Neutrophil-to-lymphocyte ratio has predictive value for lymph node involvement and distant metastasis in nasopharyngeal carcinoma patients

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Abstract

Background Systemic inflammatory responses play important roles in the development and progression of cancer, including the propensity for metastasis. High neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are associated with poor prognosis in nasopharyngeal carcinoma (NPC), but the underlying reasons are unclear. Here, we investigated the associations between a range of hematological parameters and T, N, and M stage in patients with NPC. **Methods** This was a retrospective study of 834 patients treated at our hospital for NPC between November 2011 and December 2012. A control group consisted of 647 healthy volunteers. Associations between disease stage and pretreatment laboratory data including absolute white blood cell (WBC), neutrophil, lymphocyte, and platelet counts; red cell distribution width (RDW); PLR, and NLR were analyzed using the Kruskal–Wallis test. Associations with patient survival were analyzed using the Kaplan–Meier method and log-rank test. The relationship between tumor expression of vascular-endothelial growth factor (VEGF) and circulating neutrophil counts was also evaluated in a subset of patients. **Results** NLR, PLR, RDW, total WBCs, neutrophils, and platelets were elevated, lymphocyte counts were lower, and hemoglobin was unchanged in NPC patients compared with healthy control subjects. NLR, PLR, and RDW were significantly associated with increasing N stage ($P<0.0001$, $P=0.0024$, $P<0.0001$, respectively), and NLR, WBCs, and neutrophils were significantly associated with increasing M stage ($P=0.0034$, $P=0.0157$, $P=0.0064$, respectively). Absolute neutrophil counts correlated positively with tumor VEGF expression ($P=0.0377$). An NLR of ≥ 2.26 was associated with a better 5-year distant metastasis-free survival rate compared with <2.26 (89.4% vs 85.0%, $P = 0.034$). **Conclusions** NLR is significantly associated with local and distant metastasis in NPC patients, and absolute neutrophil count is significantly related to tumor VEGF expression, suggesting that these parameters may be useful predictive biomarkers for NPC.

Introduction

Nasopharyngeal carcinoma (NPC) is rare worldwide but has reached epidemic proportions in Southeast Asia. In Southern China, the annual incidence of NPC is 15–30 cases per 100,000 people [1]. Treatment of patients with NPC is based mainly on the tumor-node-metastasis (TNM) staging system. Radiotherapy alone is generally effective for early stage NPC, while chemoradiotherapy remains the standard treatment for patients with advanced disease [2]. However, even after definitive chemoradiotherapy, 15–30% of patients eventually develop distant metastases, which is the major cause of death in NPC [3,4].

Previous work has demonstrated that a high neutrophil-to-lymphocyte ratio (NLR) is associated with distant metastasis (DM) and poor treatment outcomes in patients with NPC [5–9], suggesting that NLR may represent an easily measurable and inexpensive prognostic marker in NPC. Elevated pretreatment NLR has also been associated with poor prognosis in patients with colorectal, ovarian, gastric, breast, and lung cancers [10–14]. However, the prognostic value of other blood cell parameters in metastatic NPC and the underlying changes in cell counts that drive NLR alterations remain unclear.

In this retrospective study, we explored the relationship between immune cell counts and TNM stage in patients with NPC, with a focus on identifying potential predictors of metastasis.

Methods

Patients

The study protocol was approved by the ethics committee of Fujian Cancer Hospital, and informed consent was obtained from all patients. We reviewed the records of 834 patients treated with NPC at our hospital between November 2011 and December 2012. Pretreatment evaluations included a complete medical history, physical examination, nasopharyngoscopy examination, dental evaluation, standard laboratory tests, chest radiography or computed tomography, bone scan, and abdominal ultrasound. None of the patients had received any cancer-specific treatment at the time of data collection. Patients with evidence of infection, hematological disease, renal dysfunction, or hyperpyrexia were excluded from the study. A diagnosis of NPC was made through endoscopy-guided biopsy. Disease staging was defined according to the criteria of the 8th edition of the American Joint Committee on Cancer staging system [15]. The control group consisted of 647 healthy subjects recruited from

Tissue samples and laboratory tests

We found the information of vascular-endothelial growth factor (VEGF) expression from 834 patients. A total of 62 patients were collected tumor samples and immediately frozen in liquid nitrogen and stored at -80°C until analysis, 18 (29%) were determined to be positive by immunohistochemistry (score >3) or fluorescence *in situ* hybridization.

Laboratory data, including total white blood cell (WBC), neutrophil, lymphocyte, monocyte, and platelet counts; NLR and PLR ratios (based on counts per μL of whole blood); hemoglobin (Hb) level; and red cell width distribution (RDW), were measured as part of the automated complete blood count on a Sysmex XE-2100 analyzer (Sysmex Corporation, Japan).

