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Clinical Paper
Head and Neck Oncology

Multivariate analysis of preoperative and postoperative neutrophil-to-lymphocyte ratio as an indicator of head and neck squamous cell carcinoma outcome

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Abstract. Recent publications have highlighted a greater utility of routine blood tests in patients with various cancers than previously assumed. It appears that the neutrophil-to-lymphocyte ratio (NLR) may be a good predictive biomarker for overall survival (OS) and disease-free survival (DFS). Preoperative and postoperative NLR data for patients with head and neck cancers have yet to be established. The aim of this study was to evaluate the preoperative and postoperative NLR in 182 patients with head and neck squamous cell carcinoma and to determine the association of NLR with OS and DFS. The statistical analysis of OS and DFS and their predictors was performed using Kaplan–Meier survival analysis and multivariate Cox proportional hazards regression analysis, with factors including age, sex, alcohol and tobacco use, tumour location, treatment after surgery, and lymphocyte and neutrophil counts. Longer OS was significantly associated with not consuming alcohol, preoperative neutrophil and lymphocyte counts, preoperative NLR, and the difference between the preoperative and postoperative NLR ($P = 0.016$). Longer DFS was significantly associated with not consuming alcohol, preoperative neutrophil and lymphocyte counts, postoperative NLR, and the difference between preoperative and postoperative NLR ($P = 0.028$).

Key words: neutrophils; neutrophil-to-lymphocyte ratio; HNSCC.

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Head and neck cancers are a heterogeneous group of tumours with varying aetiology. More than 90% of head and neck cancers are squamous cell carcinomas[1,2]. The global incidence of all new head and neck cancer cases has been reported to be between 400,000 and 600,000 per year and the mortality rate between 223,000 and 300,000 deaths per year[3]. Risk factors for head and neck cancers are tobacco and alcohol intake, poor diet, and infection with human papillomavirus (HPV) or Epstein–Barr virus (EBV). Recent reports have shown an increase in head and neck cancer incidence with significantly higher mortality in developing countries[4]. These cancers are very aggressive and can develop distant metastases even after effective local therapy[5–7]. For this reason, predictive biomarkers that may direct the clinical decision for follow-up and diagnostics are of crucial significance.

Current models for predicting survival and the efficacy of therapy in patients with head and neck cancer are still inadequate; therefore, available biomarkers are being re-evaluated in order to improve their informativeness. Inflammation is a significant moderator of carcinogenesis, also associated with poorer disease-free survival (DFS) and overall survival (OS)[8]. Recently, it has been shown that the preoperative neutrophil-to-lymphocyte ratio (NLR) may predict a poor response to treatment, DFS, and OS in patients with many cancer types, including small cell lung carcinoma, oesophageal carcinoma, pancreatic adenocarcinoma, and head and neck cancers[9–18].

Neutrophils take part in the elimination of pathogens by phagocytosis – the generation of reactive oxygen species – via phagocyte NADPH oxidase, the release of antimicrobial and cytotoxic compounds, formation of neutrophil extracellular traps, and secretion of chemokines and cytokines[19]. An increased neutrophil level is characteristic of many cancer types, as they promote disease progression by releasing matrix metalloproteinase 9 (MMP9)[20]. Neutrophils themselves are also a significant source of hepatocyte growth factor, which has been implicated in the regulation of mitogenesis, motogenesis, and morphogenesis of epithelial and endothelial cells[21]. NLR has recently been associated with the metabolic tumour volume in patients with oesophageal squamous cell cancer[22].

In recent years, the cut-off values of the NLR for different cancer types have been estimated on several occasions. The pretreatment NLR cut-off value for the prediction of OS in patients with colorectal cancer was determined to be >5[23]. The median NLR cut-off value for the prediction of DFS was suggested to be >4 in a systematic meta-analysis of publications investigating the association of NLR and DFS in solid tumours[11].

Currently, preoperative and postoperative NLR data for patients with head and neck squamous cell carcinoma (HNSCC) are lacking. Therefore, the aim of this retrospective study was to establish preoperative and postoperative NLR levels for HNSCC, together with their association with DFS and OS. Additionally, multivariate analysis was used to determine the impact of alcohol and tobacco intake, patient age, tumour location, cancer stage, and therapy on the capability of the NLR to predict the disease outcome.

