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Significance of neutrophil to lymphocyte ratio as a predictor of outcome in head and neck cancer treated with definitive chemoradiation

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ABSTRACT

Background: The role of host immune system in carcinogenesis and response to treatment is increasingly studied, including predictive potential of circulating neutrophils and lymphocytes. The objective of the study was to evaluate the prognostic value of pre- and post-treatment neutrophil-to-lymphocyte (NLR) for treatment outcome in patients diagnosed with squamous cell carcinoma of head and neck (HNSCC) treated with definitive chemoradiation.

Materials and methods: Electronic medical records of patients were evaluated and NLR was calculated. Cox regression was used to assess the impact of selected variables on overall survival (OS), disease specific survival (DSS), progression free survival (PFS) and distant failure free survival (DFFS). Logistic regression was used to estimate odds ratios of complete response with NLR.

Results: 317 patients' records were included in the study. Increases in both pre-and post-NLR were associated with decreased OS in univariable analysis [hazard ratio (HR): 2.26 (1.25–4.07), p = 0.0068 and HR: 1.57 (1.03–2.37), p = 0.035 respectively). Post-NLR remained significant for OS in multivariable analysis [HR: 1.93 (1.22–3.1), p = 0.005] as well as for unfavorable DSS [HR: 2.31 (1.22–4.4), p = 0.01]. Pre-treatment NLR and nodal status correlated with shorter DFFS in multivariable analysis [HR 4.1 (1.14–14), p = 0.03 and HR 5.3: (1.62–18), p = 0.0062, respectively]. Strong correlation of increased both pre- and post-NLR with probability of clinical tumor response (CR) was found [odds ratio (OR): 0.23 (0.08–0.6), p = 0.003, and OR: 0.39 (0.2–0.8), p = 0.01 respectively].

Conclusion: NLR evaluated before and post treatment was a strong predictor of unfavorable treatment outcome and can be used for risk evaluation and clinical decision about treatment and post-treatment surveillance.

Key words: neutrophil-to-lymphocyte ratio; head and neck cancer; prediction

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Introduction

Head and neck cancer remains a global concern and its incidence is increasing. At the same time, gradual change in etiology is observed as exemplified by HPV driven oropharyngeal cancer [1]. Numerous studies demonstrated the key role of host immune system in carcinogenesis [2–6] and response to treatment of many solid tumors, including lung, head and neck, breast, osteosarco-

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ma and prostate. [7–12] Chronic inflammation is considered to be a facilitator of tumorigenesis [13]; however, the role of the immune system and its components in host-tumor interactions is complex. For example, neutrophils, essential for innate immunity, are recruited from the bone marrow by the tumor and facilitate angiogenesis and distant metastasis [2]. Recruitment of neutrophils from bone marrow and radiotherapy both decrease the number of lymphocytes in circulating blood and their antitumor activity. This phenomenon translates to increased post-treatment neutrophil-to-lymphocyte ratio (post-NLR). On the other hand, neutrophils can also help in tumor elimination, depending on their phenotype. [14]

Assessment of neutrophil and lymphocytes circulating in blood, easily obtained from standard complete blood count (CBC) can be exploited for clinically useful evaluation of host immune response. The interaction and balance between neutrophils and lymphocytes in CBC expressed as a neutrophil-to-lymphocyte ratio (NLR) proved to be an important predictor of outcome in solid tumors. Meta-analysis based on data of 2232 patients by Hu at al. suggested that high pre-treatment NLR (pre-NLR) was associated with poor overall survival and progression free survival in patients with hypopharyngeal cancer [15]. In a meta-analysis including 5475 patients, Yu et al. confirmed previous findings that higher pre-NLR is associated with higher probability of tumor recurrence and increased mortality risk. However, optimal cut-off value to define high and low NLR is not yet established and varies across studies [16, 17] Moreover, further research is needed to explain how higher NLR translates into worse prognosis.

