

Prognostic value of changes in pre- and postoperative inflammatory blood markers in HPV-negative head and neck squamous cell carcinomas

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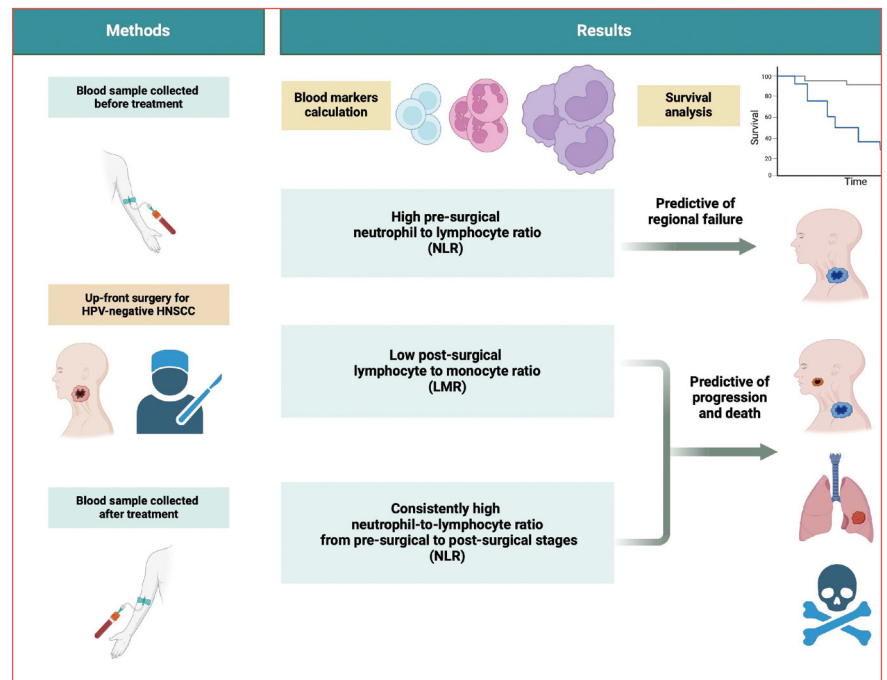
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Cover figure. Role of pre- and postoperative inflammatory blood markers in the prognosis of patients with HPV-negative head and neck cancer treated with surgery.

Summary

Objective. Neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) are inflammatory markers easily obtained from a routine complete blood count, and their preoperative values have recently been correlated with oncological outcomes in patients with HPV-negative head and neck squamous cell carcinoma (HNSCC). The aim of this study is to evaluate the prognostic value of NLR and LMR before and after treatment in patients with HPV-negative HNSCC undergoing up-front surgical treatment. **Methods.** This multicentric retrospective study was performed on a consecutive cohort of patients treated by upfront surgery for HPV-negative HNSCC between April 2004 and June 2018. Only patients whose pre- and postoperative NLR and LMR were available were included. Their association with local, regional and distant failure, progression-free survival (PFS) and overall survival (OS) was calculated.

Results. A total of 493 patients (mean age 68 years) were enrolled. The mean follow-up time was 54 months. Pre-surgical NLR ≥ 3.76 was associated with a high risk of regional failure (HR = 2.21, 95% CI: 1.08-5.55), disease progression (HR = 1.55, 95% CI: 1.07-2.25) and death (HR = 1.40, 95% CI: 0.94-2.10). A post-surgical LMR < 2.92 had a significant impact on disease progression (HR = 1.92, 95% CI: 1.13-3.28) and OS (HR = 2.98, 95% CI: 1.53-5.81). Patients with stable NLR ≥ 3.76 in the pre- and postoperative period had worse OS and PFS.

Conclusions. Our results support that pre- and postoperative NLR and LMR can be useful in identifying patients at risk of local, regional, or distant recurrence who may require closer follow-up or more aggressive treatment.