Subjects and methods

Study population

Data were retrieved from the patient charts at the Department of Otorhinolaryngology, Clinic for Tumours, Zagreb, Croatia. The study population consisted predominantly of male HNSCC patients (156 male patients, 26 female patients). The preoperative and postoperative NLR values were associated with demographic characteristics, lifestyle characteristics, OS, and DFS. For the purpose of the analysis, the TNM (tumour–node–metastasis) stage of disease was dichotomized as follows: stages I and II were designated as early stage, whereas stages III and IV were designated as advanced stage; this was done following classification according to the criteria of the TNM Classification of Malignant Tumours eighth edition[24]. With regard to treatment, all patients were initially treated with surgery, after which some patients received concomitant radiotherapy and some also chemotherapy. The localization of the cancer was dichotomized as 'oral cavity' (anterior two-thirds of the tongue, gingiva and alveolar ridge, hard palate and buccal mucosa) and 'oropharynx' (soft palate, pharynx, and tonsils). The treatment type was dichotomized as follows: patients with stage I or II disease were treated only with surgery; patients with stage III or IV disease were treated after surgery with adjuvant radiotherapy. Those with involved resection margins and/or extranodal spread of disease (histologically confirmed) received concomitant cisplatin chemotherapy at a daily dose of 100 mg/m^2 of body surface, every 3 weeks. The patients' HPV status was not investigated, as the study period started 16 years ago, at a time when HPV status was not part of routine diagnostics.

Patients with TNM stage I and II disease were treated by surgery. Those with stage III and IV disease received adjuvant therapy after surgery, using three-dimensional conformal radiotherapy. The target volume encompassed the lymph node regions bilaterally to a prescribed dose of 50 Gy and the tumour bed to a prescribed dose of 60 Gy (with or without a 'booster' dose of 6 Gy) and 6 (or 18) MV photons with a linear accelerator (ARTISTE or ONCOR; Siemens Medical Solutions USA, Inc.). Patients were irradiated over the course of 6 to 6.5 weeks with daily doses of 2 Gy.

DFS was defined as the period after surgery during which the patient had no sign of cancer recurrence. OS was defined as the period from the date of surgery to individual death from any cause or the last follow-up.

Laboratory measurements

Blood samples were taken 1 week before surgery and postoperatively 7 days after surgery. The exclusion criteria were patients with missing clinical data (none) and positive surgical margins (none). Cancer patients had no autoimmune disorders or haematological disorders, were not on any ongoing immune-modulating medications, and had no previous history of malignant disease. The Ethics Committee of the Clinical Hospital ''Sisters of Mercy'', University Hospital Centre approved the study.

Preoperative and postoperative serum neutrophils and lymphocytes were extracted from blood counts of blood samples using a fully automated five-part differential haematology analyzer (Sysmex XN-1000; Sysmex, Kobe, Japan). The time of blood sampling (7 days after surgery) ensured that the process of wound healing did not affect the results. The maximum follow-up period was 202.9 months (mean 102.1 months). The NLR was calculated by dividing the neutrophil count by the lymphocyte count. In addition, the difference between the preoperative and postoperative NLR (DiffNLR) was introduced in order to test its predictive capacity for DFS and OS.

Statistical analysis

The statistical analysis was conducted using Statistica data analysis software system version 12 (StatSoft, Inc., Tulsa, OK, USA) and MedCalc statistical software version 16.8.4 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2016). Categorical variables were recorded as numbers and

proportions (%). Quantitative variables were tested for normality of distribution using the Kolmogorov–Smirnov test and recorded as the mean and standard deviation (SD) or median and interquartile range (IQR), depending on the type of data distribution. Kaplan–Meier survival analysis was used to determine OS and DFS. Associations of OS and DFS with possible predictors were analyzed using Cox proportional hazards analysis with a backward stepwise approach. All of the tests were two-tailed, and $P < 0.05$ was used to indicate significance in all analyses.

Results

This study included the results of 182 patients treated for HNSCC (85.7% male), whose mean age was 60.0 years (range 23.4–86.3 years). The baseline characteristics of the study population are presented in Table 1. The analysis included pre- and postoperative measurements of neutrophil and lymphocyte counts, together with the NLR and DiffNLR. DFS was a median of 29.7 months (IQR 11.8–80.9 months) and OS was a median of 31.4 months (IQR 14.8–83.8 months) (Kaplan–Meier survival analysis).

Table 2 presents the preoperative and postoperative neutrophil count, lymphocyte count, NLR, and DiffNLR values.

Significant independent associations with OS and DFS are presented in Tables 3 and 4. As seen in Table 3, not consuming alcohol significantly lowered the risk of death by 45% ($P = 0.030$), the preoperative neutrophil count lowered the risk of death by 16% per each 1×10^9/l ($P = 0.030$), and the preoperative lymphocyte count marginally increased the risk of death by 49% per each 1×10^9/l ($P = 0.059$). The risk of death was significantly increased by 38% for a unit change in the preoperative NLR ($P = 0.002$), and was marginally increased by 4% for a unit change in DiffNLR ($P = 0.081$).

