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Blood Markers Predicting Clinically Occult Lymph Node Metastasis in Head and Neck Squamous Cell Carcinoma

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Keywords

Head and neck oncology · Head and neck cancer · Cervical nodal metastasis

Abstract

Introduction: The presence of cervical lymph node metastases is an unfavorable prognostic factor in head and neck squamous cell carcinoma (HNSCC) and a potential cause of treatment failure. Occult lymph node metastasis occurs in approximately 15–20% of HNSCC patients with a clinically negative neck (cN0), greatly impacting on their prognosis.

The present study aimed to investigate the role of pre-treatment peripheral blood markers in predicting clinically occult cervical lymph node metastasis. **Methods:** This multicenter, retrospective study was performed in a cohort of 472 patients diagnosed with cN0 HNSCC who underwent up-front surgery. Baseline neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), systemic inflammatory marker (SIM), and systemic immune-inflammation index (SII) were calculated from available blood parameters. **Results:** Oropharyngeal and oral cancers, locally advanced stage, moderately (G2), and poorly (G3) differentiated grade were

associated with an increased risk of pathological lymph node involvement. NLR, LMR, PLR, SIM, and SII were significantly associated at multivariable analysis. NLR >2.12 was the most reliable at predicting occult lymph node metastasis (OR = 5.22; 95% CI: 2.14–12.75). We describe a predictive score integrating cancer site, local stage, and NLR which is effective at predicting positive lymph node pathological status. **Conclusions:** The present study provides evidence that pre-treatment peripheral blood markers, in particular NLR, represent reliable predictors of clinically occult cervical lymph node metastasis in cN0 HNSCC. Therefore, the present study provides a novel useful predictive score for directing the elective management of the neck in patients with cN0 HNSCC.

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Introduction

The presence of cervical lymph node metastasis is a well-known unfavorable prognostic factor in head and neck squamous cell carcinoma (HNSCC), associated with a poor prognosis and an increased risk of regional recurrence and distant metastasis [1, 2]. Clinically occult lymph node metastases (defined as a regional localization undetected by clinical or radiographic examination) occur in approximately 15–20% of cases with a clinically negative neck (cN0), and it is a potential cause of treatment failure [3]. For patients affected by cN0 HNSCC, the management remains controversial: the choice between elective treatment (neck dissection or radiotherapy) or “watchful waiting” [4] is probability-based and depends on primary site location, primary tumor size, and its features [5, 6].

In recent years, the interaction between tumor microenvironment and systemic inflammatory status has been widely investigated, highlighting the association between circulating blood cell counts and clinical features and prognosis in HNSCC [7]. These findings have contributed to the view of cancer as a systemic disease, inducing systemic immune changes in peripheral blood cells to promote progression [8], and provided a rationale for the role of peripheral blood cells in enhancing tumor cell migration, invasive local spread, and regional metastasis and recurrence [9–11]. Moreover, immunological biomarkers for predicting prognostic information were provided, to facilitate clinical decision-making [12]. In spite of all the literature demonstrating the association between lymph node status and inflammatory indices (especially neutrophil-to-lymphocyte ratio [NLR]) [13, 14], only a little evidence has described the association of NLR with pathological lymph node status in a population

clinically N0, in oral and laryngeal cancer only [15–18]. To our knowledge, this focus on clinically occult metastases has never been replicated in the setting of oropharyngeal or hypopharyngeal HNSCC.

The present study aimed to investigate the role of pre-treatment peripheral blood markers in predicting clinically occult cervical lymph node metastasis in a population of patients affected by HPV-negative HNSCC. This evidence would provide a useful instrument for directing the management of the clinically negative neck in patients affected by HNSCC.

Materials and Methods

In accordance with Italian regulation, written informed consent was obtained for participation in this study from all alive patients, while it was waived for those who were already dead. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Treviso and Belluno provinces (Date: March 23, 2020/No. 773/CE Marca).