Key words: head and neck cancer, blood markers, inflammatory system, overall survival, NLR, LMR

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Introduction

Head and neck squamous cell carcinomas (HNSCC) is the sixth most prevalent cancer. It affects around 890,000 people annually and caused approximately 450,000 deaths in 2018 ¹. In a 10-year analysis, the overall survival (OS) rate differed significantly by site. The most favourable prognosis was observed in p16+ oropharyngeal tumours with an OS rate of 87%, followed by tumours in the oral cavity at 69%, larynx at 67%, p16- oropharyngeal tumours at 56%, and hypopharynx at 51%². Treatment options for SCC include surgery, radiation therapy, chemotherapy, or a combination of these. The type of treatment is largely determined by the traits of the presenting tumour, namely TNM stage, histological type, grade, and location, which in turn determine prognosis ^{3,4}. Improved risk stratification is needed, considering the poor survival rates, to identify patients who have a higher risk of recurrence and to accordingly tailor treatment and surveillance ⁵. The German pathologist Rudolf Virchow was the first to put forward an association between cancer and inflammation in 1863, as he observed that tumours were always accompanied by inflammatory cells, but his theory did not gain much attention until the century that followed ^{6,7}. Recent studies have confirmed the importance of the systemic inflammatory response in tumour cell invasion by promoting tumour cell proliferation, microvascular regeneration, and tumour metastasis ^{8,9}. The tumour immune microenvironment (TIM) is the immune infiltrate that emerges during tumour growth and several reports have verified its major role in cancer progression ¹⁰. The TIM includes various tumour-infiltrating lymphocytes (TILs) that have a large effect on cancer invasion and metastasis. A new pro-tumour ability of circulating neutrophils has been discovered recently, wherein they are able to trap circulating tumour cells (CTCs) and promote metastasis by facilitating

their extravasation. These neutrophils are referred to as tumour-associated neutrophils (TANs) and are also known for their ability to infiltrate cancerous tissue. TANs play a crucial role in promoting tumour progression through various mechanisms, such as proliferation, angiogenesis, and interactions with other immune cells ¹¹. Both tumour cells and the tumour microenvironment promote a systemic inflammatory response that modifies the circulating counts of lymphocytes, neutrophils, monocytes and platelets; such values may be associated with cancer prognosis when taken individually or as ratios ^{12,13}. In patients with HNSCC, decreased levels of CD3⁺, CD4⁺, and CD8⁺ cells are observed in peripheral blood, and CD4⁺ levels are found to be correlated with disease stage ¹⁴. Surgical trauma can cause significant changes in the body, leading to metabolic, haemodynamic, and immune alterations during the postoperative period ¹⁵. The initial systemic inflammatory response is mediated by the innate immune system, while the anti-inflammatory response becomes more pronounced as the injury site begins to heal, primarily mediated by the adaptive immune system. After major surgery, both innate and cell-mediated immunity functions are significantly impaired. Surgery to treat HNSCC leads to multiple changes in the patient's blood profile, including anaemia, leukocytosis, lymphopenia, thrombocytosis, increased neutrophils, C-reactive protein, erythrocyte sedimentation rate, and decreased albumin levels. It has been observed that there is a decrease in the levels of various subpopulations of lymphocytes, including B lymphocytes, T helper cells, NK cells, and naïve T helper cells ¹⁶. The neutrophil-to-lymphocyte ratio (NLR) is a recent marker of host inflammation, which resembles the relation between circulating neutrophil and lymphocyte counts⁴ as well as lymphocyte-to-monocyte ratio (LMR) ^{17,18} that can be easily calculated from a routine complete blood count (CBC) ¹⁹. These inflammatory markers have been reported

to be prognostic indicators of survival and to be associated with clinical outcomes in patients with various types of cancer, including ovarian cancer ²⁰, colorectal cancer ²¹, and head and neck cancer ^{22,23}.

We have previously reported that pre-treatment NLR and LMR correlate with oncological outcomes in patients with HPV-negative head and neck cancer ²⁴. These markers may change after treatment, and it is interesting to evaluate how the trajectories of such markers impact cancer outcomes. The aim of this study was therefore to investigate the prognostic value of pre- and post-treatment inflammatory NLR and LMR in patients receiving upfront surgery for HPV-negative HNSCC, with a focus on their trajectories.

Materials and methods

This is a retrospective multicentric study, whose design was described elsewhere ²⁵. Briefly, the cohort enrolled 1001 consecutive patients diagnosed with primary HNSCC who underwent upfront surgery from April 2004 to June 2018. The study network included General and University Hospitals in Northern Italy, located in Brescia, Ferrara, Padua, Pavia, Pordenone, Treviso, Trieste, and Verona.

