Prognostic Role of Neutrophil-to-Lymphocyte Ratio in Patients with Human Papillomavirus–Positive Oropharyngeal Cancer

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Abstract

Objective. To investigate the prognostic impact of the neutrophil-to-lymphocyte ratio (NLR) for human papillomavirus–positive oropharyngeal cancer (HPV+ OPC).

Study Design. Retrospective institutional database analysis.

Setting. Tertiary referral medical center.

Material and Methods. In total, 104 patients with HPV+ OPC were enrolled. From the blood laboratory data checked within 4 weeks before initiation of primary treatment, NLR was calculated. The association between clinicopathological characteristics and NLR was analyzed, and the prognostic role was evaluated based on overall survival (OS) and disease-free survival (DFS).

Results. According to the cutoff value (2.42) for NLR, the patients were classified into the low NLR group (n = 61) or the high NLR group (n = 43). High NLR was associated with a higher rate of advanced T classification (P = .007) and diabetes mellitus (P = .01). The proportion of surgery-based treatment was lower in the high NLR group (20.9% vs 42.6%, P = .02). The high NLR group showed a lower 5-year OS rate (85.3% vs 96.3%, P = .09) and a lower 5-year DFS rate (68.1% vs 94.7%, P = .01) than those in the low NLR group. Multivariate analysis showed that advanced N classification was a significant predictor for worse 5-year OS (hazard ratio [HR], 17.40; 95% confidence interval [CI], 2.36-128.29) and that both advanced N classification (HR, 7.78; 95% CI, 2.33-25.93) and high NLR (HR, 4.16; 95% CI, 1.24-13.95) were important prognosticators for worse 5-year DFS.

Conclusion. Elevated pretreatment NLR was associated with poor DFS in patients with HPV+ OPC.

Keywords

head and neck, oropharynx, neutrophil, lymphocyte, prognosis

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patients with HPV+ OPC. More important, we investigated the prognostic impact of the NLR for HPV+ OPC.

Materials and Methods

Study Population

This study was approved by the Institutional Review Board of Samsung Medical Center. In total, 104 patients with HPV+ OPC who had been treated in our institution from November 2004 to December 2016 were enrolled in this retrospective cohort study. All patients were treated with curative intent and followed up for more than 6 months (range, 6-120 months). Patients with immunocompromised conditions, under immunomodulatory treatments, or with hematogenous pathologies were excluded from analysis because such comorbidities could affect the systemic inflammatory conditions. Tumor HPV status was determined using p16 immunostaining and HPV DNA detection methods. All tests for tumor HPV status were prospectively performed on untreated primary tumor tissue. Diffuse (at least more than 70%) and strong nuclear and cytoplasmic staining of p16 or the detection of high-risk HPV DNA (HPV 16, 18, 33, 35, 39, 45, 51, 52, 56, or 66) were defined as positive HPV status.

Treatment was determined based on TNM stage, adverse risk factors such as multiple lymph node metastasis, perineural invasion (PNI), lymphovascular invasion (LVI), extranodal extension (ENE), positive or close resection margin (RM), and patient’s performance status. Sixty-six patients were treated with concurrent chemoradiation (CCRT). For those patients, a total dose of 60 to 70 Gy (median, 68.4 Gy) was delivered to gross tumor volume, including primary tumor and metastatic lymph nodes concurrently with cisplatin-based chemotherapy. Three patients were treated with definitive radiation (RT) alone, which was determined based on patients’ performance status and patients’ decision. Thirty-five patients were treated with surgery followed by adjuvant RT (n = 13) or adjuvant CCRT (n = 22). Wide resection of oropharyngeal tumor and lymph node dissection were performed. Adjuvant RT was delivered to 10 patients who had a microscopic positive resection margin (RM) with a total dose of 66 to 68.4 Gy in 30 fractions and to 25 patients who had negative RM with a total dose of 59.4 to 60 Gy in 27 to 30.

Blood laboratory data checked within 4 weeks before initiation of primary treatment were obtained. NLR was calculated from neutrophil count and lymphocyte count. Demographic and clinicopathological characteristics were obtained from patients’ medical records and included age at diagnosis, sex, smoking status (never, former, and current), alcohol use, underlying diseases (diabetes mellitus, chronic renal failure, chronic hepatitis/liver cirrhosis), major cardiovascular/cerebrovascular events (myocardial infarct, angina pectoris, cerebral infarct), tumor grade (well differentiated, moderately differentiated, or poorly differentiated/undifferentiated), location of the primary tumor (palatine tonsil, base of tongue, posterior pharyngeal wall), and TNM classification.