Statistical analysis

Receiver operating characteristic (ROC) curves were constructed to select cut-off values for stratification of patients by pretreatment NLR. Data are expressed as the median and interquartile range (IQR) or mean and 95% confidence interval (CI), as appropriate. Laboratory parameters in patient and control groups were compared with *t* tests for variables with normal distribution or with the Mann–Whitney U test for abnormally distributed variables. The associations between laboratory parameters and tumor stages were assessed using the Kruskal–Wallis test. DM-free survival curves were constructed using the Kaplan–Meier method and analyzed with a log-rank test. Statistical significance was defined as $P < 0.05$.

(two-tailed). All analyses were carried out using Prism 5 (GraphPad Software, La Jolla, CA, USA) and SPSS version 17 (SPSS, Chicago, IL, USA) software.

Results

Patient characteristics

Detailed information on the clinicopathological characteristics of NPC patients is shown in Table 1. A total of 834 patients were enrolled, of which 612 (73.4%) were men. The median age at the time of surgery was 59 years (range 13 to 80 years). Evaluation of TNM stages revealed the following distribution: stage I, 0.8% (7 patients); stage II, 5.9% (49 patients); stage III, 49.3 % (411 patients), and stage IV, 44.0% (367 patients). Classification according to the TNM stage revealed that 261 (31.3%) patients had deep tumor infiltration (T), 776 (93.0%) had lymph node invasion (N stage 1/2/3), and 49 (5.9%) had DM (M1).

Laboratory parameters in NPC patients and healthy control subjects

The laboratory parameters of the 834 patients with NPC and 647 healthy subjects are shown in Table 2. Compared with the control group, NPC patients had significantly higher total WBC, neutrophil, and platelet counts, and significantly lower lymphocyte counts. The median NLR, PLR, and RDW were also significantly higher in NPC patients compared with control subjects, but there was no difference in Hb levels.

Association between hematologic markers and TNM stages in NPC patients

Next, we examined the relationship between the parameters that were significantly altered in NPC patients (NLR, PLR, WBC, RDW, cell counts) and the T, N, and M stage (Fig. 1). Kruskal–Wallis analysis revealed that NLR ($P<0.0001$), PLR ($P=0.0038$), and RDW ($P<0.0001$) were significantly associated with increasing N stage, whereas NLR and WBC were significantly higher in M1 compared with the M0 subgroup ($P=0.0034$ and $P=0.0157$, respectively) (Fig. 1). None of these parameters were significantly associated with the T stage (Fig. 1). The median neutrophil and platelet counts increased with advancing N stage ($P=0.0032$ and $P=0.0164$, respectively), whereas the lymphocyte count was not associated ($P=0.1653$) (Table 3). Neutrophil counts were significantly higher in patients with DM compared with the rest of the group (M0 vs M1, $P=0.0034$) (Table 3).

Association between hematologic markers and overall disease stage in NPC patients

To evaluate the relationship between the disease stage and the hematological parameters, we segregated the patients into stage I/II, III, and IV subgroups (Fig. 2). Kruskal–Wallis analysis revealed that NLR

($P=0.0015$), PLR ($P=0.0008$), and RDW ($P<0.0001$) increased significantly with advancing disease (Fig. 2A–C). Absolute neutrophil and platelet counts, but not lymphocyte counts, also showed significant increases in parallel with the disease stage ($P=0.0124$, $P<0.0001$, and $P=0.313$, respectively) (Fig. 2D–F).

Values of NLR, PLR, and RDW for predicting DM-free survival of NPC patients

To assess the association with DM-free survival, we dichotomized the 834 NPC patients into groups based on NLR, PLR, RDW, and WBC cut-off values of 2.26, 87.6, 12.7, and 6.9, respectively, based on ROC curve analyses (data not shown). As shown in by the Kaplan–Meier analysis in Fig. 3, we found that patients with high baseline $\text{NLR} \geq 2.26$ had worse DM-free survival in comparison with the patients with low baseline $\text{NLR} < 2.26$, ($P=0.034$, HR: 1.5, CI: 1.1–1.9). However, no significant associations between DM-free survival and PLR, RDW, or WBC were detected (Fig. 3B–D).

Absolute neutrophil count is positively associated with tumor VEGF expression in NPC patients

VEGF production by tumors is known to be involved in the recruitment of pro-angiogenic neutrophils, which is associated with the development of DM [16,17]. Therefore, to determine whether the observed stage-dependent increase in neutrophil counts may be related to DM formation, we examined the association between neutrophils and VEGF expression in the patients. Kruskal–Wallis analysis revealed that the neutrophil count was significantly higher in patients with tumors that expressed VEGF ($P=0.0377$, Fig. 4), suggesting that tumor VEGF production may contribute to the rise in neutrophils.