Data were derived from a cohort of 1,001 consecutive patients diagnosed with HNSCC who underwent up-front surgery on primary tumor and regional lymph nodes from April 2004 to June 2018. The study network included General and University Hospitals in north-eastern Italy, located in Treviso, Padova, Verona, Trieste, Brescia, Pordenone, Ferrara, and Pavia. Inclusion criteria for the present study were (1) HNSCC arising from the oral cavity, oropharynx, hypopharynx, or larynx; (2) neck staged as cN0 after physical and radiological examinations. In detail, contrast enhanced computer tomography and magnetic resonance imaging were performed in all patients, while ultrasound neck examination (with eventual ultrasound-guided fine needle aspiration cytology) and FDG-PET scan were indicated only in doubtful cases, in cases of high risk of distant metastases, or in cases of unknown primary tumors; (3) curative up-front surgery as the primary treatment modality, including elective neck dissection. The indication for elective surgical treatment of the neck was uniformly placed by the operators, taking into account the risk factors for regional metastasis (e.g., primary site, tumor size, histological grade at biopsy). The levels of dissection were decided by the operator according to the location of the primary tumor and its stage.

Patients were specifically excluded if (1) they were diagnosed with nasopharyngeal carcinoma; (2) they had any coexisting conditions that could result in altered inflammatory parameters; (3) they had previous malignancy or additional synchronous primary tumors; (4) their pre-treatment blood test results were not available; (5) they had metastatic disease; or (6) they had HPV-driven SCC. Following exclusions, 472 patients were included in the final analysis.

Participants and Data

Medical records were reviewed to collect the clinical characteristics of enrolled patients. Baseline characteristics, including clinical stage (obtained by clinical and endoscopic evaluation, radiological assessment), histology, and grade were retrieved. For oropharyngeal carcinomas, HPV status was assessed by p16

immunostaining and/or HPV-DNA. Blood parameters were collected at baseline, including platelets ($10^3/\mu\text{L}$), neutrophils ($10^3/\mu\text{L}$), lymphocytes ($10^3/\mu\text{L}$), and monocytes ($10^3/\mu\text{L}$). From these parameters, five inflammatory indices were calculated: NLR, platelet-to-lymphocyte ratio (PLR) [12], lymphocyte-to-monocyte ratio (LMR) [19], systemic inflammatory marker (SIM) [20], and systemic immune-inflammation index (SII) [21].

Statistics

A case-control design was employed, comparing 70 patients with confirmed occult cervical lymph node metastasis (cases) and 402 metastasis-free patients (pN0) who served as controls. The risk of occult metastasis (pN1 + pN2) was evaluated through odds ratio (OR) and corresponding 95% confidence interval (CI), estimated using an unconditional logistic regression model.

Blood markers were dichotomized according to optimal cut-off, estimated through receiver operating curve (ROC) analysis. The association between blood markers and the risk of occult metastasis was estimated with 2 different models: (1) multivariable “Model 1,” including sociodemographic and clinical characteristics significant at the univariate analysis (e.g., gender, age, cancer site, cT, and grading); (2) multivariable “Model 2,” further adjusted for all blood markers.

To develop and validate an integrated prognostic score to predict the risk of occult metastasis, training and validation datasets were drawn representing 70% and 30% of the whole dataset, respectively. Candidate predictors with $p < 0.05$ from univariate analysis (Table 1) were investigated in the training dataset to select independent predictors and to estimate their relative importance [22]. A bootstrap stepwise regression model was performed, in which 500 random samples were generated with replacement from the training dataset. The frequency of occurrence of each predictor was accounted for, and a variable was retained for score development when it occurred in at least 75% of the bootstrap models: for each variable in the final model, β coefficient was estimated as median values among all bootstrap models. β coefficients were then used to assign integer score points of each risk factor according to a linear transformation of the corresponding β coefficient (i.e., divided by the lowest β value among all predictors). The score was calculated by summing up the score points for each predictor, with a range from 0 to 4. Predictive performance was then tested in the validation set. The optimal cut-off for pN2 prediction was assessed through ROC analysis. Sensitivity, specificity, accuracy, and predictive values, with corresponding 95% CIs, were calculated for the optimal cut-off.

Results

Population

A total of 472 patients met the inclusion criteria. The majority of patients were male (74.2%), equally divided into locally advanced cT3–4 (47.9%) or early cT1–2 (52.1%) tumors (Table 1). The primary site of the tumor was the oral cavity in 47.2%, the larynx in 41.7%, and the oro-hypopharynx in 11.0%. Pathological lymph node involvement was more frequent in oral cavity cancer (OR = 3.70; 95% CI: 1.77–7.74) and oro-hypopharyngeal

cancer (OR = 6.99; 95% CI: 2.78–17.56) than in laryngeal cancer. Furthermore, the risk of pathological lymph node involvement was independently associated with advanced T stage (OR = 2.21; 95% CI: 1.10–4.41 for cT3 and OR = 2.62; 95% CI: 1.36–5.06 for cT4 stage) and tumor grade (OR = 5.20; 95% CI: 1.21–22.41 for G2 and OR = 5.92; 95% CI: 1.30–27.03 for G3).

