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Neutrophil-to-lymphocyte ratio and overall survival in all sites of head and neck squamous cell carcinoma

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Abstract

Background—Current prognostic criteria are insufficient in predicting outcomes in head and neck cancers, necessitating new, readily available biomarkers

Methods—Pretreatment neutrophil and lymphocyte counts and their ratio (NLR) were retrospectively investigated for correlation with overall survival while controlling for demographic and clinical confounders.

Results—Patients in the highest tertile of neutrophil counts and those in the lowest tertile of lymphocytes experienced shorter survival than the rest of the population. Patients in the highest tertile of the NLR were at a higher risk compared to those in the lowest tertile after multivariate analysis (HR=2.39, p=0.0001). Additionally, NLR was lower in patients with Human Papilloma Virus (HPV) positive tumors compared to HPV negative ones and predicted survival in both tumor types.

Conclusions—Neutrophil and lymphocyte counts are strong biomarkers with opposing prognostic significance and the NLR is a robust predictor of overall survival in oral, pharyngeal and laryngeal squamous cell carcinomas.

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Author Contributions: S.R. collected the data. K.W. performed statistical analysis. S.R., K.W. and Z.L. prepared the manuscript and figures. J.M.W., T.A.D. and A.J.A contributed to writing and discussion. Z.L. supervised the study.

Keywords

Neutrophil; lymphocyte; head; neck; cancer

Introduction

Head and neck cancer affects more than half a million new cases annually, ranking it as the 5th most common cancer worldwide [(1)], and cancers of squamous mucosa constitute the vast majority of cases. Major risk factors for head and neck squamous cell carcinoma (HNSCC) include smoking, alcohol consumption [(2)] and infection with oncogenic strains of HPV [(3)]. Disease stage is the single most predictive prognostic factor, including primary tumor size, lymph node involvement and distant metastasis, in addition to extra-capsular and perineural invasion [(4, 5)]. However, these parameters are not sufficient for identifying patients at highest risk for recurrence, as there is significant heterogeneity in outcome within single stage groups. Additional prognostic information based on readily available markers such as hematological parameters would be of obvious clinical utility.

The relation between cancer and the immune system has been increasingly recognized over the past three decades [(6)]. While immune-surveillance is a strong line of defense by which transformed cells are cleared by cells like lymphocytes and natural killer cells, chronic inflammation is an established risk factor for developing several types of cancer including colon cancer, hepatocellular carcinoma and gastric cancer [(6)]. In addition, the tumor microenvironment is infiltrated by a heterogeneous population of immune cells, each playing a different role in the cross-talk between cancer cells and the host, either favoring or suppressing tumor progression. For example, a subset of myeloid cells which is expanded in cancer patients are myeloid-derived suppressor cells (MDSCs). These are immature myeloid cells of granulocytic or monocytic lineages that are elevated in cancer [(7)]. MDSCs are capable of suppressing anti-tumor T cell activity and promoting tumor angiogenesis [(7)]. In fact, higher numbers of circulating MDSCs is a poor prognostic indicator in esophageal, gastric and pancreatic cancers [(8)]. On the other hand, higher lymphocyte infiltration in the tumor (tumor-infiltrating lymphocytes, TILs) is a good prognostic indicator in HNSCC [(9)]. In turn, cancer cells modify the behavior of neutrophils by inducing the release of cytokines and metalloproteinases, increasing their chemotactic potential and inhibiting apoptosis [(10, 11)], which perpetuates cancer-associated inflammation. This suggests that different subsets of the inflammatory arsenal play opposing roles in shaping cancer behavior.