For the present analysis, the inclusion criteria were: (a) HNSCC arising from the oral cavity, oropharynx, hypopharynx, or larynx; (b) curative upfront surgery as the primary treatment modality; (c) availability of preoperative neutrophils, lymphocytes, monocytes, and platelet count, i.e., the blood parameters necessary for the calculation of the inflammatory blood markers under investigation; (d) availability of the same blood parameters evaluated 3-6 months after the completion of the treatment.

Patients were specifically excluded if: (a) diagnosed with nasopharyngeal carcinoma or T1 glottic SCC; (b) had any coexisting conditions or haematological conditions that could alter inflammatory parameters; (c) had a previous malignancy or additional synchronous primary tumours; (d) pre- or post-treatment blood test results were not available; (e) had metastatic disease; and (f) had HPV-positive disease.

Participants and data

Socio-demographic and clinical data were retrieved from medical records, including gender, age, smoking habits, drinking habits, cancer site, clinical and pathological TNM staging (7th edition), grading, surgical margins and extranodal extension were retrieved. For oropharyngeal carcinomas, HPV status was assessed by p16 immunostaining and/or HPV-PCR. Haemoglobin (Hb, g/L), platelets ($10^3/\mu\text{L}$), neutrophils ($10^3/\mu\text{L}$), lymphocytes ($10^3/\mu\text{L}$), and monocytes

($10^3/\mu\text{L}$) were collected at baseline before surgical treatment and at 3-6 months after the end of treatment.

Patients were routinely followed-up according to consensus guidelines with endoscopic examination of the upper aero-digestive tract every 1-3 months for the first year, 3-4 months during the second year, 4-6 months during the third year, and every 6 months thereafter. A chest computed tomography scan was performed annually in patients with a history of smoking ≥ 20 pack/years. Additional dedicated head and neck imaging was acquired based on clinical features and local protocol. No patient was lost to follow-up.

Inflammatory blood markers

Using pre- and post-treatment blood parameters, we investigated two indexes: 1) NLR, calculated as $\text{NLR} = \text{neutrophils/lymphocytes}$; 2) LMR, calculated as $\text{LMR} = \text{lymphocytes/monocytes}$. A comparison between pre- and postoperative values was also performed.

Statistical methods

Blood parameters were reported as median values and interquartile range (IQR); changes between pre- and postoperative values were evaluated through the Wilcoxon signed-rank test. Correlation between pre- and postoperative indices was evaluated with Spearman's correlation coefficient (r).

The following outcomes were assessed: a) OS defined as death from any cause; b) progression-free survival (PFS) defined as the time from surgery to any type of recurrence/progression or death from any cause; c) local failure as expression of local recurrence; d) regional failure as expression of regional recurrence; e) distant failure as expression of distant metastases. Time at risk was calculated from the date of surgery to the date of the event, date of death, or last follow-up. Survival probabilities were calculated according to Kaplan-Meier method; differences across strata were evaluated through the log-rank test. To account for competing risks, local, regional, and distant failures were evaluated through cumulative incidence, and differences according to blood parameters were tested using Gray's test. Hazard ratios (HR) and the corresponding 95% confidence intervals (CI) were calculated using Cox proportional hazards models, adjusting for study centre, gender, and age, plus clinically relevant covariates (i.e., pT, pN, surgical margins, extranodal extension, adjuvant [chemo]radiotherapy). For local, regional, and distant recurrence, risk estimates were adjusted for competing risk, according to the Fine-Gray model. Blood parameters and inflammatory indexes were categorised in 3 levels, according to cut-offs previously re-

Table I. Distribution of 495 patients according to socio-demographic and clinical characteristics.