Inflammatory Indices and Occult Metastasis

For each inflammatory blood marker, the optimal cut-off for the prediction of pathological lymph node involvement was estimated through a ROC analysis. All markers reported high sensitivity and negative predictive value, but low specificity and positive predictive value (Table 2). $\text{NLR} \geq 2.12$ reported the highest accuracy (54.3%; 95% CI: 49.6–58.9%).

The association between inflammatory indices and pathological lymph node involvement is reported in Table 3. A statistically significant association emerged for all of the considered indices, though a higher risk was found for $\text{NLR} \geq 2.12$ (OR = 5.22; 95% CI: 2.14–12.75); however, after mutual adjustment (multivariable model 2), only $\text{NLR} \geq 2.12$ remained independently associated to pN1–pN2 (OR = 3.94; 95% CI: 1.33–11.65).

Analysis stratified for cancer site (Table 4) confirmed $\text{NLR} \geq 2.12$ as the strongest predictor of pathological lymph involvement in oral, oropharyngeal, and hypopharyngeal cancers (OR = 8.03; 95% CI: 1.53–42.07). Conversely, no statistically significant associations were found in laryngeal cancer.

Prognostic Score

Variables significantly associated with pathological lymph node involvement in the multivariable model (namely, cancer site, cT, grading, and NLR) were used to develop and integrate a prognostic score in the training dataset. Three predictors were found to be independently associated with pathological lymph node involvement, assigning 1 point for cancer localized in the oral cavity, for cT3 or cT4, and $\text{NLR} \geq 2.12$, and 2 points for cancer localized in the oropharynx or hypopharynx (Table 5). The score was calculated by totaling the points for each predictor, ranging from 0 to 4.

The score's predictive performance was then tested in the validation set (shown in Fig. 1). A sufficient classification accuracy was found (AUC = 0.776), with the optimal cut-off being ≥ 3 . The score reported good specificity (83.8%; 95% CI: 78.7–88.1), but poor sensitivity (58.7%; 95% CI: 43.2–73.0); consequently, the score had a high negative predictive value (91.9%; 95% CI: 87.7–95.1). Patients with score 3 or 4 had a 5-fold higher

Table 1. Odds ratio (OR) and corresponding confidence interval (CI) for pathological lymph node involvement in 472 cN0 patients with head and neck cancer according to sociodemographic and clinical characteristics

	pN1–pN2		pN0		Univariate	Multivariable
	<i>n</i>	(%)	<i>n</i>	(%)	OR (95% CI) ^a	OR (95% CI) ^b
Gender						
Female	19	(14.5)	112	(85.5)	Reference	Reference
Male	51	(15.0)	290	(85.0)	1.04 (0.59–1.83)	1.11 (0.59–2.08)
Age, years						
<65	35	(19.3)	146	(80.7)	Reference	Reference
65–74	21	(13.0)	141	(87.0)	0.62 (0.35–1.12)	0.84 (0.46–1.59)
≥75	14	(10.9)	115	(89.2)	0.51 (0.26–0.99)	0.62 (0.30–1.26)
Smoking habits						
Never	19	(17.1)	92	(82.9)	Reference	Reference
Ever	47	(14.7)	273	(85.3)	0.83 (0.47–1.49)	0.73 (0.36–1.44)
Missing	4	(9.8)	37	(90.2)		
Drinking habits						
Never	31	(13.1)	205	(86.9)	Reference	Reference
Ever	31	(18.5)	137	(81.6)	1.50 (0.87–2.58)	0.98 (0.53–1.82)
Missing	8	(11.8)	60	(88.2)		
Cancer site						
Oral cavity	37	(16.6)	186	(83.4)	2.25 (1.21–4.19)	3.70 (1.77–7.74)
Oro/hypopharynx	17	(32.7)	35	(67.3)	5.50 (2.53–11.90)	6.99 (2.78–17.56)
Larynx	16	(8.1)	181	(91.9)	Reference	Reference
cT ^c						
cT1–cT2	25	(10.2)	221	(89.8)	Reference	Reference
cT3	20	(16.3)	103	(83.7)	1.72 (0.91–3.23)	2.21 (1.10–4.41)
cT4	24	(23.8)	77	(76.2)	2.68 (1.43–4.99)	2.62 (1.36–5.06)
Grade						
G1	2	(3.0)	64	(97.0)	Reference	Reference
G2	44	(16.8)	218	(83.2)	5.40 (1.27–22.89)	5.20 (1.21–22.41)
G3	22	(19.3)	92	(80.7)	6.00 (1.35–26.67)	5.92 (1.30–27.03)
Missing	2	(6.7)	28	(93.3)		