Importantly, it was recently shown that circulating hematopoietic stem and progenitor cells (HSPCs) in cancer are predominantly myeloid-based. The frequency of circulating hematopoietic precursors with lymphoid potential was significantly reduced in cancer patients, along with preferential expansion of granulocytic progenitor cells [(12)]. In particular, the number of granulocyte-monocyte progenitors (GMP) in circulation correlated with progression-free survival, and their expansion was shown to be induced by cancer-secreted cytokines such as IL-6, GM-CSF and G-CSF [(12)]. In light of the above, it is useful to seek a readily available clinical indicator of the myeloid-lymphoid status of the patients' hematopoietic system, and take advantage of that to predict prognosis. This makes

peripheral blood neutrophil and lymphocyte counts attractive prognostic entities to be utilized as a gauge of the myeloid-lymphoid stoichiometry. Recent work has indeed shown a negative prognostic value of higher neutrophil-to-lymphocyte ratios in multiple sites of head and neck cancer [13-18]. However, these studies had a limited sample size, were non-comprehensive in the anatomic sites studied, and/or evaluated the neutrophil-to-lymphocyte ratio (NLR) without analyzing its individual components. Here, we investigate the prognostic significance of neutrophils and lymphocytes independently, as well as the neutrophil-to-lymphocyte ratio in a large cohort of oral, pharyngeal and laryngeal cancer patients.

Subjects and Methods

Patient inclusion and exclusion criteria

The Cancer Registry at Hollings Cancer Center, Medical University of South Carolina (MUSC) was used to identify all cases of HNSCC (squamous cell cancers of the oral cavity, pharynx and larynx). The study population was comprised of histologically confirmed cases diagnosed between January 1, 2000 and June 30, 2012. After excluding patients diagnosed with a second primary cancer between January 1, 1993 (the earliest date recorded) and June 30, 2013, there were 1376 patients available for the study. Patients without recorded neutrophil or lymphocyte counts (n=832) or abnormally high neutrophil count suggestive of lab error (n=1) were also excluded, resulting in a total of 543 patients available for analyses. The follow-up period ranged between 2 weeks and 156 months, with a median of 64.4 months.

Data collection

The MUSC Institutional Review Board approved all study activities. For each case, we abstracted data on demographic characteristics, clinical and pathological variables at diagnosis, treatment received and outcome using two different data sources: the Hollings Cancer Center (HCC) cancer registry and the MUSC Clinical Data Warehouse (CDW). The registry is part of a state mandated data system that collects cancer incidence on all cases in South Carolina. The CDW is a single, secure and integrated database extracted from the MUSC OACIS Clinical Data Repository, which includes patient demographics, ICD-coded diagnoses, ICD-coded procedures, medications and laboratory test results. These databases were subsequently linked, in a blinded fashion, through an honest broker at the CDW, and entered into a secured study database.

Independent variables obtained from the CDW included socio-demographic characteristics (age at diagnosis, sex and race), pre-treatment hematologic parameters (white blood cell count, neutrophil percent, absolute neutrophil count, lymphocyte percent, absolute lymphocyte count, hemoglobin level) and lifestyle factors including smoking status (never, former and current) and alcohol use (never, former and current). Tumor-related variables obtained from the registry included tumor grade (well-differentiated, moderately differentiated or poorly differentiated/undifferentiated), location of the primary tumor (oral cavity, pharynx, larynx), TNM stage (I, II, III, IV), all first-line therapies (chemotherapy,

surgery, radiation and/or other) and the p16 (HPV) status. p16, assayed by immunohistochemistry, was used as a surrogate marker for HPV.

Data analysis

The main study outcome was overall survival. Survival time was calculated as the time from diagnosis with HNSCC to death from any cause through July 19, 2013. Subjects alive as of this date were censored at the end of follow-up. Kaplan-Meier methods were used to generate median survival time and corresponding 95% confidence intervals for tertiles of each of the hematologic parameters (including NLR). Kaplan-Meier curves were plotted to graphically assess the relationship between hematologic parameters (described above) and survival. The log-rank test was used to assess statistical significance.

We evaluated the association between each of the hematologic parameters and survival by fitting univariate Cox Proportional Hazards (CPH) regression models. We also studied a variable shown to be prognostic of survival in other cancer types: the neutrophil-to-lymphocyte ratio (NLR) which is the ratio of the absolute neutrophil count divided by the absolute lymphocyte count. To control for potential confounding variables, we performed univariate CPH for all other variables in Table 1 (i.e., age, sex, race, smoking status, alcohol use, treatment, tumor grade, tumor site and clinical stage). Multivariable CPH models were then fit for the NLR variable (continuous) and all other variables that were found to have a p-value of < 0.20 in the univariate analysis. Variables were retained in the final CPH model if they were significant at $p<0.05$.