	N	(%)
Gender		
Female	127	(25.8)
Male	366	(74.2)
Age (years)		
< 60	113	(22.9)
60-69	160	(32.5)
70-79	152	(30.8)
≥ 80	68	(13.8)
Smoking habits		
Never	81	(16.4)
Ever	372	(75.5)
Missing	40	(8.1)
Drinking habits		
Never	261	(52.9)
Ever	164	(33.3)
Missing	68	(13.8)
Cancer site		
Oral cavity	221	(44.9)
Oropharynx	59	(12)
Hypopharynx	26	(5.3)
Larynx	187	(37.9)
pT		
pT1	51	(10.3)
pT2	174	(35.3)
pT3	131	(26.6)
pT4	131	(26.6)
Missing	6	(1.2)
pN		
pN0	290	(58.8)
pN1	64	(13)
pN2-pN3	134	(27.2)
Missing	5	(1)
TNM stage		
I-II	148	(30)
III	122	(24.8)
IV	217	(44)
Missing	6	(1.2)
Grading		
G1	57	(11.6)
G2	277	(56.2)
G3	142	(28.8)
Missing	17	(3.5)
Surgical margins		

Negative	353	(71.6)
Close/positive	128	(26)
Missing	12	(2.4)
Extracapsular extension		
Absent	423	(85.8)
Present	60	(12.2)
Missing	10	(2)

ported ²⁴. Statistical analysis was performed using SAS 9.4 and R software 4.0.2 (The R foundation for statistical computing, Vienna, Austria).

Results

A total of 493 patients were included (median age, 68 years; interquartile range [IQR], 60-75 years). Demographic and clinical characteristics of the cohort are shown in Table I. Most patients were males (n = 366, 74.2%), with stage III-IV cancer (n = 339, 68.8%) and with moderately differentiated SCC (n = 277, 56.2%). Negative surgical margins were achieved in 353 patients (71.6%) and extracapsular extension was absent in 423 patients (85.8%). During a median follow-up of 54 months (IQR, 34-83 months), 187 patients died. Seventy patients had a local recurrence, while 68 experienced a regional recurrence, and 60 distant metastases. Baseline blood samples were obtained at a median of 20 days before surgery (IQR, 11-33 days), whereas postoperative blood samples were taken at a median of 152 days after surgery (IQR, 92-219 days).

Figure 1 shows changes in pre- and postoperative blood parameters. After surgery, a significant increase in NLR and a decrease in LMR was observed. Although significant, the variation in monocyte value is clinically negligible (median variation, $-0.02 \times 10^3/\mu\text{L}$), so that the decrease in LMR is mainly due to the decrease in lymphocytes (median variation, $-0.40 \times 10^3/\mu\text{L}$). Pre- and postoperative indices were moderately correlated, being $r = 0.32$ for NLR and $r = 0.36$ for LMR.

Table II shows the correlation between inflammatory index and patient outcomes. Preoperative NLR and LMR were confirmed to be prognostic markers, with similar HRs than in the wider study population ²⁴. Specifically, $\text{NLR} \geq 3.76$ was associated with high risk of regional failure (HR = 2.21, 95% CI: 1.08-5.55), disease progression (HR = 1.55, 95% CI: 1.07-2.25), and death (HR = 1.40, 95% CI: 0.94-2.10). No significant associations emerged for preoperative LMR, though the risk magnitude was similar to that reported previously ²⁴. A postoperative $\text{LMR} < 2.92$ had a significant

impact on disease progression (HR = 1.92, 95% CI: 1.13-3.28) and OS (HR = 2.98, 95% CI: 1.53-5.81). The same inflammatory indices with blood markers evaluated between one and 6 months after surgery are summarised in Supplementary Table I.

Patients with stable NLR ≥ 3.76 in the pre- and postoperative period had worse OS (10-year OS, 36.7%) and PFS (10-year PFS, 27.3%; Figure 2), which was much lower than those in patients with stable NLR < 3.76 (10-year OS, 53.3% and 10-year PFS, 48.1%). After adjusting for potential confounders, the HR for death and progression associated with stable NLR ≥ 3.76 were 1.87 (95% CI, 1.17-2.99) and 2.06 (95% CI, 1.35-3.14), respectively (Tab. III). Patients who had increased NLR level after surgery showed a risk of death or recurrence similar to that reported by patients with stable NLR < 3.76 . Similar trends were found for a persistent LMR < 2.92 (Fig. 2, Tab. III). Interestingly, patients with a decline in LMR after surgery had an increased risk of death (HR, 1.73; 95% CI, 1.14-2.63).

Discussion

The results of the present study indicate that preoperative high NLR and low LMR are negative prognostic factors in patients who underwent up-front surgery for HPV-negative HNSCC. Interestingly, the risk of death and progression increases when NLR and LMR levels persisted after surgery.