Prognosis was evaluated based on overall survival (OS) and disease-free survival (DFS). OS was calculated from the date of diagnosis to the date of death. In this study, DFS was defined as survival from the diagnosis to the first detection of any recurrence (locoregional recurrence or distant metastasis) or disease progression or death. Second primary tumor was evaluated separately. Both synchronous malignancies occurring within 6 months and metachronous malignancies that had developed after 6 months were included in the second primary tumor.

Statistical Analyses

Chi-square test and independent t test were used to compare categorical variables and continuous variables, respectively. OS and DFS were assessed using Kaplan-Meier estimates. Log-rank test was used to assess the equality of the survivor function between different groups. Cox proportional hazards model with 95% confidence interval (CI) was used for the multivariate analysis to assess the effect of the NLR and other clinical/demographic factors. SPSS software for Windows, version 17.0 (SPSS, Inc, an IBM Company, Chicago, Illinois) was used for statistical analysis. All tests were 2-sided, and P < .05 was considered statistical significant.

Results

The mean age of all patients was 57.2 years. Most patients were male (86.5%, 90/104), with females accounting for 13.5% (14/104). Of the patients, 68.3% (71/102) were current or former smokers. Comorbidities of the study cohort included 16 with diabetes mellitus, 1 with chronic renal failure, 8 with chronic hepatitis/liver cirrhosis, and 6 with major cardiovascular diseases. The most common primary site was the tonsil, followed by the base of the tongue. Sixty-nine patients had undergone RT-based treatment, whereas 35 patients had undergone surgery-based treatment. In the entire study cohort, the 5-year OS rate and 5-year DFS rate were 91.7% and 82.8%, respectively.

The value of pretreatment NLR ranged from 0.65 to 22.66 in all patients. The optimal cutoff value of pretreatment NLR was determined using receiver operating characteristic (ROC) curve analysis (Figure 1). With the area under the ROC curve for NLR being 0.643 (95% CI, 0.428-0.858; P = .19) and the c-index being 0.659 (95% CI, 0.520-0.798; P = .04), an NLR value of 2.42 was identified as the cutoff value for predicting DFS, with a sensitivity of 78.4% and a specificity of 64.7%. According to the cutoff values for NLR, the patients were classified into the low NLR group (NLR ≤2.42) or the high NLR group (NLR >2.42). Sixty-one patients (58.7%) were classified in the low NLR group and 43 patients (41.3%) in the high NLR group. Comparisons of demographic and clinical factors of the 2 NLR groups (Table I) revealed an association of high NLR with a higher rate of advanced T classification (P = .007) and diabetes mellitus (P = .01). The proportion of surgery-based treatment was lower in the high NLR group (20.9% vs 42.6%, P = .02).
Locoregional failure rate was 4.9% (3/61) in the low NLR group and 11.6% (5/43) in the high NLR group. All 4 distant metastases developed in the high NLR group (4/43).

Frequency of second primary tumor was 4.9% (3/61) in the low NLR group and 2.3% (1/43) in the high NLR group. Univariate analysis showed that advanced N classification was associated with worse 5-year OS than early N classification ($P < .001$), whereas high NLR only showed a tendency toward a significance for worse 5-year OS than low NLR ($P = .09$; Table 2).

Concerning DFS, both advanced N classification and high NLR had a significant association with worse 5-year DFS ($P = .001$ and .01, respectively). Multivariate analysis showed that advanced N classification was a significant predictor for worse 5-year OS (hazard ratio [HR], 7.78; 95% CI, 2.33-25.93; $P = .001$; NLR: HR, 4.16; 95% CI, 1.24-13.95; $P = .02$; Table 3). T classification and treatment modalities did not show any association or significance as predictors for 5-year OS and DFS in patients with HPV+ OPC.

Kaplan-Meyer estimates showed that the 5-year OS rate was lower in the high NLR group than in the low NLR group (85.3% vs 96.3%), although there was no statistical significance (log rank $P = .09$; Figure 2A). On the contrary, the 5-year DFS rate was significantly lower in the high NLR group than in the low NLR group (68.1% vs 94.7%, log rank $P = .01$; Figure 2B).

**Discussion**

This study attempts to address the prognostic value of the NLR for oncologic outcomes in the patients with HPV+ OPC, treated by RT-based or surgery-based modality. Although it is well accepted that patients with HPV+ OPC have better survival rates and prognoses than patients with HPV− OPC, a small subset of patients will have recurrent or residual cancer after curative treatments. The aim of the current study is to investigate a clinically relevant prognosticator to help identify that subset of patients within those with HPV+ OPC.