Results

Neutrophil and lymphocyte absolute counts are inversely correlated

It is unknown whether a correlation exists between the neutrophil and lymphocyte counts in head and neck cancer. Both being products of leukopoiesis, it is reasonable to think that both lineages reflect the hematopoietic status of the white blood cell progenitors in the bone marrow, thus, conferring a positive correlation. On the other hand, it is likely that the hematopoietic system assumes a myeloid or lymphoid skewed phenotype, leading to preferentially higher lymphoid or myeloid cells, one at the expense of the other. Interestingly, plotting the absolute lymphocyte and neutrophil counts demonstrates that higher absolute neutrophil numbers correlate with lower lymphocyte counts [r= -0.11, p=0.009, Figure 1].

Neutrophils correlate with shorter overall survival in head and neck squamous cell carcinoma

To determine the relationship between neutrophils and survival, each of neutrophil percent and absolute neutrophil count was analyzed as a continuous variable. In our patient population, an increase of 10^3 neutrophils/μL resulted in 7% increase in the hazard of death (Table 2). In turn, each increase of 1% in the neutrophil percentage conferred an increase of 1.0% in the hazard [95% CI 0-2%, p=0.01]. Upon categorizing the neutrophil numbers into tertiles, patients in the highest tertile were at a significantly higher risk than those in the

lowest and middle tertiles (Table 2, Figure 2). A similar pattern was observed in the multivariate analysis (Table 2).

Lymphocytes positively correlate with overall survival

Lymphocytes were associated with longer overall survival; the risk of death decreased by 19% for every increase of 10^3 lymphocyte/μL (Table 2). Similarly, 1% increase in the lymphocyte percentage decreased the risk by 2% [95% CI 0-3%, p=0.007]. Patients with lymphocyte counts in the middle and upper tertiles had a lower risk of death compared to those in the lowest tertile (Table 2, Figure 3). Results were similar in the multivariable analysis model (Table 2).

Higher neutrophil-to-lymphocyte ratio is a poor prognostic marker in head and neck squamous cell carcinoma

Given the strong and opposing prognostic values observed for neutrophils and lymphocytes, it stands to reason that a higher neutrophil-to-lymphocyte ratio (NLR) would be associated with shorter overall survival. For each 1 unit increase in the NLR, the risk of death increased by 4% (table 2). Patients with an NLR in the highest tertile had a significantly higher risk than those in the middle and lowest tertiles (Table 2, Figure 4). Similar results were observed after multivariate analysis (Table 2).

Neutrophil-to-lymphocyte ratio is lower in p16+ tumors than p16- ones and confers prognostic significance in both tumor types

Analysis of smoking status and alcohol consumption so far demonstrated no correlation between NLR and any of those parameters (Table 1, no statistical significance and a negligible magnitude of correlation). Next, we decided to investigate the correlation between HPV (p16) status and NLR as well as the predictive value of NLR in HPV+ and HPV- tumors. HPV status and NLR data were available for 89 patients (51 HPV- and 38 HPV+ tumors). Interestingly, NLR was significantly lower in HPV (p16) positive patients (NLR=2.73) compared to HPV (p16) negative ones (NLR=4.75, p=0.039, Figure 5A).

Given the strong prognostic value of NLR in the whole HNSCC population, it was further evaluated in both p16 positive and p16 negative patients. Using the continuous model, we found that within p16 negative patients, an increase of the NLR by 1 unit resulted in 7% increase in the risk of death (p=0.01, 95% CI 1.7-12.9%). p16 positive patients showed a similar trend without reaching statistical significance, likely due to the small sample size; the risk of death increased by 54.9% for every 1 unit increase in NLR (p=0.2, 95% CI -22-304%). Log-rank test within HPV (p16)+ patients shows that those in the highest tertile have twice the risk compared to the lower two tertiles (p=0.083, Figure 5B). The results in the HPV+ group did not reach statistical significance, however, likely due to the small sample size.

