Int. J. Oral Maxillofac. Surg. 2018; xxx: xxx–xxx
https://doi.org/10.1016/j.ijom.2018.02.011, available online at https://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

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

G. Muhaxheri[1], V. Vucicevic Boras[1], A. Fucic[2], D. Plavec[3], M. Sekerija[4], M. Filipovic[5], K. Grsic[6], B. Stubljar[6], T. Krnic[6], B. Vrdoljak[5]

[1]Department of Oral Medicine, School of Dentistry, University of Zagreb, Zagreb, Croatia; [2]Institute for Medical Research, Zagreb, Croatia; [3]Childrens Hospital Srebrnjak, Zagreb, Croatia; [4]Institute for Public Health, Zagreb, Croatia; [5]School of Medicine, University of Zagreb, Zagreb, Croatia; [6]Clinic for Tumours, Zagreb, Croatia

G. Muhaxheri, V. Vucicevic Boras, A. Fucic, D. Plavec, M. Sekerija, M. Filipovic, K. Grsic, B. Stubljar, T. Krnic, B. Vrdoljak: Multivariate analysis of preoperative and postoperative neutrophil-to-lymphocyte ratio as an indicator of head and neck squamous cell carcinoma outcome. Int. J. Oral Maxillofac. Surg. 2018; xxx: xxx–xxx.

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.

Accepted for publication 26 February 2018

0901-5027/000001+06

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

Please cite this article in press as: Muhaxheri G, et al. Multivariate analysis of preoperative and postoperative neutrophil-to-lymphocyte ratio as an indicator of head and neck squamous cell carcinoma outcome, *Int J Oral Maxillofac Surg* (2018), https://doi.org/10.1016/j.