Lately there is great interest in easily obtained inflammatory biomarkers that have the ability to predict the prognosis in patients with cancer^{26,27}. Currently, high-risk HPV is considered a critical prognostic factor in HNSCC, and has been included in the newest TNM stage of oropharyngeal cancers. Reliable biomarkers for prognostic stratification are lacking in staging of non-HPV-related cancers. To address these issues and to analyse a more homogeneous population, in our study we selected only HPV-negative SCC. Inflammatory cells and their mediators are a prognostic tool as evidence of the immunity of the host response to cancer progression⁸. The tumour microenvironment (TME) is characterised by an important inflammatory component that can induce inflammatory reactions through different mechanisms²⁸. Furthermore, there is a significant dialogue between the mediators and cytokines in the local TME and in the peripheral circulating compartment²⁹. Macrophages, the predominant cell type in chronic inflammation, produce reactive oxygen and nitrogen species to combat infections. However, their persistent presence in the microenvironment can lead

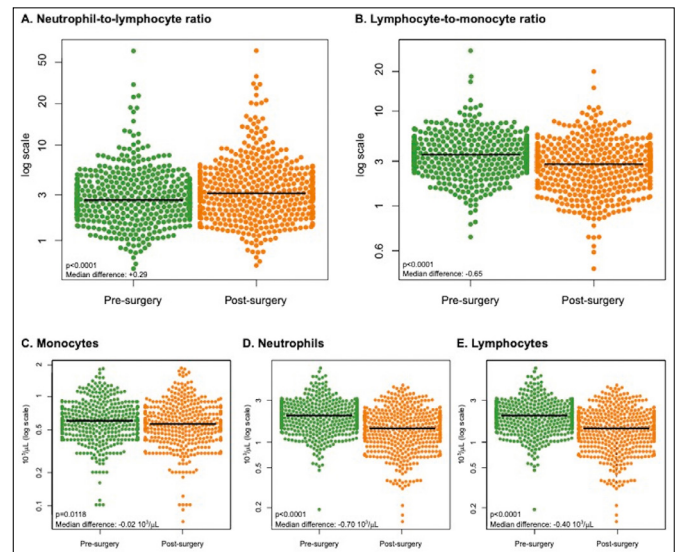


Figure 1. Pre- and post-treatment neutrophil-to-lymphocyte ratio (A), lymphocyte-to-monocyte ratio (B), and selected blood markers (C-D-E).

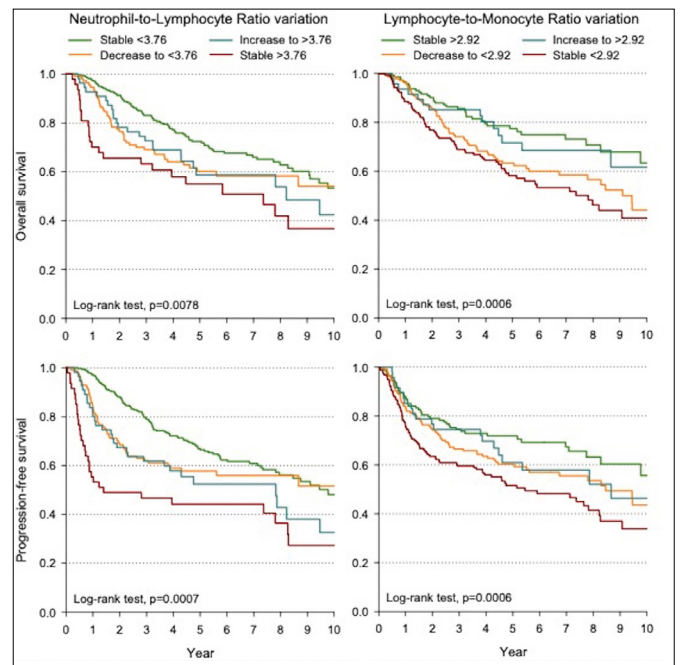


Figure 2. Kaplan-Meier estimates of overall survival and progression-free survival according to pre- and post-treatment variations in neutrophil-to-lymphocyte ratio and lymphocyte-to-monocyte ratio.

to harmful effects such as DNA mutations that promote cancer. Chemicals like TNF- α and macrophage migration inhibitory factor, released by macrophages and T lymphocytes, can exacerbate DNA damage and hinder pro-

Table II. Hazard ratio (HR) and corresponding 95% confidence interval (CI)^a for local failure, regional failure, progression, and death according to the pre- and postoperative blood markers.