Discussion

Here we report findings of the largest study to date analyzing the relationship of hematologic markers with survival outcomes in head and neck cancer. The size of the presented study

also is unique in its ability to examine the relationship of hematologic markers to outcomes in several disease sites for a complex cancer in which tumor location is uniquely important. Our study establishes absolute levels of neutrophils and lymphocytes measured in circulation as independent prognostic variables in cancers of the oral cavity, pharynx, and larynx. Furthermore, we observed that the numbers of neutrophils and lymphocytes were inversely correlated and that neutrophil-to-lymphocyte ratio was an indicator of poor prognosis. Based on these findings it is conceivable that the white blood cell differential in HNSCC is preferentially skewed towards either a myeloid or a lymphoid lineage, with the lymphoid preponderance being associated with better disease outcomes. We also observed a slight, but statistically significant, increase in the mean age in the upper 2 tertiles of NLR, which is in agreement with previous large scale reports [19]. Interestingly, more oral cancer cases were observed in the 2nd and 3rd tertiles; whether this is contributed by different flora, concomitant periodontal disease, smoking or other factors remains to be elucidated. Nevertheless, multivariate analysis demonstrated that controlling for both factors (among others) did not affect the prognostic value of NLR.

It is noteworthy that the neutrophil and/or lymphocyte counts could be influenced by comorbid diseases in cancer patients such as chronic inflammatory and autoimmune diseases, along with the intake of medications such as steroids associated with such conditions. In our patient population, 88/543 patients (16.2%) were on steroids. Steroid intake was not associated with lymphocyte counts [Any versus no steroid use: 1.67 versus $1.70 \times 10^3/\mu L$, p=0.74], but consistent with previous reports[20], steroid intake was significantly associated with higher neutrophil counts [Any versus no steroid use: 6.82 versus $5.49 \times 10^3/\mu L$, p=0.0005) and higher NLR [Any versus no steroid use: 5.88 versus 4.33, p=0.008]. Univariate analysis showed that the association between higher NLR and worse overall survival persisted both in patients who received steroids [RR by tertile: 1.0 (lowest), 1.30 (middle), 1.72 (highest), p=0.071] and those who did not [RR by tertile: 1.0 (lowest), 1.27 (middle), 2.04 (highest), p=0.008]. In addition, 145/543 (26.7%) patients received non-steroidal anti-inflammatory drugs (NSAIDs), and similarly, higher NLR was associated with worse survival in both NSAID users and non-users.

Inflammation contributes to cancer initiation and progression. For example, chronic infections are established etiological factors for a number of human cancers: Hepatitis B and C and hepatomas, H. pylori and gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphomas, HPV and oral and cervical cancer and many others [21]. Similarly, chronic inflammation in inflammatory bowel disease and chronic hepatitis increases the risk for colorectal and hepatocellular carcinomas, respectively. The inflammatory microenvironment induces genotoxic stress via multiple mechanisms, including reactive oxygen species and induction of activation-induced cytidine deaminase (AID) [22]. Inflammatory cells also propagate transformed cells by releasing cytokines which turn on pro-tumorigenic signals such as the Wnt and NF-κB pathways [23, 24]. Relevant to HNSCC is the role of inflammation in tobacco-induced carcinogenesis, where tobacco induces chronic inflammation by activating the NF-κB and MAP kinase pathways, thus contributing to its ability to induce lung cancer in mice [25]. All of that highlights the cross-talk between cancer and the inflammatory microenvironment, and its implications on tumor behavior. That being said, it is important to point that the roles of different cellular subsets in dictating

cancer behavior have been extensively evaluated. For example, tumor-associated macrophages promote tumor progression in preclinical models [26-28]. Similarly, regulatory CD4+ T cells contribute to the progression and metastasis in a lymphoma pre-clinical model [29], and predict worse outcomes in ovarian, breast and liver cancers [30-32]. On the other hand, tumor-reactive cytotoxic T cells and subsets of helper T lymphocytes are being investigated for the clearance of established tumors in mice and humans [33-36].