Pretreatment NLR was a prognostic marker for DFS in patients with HPV+ OPC, even though it was not sufficient for OS. Patients with HPV+ OPC with a high NLR had a worse 5-year DFS than patients with a low NLR, regardless of treatment modalities. The outcomes of the present study are similar to those of the previous study, which investigated the prognostic value of absolute numbers of

**Figure 1.** Receiver operating characteristic analysis of neutrophil-to-lymphocyte ratio (NLR) for prediction of disease-free survival (DFS). An NLR value of 2.42 was identified as the cutoff value for predicting DFS, with a sensitivity of 78.4% and a specificity of 64.7%. AUC, area under the curve.

**Table 1.** Clinicopathological and Demographic Characteristics According to the Pretreatment NLR Status in HPV+ Oropharyngeal Cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low NLR</th>
<th>High NLR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>53 (86.9)</td>
<td>37 (86.0)</td>
<td>.902</td>
</tr>
<tr>
<td>Female</td>
<td>8 (13.1)</td>
<td>6 (14.0)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>57.1 ± 8.4</td>
<td>57.3 ± 10.2</td>
<td>.933</td>
</tr>
<tr>
<td>Smoking status, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>17 (27.9)</td>
<td>14 (34.1)</td>
<td>.792</td>
</tr>
<tr>
<td>Former</td>
<td>25 (41.0)</td>
<td>15 (36.6)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>19 (31.1)</td>
<td>12 (29.3)</td>
<td></td>
</tr>
<tr>
<td>DM, No. (%)</td>
<td>5 (8.2)</td>
<td>11 (25.6)</td>
<td>.016</td>
</tr>
<tr>
<td>CRF, No. (%)</td>
<td>0 (0)</td>
<td>1 (2.3)</td>
<td>.231</td>
</tr>
<tr>
<td>LC/hepatitis, No. (%)</td>
<td>5 (8.2)</td>
<td>3 (7.0)</td>
<td>.818</td>
</tr>
<tr>
<td>CVA/CVD, No. (%)</td>
<td>1 (1.6)</td>
<td>5 (11.6)</td>
<td>.079</td>
</tr>
<tr>
<td>Primary site, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonsil</td>
<td>50 (82.0)</td>
<td>37 (86.0)</td>
<td>.580</td>
</tr>
<tr>
<td>BOT</td>
<td>11 (18.0)</td>
<td>6 (14.0)</td>
<td></td>
</tr>
<tr>
<td>Treatment, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCRT</td>
<td>35 (57.4)</td>
<td>34 (79.1)</td>
<td>.021</td>
</tr>
<tr>
<td>Op + CCRT</td>
<td>26 (42.6)</td>
<td>9 (20.9)</td>
<td></td>
</tr>
<tr>
<td>Differentiation, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/D</td>
<td>4 (6.7)</td>
<td>4 (9.3)</td>
<td>.285</td>
</tr>
<tr>
<td>M/D</td>
<td>45 (75.0)</td>
<td>26 (60.5)</td>
<td></td>
</tr>
<tr>
<td>P/D</td>
<td>11 (18.3)</td>
<td>13 (30.2)</td>
<td></td>
</tr>
<tr>
<td>T classification, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1-2</td>
<td>56 (91.8)</td>
<td>31 (72.1)</td>
<td>.007</td>
</tr>
<tr>
<td>T3-4</td>
<td>5 (8.2)</td>
<td>12 (27.9)</td>
<td></td>
</tr>
<tr>
<td>N classification, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1-2b</td>
<td>52 (85.2)</td>
<td>34 (79.1)</td>
<td>.412</td>
</tr>
<tr>
<td>N2c-3</td>
<td>9 (14.8)</td>
<td>9 (20.9)</td>
<td></td>
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Abbreviations: BOT, base of tongue; CCRT, concurrent chemoradiation; CRF, chronic renal failure; CVA, cerebrovascular attack; CVD, cardiovascular disease; DM, diabetes mellitus; LC, liver cirrhosis; M/D, moderately differentiated; NLR, neutrophil-to-lymphocyte ratio; Op, operation; P/D, poorly differentiated; SD, standard deviation; W/D, well differentiated.

$a$Smoking status was available for 102 patients.

$^b$Tumor differentiation was available for 103 patients.