As seen in Table 4, not consuming alcohol significantly lowered the risk of disease relapse by 42% ($P = 0.041$), the preoperative neutrophil count lowered the risk of disease relapse by 13% per each 1×10^9/l ($P = 0.049$), and the preoperative lymphocyte count significantly increased the risk of disease relapse by 53% per each 1×10^9/l ($P = 0.026$). There was a significant increase in the risk of disease relapse by 34% for a unit change in the postoperative NLR ($P = 0.002$), and a significant decrease in this risk by 22% for a unit change in DiffNLR ($P = 0.008$).

Tumour location, tumour stage, smoking, age, and type of therapy were not significantly associated with either DFS or OS.

Radiotherapy or combined radiotherapy with chemotherapy had no impact on the biomarkers measured.

Table 1. Baseline characteristics of the study patients ($N = 182$)[a].

Characteristic	
Age, years, mean ± SD (range)	60.0 ± 9.7 (23.4–86.3)
Sex	
Male	156 (85.7%)
Female	26 (14.3%)
Alcohol consumption	136 (74.7%)
Smoking habit	
Non-smokers	20 (11.0%)
Active smokers	120 (65.9%)
Ex-smokers	25 (13.7%)
Missing data	17 (9.3%)
Tumour location	
Oral	132 (72.5%)
Pharyngeal	48 (27.5%)
TNM stage	
1	21 (11.5%)
2	91 (50.0%)
3	48 (26.3%)
4	22 (12.1%)
Treatment after surgery	
No additional treatment	34 (18.7%)
Radiotherapy	143 (78.6%)
Radio- and chemotherapy	5 (2.7%)
Relapse	124 (68.1%)
Death	122 (67.0%)

SD, standard deviation.
[a] Results are presented as the number and percentage of patients, unless stated otherwise.

Discussion

This study showed that preoperative and postoperative neutrophil counts and NLR can be predictive biomarkers for DFS and OS in HNSCC. This study used multivariate analysis, which enabled the testing of neutrophil count, NLR, and DiffNLR as predictive biomarkers for DFS and OS by including alcohol consumption, patient age, smoking, tumour stage, and therapy type as modifying parameters. The finding that the difference in NLR before and after surgery was a significant predictor of OS and DFS appears to be novel. Not consuming alcohol was significantly associated with longer DFS and better OS.

Lymphocytes are the most significant components of the adaptive immune system, which when infiltrated into the tumour indicate the generation of an effective anti-tumour cellular immune response[25]. Increased NLR has been associated with an increase in the peri-tumoural infiltration of macrophages and an increase in interleukin 17, interleukin 6, interleukin 8, tumour growth promoting factors, vascular endothelial growth factor, hepatocyte growth factor, and matrix metalloproteinases, all of which form tumour microenvironments[26].

Previous studies have shown an association between a low peripheral blood lymphocyte count and shorter survival of patients with different types of cancer[27,28]. However, other cell types also involved in the immunological response have been shown to play a significant role in the progression of cancer. Thus, it has been reported that neutrophils support metastasis by producing leukotrienes, which enable the colonization of distant tissues with cancer cells[29]. Both neutrophil levels and the NLR have been shown to be prognostic factors in nasopharyngeal cancer independent of OS and DFS[30]. Tsai et al. reported that neutrophil counts and NLR increased with the advancement of the clinical stage (i.e., T4) and poorer tumour differentiation in patients with oral cancer, which was also accompanied by a decrease in the lymphocyte count[31].

The location of the tumour was not associated with preoperative NLR, postoperative NLR, DiffNLR, OS, or DFS, which is in accordance with published data[32].

The results of this study confirm those of a study by Rachidi et al., which showed that the NLR is a robust predictor of OS in oral, pharyngeal, and laryngeal squamous cell carcinomas[33]. Perisanidis et al. analyzed 97 patients with oral cancer undergoing preoperative chemo-radiotherapy in terms of DFS[34]. These authors reported that a high pre-treatment NLR is a significant independent predictor of shorter DFS in patients with oral cancer

Table 3. Covariates depicted for the model of overall survival (OS); $P = 0.016$ for the model, Cox proportional hazards model.