Previous studies have also investigated the significance of NLR in HNSCC, where higher NLR predicted shorter disease-specific and overall survivals [16, 17]. However, these studies were limited in their sample sizes or their anatomic sites, and the results of neutrophils and lymphocytes as independent markers were either not studied or did not reach statistical significance. Here, we included all three sites of HNSCC (oral, pharyngeal and laryngeal) with a considerably larger sample size. In addition, each of neutrophils and lymphocytes were independently investigated and their predictability of survival was statistically significant. Importantly, the interaction between NLR and HPV status is an important finding in this study, although the small number of patients with known HPV status limited the robustness of our analysis. It is also important to determine whether the predictability of the NLR is affected by the HPV status of the tumor. Our analysis demonstrated a bigger magnitude of correlation between NLR and survival within the HPV+ group than that seen in the HPV- group. However, given the relatively small sample sizes, it is warranted that this aspect is further investigated in future studies. In conclusion, this analysis demonstrates the clinical significance of the two major circulating cell types, neutrophils and lymphocytes, their prognostic usefulness in the 3 subsets of head and neck squamous cell carcinoma, and the correlation of NLR with HPV status. Further retrospective and prospective studies on the efficacy of treatment when stratified by NLR category are warranted in order to obtain more refined risk stratification for the potential determination of therapy in cancers of the head and neck.

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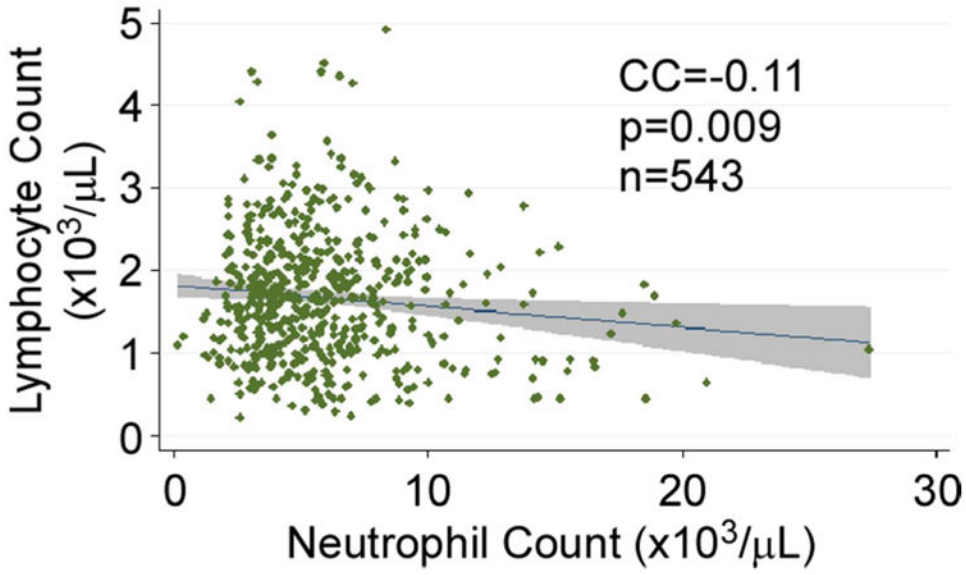

Figure 1. Absolute neutrophil counts are inversely associated with absolute lymphocyte counts

Absolute counts of neutrophils and lymphocytes in the peripheral venous blood of 543 HNSCC patients were plotted and correlation was assessed using linear regression analysis (p=0.009).