The objective of this study was to evaluate the prognostic value of pre- and post-treatment neutrophil-to-lymphocyte ratio (pre-NLR, post-NLR) for treatment outcomes: overall survival, disease specific survival, progression free survival, distant failure free survival and complete response in patients diagnosed with squamous cell carcinoma of head and neck (HNSCC) treated with definitive radiotherapy or chemoradiation

Materials and methods

Electronic medical records of consecutive patients diagnosed with newly diagnosed head and neck squamous cell carcinoma (HNSCC) were retrospectively collected and evaluated. 331 patients were initially included into the study according to the inclusion criteria: primary site of cancer: nasopharynx, oropharynx, hypopharynx, larynx or oral cavity, treatment with definitive chemoradiation or radiation, performance status Eastern Cooperative Oncology Group (ECOG 0–1), no surgery other than biopsy before definitive treatment, follow up time equal or longer than 2 years, known survival status and available CBC before and after treatment.

All patients were treated with curative intent at the Greater Poland Cancer Center between 1 January 2010 and 26 January 2021 with intensity-modulated radiation therapy (IMRT) to total dose of 70 Gy in 2 Gy daily fraction, with or without concomitant cisplatin in dose of 100 mg/m^2 every three weeks, up to 3 courses or 40 mg/m^2 weekly up to 6 courses. Patients with active infection before start of treatment, chronic inflammatory disease or steroid use were excluded from this study.

Included patients were followed up according to standard institutional procedure: evaluation by an ear, nose, and throat (ENT) surgeon and radiation oncologist in the first and third month after completion of the treatment and every 3 months for the first 2 years and afterwards every 6 months. First computed tomography (CT) scan for response assessment was performed 3 months after treatment or earlier if treatment failure was suspected.

14 patients were excluded due to missing survival data, leaving 317 patients available for analysis. Follow up and survival analysis was censored on 26 January 2023.

The primary objective of this study was to determine correlation of pre- and post-treatment neutrophil-to-lymphocyte ratio (pre-NLR, post-NLR), calculated as the ratio of absolute neutrophil count to absolute lymphocyte count obtained directly before start and at the end of radiotherapy with overall survival (OS) of included patients. Secondary outcomes were correlation of both NLR variables with disease specific survival (DSS), locoregional progression free survival (PFS) and distant failure free survival (DFFS).

Follow up time (FU) was defined as the time between the end of treatment and the last physical visit. Overall survival (OS) time was defined as the time between the end of treatment and date of

death from any reason or date of censoring (26 January 2023), whichever came first. Disease specific survival (DSS) was defined as the time between the end of treatment and date of death from cancer. Progression free survival (PFS) was defined as the time between the end of treatment date and date of confirmed diagnosis of locoregional failure. Distant failure free survival (DFFS) was defined as the time between the end of treatment and date of confirmation of distant failure. Separate analysis was performed for a subgroup of patients with oropharyngeal cancer.

Variables assessed as important for outcomes were age, gender, active tobacco smoking, T and N stage [the 7th edition of the American Joint Committee on Cancer (AJCC7)], tumor primary site, chemoradiation *vs.* radiation, pre-NLR and post-NLR and p16 status for oropharyngeal cancer.

Ethical approval for this study was waived by the Ethics Committee of the Poznan University of Medical Sciences (KB-291/23) due to the retrospective nature of the study.

Statistical analysis

Both pre- and post-NLR were log-transformed and treated as continuous variables. Survival outcomes were analyzed using the Cox proportional hazards model and logistic regression was used for binary outcomes (CR). A two-tailed p value of < 0.05 was deemed statistically significant. Statistical analysis was performed using the R software.

Results

Patient and disease characteristics

317 patients were available for analysis. 231 patients were male. The most common primary sites were oropharynx (46%) and larynx (28%) (Tab. 1).

Median FU was 27.8 months (0–139.2). 199 patients died (62.8%) including 102 cancer related deaths (32.2%) and 17 (5.4%) deaths from unknown causes. 118 (37.2%) patients were still alive at the time of analysis. Median overall survival for the whole group was 34.2 months (0–135). 58 (18.3%) locoregional failures, diagnosed as progression of residual tumor or recurrence after complete response of primary and/or lymph nodes at least 6 months after completion of the treatment and 39 (12.3%) metastatic progressions of the disease were diagnosed. Complete response of the primary evaluated as residual tumor 3 months after treatment completion was observed in 226 patients, whereas partial response, stable disease or progression during treatment were diagnosed in 87 patients. Data for 4 patients was missing.

