and either death due to any cause or the last follow-up visit. Optimal cutoff values for NLR, PLR, and LMR were determined from receiver-operating characteristic curves. Multivariate Cox regression analysis was performed to identify independent predictors of OS and RFS. A backward stepwise method was used to select variables for multivariate analysis. All statistical analyses were performed using the JMP Pro 15 software (SAS Institute Inc., Cary, NC, USA). A P-value <0.05 was considered statistically significant.

**Results**

**Blood sampling data**

Table 2 presents the blood sampling data. The mean NLR, PLR, and LMR (± standard deviation) values were 2.1 ± 1.1, 128.4 ± 48.7, and 5.7 ± 2.6, respectively.

| Variable                  | n = 185    |
|---------------------------|------------|
| Neutrophil count (mean ± SD, /µL) | 3540.9 ± 1305.0 |
| Lymphocyte count (mean ± SD, /µL) | 1905.1 ± 710.0 |
| Monocyte count (mean ± SD, /µL) | 371.1 ± 149.9 |
| Platelet count (mean ± SD, /µL) | 222.9 ± 63.3 |
| NLR (mean ± SD)           | 2.1 ± 1.1  |
| PLR (mean ± SD)           | 128.4 ± 48.7 |
| LMR (mean ± SD)           | 5.7 ± 2.6  |

Values are shown as mean ± standard deviation. LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SD, standard deviation

**Clinical characteristics of patients according**
**to pathological LN metastasis**
Table 3 shows the clinical characteristics of patients according to the detection of pathological LN metastasis. The features that showed significant between-group differences according to pathological LN metastasis were the length of the primary tumor, depth of tumor invasion on EUS, NLR, PLR, and LMR.
| Parameter                        | pN0 (n = 140) | pN+ (n = 45) | P-value |
|---------------------------------|---------------|--------------|---------|
| Age (mean ± SD, years)          | 64.4 ± 7.7    | 62.5 ± 8.2   | 0.1639  |
| Sex                             |               |              |         |
| Male                            | 119 (85.0%)   | 40 (88.9%)   | 0.5138  |
| Female                          | 21 (15.0%)    | 5 (11.1%)    |         |
| Performance status              |               |              |         |
| 0                               | 111 (79.3%)   | 39 (86.7%)   | 0.2714  |
| 1                               | 29 (20.7%)    | 6 (13.3%)    |         |
| Tumor marker                    |               |              |         |
| SCC (mean ± SD, ng/mL)          | 1.2 ± 1.0     | 1.1 ± 0.5    | 0.2942  |
| CEA (mean ± SD, ng/mL)          | 3.2 ± 2.0     | 2.9 ± 1.8    | 0.4387  |
| Primary tumor location          |               |              |         |
| Upper                           | 27 (19.3%)    | 8 (17.8%)    | 0.5648  |
| Middle                          | 74 (52.9%)    | 22 (48.9%)   |         |
| Lower                           | 39 (27.8%)    | 15 (33.3%)   |         |
| Macromosaic tumor type          |               |              |         |
| Elevated                        | 15 (9.9%)     | 6 (12.8%)    | 0.8254  |
| Depressed                       | 123 (89.1%)   | 41 (87.2%)   |         |
| Primary tumor length (mean ± SD, mm) | 31.4 ± 16.8 | 43.7 ± 17.5 | <.0001  |
| Tumor invasion depth measured with EUS |          |              |         |
| EP-LPM                          | 18 (12.8%)    | 0            | 0.0014  |
| MM-SM1                          | 33 (23.6%)    | 4 (8.9%)     |         |
| SM2-SM3                         | 89 (63.6%)    | 41 (91.1%)   |         |
| NLR (mean ± SD)                 | 1.7 ± 0.7     | 3.1 ± 1.3    | <.0001  |