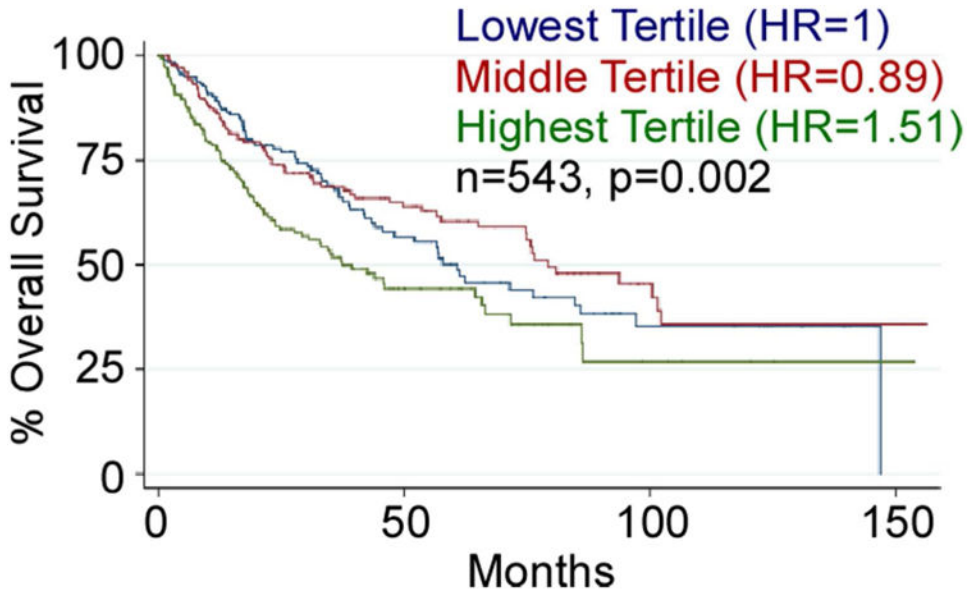

Figure 2. Higher absolute neutrophil count is associated with shorter overall survival

HNSCC patients (n=543) were divided into 3 tertiles based on their absolute neutrophil count, each curve represents one tertile and the p-value is obtained by log-rank test (p=0.002).

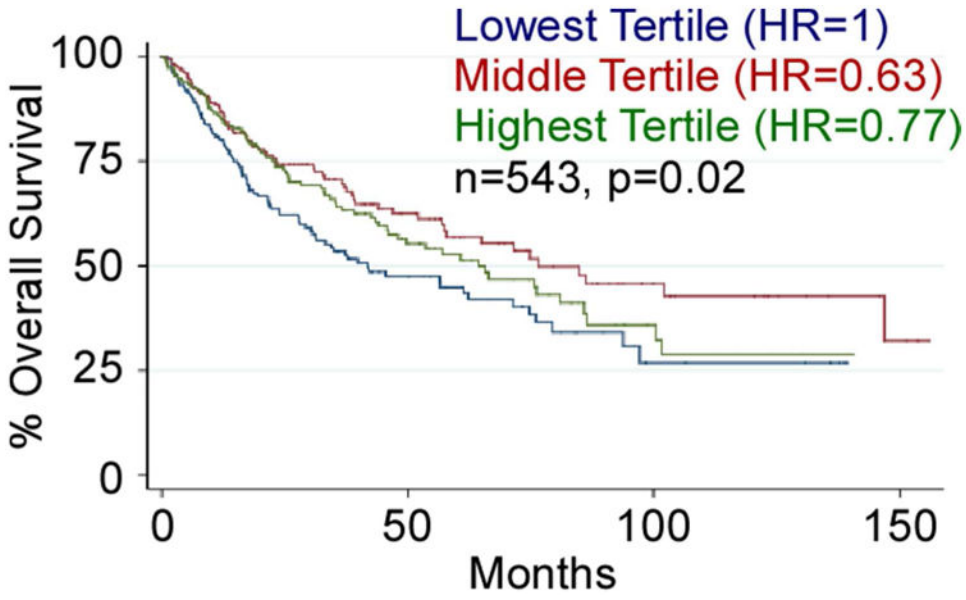

Figure 3. Higher absolute lymphocyte count is associated with longer overall survival

HNSCC patients (n=543) were divided into 3 tertiles based on their absolute lymphocyte count, each curve represents one tertile and the p-value is obtained by log-rank test (p=0.02).