Overall survival

2-year overall survival reached 60.1% (190 patients) (Fig. 1). The median PFS was 28.6 months (range 0–185.9) and median DFFS — 32.13 months (range 0–186).

In univariate analysis both pre-NLR and post-NLR were significant for OS [hazard ratio (HR): 2.26 (1.25–4.07), p = 0.0068 and HR: 1.57 (1.03–2.37), p = 0.035 respectively) as well as site of primary tumor in larynx [HR: 2 (1.05–3.81), p = 0.03) and oral cavity [HR: 3.46 (1.74–6.87), p = 0.00034) and stage N2 [HR: 1.55 (1.1–2.18), p = 0.01) (Tab. 2).

In multivariable analysis post-NLR was associated with OS [HR: 1.93 (1.22–3.1), p = 0.005) together with age [HR: 1.03 (1.01–1.1), p = 0.002], stage N2 [HR: 1.63 (1.11–2.4), p = 0.013) and oral cavity cancer as a primary site of the tumor [HR: 2.67 (1.29–5.5), p = 0.008) (Fig. 2).

Disease specific survival

All variables were also evaluated for correlation with disease specific survival (DSS). Higher pre- and post-NLR [HR: 3.1 (1.38–6.95), p = 0.006 and HR: 2.43 (1.36–4.36), p = 0.003 respectively] and N2 stage [HR: 3.07 (1.69–5.55), p = 0.0002) and oral cavity primary [HR: 3.19 (1.35–7.52), p = 0.008] were all associated with worse DSS in univariable analysis (Tab. S1 — Supplementary File).

In multivariable analysis post-NLR remained significant [HR: 2.31 (1.22–4.4), p = 0.01) together with N2 stage [HR: 2.69 (1.44–5), p = 0.002] and primary site oral cavity [HR: 3.55 (1.42–8.9), p = 0.007] (Fig. S1 — Supplementary File).

Treatment failure

Secondary endpoints in this study were time to locoregional failure (PFS) and time to distant failure (DFFS).

None of the tested variables was significant in univariable analysis of locoregional PFS, except primary site oral cavity [HR: 2.69 (1.03–7.04),

Table 1. Patient characteristics

Characteristic (n = 317)	Value (%)
Age (years)	
Median	60
Range	20–81
Gender	
Male	237 (74.7)
Female	80 (25.2)
Smoking	
Yes	242
No	75
Follow up (months)	
Median FU	27.8
Range	0–139
Primary site	
Nasopharynx	24 (7.6)
Oropharynx	134 (42.3)
Hypopharynx	34 (10.7)
Oral cavity	39 (12.3)
Larynx	86 (27.1)
Tumor classification	
T1	13 (4.4)
T2	87 (27.5)
T3	91 (29.1)
T4	123 (38.9)
Nodal classification	
N0	85 (26.8)
N1	50 (15.8)
N2	168 (53)
N3	14 (4.4)

Characteristic (n = 317)	Value (%)
Stage AJCC v.7	
I	3 (0.9)
II	30(9.5)
III	68 (21.6)
IV	216 (68.1)
HPV status	
Positive	30 (9.5)
Negative	44 (13.9)
Unknown	243 (76.70)
Treatment	
RT	72(22.7)
RTCT	245(77.3)
Treatment outcome	
CR	226 (71.3)
PR, SD, PRO	87 (24.4)
Unknown	4 (1.3)
pre-NLR	
Median	2.51
Range	(0.34–22.05)
post-NLR	
Median	6.91
Range	(0.19–147.78)
Unknown	41

FU — follow up; AJCC — American Joint Committee on Cancer; HPV — human papilloma virus; RT — radiotherapy; RTCT — radiochemotherapy; CR — clinical tumor response; PR — partial response; SD — stable disease, PRO — progression; pre-NLR — pre-treatment neutrophil-to-lymphocyte ratio; post-NLR — post-treatment neutrophil-to-lymphocyte ratio