Values are shown as the number (percentage) or mean ± standard deviation. CEA, carcinoembryonic antigen; EP, epithelium; EUS, endoscopic ultrasound; LMR, lymphocyte-to-monocyte ratio; LPM, lamina propria mucosa; MM, muscularis mucosa; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SCC, squamous cell carcinoma-related antigen; SD, standard deviation; SM, submucosa.
| Parameter            | pN0 (n = 140) | pN+ (n = 45) | P-value |
|----------------------|---------------|--------------|---------|
| PLR (mean ± SD)      | 119.1 ± 43.7  | 157.1 ± 52.8 | <.0001  |
| LMR (mean ± SD)      | 6.0 ± 2.6     | 4.5 ± 2.1    | 0.0001  |

Values are shown as the number (percentage) or mean ± standard deviation. CEA, carcinoembryonic antigen; EP, epithelium; EUS, endoscopic ultrasound; LMR, lymphocyte-to-monocyte ratio; LPM, lamina propria mucosa; MM, muscularis mucosa; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SCC, squamous cell carcinoma-related antigen; SD, standard deviation; SM, submucosa

**Optimal NLR, PLR, and LMR cut-off values**

The optimal NLR, PLR, and LMR cut-off values for predicting pathological LN metastasis were 2.1, 122, and 4.8, respectively (Table 4).

| Variables | AUC     | 95% CI   | P-value | Cut-off value |
|-----------|---------|----------|---------|---------------|
| NLR       | 0.8897  | 0.83–0.93| <.0001  | 2.1           |
| PLR       | 0.7241  | 0.63–0.80| <.0001  | 122           |
| LMR       | 0.7018  | 0.60–0.78| <.0001  | 4.8           |

AUC, area under the curve; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio

The NLR, PLR, and LMR were good predictive markers of pathologic LN metastasis; 57.1% of patients with an NLR ≥ 2.1 were positive for pathologic LN metastasis, compared with only 4.4% of patients with an NLR < 2.1 (sensitivity, 0.57; specificity, 0.95; accuracy, 0.81; P < 0.0001). Similarly, 38.3% of patients with a PLR ≥ 122 had positive pathologic LNs, compared with only 9.9% of patients with a PLR < 122 (sensitivity, 0.38; specificity, 0.90; accuracy, 0.63; P < 0.0001). Likewise, 41.1% of patients with an LMR < 4.8 had positive pathologic LNs, compared with only 13.4% of patients with an LMR ≥ 4.8 (sensitivity, 0.41; specificity, 0.86; accuracy, 0.68; P < 0.0001) (Table 5).
### Table 5
Prediction of pathological lymph node metastasis based on the optimal NLR, PLR, and LMR cut-off values

|          | pN0 (n = 140) | pN+ (n = 45) | P-value |
|----------|---------------|--------------|---------|
| **NLR**  |               |              |         |
| <2.1     | 115           | 110 (95.6%)  | 5 (4.4%)| <.0001 |
| ≥2.1     | 70            | 30 (42.9%)   | 40 (57.1%) |     |
| **PLR**  |               |              |         |
| <122     | 91            | 82 (90.1%)   | 9 (9.9%)| <.0001 |
| ≥122     | 94            | 58 (61.7%)   | 36 (38.3%) |     |
| **LMR**  |               |              |         |
| ≥4.8     | 112           | 97 (86.6%)   | 15 (13.4%)| <.0001 |
| <4.8     | 73            | 43 (58.9%)   | 30 (41.1%) |     |

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

### Univariate and multivariate analyses of predictors of pathological LN metastasis

Univariate analysis identified the length of the primary tumor, depth of tumor invasion on EUS, NLR, PLR, and LMR as statistically significant prognostic factors.