Discussion

Elevated NLR has previously been reported to be a risk factor for DM and an indicator of poor prognosis in NPC patients [5–9], although the reasons for this are obscure. In this study, we investigated a number of hematological parameters and found that several showed increases significantly associated with the N and M stage, but not the T stage. The change in NLR was driven by an increase in the absolute number of neutrophils, which correlated positively with VEGF expression in NPC tissues.

Of the parameters examined here, both NLR and PLR showed an association with advanced lymph node involvement (N3), but only NLR was significantly associated with DM and DM-free survival. This finding is consistent with other similar studies of head and neck cancers, which showed that PLR was not significantly associated with survival [18–20]. However, increasing evidence suggests that PLR is a useful prognostic factor for lung and colon cancer [21, 22]. This apparent discrepancy may be caused by tumor heterogeneity, among other factors. Taken together, our results implicate a neutrophil-dependent inflammatory response in the development of NPC metastasis.

Our finding here that absolute neutrophil counts increase with advancing NPC is consistent with previous studies showing that myeloid cells facilitate tumor advancement by promoting an inflammatory tumor microenvironment required for epithelial–mesenchymal transition, intravasation, and tumor metastasis

[23]. Neutrophils can release growth factors such as VEGF and specific proteases [24, 25] and, consistent with this, we confirmed that VEGF expression in NPC tissues was positively associated with the neutrophil count.

We found that NPC patients had significantly lower lymphocyte counts compared with the healthy control group; however, there was no obvious association of lymphocyte count with either advancing disease stage or the development of metastases. Lymphocytes play an essential role in the anti-tumor immune response, impeding tumor cell proliferation and migration and inducing cell death [26]. Thus, although lymphocytes affect tumor growth, they likely have little influence on metastasis in NPC.

VEGF is a tumor-produced growth factor that contributes to tumor angiogenesis and metastasis [27–31], and high VEGF expression has previously been associated with poor prognosis in patients with NPC [32]. The anti-VEGF monoclonal antibody bevacizumab is currently used to treat NPC [33]; however, its precise curative effect and therapeutic index is unclear. The association between neutrophil counts and tumor VEGF expression identified in the present study suggests that these parameters could be potential evaluation markers for locally advanced and metastatic NPC.

There are several limitations to this study. First, it was retrospective. Second, the number of patients with stages I and II NPC was low (7 and 49 patients, respectively), which may have reduced the statistical power for subgroup analysis. Thus, our observations must be validated in a large-scale prospective study. Moreover, further work is required to determine whether NLR can serve as a robust predictive biomarker for NPC therapy.

In summary, we identified a stage-dependent increase in absolute neutrophil counts in patients with NPC, providing an explanation for the finding that NLR is an independent prognostic factor for DM in this patient population. Neutrophil counts also correlated positively with tumor VEGF expression, and may have utility as a prognostic marker for the development of locally advanced and metastatic NPC.

Abbreviations

AJCC: American Joint Committee on Cancer; DMFS: Distant metastasis-free survival; NLR: Neutrophil-to-lymphocyte ratio; NPC: Nasopharyngeal carcinoma; OS: overall survival; ROC: Receiver operating characteristic; TNM: tumour-Node-Metastasis.

Declarations

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Authors' contributions

XYM, TZL and WDB acquired and analyzed data. JJ and LW analyzed data. YC conceived and designed studies. JFZ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted in compliance with institutional policy to protect patient's private information, and was approved by the Institutional Review Board of Fujian Cancer Hospital. As the current study was a retrospective assessment of routine data, the ethics committee of our Cancer Center waived the need for individual informed consent. Consent for publication not applicable.

Competing interests

The authors declare no competing financial interests.

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Figures

Figure 1

Analysis of blood parameters in NPC patients according to T, N, and M stages. (a) NLR, (b) PLR, (c) WBC, and (d) RDW in 834 NPC patients grouped by T, N, and M stages.

Figure 2

Analysis of blood parameters in NPC patients according to overall disease stage. (a) NLR, (b) PLR, (c) RDW, (d) neutrophil count, (e) platelet count, and (f) lymphocyte count in 834 NPC patients grouped by stage I/II, III, and IV disease. Cell counts are expressed as the number $\times 10^9/L$.

Figure 3

Kaplan–Meier DM-free survival curves for NPC patients stratified by hematological parameters. Patients were assigned to two groups based on cut-off values for (a) NLR, (b) PLR, (c) RDW, and (d) WBC, as determined by ROC curve analysis. Group sizes for the $\geq$ and $<$ cut-off values, respectively, were (a) ≥ 2.26 and < 2.26 , (b) ≥ 87.6 and < 87.6 (c) ≥ 12.7 and < 12.7 , and (d) ≥ 6.9 and < 6.9 .

Figure 4

Correlation between the neutrophil count and tumor VEGF expression in NPC patients. NPC tissue samples from 62 patients were analyzed for VEGF mRNA by fluorescence in situ hybridization or immunohistochemistry. Neutrophils are expressed as the number $\times 10^9/L$. $P=0.0377$.

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