	Pts	Local failure		Regional failure		Progression/death		Death	
		Events	HR (95% CI) ^b	Events	HR (95% CI) ^b	Events	HR (95% CI)	Events	HR (95% CI)
Preoperative									
Neutrophil-to-Lymphocyte Ratio									
< 2.10	178	27	Ref	15	Ref	65	Ref	57	Ref
2.10 to < 3.76	213	26	0.63 (0.36-1.11)	35	1.80 (0.99-3.30)	94	0.94 (0.67-1.31)	81	0.92 (0.65-1.32)
≥ 3.76	102	17	0.88 (0.44-1.77)	18	2.21 (1.08-5.55)	60	1.55 (1.07-2.25)	49	1.40 (0.94-2.10)
Lymphocyte-to-Monocyte Ratio									
≥ 4.28	101	9	Ref	9	Ref	33	Ref	28	Ref
2.92 to < 4.28	188	29	1.64 (0.75-3.59)	27	1.36 (0.63-2.94)	81	1.21 (0.79-1.84)	72	1.17 (0.74-1.84)
< 2.92	204	32	1.95 (0.91-4.17)	32	1.55 (0.73-3.27)	105	1.46 (0.96-2.21)	87	1.27 (0.81-2.00)
Postoperative									
Neutrophil-to-Lymphocyte Ratio									
< 2.10	154	22	Ref	22	Ref	59	Ref	47	Ref
2.10 to < 3.76	180	19	0.51 (0.26-0.98)	25	0.76 (0.40-1.46)	82	0.97 (0.68-1.37)	72	1.13 (0.77-1.65)
≥ 3.76	159	29	0.82 (0.45-1.51)	21	0.64 (0.32-1.28)	78	1.19 (0.83-1.71)	68	1.44 (0.97-2.13)
Lymphocyte-to-Monocyte Ratio									
≥ 4.28	67	7	Ref	5	Ref	16	Ref	10	Ref
2.92 to < 4.28	125	15	0.83 (0.33-2.10)	22	1.71 (0.58-5.03)	51	1.61 (0.90-2.89)	41	2.18 (1.6-4.49)
< 2.92	301	48	1.03 (0.44-2.41)	43	1.40 (0.50-3.90)	152	1.92 (1.13-3.28)	136	2.98 (1.53-5.81)

^aEstimated from Cox proportional hazard model, adjusted for gender, age, cancer site, pT, pN, surgical margins and extracapsular extension; ^bAdjusted for competing risk according to the Fine-Gray model.

Table III. Hazard ratio (HR) and corresponding 95% confidence interval (CI)^a for local failure, regional failure, progression, and death according to blood marker variations.

Variation from pre- to postoperative	Pts	Local failure		Regional failure		Progression/Death		Death	
		Events	HR (95% CI) ^b	Events	HR (95% CI) ^b	Events	HR (95% CI)	Events	HR (95% CI)
Neutrophil-to-Lymphocyte Ratio									
Stable < 3.76	279	33	Ref	38	Ref	111	Ref	94	Ref
< 3.76 to ≥ 3.76	112	20	1.17 (0.63-2.15)	12	0.59 (0.30-1.19)	48	1.03 (0.72-1.47)	44	1.24 (0.84-1.81)
≥ 3.76 to < 3.76	55	8	1.05 (0.48-2.33)	9	1.29 (0.60-2.80)	30	1.36 (0.90-2.06)	25	1.36 (0.86-2.14)
Stable ≥ 3.76	47	9	1.43 (0.65-3.12)	9	1.30 (0.59-2.89)	30	2.06 (1.35-3.14)	24	1.87 (1.17-2.99)
Lymphocyte-to-Monocyte Ratio									
Stable ≥ 2.92	145	16	Ref	18	Ref	47	Ref	37	Ref
≥ 2.92 to < 2.92	144	22	1.01 (0.51-2.01)	18	0.78 (0.39-1.56)	67	1.31 (0.89-1.91)	63	1.73 (1.14-2.63)
< 2.92 to ≥ 2.92	47	6	1.14 (0.46-2.83)	7	1.04 (0.43-2.54)	20	1.20 (0.70-2.06)	14	1.10 (0.58-2.07)
Stable < 2.92	157	26	1.47 (0.74-2.90)	25	1.11 (0.57-2.16)	85	1.57 (1.08-2.28)	73	1.68 (1.11-2.55)