Multivariate analysis showed that the length of the primary tumor, depth of tumor invasion on EUS, and NLR were statistically significant predictors of LN metastasis (Table 6).
## Table 6
Results of univariate and multivariate analyses of factors that predict pathological lymph node metastasis

| Variable                                | Univariate | Multivariate |      |      |
|------------------------------------------|------------|--------------|------|------|
|                                          | HR         | 95% CI       | P-value | HR | 95% CI | P-value |
| Age, years (continuous)                  | 1.03       | 0.98–1.07    | 0.1639 | –  | –      | –       |
| Sex                                      |            |              |        |     |        |         |
| Female (reference)                       | 1          | –            | –      | –  | –      | –       |
| Male                                     | 1.41       | 0.49–3.99    | 0.5154 | –  | –      | –       |
| Performance status                       |            |              |        |     |        |         |
| 0 (reference)                            | 1          | –            | –      | –  | –      | –       |
| 1                                        | 1.69       | 0.65–4.39    | 0.2755 | –  | –      | –       |
| SCC (continuous)                         | 1.28       | 0.76–2.15    | 0.2942 | –  | –      | –       |
| CEA (continuous)                         | 1.07       | 0.89–1.29    | 0.4387 | –  | –      | –       |
| Primary tumor location                   |            |              |        |     |        |         |
| Middle, lower (reference)                | 1          | –            | –      | –  | –      | –       |
| Upper                                    | 1.23       | 0.56–2.73    | 0.5974 | –  | –      | –       |
| Macrossopic tumor type, elevated/depressed |        |              |        |     |        |         |
| Elevated (reference)                     | 1          | –            | –      | –  | –      | –       |
| Depressed                                | 1.13       | 0.34–3.75    | 0.7402 | –  | –      | –       |
| Length of primary tumor, <35 mm/≥35 mm   |            |              |        |     |        |         |
| <35 mm (reference)                       | 1          | –            | –      | 1  | –      | –       |
| ≥35 mm                                   | 3.35       | 1.67–6.73    | 0.0007 | 3.43| 1.29–9.12| 0.0133 |

CEA, carcinoembryonic antigen; CI, confidence interval; EUS, endoscopic ultrasonography; HR, hazard ratio; LMR, lymphocyte monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet lymphocyte ratio; SCC, squamous cell carcinoma-related antigen; SM, submucosa
### Depth of tumor invasion by EUS, ≤ SM1/SM2 ≤

| Depth of Tumor Invasion | Univariate | Multivariate |
|-------------------------|------------|--------------|
| < SM2 (reference)       | 1          | 1            |
| ≥ SM2                   | 5.87       | 1.98–17.34   |
|                         | 0.0014     | 4.14         |
|                         | 1.09–15.60 | 0.0358       |

### NLR

| NLR Value | Univariate | Multivariate |
|-----------|------------|--------------|
| < 2.1 (reference) | 1          | 1            |
| ≥ 2.1     | 29.33      | 10.64–80.82  |
|           | < .0001    | 19.93        |
|           | 6.41–61.89 | < .0001      |

### PLR

| PLR Value | Univariate | Multivariate |
|-----------|------------|--------------|
| < 122 (reference) | 1          | 1            |
| ≥ 122     | 5.65       | 2.62–13.32   |
|           | < .0001    | 1.73         |
|           | 0.56–5.29  | 0.3344       |

### LMR

| LMR Value | Univariate | Multivariate |
|-----------|------------|--------------|
| ≥ 4.8 (reference) | 1          | 1            |
| < 4.8     | 4.51       | 2.20–9.23    |
|           | < .0001    | 1.99         |
|           | 0.69–5.74  | 0.1988       |

CEA, carcinoembryonic antigen; CI, confidence interval; EUS, endoscopic ultrasonography; HR, hazard ratio; LMR, lymphocyte monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet lymphocyte ratio; SCC, squamous cell carcinoma-related antigen; SM, submucosa

## Relapse-free and overall survival according to NLR value

For analysis of the prognosis, we limited the eligible cases to the 170 patients treated between April 2003 and March 2018 because these patients were able to be followed up for at least 3 years. The five-year RFS was better in the low NLR (< 2.1) group than in the high NLR (≥ 2.1) group (91.0% vs. 64.7%, P < 0.0001; Fig. 1a), as was the five-year OS (90.9% vs. 72.4%, P < 0.0001; Fig. 1b).