^aEstimated from unconditional logistic regression model. ^bAdjusted for gender, age, cancer site, cT, and grade. ^cDatum not reported for 2 patients.

risk of pathological lymph node involvement than those with score 0, 1, or 2 (OR = 5.17; 95% CI: 1.69–15.83; data not shown).

Discussion

Nowadays, lymph node status is assessed through cross-sectional imaging (contrast enhanced computer tomography or magnetic resonance imaging), which can be integrated with FDG-PET scan or ultrasound assessment with eventual ultrasound-guided fine needle aspiration cytology, when further diagnostic investigation is needed [23]. Despite technological and scientific advancements, 15–20% of patients affected by HNSCC without clinical and radiological evidence of regional

metastasis present pathological lymph node involvement when neck dissection is performed [2]. Thus, investigating further instruments to stratify the risk of regional metastasis is paramount to improve the indication of elective neck treatment in cN0 HNSCC patients.

In the present study, the pathological lymph node status of 472 cN0 patients affected by HPV-negative HNSCC was associated with clinical and inflammatory information. Three factors increased the risk of pathological lymph node involvement, namely, cancer site, clinical T status, and NLR. Using these results, an integrated score was developed, with high specificity and negative predictive value.

According to our findings, the tumor primary site significantly influences the risk of occult lymph node metastasis, with a higher risk for oro-hypopharyngeal and

Table 2. Diagnostic performances of blood markers in predicting pN1–pN2 in cN0 patients

Blood markers	Optimal cut-off ^a	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value
		% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
NLR	≥2.12	91.3 (82.0–96.7)	37.8 (33.1–42.8)	54.3 (49.6–58.9)	20.3 (15.9–25.5)	96.2 (91.9–98.6)
PLR	≥116.1	75.4 (63.5–84.9)	45.4 (40.4–50.4)	50.2 (45.6–54.8)	19.3 (14.7–24.5)	91.4 (76.7–94.9)
LMR	<2.75	55.1 (42.6–67.1)	65.5 (60.6–70.2)	36.0 (31.7–40.6)	27.5 (20.3–35.8)	89.4 (85.3–92.7)
SIM	≥1.19	82.6 (71.6–90.7)	42.9 (37.9–47.9)	51.2 (46.7–55.9)	20.0 (15.5–25.1)	93.4 (88.8–96.6)
SII	≥599	68.1 (55.8–78.8)	58.1 (53.1–63.0)	40.4 (35.9–45.0)	22.0 (16.6–28.1)	91.3 (87.2–94.4)

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; SIM, systemic inflammatory marker; SII, systemic immune-inflammation index. ^aOptimal cut-offs were determined through ROC analysis.

Table 3. Odds ratio (OR) and corresponding confidence interval (CI) for pathological lymph node involvement in 472 cN0 patients with head and neck cancer according to blood markers

Blood markers ^a	pN1–pN2		pN0		Multivariable 1	Multivariable 2
	<i>n</i>	(%)	<i>n</i>	(%)	OR (95% CI) ^b	OR (95% CI) ^c
NLR						
<2.12	6	(3.8)	151	(96.2)	Reference	Reference
≥2.12	63	(20.3)	248	(79.7)	5.22 (2.14–12.75)	3.94 (1.33–11.65)
PLR						
<116.7	17	(8.6)	181	(91.4)	Reference	Reference
≥116.7	52	(19.3)	218	(80.7)	2.22 (1.19–4.17)	1.56 (0.73–3.31)
LMR						
≥2.75	31	(10.6)	262	(89.4)	Reference	Reference
<2.75	38	(21.6)	138	(78.4)	1.94 (1.10–3.42)	1.20 (0.62–2.30)
SIM						
<1.19	12	(6.6)	171	(93.4)	Reference	Reference
≥1.19	57	(20.0)	228	(80.0)	2.80 (1.40–5.58)	1.26 (0.49–3.25)
Systemic immune-inflammation index						
<599	22	(8.7)	232	(91.3)	Reference	Reference
≥599	47	(22.0)	167	(78.0)	2.37 (1.32–4.28)	0.89 (0.39–2.00)