^aEstimated from Cox proportional hazard model, adjusted for gender, age, cancer site, pT, pN, surgical margins and extracapsular extension; ^bAdjusted for competing risk according to the Fine-Gray model.

tective responses, leading to accumulation of mutations. Additionally, chronic inflammation is associated with

tumour progression through processes like angiogenesis and the establishment of a tumour inflammatory micro-

environment, which involves infiltration by immune cells such as tumour-associated macrophages (TAMs), mast cells, tumour-associated neutrophils (TANs), and lymphocytes, all of which contribute to tumour growth and progression^{30,31}. Numerous studies have examined the ability of lymphocytes, neutrophils, monocytes, LMR^{32,33} and NLR to predict outcomes in patients with HNSCC. A preoperative elevated NLR^{4,34-40} or a low LMR⁴¹⁻⁴⁵, as well as an increase in the absolute count of neutrophils³⁹ or monocytes^{46,47}, or a decrease in the absolute count of lymphocytes, have been associated with poorer prognosis in many studies. Neutrophils and monocytes play a role in tumour initiation, growth, proliferation, and metastasis, while lymphocytes help to inhibit tumour development and growth through immune surveillance mechanisms.

As noted above, previous retrospective studies and meta-analyses have reported that NLR and LMR may serve as independent prognostic factors in patients with HNSCC. However, these reports were based on preoperative counts, while the dynamic changes in pre- and post-treatment values of peripheral inflammatory markers and clinical outcomes of patients with HPV-negative HNSCC undergoing curative surgery has not yet been fully investigated. A study on patients with gastric cancer found that changes in LMR after 12 months could predict long-term survival⁴⁸. Another study on gastric carcinoma revealed that high NLR and low LMR levels early after surgery were associated with complications and poor short-term outcomes⁴⁹. In breast cancer, an elevated NLR, measured around 5 years after diagnosis, was an independent risk factor for late recurrence⁵⁰. For non-small cell lung cancer patients receiving nivolumab treatment, those with an NLR greater than or equal to 5 had worse OS compared to those with an NLR less than 5⁵¹. Additional research focusing on patients with HNSCC who underwent radiation therapy indicated that alterations in delta-LMR could potentially serve as a prognostic marker. Patients who had a high delta-LMR at 2, 4, and 6 weeks after starting treatment were found to have poorer OS and were less likely to be free from metastasis, in comparison to those with a low delta-LMR. The threshold values for delta-LMR at 2, 4, and 6 weeks were 1.5, 2.8, and 3, respectively⁵². A study on patients diagnosed with SCC of the oral cavity revealed that elevated post-treatment levels of platelet count and platelet-to-lymphocyte ratio serve as independent prognostic indicators of a poor outcome⁵³.

The results of the present study are in agreement with data in the literature that patients with high preoperative levels of NLR (> 3.76) are at a higher risk of regional failure (with

HR, 2.21), disease progression, and death, while those with a low preoperative LMR (< 2.92) are at a higher risk of local failure (with HR, 1.95) according to a previous study²⁴ and a meta-analysis⁴, regional failure, and disease progression. Additionally, has been found that changes in these markers after surgery were also associated with different outcomes. Increased NLR after surgery was associated with a reduction in OS, while a low LMR after surgery was associated with an increased risk of disease progression and reduced OS. This study also found that patients with persistently high NLR or low LMR before and after surgery were at increased risk of disease progression and reduced OS. Interestingly, even patients with a normal preoperative LMR but low LMR after surgery were found to have reduced OS. Overall, these findings suggest that monitoring these inflammatory markers before and after surgery may provide valuable prognostic information and help identify patients who may benefit from more aggressive treatment or closer follow-up.