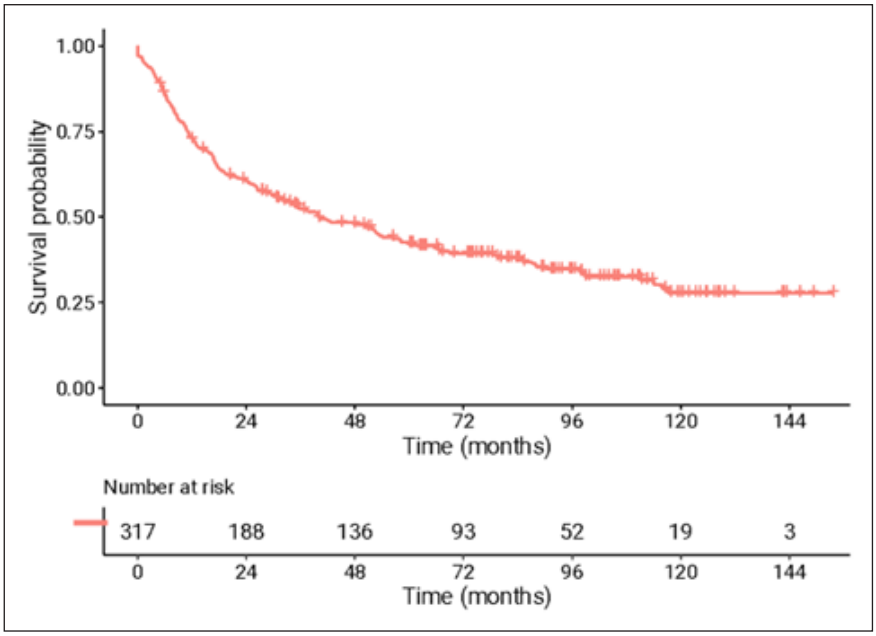

Figure 1. Kaplan-Meier plot of overall survival for the whole group

Table 2. Univariable Cox regression analysis of overall survival. Asterisk marks p-values < 0.05

Variable	Reference	HR (95% CI)	p
T STAGE	T1	T2: 1.08 (0.46–2.55) T3: 1.85 (0.79–4.29) T4: 2.07 (0.91–4.73)	0.85 0.15 0.08
N STAGE	N0	N1: 0.78 (0.48 -1.3) N2: 1.55 (1.1–2.18) N3: 1.23 (0.58–2.61)	0.38 0.01* 0.59
Age	1 year	1.02 (1–1.03)	0.04*
Site	Nasopharynx	Oropharynx: 1.32 (0.69–2.48) Hypopharynx: 1.74 (0.84–3.56) Larynx: 2 (1.05–3.81) Oral cavity: 3.46 (1.74–6.87)	0.39 0.13 0.03* 0.0004*
Treatment	RTCT *vs.* RT	0.77 (0.56–1.06)	0.11
Smoking	10 pack years	1 (0.99–1)	0.78
Stage AJCC7	AJCC stage I	AJCC7 II: HR 0.56 (0.13–2.49) AJCC7 III: HR 0.66 (0.16–2.75) AJCC7 IV: HR 0.95 (0.24–3.84)	0.45 0.56 0.94
log 10 pre-NLR	1 log NLR	2.26 (1.25–4.07)	0.0068*
log10 post-NLR	1 log NLR	1.57 (1.03–2.37)	0.035*

HR — hazard ratio; CI — confidence interval; RT — radiation therapy; RTCT — radiochemotherapy; AJCC — American Joint Committee on Cancer; pre-NLR — pre-treatment neutrophil-to-lymphocyte ratio; post-NLR — post-treatment neutrophil-to-lymphocyte ratio