We also examined the prognosis of pN0 patients by NLR. The five-year RFS was better in the low NLR (< 2.1) group than in the high NLR (≥ 2.1) group (91.6% vs. 52.1%, P < 0.0001; Fig. 1c), as was the five-year OS (91.5% vs. 65.5%, P < 0.0001; Fig. 1d).

## Discussion

LN metastasis is an independent and important prognostic factor in patients with esophageal cancer. Despite their importance, the preoperative clinical LN diagnosis and the final pathologic evaluation may not be in agreement. The presence of false-negative LNs obscures the actual status of the cancer and the
choice of treatment. EUS, CT, and FDG-PET are often used to diagnose LN; however, past studies have reported their diagnostic performance to be unsatisfactory. EUS, when combined with fine needle aspiration, has been shown to improve the sensitivity for diagnosis of LN metastasis from 84.7–96.7% [4–6]. However, this method can only evaluate LNs proximal to the esophagus and gastric wall. FDG-PET has the disadvantage of low spatial resolution, which makes it difficult to distinguish between LNs adjacent to the tumor and the tumor itself. Furthermore, FDG-PET is not suitable for diagnosis of small LN metastases. FDG-PET has been reported to detect tumors with a diameter of more than 6 mm or an area of more than 33 mm$^2$ [7, 8]. Therefore, diagnosis of small LN metastases is difficult. CT is a noninvasive modality that is widely used to evaluate invasion of esophageal cancer to the LNs. Detection of metastatic LNs on CT depends primarily on size, and the sensitivity of CT for detection of metastasis is inadequate unless LNs are larger than 10 mm [7, 9, 10]. The short-axis diameter alone is insufficient to detect normal-sized metastatic LNs and to distinguish between reactive hyperplasia and metastatic hypertrophy [11]. Previous studies have reported false-negative rates of 11–56% for LN metastases [12]. In our study, LN metastasis was found in 24.3% of cases (21.1% for pN1 and 3.2% for pN2) and included many false-negative results.

Lymphocytes act as tumor suppressors and are widely used as indicators of immunity [13, 14]. Neutrophils, platelets, and monocytes are indicators of inflammation, and systemic inflammation plays an important role in all stages of tumorigenesis. Inflammation is thought to contribute to tumor initiation through genetic mutations, genomic instability, and epigenetic changes. It also activates tissue responses that induce proliferation of precancerous cells and enhance their survival. Inflammation also stimulates angiogenesis, causes immunosuppression, and encourages a microenvironment in which malignant cells can survive and ultimately promote metastatic spread [15–17]. NLR, PLR, and LMR from these blood components have been used as indicators of the nutritional status of patients and are also attracting attention as biomarkers for assessment of immune status and tumor progression. Changes in NLR, PLR, and LMR may reflect widespread changes in the tumor microenvironment and have been recently identified as prognostic markers for cancer [18–22]. Compared with other biomarkers, these have the advantages of being inexpensive and rapid to detect with no additional costs. Apart from their prognostic value, these biomarkers are reportedly useful for predicting recurrence, the efficacy of preoperative treatment, and perioperative complications [32, 33]. In recent years, they have been found to predict LN metastasis in various types of cancer. However, there have been no relevant reports on the value of these biomarkers in esophageal cancer up until now. The optimal cut-off value for the NLR as a predictor of LN metastasis in patients with gastric cancer was 1.6 in a study by Kosuga et al. [23] and 2.0 in a study by Andrea et al. [24]; the cut-off value was 2.18 in a study of patients with endometrial cancer by Aoyama et al. [25] and 2.7 in a study of patients with bladder cancer by Andrea et al [26]. Our findings show that the NLR was the most useful predictor of LN metastasis and correlated more strongly than tumor characteristics such as the tumor diameter and depth. Herein, the optimal cut-off value was 2.1, which is similar to the previously published literature on other types of cancer.
This study showed that patients with a high pretreatment NLR were at high risk for pathological LN metastasis. The optimal cut-off value was 2.1, and 57.1% of patients with a high NLR had pathological LN metastasis, while only 4.4% of patients with a low NLR had LN metastasis. Therefore, patients with a high NLR require esophagectomy with adequate and systematic LN dissection, and furthermore may be good candidates for preoperative adjuvant therapy. On the other hand, patients with a low NLR may not have LN metastasis, and those patients who are at high risk for surgery based on comorbidities and preoperative functional tests may be considered for reduced LN dissection in surgery and aggressive endoscopic resection.