^aOptimal cut-offs were determined through ROC analysis. ^bEstimated from unconditional logistic regression model. ^cFurther adjusted for other blood markers, gender, age, cancer site, cT, and grading.

(to a lesser degree) oral primary sites as opposed to laryngeal cancer. This is possibly due to the inclusion in laryngeal cancers of those arising from the glottis, an anatomical region with poorly represented lymphatic drainage compared to the supraglottic larynx, leading to a reduced risk of lymph node metastasis for the laryngeal cancer group as a whole [24]. This difference in lymphatic drainage pattern is the reason for the primary site (and sub-site) of the tumor along with T-classification being key considerations when evaluating the need for elective

neck treatment [5], and the findings of the current study support these principles.

Our analysis was designed to investigate the association between inflammatory indices and occult pathological lymph node involvement. The inflammatory indices analyzed (NLR, LMR, PLR, SIM, and SII, all of them comprising lymphocyte count) were significantly associated with occult lymph node metastasis in both univariate and multivariable analyses. There are two possible interpretations of these results: first, that tumors with an

Table 4. Odds ratio (OR) and corresponding confidence interval (CI)^a for pathological lymph node involvement in 472 cN0 patients with head and neck cancer according to blood markers, by cancer site

Blood markers	Oral cavity, oropharynx, and hypopharynx			Larynx		
	pN1–pN2	pN0	OR (95% CI)	pN1–pN2	pN0	OR (95% CI)
NLR						
<2.12	2	76	Reference	4	75	Reference
≥2.12	51	143	8.03 (1.53–42.07)	12	105	1.51 (0.27–8.55)
PLR						
<116.1	12	88	Reference	5	93	Reference
≥116.1	41	131	1.53 (0.59–3.98)	11	87	2.11 (0.56–7.98)
LMR						
≥3.10	24	142	Reference	7	120	Reference
<3.10	29	77	1.10 (0.50–2.42)	9	61	2.12 (0.52–8.66)
SIM						
<1.19	7	93	Reference	5	78	Reference
≥1.19	46	126	1.59 (0.49–5.10)	11	102	0.81 (0.14–4.74)
SII						
<599	14	122	Reference	8	110	Reference
≥599	39	97	0.98 (0.36–2.65)	8	70	0.57 (0.12–2.77)

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; SIM, systemic inflammatory marker; SII, Systemic immune-inflammation Index. ^aEstimated from unconditional logistic regression model, adjusting for gender, age, cT, grade, and all other blood markers reported in the table.

Table 5. Score point determination in the training set

	Occurrence frequency	OR (95% CI) ^a	Score points
Cancer site	90.0%		
Oral cavity		2.77 (1.75–6.59)	1
Oro/hypopharynx		4.93 (2.22–13.77)	2
Larynx		Reference	0
NLR	95.9%		
<2.12		Reference	
≥2.12		4.33 (2.10–13.87)	1
cT	79.8%		
cT1–cT2		Reference	0
cT3		2.70 (1.77–5.75)	1
cT4		2.69 (1.72–5.66)	1

^aEstimated from unconditional logistic regression model, adjusting for all variables in the table.

increased tendency for lymph node metastasis are more able to induce systemic peripheral blood changes, resulting in lower lymphocyte and higher platelet, neutrophil, and monocyte counts [25]. This would be in line with what has been evidenced for breast cancer cytokine (IFN γ) signaling dysregulating monocyte and lympho-

cyte counts and second, that lymph node metastasis (even when clinically occult) is itself responsible for creating an immunosuppressive cytokine environment, facilitating lymphatic pathway invasion, and thus influencing peripheral blood counts [26]. An exemplificative role of these molecular interactions has been provided for