Thus, our multicentre study has some strong points. First, we included a cohort of highly selected patients who met strict inclusion criteria and were followed with regular clinical examinations as recommended by the American Cancer Society, which allowed for a more robust analysis. Second, we utilised statistical models that yielded significant results, providing valuable insights into the data. Lastly, we compared preoperative and postoperative blood values in HNSCC, which are rarely found in the literature, further contributing to the novelty of our findings. However, a number of limitations have to be acknowledged. First, the retrospective nature of the study may have introduced some bias. Second, although blood parameters were collected before surgery to avoid the impact of the surgical procedure itself on the baseline values, it was not always possible to exclude the influence of other systemic conditions, since the parameters investigated are not specific to tumours. Furthermore, the sample size was not sufficiently large to guarantee enough power when the patients were stratified for combined pre- and postoperative parameters, as the number of events in some strata was low.

Conclusions

In summary, this study highlights the importance of monitoring NLR and LMR before and after surgery to assess prognosis in patients with HPV-negative HNSCC. The findings suggest that monitoring these markers can provide valuable information on the risk of disease progression and reduced OS and may help identify patients who require

closer follow-up or more aggressive treatment. While NLR and LMR are easily calculated from standard blood tests, further studies are necessary before they can be incorporated into routine clinical practice for the management of patients with HNSCC; just as understanding the mechanisms that link inflammation and cancer progression may lead the way for the development of new therapies that target these pathways. Overall, this study provides a foundation for future research on the role of inflammatory blood markers in predicting outcomes and guiding treatment decisions in patients with HNSCC.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

TM, AM, AC, JP, DB, PBR: work conceptualisation and writing; TM, AM, GT, MT, VR, CP, MF, PN, VB, VL, FU, FT, SM, FG: data curation; JP: statistical analysis; GT, CP, PN, FT, AC, JP, DB, PBR: revision and editing. All authors have read and agree with the final version of the manuscript.

Ethical consideration

The study was conducted with the approval of the Board of Ethics of Treviso/Belluno provinces (Date March 23rd, 2020/No. 773/CE Marca). Written consent to participate was obtained from patients in accordance to Italian rules and regulations, in agreement to requirement of the Board of Ethics that approved the study.

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Appendix

Supplementary material

Supplementary Table I. Hazard ratio (HR) of death and corresponding confidence intervals (CI)^a according to blood parameters assessed before and after surgery.

	Preoperative				Postoperative			
	Pts	Deaths		HR (95% CI)	Pts	Deaths		HR (95% CI)
		n	(%)			n	(%)	
Haemoglobin ^b								
Normal/High	351	120	(34.2)	Ref.	213	61	(28.6)	Ref.
Low	142	67	(47.2)	1.35 (0.98-1.86)	280	126	(45.0)	1.62 (1.14-2.29)
Lymphocytes								
≥ 1.97	227	77	(33.9)	Ref.	104	29	(27.9)	Ref.
1.44 to < 1.97	152	63	(41.5)	1.19 (0.84-1.67)	127	47	(37.0)	1.26 (0.78-2.02)
< 1.44	114	47	(41.2)	1.23 (0.83-1.80)	262	111	(42.4)	1.20 (0.78-1.87)
Monocytes								
< 0.59	241	89	(36.9)	Ref.	268	87	(32.5)	Ref.
0.59 to < 0.78	129	48	(37.2)	0.96 (0.67-1.37)	119	55	(46.2)	1.52 (1.07-2.19)
≥ 0.78	123	50	(40.7)	0.99 (0.69-1.42)	106	45	(42.5)	1.95 (1.31-2.90)
Neutrophils								
< 4.28	217	73	(33.6)	Ref.	288	108	(37.5)	Ref.
4.28 to < 5.54	119	56	(47.1)	1.22 (0.84-1.76)	118	35	(29.7)	0.86 (0.58-1.27)
≥ 5.54	157	58	(36.9)	0.96 (0.67-1.38)	87	44	(50.6)	1.89 (1.29-2.77)
Platelets								
< 160	72	32	(44.4)	Ref.	84	37	(44.1)	Ref.
160 to < 236	201	69	(34.3)	153 (99-238)	198	69	(34.9)	132 (88-200)
≥ 236	220	86	(39.1)	114 (82-160)	211	81	(38.4)	126 (90-177)

^aEstimated from Cox proportional hazard model, adjusted for gender, age, cancer site, pT, pN, surgical margins and extracapsular extension; ^bHaemoglobin level was defined as low when < 12 g/dL in women and < 14 g/dL in men; normal when in 12-16 g/dL in women and in 14-18 g/dL in men; high otherwise.

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