The group with a high NLR also had a significantly poorer RFS and OS, further confirming that NLR is a useful prognostic factor. Interestingly, in subgroup analysis, even in the pN0 group, which is considered to have a good prognosis, the group with a high NLR had a significantly poorer RFS and OS. The NLR may also be an important predictor of recurrence in patients with pN0 disease. Therefore, it may be a prognostic factor even in very early-stage esophageal cancer. It is possible that the immune system in patients with a high NLR value, i.e., those with impaired immunity because of cancer, may not be able to destroy microscopic cancer cells that are not clinically recognized as LN metastasis, leading to postoperative recurrence. However, further research is needed to elucidate this problem.

This study had several limitations. First, it had a retrospective design. Second, the number of patients was somewhat small, and the study was conducted at a single institution. Therefore, larger prospective studies are required in the future to confirm our findings regarding the usefulness of the NLR as a predictor of pathological LN metastasis and the prognosis in patients with clinical stage T1N0 ESCC.

**Conclusions**

Among the various blood biomarkers investigated in this study, the NLR was the most useful predictor of pathological LN metastasis. Furthermore, the NLR was strongly correlated with prognosis and may be useful in predicting recurrence. NLR may be a useful tool in the evaluation of LN metastasis, which is difficult to predict by imaging, even for clinical stage ESCC. Patients with a high NLR are at high risk for LN metastasis and require systematic LN dissection. Furthermore, these patients may be good candidates for preoperative adjuvant therapy.

**List Of Abbreviations**

CT, computed tomography; ESCC, esophageal squamous cell carcinoma; EUS, endoscopic ultrasound; FDG, fluorodeoxyglucose; LMR, lymphocyte-monocyte ratio; LN, lymph node; NLR, neutrophil-lymphocyte ratio; OS, overall survival; PET, positron emission tomography; PLR, platelet-lymphocyte ratio; RFS, relapse-free survival; T1a-EP, carcinoma in situ; T1a-LPM, tumor invasion of lamina propria mucosa; T1a-MM, tumor invasion of muscularis mucosa; T1b-SM1, tumor invasion of upper submucosal layer; T1b-SM2, tumor invasion of middle submucosal layer; T1b-SM3, tumor invasion of lower submucosal layer...
Declarations

Ethics approval and consent to participate

In the study, all methods were carried out in accordance with relevant guidelines and regulations. The study was approved by the Institutional Review Board of Hiroshima University (approval no. E-2225). Informed consent was obtained from all subjects or their legal guardians for participating in the current study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest relevant to this article.

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Authors' contributions

MOh and YH drafted the manuscript. MOh, YH, ME, YI, TK, TY, RH and NK contributed to patient care. MOh, MK, JH, and MH performed the literature search. MOh, YH, ME, YI, TK, TY, RH, NK, and MOk critically revised the manuscript. All authors have read and approved the final version of the manuscript.

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**Figures**

**Figure 1.**

RFS and OS in all patients and in patients without pathological LN metastasis. For all patients, (a) the five-year RFS and (b) the five-year OS were better in the group with a low NLR than in the group with a
high NLR (P < 0.0001). For patients without pathological LN metastasis, (c) the five-year RFS and (d) the five-year OS were better in the group with a low NLR than in the group with a high NLR (P < 0.0001). LN, lymph node; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; RFS, relapse-free survival.