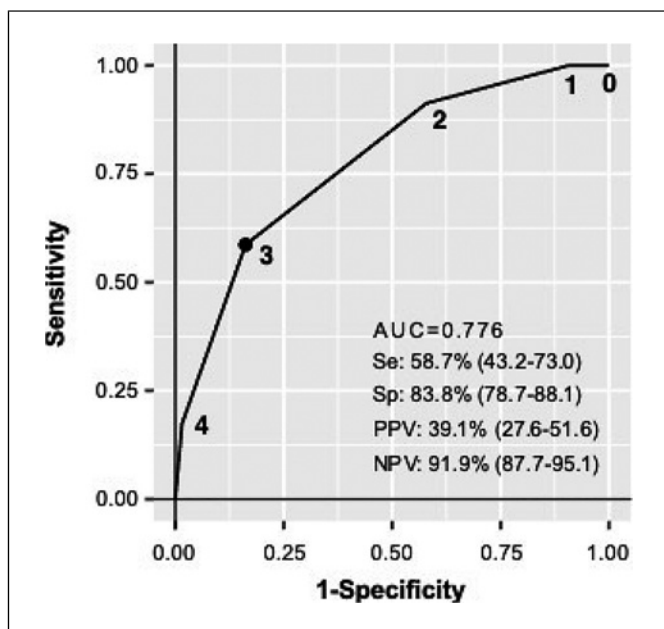


Fig. 1. Receiver operating curve (ROC) for predicting score in the validation set. Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

colorectal cancer, in which patients with lymph node metastasis had a significant increase in CD4+Foxp3+ Tregs compared to patients with no lymph node metastasis, associated with functional impairment of CD8+ T cells [27].

Among inflammatory indices, NLR was the most reliable at predicting occult lymph node metastasis. When stratified according to the primary site, this remained the case for oro-hypopharyngeal and oral cavity cancers, but not for laryngeal cancer. This may be a reflection of the previously discussed differences in lymphatic drainage and propensity for occult lymph node metastasis in laryngeal cancer as opposed to HNSCC from other primary sites. Moreover, corticosteroid therapy is frequently used for the management of respiratory distress associated with laryngeal cancer, and is itself a well-known cause of leukocyte count dysregulation with lymphocyte count reduction, which may result in a degree of bias in the data for laryngeal cancers.

Finally, the present study proposes a predictive score integrating the three pieces of information most significantly associated with clinically occult lymph node metastasis: cancer site, local stage, and NLR. This predictive score is characterized by low sensitivity and positive predictive value, but high specificity and negative predictive value. Therefore, the score is a reliable tool in

the prediction of the absence of occult pathological lymph node involvement when it is ≤ 2 : indeed, the probability of pT1–pT2 is about 8% among patients with score 0, 1, or 2. This score may be a useful tool for the management of cN0 patients with a borderline risk of occult lymph node metastasis (balancing clinical, radiological, instrumental, and pathological information). In these patients, a score ≤ 2 might suggest clinical-instrumental neck monitoring. Furthermore, even when the indication for elective neck treatment has already been met, a low score could lead the surgeon to be more conservative.

Some limitations of the current study must be acknowledged. First, data on the depth of invasion were not available for oral cavity cancers, thus we were unable to stratify according to this important variable. In the case of oropharyngeal cancers, which were under-represented in our study population (11% of the total), we excluded HPV-positive tumors as these made up only 9.7% of all oropharyngeal cancers. This means that our results may not be applicable to patients with HPV-positive oropharyngeal SCC. The retrospective design of this study may have biased the results due to potential selection and information bias. However, selection bias has been limited by the inclusion of all consecutive patients and by the application of strict inclusion criteria. Regarding information bias, blood parameters were collected pre-operatively from medical charts, avoiding the influence of surgery itself on the baseline values. A slight difference in the selection of patients affected by low-stage carcinomas undergoing a neck dissection might have been present. Regarding the definition of cN0 patients, a bias might be represented by the indication of the ultrasound neck examination: in many centers, this examination was indicated only in case of doubt findings in the cross-sectional imaging, but this indication was operator-dependent as in some cases FDG-PET scan might have been chosen instead. Anyway, participating centers were selected to keep the management strategy as homogeneous as possible, limiting this bias. Finally, although the sample size is large in comparison to previous work on the subject, it may be not sufficiently large for the creation and validation of a score, although a bootstrap procedure was implemented to increase the estimates' robustness.

Conclusions

Pre-treatment peripheral blood markers, in particular NLR, represent a useful predictor of clinically occult cervical lymph node metastasis in HNSCC.