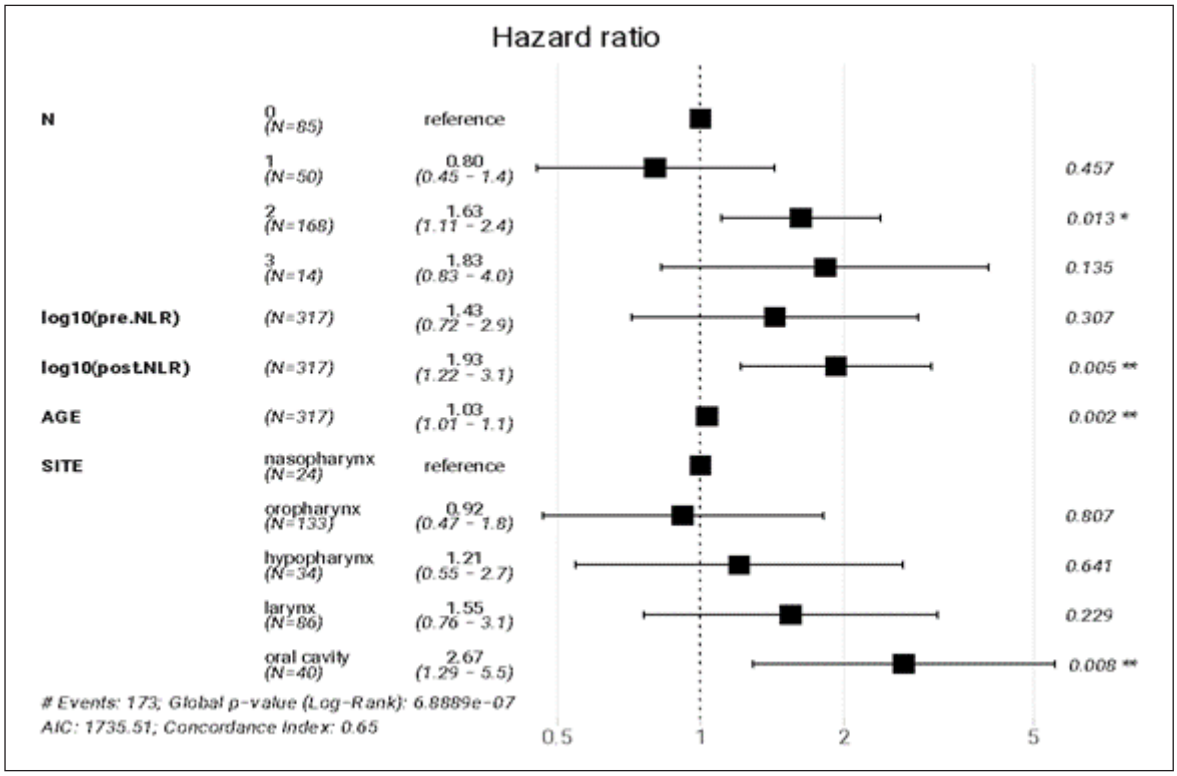

Figure 2. Multivariable Cox regression analysis of overall survival. Asterisk marks p-values < 0.05

p = 0.04) (Tab. S2 — Supplementary File). Consequently, no multivariable analysis was conducted.

Distant failure

Higher pre-NLR [HR: 4.52 (1.22–16.8), p = 0.02] and N stage [HR: 5.42 (1.64–17.8), p = 0.005] were significantly associated with higher distant failure risk in univariable analysis (Tab. S3, Supplementary File). Both variables remained significant in multivariable analysis [HR: 4.1 (1.14–14), p = 0.03 and HR: 5.3 (1.62–18), p = 0.0062, respectively) (Fig. S2 — Supplementary File).

Oropharyngeal cancer

Additionally, a subgroup of patients with oropharyngeal cancer was analyzed for OS and DSS. Univariable analysis revealed significant correlation of pre-NLR, N2 stage with OS [HR: 3.19 (1.21–8.44), p = 0.02 and [HR: 2.95 (1.26–6.88), p = 0.01, respectively] as well as smoking [HR: 1.03 (1–1.05), p = 0.002] (Tab. 3). p16 status was included in analysis in this group of patients and, as expected, was strongly correlated with OS [HR: 0.25 (0.09–0.71), p = 0.0087]. However, these results should be considered with caution due to the high number of missing data.

Only pre-NLR and smoking remained significant in multivariable analysis [HR: 3.06 (1.11–8.4), p = 0.03, HR: 1.02 (1–1), p = 0.015) (Fig. S3 — Supplementary File). p16 was not included in multivariable analysis due to a small number of observations.

In univariable analysis of variables significant for DSS and PFS, only pre-NLR was significantly correlated with increased risk of unfavourable outcome [HR: 7.4 (1.8–29.2), p = 0.004 and HR: 10.1 (1.8–56.6), p = 0.008 respectively]. None of the evaluated variables proved to be significant for DFFS in univariable analysis.