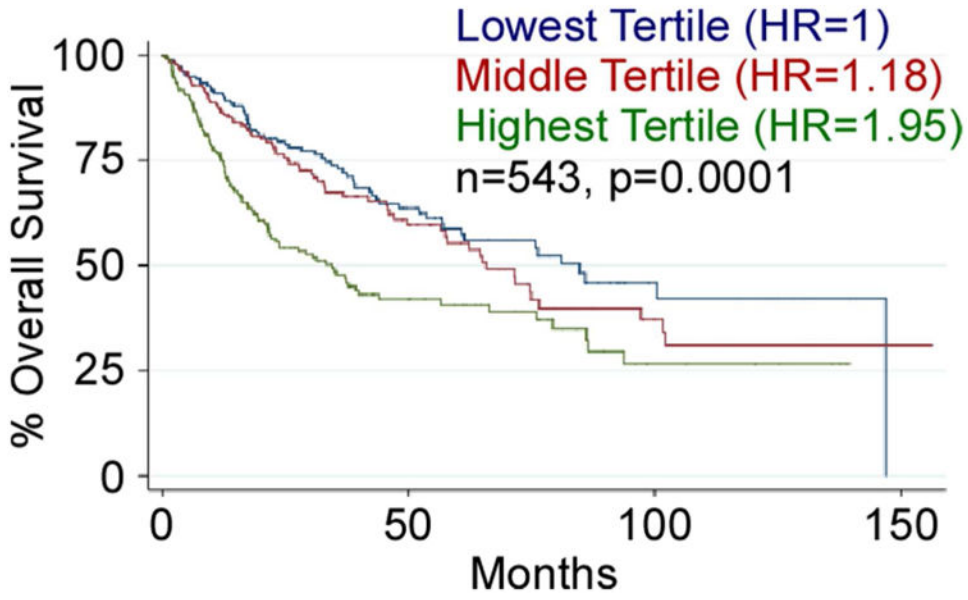

Figure 4. Higher neutrophil-to-lymphocyte ratio (NLR) is associated with lower overall survival

HNSCC patients (n=543) were stratified into lowest, middle and highest tertiles based on their NLR. Each curve represents one tertile and the p-value is obtained by log-rank test (p=0.0001).

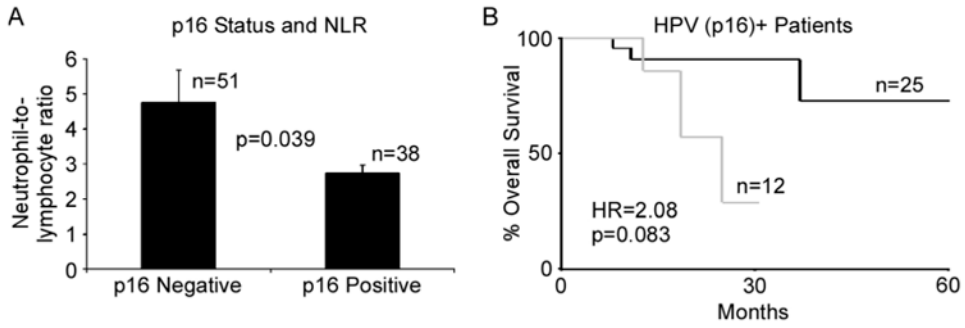

Figure 5. Neutrophil-to-lymphocyte ratio is lower in HPV (p16)+ tumors than HPV (p16)-ones and confers prognostic significance in both tumor types

A. Patients with p16+ cancer have lower NLR than those with p16- ones (p=0.039). B. Patients in the highest tertile have shorter overall survival than those in the other lowest 2 tertiles combined (HR=2.08, p=0.083). HR: Hazard ratio.

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Table 1

Demographic and clinical characteristics within tertiles of neutrophil-to-lymphocyte ratio

Variable	Number of patients	Mean (SD)	Tertile 1 <2.36	Tertile 2 2.36-4.39	Tertile 3 >4.39	p-value
Age—yrs. Mean (SD)	543	58.8 (10.9)	57.0 (9.7)	59.6 (11.9)	59.9 (10.8)	0.02
Sex						
Male—no. (%)	420 (77)		136 (75.1)	136 (75.1)	148 (81.8)	0.22
Race—no. (%)						0.05
Caucasian	405 (74.5)		124 (68.5)	138 (76.2)	143 (79.0)	
African American	132 (24.3)		56 (30.9)	39 (21.6)	37 (20.4)	
Other	6 (1.1)		1 (0.5)	4 (2.2)	1 (0.5)	
Smoker—no. (%)						0.17
Never	83 (16.3)		31 (18.2)	29 (17.3)	23 (13.5)	
Former	175 (34.4)		49 (28.8)	67 (39.9)	59 (34.5)	
Current	251 (49.3)		90 (52.9)	72 (42.9)	89 (52.1)	
Alcohol – no. (%)						0.06
Never	146 (29.3)		52 (31.0)	42 (25.3)	52 (31.5)	
Former	73 (14.6)		18 (10.7)	35 (21.1)	20 (12.1)	
Current	280 (56.1)		98 (58.3)	89 (53.6)	93 (56.4)	
Stage no. (%)						0.40
I	43 (9.5)		15 (10.3)	17 (11.3)	11 (7.1)	
II	42 (9.3)		12 (8.2)	14 (9.3)	16 (10.3)	
III	89 (19.7)		26 (17.8)	24 (15.9)	39 (25.2)	
IV	278 (61.5)		93 (63.7)	96 (63.6)	89 (57.4)	
Grade no. (%)						0.62
Low	301 (77.8)		98 (76.6)	95 (76.0)	108 (80.6)	
High	86 (22.2)		30 (23.4)	30 (24.0)	26 (19.4)	
Primary Site no. (%)						0.02
Oral	251		71 (39.2)	89 (49.2)	91 (50.3)	
Pharynx	170		67 (37.0)	60 (33.2)	43 (23.8)	
Larynx	122		43 (23.8)	32 (23.8)	47 (26.0)	