Using this and other parameters, we describe a novel useful predictive score for directing the management of the clinically negative neck in patients with cN0 HNSCC.

Statement of Ethics

In accordance with Italian regulation, written informed consent was obtained for participation in this study from all alive patients, while it was waived for those who were already dead. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Treviso and Belluno provinces (Date: March 23, 2020/No. 773/CE Marca).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Piergiorgio Gaudioso: conception and design of the study, acquisition of data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published; Daniele Borsetto: design of the study, interpretation of data, revised the article critically for important intellectual content, and gave final approval of the version to be published. Jerry Polesel, Fabiola Giudici: analysis and interpretation of data, revised the article critically for important intellectual content, and gave final approval of the version to be published; Giancarlo Tirelli, Enzo Emanuelli, Anna Menegaldo, Gabriele Molteni, Piero Nicolai, Michele Tomasoni, Claudia Montenegro, Cesare Piazza, Paolo Bossi, Andrea Ciorba, Pietro Canzi, Vittorio Giacomarra: substantial contributions to the collection of data, revised the article critically for important intellectual content, and gave final approval of the version to be published; Jonathan Fussey: linguistic revision, revised the article critically for important intellectual content, gave final approval of the version to be published; Paolo Boscolo-Rizzo: conception and design of the study, acquisition of data, interpretation of data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

- 1 Ferlito A, Rinaldo A, Devaney KO, MacLennan K, Myers JN, Petruzzelli GJ, et al. Prognostic significance of microscopic and macroscopic extracapsular spread from metastatic tumor in the cervical lymph nodes. *Oral Oncol.* 2002;38(8):747–51.
- 2 de Bondt RBJ, Nelemans PJ, Hofman PAM, Casselman JW, Kremer B, van Engelshoven JMA, et al. Detection of lymph node metastases in head and neck cancer: a meta-analysis comparing US, USgFNAC, CT and MR imaging. *Eur J Radiol.* 2007;64(2):266–72.
- 3 Ferlito A, Shaha AR, Rinaldo A. The incidence of lymph node micrometastases in patients pathologically staged N0 in cancer of oral cavity and oropharynx. *Oral Oncol.* 2002;38(1):3–5.
- 4 Wei WI, Ferlito A, Rinaldo A, Gourin CG, Lowry J, Ho WK, et al. Management of the N0 neck: reference or preference. *Oral Oncol.* 2006;42(2):115–22.
- 5 Sanabria A, Shah JP, Medina JE, Olsen KD, Robbins KT, Silver CE, et al. Incidence of occult lymph node metastasis in primary larynx squamous cell carcinoma, by subsite, t classification and neck level: a systematic review. *Cancers.* 2020;12(4):1059.
- 6 van den Brekel MWM, Castelijns JA, Stel HV, Golding RP, Meyer CJL, Snow GB. Modern imaging techniques and ultrasound-guided aspiration cytology for the assessment of neck node metastases: a prospective comparative study. *Eur Arch Oto-Rhino-Laryngol.* 1993;250(1):11–7.
- 7 Mascarella MA, Mannard E, Silva SD, Zeitouni A. Neutrophil-to-lymphocyte ratio in head and neck cancer prognosis: a systematic review and meta-analysis. *Head Neck.* 2018;40(5):1091–100.
- 8 Wang L, Simons DL, Lu X, Tu TY, Avalos C, Chang AY, et al. Breast cancer induces systemic immune changes on cytokine signaling in peripheral blood monocytes and lymphocytes. *EBioMedicine.* 2020;52:102631.
- 9 Liang W, Ferrara N. The complex role of Neutrophils in tumor angiogenesis and metastasis. *Cancer Immunol Res.* 2016;4(2):83–91.
- 10 Chen L, Zhang F, Sheng XG, Zhang SQ, Chen YT, Liu BW. Peripheral platelet/lymphocyte ratio predicts lymph node metastasis and acts as a superior prognostic factor for cervical cancer when combined with neutrophil: lymphocyte. *Medicine.* 2016;95(32):e4381.
- 11 Chen MF, Tsai MS, Chen WC, Chen PT. Predictive value of the pretreatment neutrophil-to-lymphocyte ratio in head and neck squamous cell carcinoma. *J Clin Med.* 2018;7(10):294.
- 12 Szilasi Z, Jóna V, Zrubka Z, Mezei T, Vass T, Merkel K, et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as prognostic markers of survival in patients with head and neck tumours – results of a retrospective multicentric study. *Int J Environ Res Public Health.* 2020;17(5):1742.
- 13 Yang L, Huang Y, Zhou L, Dai Y, Hu G. High pretreatment neutrophil-to-lymphocyte ratio as a predictor of poor survival prognosis in head and neck squamous cell carcinoma: systematic review and meta-analysis. *Head Neck.* 2019;41(5):1525–35.
- 14 De Felice F, Tombolini M, Abate G, Salerno F, Bulzonetti N, Tombolini V, et al. Prognostic significance of the neutrophil/lymphocyte ratio in patients with non-human papilloma virus-related oropharyngeal cancer: a retrospective cohort study. *Oncology.* 2019;96(1):8–13.
- 15 Abbate V, Dell'Aversana Orabona G, Salzano G, Bonavolontà P, Maglitti F, Romano A, et al. Pre-treatment Neutrophil-to-Lymphocyte Ratio as a predictor for occult cervical metastasis in early stage (T1-T2 cN0) squamous cell carcinoma of the oral tongue. *Surg Oncol.* 2018;27(3):503–7.