Complete response

In the analyzed dataset, complete radiological and clinical tumor response (CR) was confirmed in 226 patients, while partial response (PR), stable disease (SD) or progression were diagnosed in 87 patients. Strong negative correlation of pre- and post-NLR with probability of CR was found [odds ratio (OR): 0.23 (0.08–0.6), p = 0.003, and OR: 0.39 (0.2–0.8), p = 0.01, respectively) for the whole group. For the oropharyngeal cancer subgroup only pre-NLR was significantly correlated with CR [OR: 0.18 (0.04–0.8), p = 0.03).

Discussion

Results of research on the key role of the immune system in carcinogenesis and cancer treatment caused an increased interest in studying components of this system, including neutrophils and lymphocytes circulating in the blood [2, 3, 18].

Table 3. Univariable Cox regression analysis of overall survival in oropharyngeal cancer subgroup. Asterisk marks p values < 0.05

Variable	Reference	HR (95% CI)	p
T STAGE	T1	T2: 0.5 (0.11–2.31) T3: 1.4 (0.32–6) T4: 1.45 (0.35–6)	0.37 0.64 0.61
N STAGE	N0	N1: 1.76 (0.65–4.76) N2: 2.95 (1.26–6.88) N3: 1.83 (0.45–7.36)	0.26 0.01* 0.39
Age	1 year	1.01 (0.98–1.04)	0.29
Tretament	RTCT *vs* RT	0.66 (0.36–1.18)	0.54
Smoking	10 pack years	1.03 (1–1.05)	0.002*
log 10 pre-NLR	1 log NLR	3.19 (1.21–8.44)	0.02*
log10 post-NLR	1 log NLR	1.06 (0.54–2.19)	0.79
p16	Positive *vs.* negative	0.25 (0.09–0.71)	0.0087*

HR — hazard ratio; CI — confidence interval; RT — radiation therapy; RTCT — radiochemotherapy; pre-NLR — pre-treatment neutrophil-to-lymphocyte ratio; post-NLR — post-treatment neutrophil-to-lymphocyte ratio

Numerous studies investigated impact of NLR on outcome in non-small cell lung cancer [7, 8], but there is also growing evidence of negative impact of increased NLR on survival in other solid tumors [19, 20], including squamous cell carcinoma of head and neck [21–28]. A clear advantage of studying NLR and other components of CBC is their availability from routinely collected blood samples, low cost of analysis and lack of additional burden for patients. Results have a potential for clinical application and risk assessment. However, in the light of the latest basic research of the complex role of the immune system, translation of these results into clinical practice might not be straightforward.

Balance between neutrophils circulating in blood and maturating in bone marrow is related to chronic inflammation, which is cited as one of the hallmarks of cancer [29]. Circulating neutrophils have a complex influence on carcinogenesis. They facilitate proliferation of tumor cells by decreasing the impact of the host immune system and induce angiogenesis. [30] Neutrophils can promote the spread of cancer cells outside blood vessels, as well as support formation of metastases by inhibiting natural killer function (NK) [2, 30, 31]. Ongoing and further studies focus on identification of neutrophils subpopulations, like for example polymorphonuclear myeloid - derived suppressor cells (PMN-MDSC), and insight in their role in carcinogenesis and resistance for cancer therapies [32].

The complex and still not fully studied interplay of the immune system with host and cancer reflects the role of tumor associated neutrophils (TANs), which increase anti-tumor immune response by interacting with CD8+ lymphocytes. Also, TANs induced by radiotherapy increase production of reactive oxygen species (ROS), leading to increased rate of apoptosis of tumor cells. On the other hand, different phenotypes of TANs were recently discovered, which might exhibit different impacts on tumorigenesis. Their role is dynamic and their impact on the course of disease still needs to be studied. [18, 30] Moreover, immune system components localized in the tumor microenvironment (TME) play a very important role in increasing the benefit of radiotherapy, including potential abscopal effect [33].

Lymphocytes are another important component of the immune system involved in response to cancer. It is acknowledged that "hot tumors" — infiltrated by tumor-infiltrating lymphocytes (TILs) — have a more favorable prognosis than "cold tumors" [5, 34]. Lymphocytes are also very sensitive to irradiation and chronic decrease in lymphocyte population is often seen after radiotherapy and chemoradiation.