$^c$AJCC 7th edition.
circulating neutrophils, monocytes, or lymphocytes for the oncologic outcomes of patients with HPV+ and HPV− oropharyngeal cancer, treated with RT alone or CRT.5 What they found was that only in the HPV+ population, high circulating neutrophil count or monocyte count independently predicted inferior OS and recurrence-free survival (RFS), which was not apparent in HPV− patients. Otherwise, this study demonstrated novel data in several aspects. First, we used the NLR as a candidate prognosticator. NLR is a combination of counts of neutrophils and lymphocytes in the peripheral circulation. It is considered to more accurately reflect the systemic inflammatory response compared with each single factor. Second, the study population was also different between the current study and the previous one, in that heterogeneous treatment modalities (RT based or surgery based) were used in our study. Last, ROC curve analysis was used to identify the optimal cutoff value of the NLR as a prognostic impact, with DFS as a reference. In the previous study, the median value of each circulating lymphocyte count (CLC), circulating monocyte count (CMC), and circulating neutrophil count (CNC) was used to dichotomize the patients into high and low groups.

The mechanisms underlying the association of systemic inflammation and cancer prognosis are not clearly understood yet. However, it is interesting that recently, surrogate markers of systemic inflammation, such as neutrophil, lymphocyte, and platelet counts, either alone or expressed as ratios, have been associated with prognosis in various cancers.16-23 Systemic inflammatory responses like neutrophilia can suppress the antitumor, cytolytic activity of immune cells, such as activated T cells and natural killer cells.24,25

Among many markers, NLR represents the influence of 2 opposing factors—neutrophils and lymphocytes—and it has been shown to have superior prognostic value compared with individual leukocyte counts in several studies.13,14 Its superior stability has been ascribed to various physiological conditions and in vitro handling of blood samples.13,26

Also for head and neck cancers, a growing body of evidence has shown an association between white blood cell counts (or their ratio) and survival parameters.5,13-15,27-30 However, the magnitude of prognostic impacts of the NLR in head and neck cancer is inconsistent, and specifically, the prognostic impact of the NLR can differ according to the HPV status of tumors. One study demonstrated that a high neutrophil count and a low lymphocyte count were associated with inferior survival in patients with HPV+ OPC, an association that was not apparent in HPV− patients.5 In another study, the NLR was significantly lower in patients with HPV+ head and neck cancer (NLR = 2.73) compared to the HPV− counterpart (NLR = 4.75; P = .03).14 It was also shown that an increase of the NLR by 1 unit resulted in a 7% increase in the risk of death by disease increased by 11%. On the contrary, the risk of death increased by 54.9% for every 1-unit increase in the NLR in the HPV+ group, although it did not reach statistical significance. In a recent study, it was reported that the NLR lost its prognostic significance when HPV status was incorporated into multivariate models.15 All these data support that HPV+ head and neck cancer has an entirely different pathogenesis and immunologic profile compared to the HPV− counterpart.31,32

When we analyzed our data using the NLR as a continuous variable, the NLR still maintained a significant impact on DFS (HR, 1.11; 95% CI, 1.01-1.22; P = .03). Specifically, for each 1-unit increase in the NLR, the risk of death by disease increased by 11%. On the contrary, the NLR did not have a significant impact on OS (HR, 1.06; 95% CI, 0.92-1.23; P = .39) as a continuous variable. In an effort to find another optimal cutoff, we also performed the analysis using the cutoff value of 4 as proposed in a recent meta-analysis on the prognostic role of the NLR in solid tumors.33 As a result, it was shown that a high NLR was significantly associated with worse OS as well as worse
DFS ($P = .022$ and .043, respectively). However, by selecting 4 as a cutoff on the ROC curve, AUC, sensitivity, and specificity were decreased compared to those in the current study: AUC of 0.583 (95% CI, 0.3937-0.7727), sensitivity of 40.16%, and specificity of 76.47%, respectively. The c-index with a cutoff value 4 was 0.6426 (95% CI, 0.49-0.79) for DFS, which was also lower than the c-index with a cutoff value of 2.42. Moreover, the cutoff value of 4 is somewhat lopsided and far from the median of 2.31 in our cohort. This discrepancy can be attributable to the fact that the distribution of the NLR can be different according to the disease and that the NLR might be lower in HPV-positive cancer than in HPV-negative cancer, as suggested in a previous study.14