- 16 Li Y, Liu K, Ke Y, Zeng Y, Chen M, Li W, et al. Risk factors analysis of pathologically confirmed cervical lymph nodes metastasis in oral squamous cell carcinoma patients with clinically negative cervical lymph node: results from a cancer center of central China. *J Cancer*. 2019;10(13):3062–9.
- 17 Salzano G, Perri F, Maglito F, Togo G, De Fazio GR, Apolito M, et al. Pre-treatment neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as predictors of occult cervical metastasis in clinically negative neck supraglottic and glottic cancer. *J Pers Med*. 2021;11(12):1252.
- 18 Ventura E, Barros J, Salgado I, Millán A, Vilares M, Zagalo C, et al. Pretreatment blood markers in the prediction of occult neck metastasis: a 10-year retrospective study. *Cureus*. 2021;13(7):e16641.
- 19 Kano S, Homma A, Hatakeyama H, Mizumachi T, Sakashita T, Kakizaki T, et al. Pretreatment lymphocyte-to-monocyte ratio as an independent prognostic factor for head and neck cancer. *Head Neck*. 2017;39(2):247–53.
- 20 Zhou S, Yuan H, Wang J, Hu X, Liu F, Zhang Y, et al. Prognostic value of systemic inflammatory marker in patients with head and neck squamous cell carcinoma undergoing surgical resection. *Future Oncol*. 2020;16(10):559–71.
- 21 Diao P, Wu Y, Li J, Zhang W, Huang R, Zhou C, et al. Preoperative systemic immune-inflammation index predicts prognosis of patients with oral squamous cell carcinoma after curative resection. *J Transl Med*. 2018;16(1):365.
- 22 Hong W, Lillemoe KD, Pan S, Zimmer V, Kontopantelis E, Stock S, et al. Development and validation of a risk prediction score for severe acute pancreatitis. *J Transl Med*. 2019;17(1):146.
- 23 Bhattacharya K, Mahajan A, Vaish R, Rane S, Shukla S, D'Cruz AK. Imaging of neck nodes in head and neck cancers – a comprehensive update. *Clin Oncol*. 2023;35(7):429–45.
- 24 Welsh LW, Welsh JJ, Rizzo TA. Laryngeal spaces and lymphatics: current anatomic concepts. *Ann Otol Rhinol Laryngol Suppl*. 1983;105:19–31.
- 25 Valero C, Pardo L, López M, García J, Camacho M, Quer M, et al. Pretreatment count of peripheral neutrophils, monocytes, and lymphocytes as independent prognostic factor in patients with head and neck cancer. *Head Neck*. 2017;39(2):219–26.
- 26 Pereira ER, Jones D, Jung K, Padera TP. The lymph node microenvironment and its role in the progression of metastatic cancer. *Semin Cell Dev Biol*. 2015;38:98–105.
- 27 Deng L, Zhang H, Luan Y, Zhang J, Xing Q, Dong S, et al. Accumulation of Foxp3+ T regulatory cells in draining lymph nodes correlates with disease progression and immune suppression in colorectal cancer patients. *Clin Cancer Res*. 2010;16(16):4105–12.