Variable	Number of patients	Mean (SD)	Tertile 1 <2.36	Tertile 2 2.36-4.39	Tertile 3 >4.39	p-value
Hematological Parameters—Mean (SD)						
NLR	543	4.8 (5.1)	--			
Neutrophils	543	5.9 (3.3)	--			
Lymphocytes	543	1.6 (0.7)	--			
White Blood Count	543	8.1 (3.0)	7.2 (2.3)	8.0 (2.3)	9.2 (3.7)	0.0001
Platelets	543	255.8 (88.2)	243.0 (78.2)	262.5 (82.8)	262.0 (100.9)	0.06
Hemoglobin	543	13.4 (1.8)	13.8 (1.6)	13.5 (1.7)	13.1 (2.0)	0.0004

SD: Standard deviation. NLR: Neutrophil-to-lymphocyte ratio

Table 2

Relative hazards and survival times as a function of absolute neutrophil and lymphocyte counts and neutrophil-to-lymphocyte ratio

Variable	Survival Months, 95% CI N=543	HR, 95% CI (Univariate) N=543	HR[1], 95% CI (Adjusted) N=406
Neutrophil Count			
Lowest Tertile	60.7 (44.0-85.5)	1.0 (Reference)	1.0 (Reference)
Middle Tertile	79.3 (65.1-102.2)	0.89 (0.64-1.24)	1.04 (0.69-1.57)
Highest Tertile	37.3 (27.6- 65.7)	1.51 (1.10-20.6)	1.65 (1.11-2.44)
Continuous[2]		1.07 (1.04-1.11)	1.08 (1.04-1.13)
p-value[3]	0.002	0.0001	0.002
Lymphocyte Count			
Lowest Tertile	41.8 (30.6-71.5)	1.0 (Reference)	1.0 (Reference)
Middle Tertile	76.5 (57.4-NA)	0.63 (0.46-0.87)	0.59 [0.40-0.86]
Highest Tertile	64.4 (45.8-85.8)	0.77 (90.56-1.04)	0.56 [0.40-0.86]
Continuous[2]		0.81 (0.68-0.96)	0.68 (0.56-0.83)
p-value[3]	0.02	0.02	0.02
NLR			
Lowest Tertile	84.8 (56.9-NA)	1.0 (Reference)	1.0 (Reference)
Middle Tertile	65.7 (56.5-97.2)	1.18 (0.84-1.65)	1.13 (0.74-1.73)
Highest Tertile	34.4 (21.5-56.6)	1.95 (1.42-2.67)	2.39 (1.62-3.53)
Continuous[2]		1.04 (1.02-1.06)	1.04 (1.02-1.07)
p-value[3]	0.0001	0.001	0.0001

NLR: Neutrophil-to-lymphocyte ratio. CI: Confidence interval.

[1]HR for tertiles is adjusted for age, race, site, stage, smoking, and treatment (n= 406).

[2]HR for each continuous variable is defined as every $10^3/\mu L$ increase in neutrophils and lymphocytes and 1 unit increase in NLR adjusted for age, race, site, stage, smoking and treatment.

[3]p-values are derived from the log-rank test or from the continuous variable in CPH model.

NA: Not applicable.