Inflammation has long been associated with tumor development and progression. Proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor–α, which is released by tumor cells and infiltrating host immune cells, stimulate the release of inflammatory cells from the bone marrow, resulting in an elevation of circulating inflammatory cells and infiltration of those cells in the tumor microenvironment.34 In turn, it has been shown that tumor-infiltrating neutrophils can promote angiogenesis and metastasis of tumors.35 Similarly, increased systemic inflammatory response, such as neutrophilia, can suppress the antitumor, cytolytic activity of immune cells, such as activated T cells and natural killer cells.25 Also, it is known to be associated with the elevation of several cytokines and tumor growth-promoting factors, including vascular endothelial growth factor, hepatocyte growth factor, IL-6, and IL-8. Therefore, a high peripheral neutrophil level may indicate a cancer-related inflammation and can be related to poor prognosis. In contrast, lymphocytes are crucial components of effective antitumor immune response. As a result of cancer immune suppression, peripheral lymphocyte count is generally decreased in patients with cancer.26 A low peripheral lymphocyte level may indicate a poorer lymphocyte-mediated immune response to tumors and

Table 3. Multivariate Analysis of Overall Survival and Disease-Free Survival.

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
<th></th>
<th>DFS</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>$P$ Value</td>
<td>HR</td>
</tr>
<tr>
<td>DM</td>
<td>2.41</td>
<td>0.20-28.74</td>
<td>.486</td>
<td>2.07</td>
</tr>
<tr>
<td>T3-4 (vs T1-2)$^a$</td>
<td>0.49</td>
<td>0.05-4.60</td>
<td>.532</td>
<td>0.41</td>
</tr>
<tr>
<td>N2c-3 (vs N1-2b)$^a$</td>
<td>17.40</td>
<td>2.36-128.29</td>
<td>.005</td>
<td>7.78</td>
</tr>
<tr>
<td>Op + CCRT (vs CCRT)</td>
<td>0.94</td>
<td>0.15-5.78</td>
<td>.951</td>
<td>0.97</td>
</tr>
<tr>
<td>NLR $&gt;2.42$ (vs $\leq 2.42$)</td>
<td>3.32</td>
<td>0.58-19.15</td>
<td>.179</td>
<td>4.16</td>
</tr>
</tbody>
</table>

Abbreviations: CCRT, concurrent chemoradiation; DFS, disease-free survival; CI, confidence interval; DM, diabetes mellitus; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; Op, operation; OS, overall survival.

$^a$AJCC 7th edition.

Figure 2. Overall survival (OS) and disease-free survival (DFS) according to neutrophil-to-lymphocyte ratio (NLR) group. Five-year OS rate was lower in the high NLR group than in the low NLR group (85.3% vs 96.3%, $P = .093$). Five-year DFS rate was significantly low in the high NLR group than in the low NLR group (68.1% vs 94.7%, $P = .013$).
suggests poor prognosis. Tumor regressions following antitumor immunotherapy, such as therapeutic PD-1 blockade, require an immunogenic tumor microenvironment, including lymphocyte infiltration. Low lymphocyte counts can indicate immunosuppression in patients with cancer and may be related to the limited effect of cancer immunotherapy.

Certain limitations in this study should be addressed. First, study patients were derived from a single institution with a retrospective analysis; therefore, selection bias could have affected the outcomes in this study. Second, substantial heterogeneity within the study cohort, such as treatment modalities or initial cancer staging, could be confounding factors, hampering the clear interpretation of study outcome. Third, the prognostic value of the NLR needs to be validated in a separate set of patients with HPV+ OPC to increase its significance, which was not feasible in the current study.

In conclusion, the present study demonstrated that elevated pretreatment NLR was associated with poor DFS in patients with HPV+ OPC, treated with RT-based or surgery-based modalities. The NLR may thus be a clinically relevant prognostic biomarker in HPV+ OPC, although the underlying mechanism is unclear. Well-designed, large-scale studies might help to clarify the prognostic value of the NLR in HPV+ OPC.

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Author Contributions
Yoon Kyoung So, conception or design of the work; the acquisition, analysis, or interpretation of data for the work; drafting the work; final approval; accountability for the work; GilJoon Lee, conception or design of the work, drafting the work, final approval, accountability for the work; Dongryul Oh, the acquisition, analysis, or interpretation of data for the work; drafting the work; final approval; accountability for the work; Sunju Byeon, the acquisition, analysis, or interpretation of data for the work; drafting the work; final approval; accountability for the work; Woori Park, the acquisition, analysis, or interpretation of data for the work; drafting the work; final approval; accountability for the work; Man Ki Chung, conception or design of the work, drafting the work, final approval, accountability for the work.

Disclosures
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