Covariate	b	SE	Wald	*P*-value	HR	95% CI of HR
Not consuming alcohol	−0.601	0.276	4.734	0.03	0.548	0.319 to 0.948
Preoperative neutrophil count	−0.17	0.078	4.691	0.03	0.844	0.724 to 0.984
Preoperative lymphocyte count	0.397	0.21	3.554	0.059	1.487	0.984 to 2.245
Preoperative NLR	0.319	0.101	9.954	0.002	1.375	1.128 to 1.676
DiffNLR[a]	0.043	0.024	3.054	0.081	1.044	0.995 to 1.095

E, standard error; HR, hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio.
[a] Difference between preoperative and postoperative NLR.

Table 2. Neutrophil count, lymphocyte count, and NLR before and after surgery, and the difference in NLR.

Characteristic	Mean ± SD or median (IQR)	Range
Preoperative		
Neutrophil count, $\times 10^9$/l	5.11 ± 1.88	1.33 to 10.63
Lymphocyte count, $\times 10^9$/l	2.11 ± 0.78	0.44 to 5.89
NLR	2.27 (1.77, 3.19)	0.51 to 12.39
Postoperative		
Neutrophil count, $\times 10^9$/l	6.34 (4.77, 8.10)	1.86 to 19.70
Lymphocyte count, $\times 10^9$/l	1.72 (1.25, 2.20)	0.44 to 5.59
NLR	3.80 (2.53, 5.33)	0.00 to 38.41
DiffNLR[a]	1.06 (−0.02, 2.33)	−9.09 to 34.06

NLR, neutrophil-to-lymphocyte ratio; SD, standard deviation; IQR, interquartile range.
[a] Difference between preoperative and postoperative NLR.

Table 4. Covariates depicted for the model of disease-free survival (DFS); $P = 0.028$ for the model, Cox proportional hazards model.

Covariate	b	SE	Wald	*P*-value	HR	95% CI of HR
Not consuming alcohol	−0.546	0.267	4.179	0.041	0.579	0.343 to 0.978
Preoperative neutrophil count	−0.144	0.073	3.845	0.049	0.866	0.750 to 0.999
Preoperative lymphocyte count	0.428	0.192	4.975	0.026	1.534	1.053 to 2.235
Postoperative NLR	0.29	0.096	9.145	0.002	1.336	1.107 to 1.613
DiffNLR[a]	−0.245	0.093	6.943	0.008	0.783	0.653 to 0.939

SE, standard error; HR, hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio.
[a] Difference between preoperative and postoperative NLR.

receiving preoperative chemo-radiotherapy[34]. Fang et al. analyzed data from 226 patients with oral cancer and reported that elevated NLR was significantly associated with tumour status, nodal metastasis, tumour depth, DFS, and OS[35]. The effect of the NLR on DFS and OS was shown to exist even after adjusting data for tumour status, lymph node metastasis, and tumour cell differentiation. Song et al. showed that a high preoperative NLR was associated with increased wound complications and poorer survival in patients with hypopharyngeal squamous cell carcinoma after radical resection[36]. The present study results are in concordance with these previous studies, which have suggested that the preoperative NLR is an independent predictor of head and neck cancer recurrence[37–39], even after other lifestyle and demographic data are included in the analysis.

A recent study involving a healthy population of adult, non-geriatric subjects estimated normal NLR values to be between 0.78 and 3.53, adding significant value to the application of the NLR[40]. However, the control range obtained did not take into account smoking habit or alcohol intake. It is interesting to note that in the present study, in which 70–80% of the subjects were alcohol consumers and smokers, the mean postoperative NLR was higher (group value) than the suggested control range values. Alcohol intake has been associated with neutropenia, and neutrophils have also been shown to be hypo-responsive in cases of exposure to alcohol due to impaired phagocytosis and superoxide generation in humans and in animal models[41,42]. Additionally, the heterogeneous impact of alcohol on OS related to treatment and primary site shows the need for further investigation in this study[43]. However, the effect of smoking on the parameters measured was not of great significance, as it is known that in contrast to alcohol, smoking causes an increase in neutrophil counts[44]. The present study results confirm the importance of taking alcohol consumption and smoking into consideration in studies assessing the NLR.

This study showed the neutrophil count, the NLR, and DiffNLR to be significantly associated with DFS and OS in HNSCC. Future studies should be performed in order to determine whether the use of focused medical surveillance protocols depending on neutrophil counts, the NLR, and DiffNLR in these patients might increase DFS or OS. Finally, a significant association between alcohol consumption and tumour site with regard to the DFS and OS was noticed. Studies of the same design as the present study should be performed in other cohorts in order to define cut-off values for HNSCC and to include the use of these parameters in clinical practice.

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Competing interests

We wish to confirm that there are no known conflicts of interest associated with this publication.

Ethical approval

Ethical approval was obtained from the School of Dental Medicine, Zagreb, Croatia.

Patient consent

Not required.

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