In this study, the impact of changes of pre- and post-treatment NLR was retrospectively evaluated in a group of patients treated for head and neck cancer with curative intent. The study demonstrated that NLR is a strong predictor of overall survival and cancer related death, supporting previous research [21, 22, 24]. In univariable analysis the increase of NLR at both time points was significant for cancer related death, with post-NLR remaining significant when adjusted for other factors in multivariable analysis. Pretreatment NLR also showed significant impact on the risk of distant metastases, which is in line with previous studies [27, 35]. The involvement of regional lymph nodes was a significant factor together with increased NLR in both OS and DFFS analyses, providing evidence for the suggested complex role of the immune system in pro- and anticancer response.

Results of research of impact of NLR on locoregional failure are conflicting. Concurrent with Bojaxiu, this study did not confirm decrease of time to locoregional failure in patients with higher pre- and post-NLR for the whole group of patients [22]. However, meta-analyses conducted by Yang and Yu [35, 36] suggests shorter time to locoregional failure for those with higher NLR. The reason could be the ethnicity of included patients, as according to Yang higher NLR has a negative impact on time to failure in Asian population with higher rate of nasopharyngeal cancer, whereas such impact was not observed in included studies with non-Asian patients [36].

However, in this study both pre- and post-treatment NLR were significant for locoregional failure in a subgroup of patients diagnosed with oropharyngeal cancer. These findings are in line with results of Ng and Gorphe studies [27, 37] and support the hypothesis that the immune system plays a key role in the etiology of p16 positive cancer [38]. P16 was not included in multivariable analysis in this study due to the relatively low number of patients with a known p16 status. Assessment of the p16 status became a standard in our center in 2018. Active smoking was a significant predictor of OS only

for oropharyngeal cancer, which was also reported by Ng [27]. Active tobacco use has been shown to influence tumor immune microenvironment via suppression of IFN pathways, and decreasing the number of cytotoxic T lymphocytes in patients with head and neck cancer [39].

A novel finding of this study is the negative correlation of probability of complete response with increased pre-NLR level, which in this context likely reflects the different characteristics of tumor microenvironment (TIM). Infiltration of tumor by lymphocytes ("hot tumors") was previously shown to correlate with better prognosis in comparison with "cold" tumors [34], where depletion and inhibition of lymphocyte population by the excess of neutrophils recruited from bone marrow protect tumors from infiltration by NK and T lymphocytes. These findings were also confirmed for oropharyngeal cancer, as well as for osteosarcoma and prostate [11, 12].

The NLR ratio could be potentially used in the clinic as an additional factor in planning treatment strategy for individual patients, including closer surveillance after treatment for high-risk patients. Many studies made an attempt to stratify the patients according to NLR ratio, generating risk groups or nomograms. Indeed, such an approach makes this parameter easier for use in daily practice; however, there is no consensus in available literature regarding valid threshold separating risk groups. Value varies from 3 to 5 and above, depending on the study [16, 17, 23]. Thus, in this study we did not make an attempt to find a threshold value but used the hazard ratio to assess individual patient's risk.

The study has several limitations. First, the dataset was collected retrospectively. However, all of the included patients were treated according to the same therapeutic and supportive care procedures, making this group of patients uniquely homogenous and decreasing the probability of selection bias. The study also failed to demonstrate significant correlation of NLR with outcome in multivariable analysis if adjusted for p16 status due to a high number of missing observations.

An interesting novel finding is the negative impact of high pre- and post-NLR on complete response, both in the entire group and in patients diagnosed with oropharyngeal cancer. These findings support the concept of the role of the immune system and neutrophil subpopulations in the natural history of cancer and treatment response.

Conclusions

Neutrophil-to-Lymphocyte Ratio evaluated before and post chemoradiation of head and neck cancer was a strong predictor of unfavorable treatment outcome for the whole group of patients and, if confirmed in prospective studies, could be used for risk evaluation and clinical decision about treatment as well as for selection of high-risk patients for closer post-treatment surveillance. Further studies are necessary to translate the complex role of immune system components in pro- and anti-tumor activity and exploit it for individualization of the treatment.

Conflict of interests

None declared.

Funding

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