Prognostic value of preoperative inflammatory ratios in early glottic cancer treated with transoral laser surgery.

Authors:

Dr. Luis Juesas-Iglesias[1], 0000-0002-8345-4168, Contributions: data collection, analysis and interpretation of the data and manuscript drafting.

Dr. Mario Sánchez-Canteli[1,2], 0000-0003-0343-0041, Contributions: data collection and analysis and interpretation of the data.

Dr. Daniel Pedregal Mallo[1], 0000-0001-8899-7957, Contributions: analysis and interpretation of the data and critical revision.

Dra. María Otero-Rosales[1], 0000-0003-3369-0821, Contributions: analysis and interpretation of the data and critical revision.

Dr. Fernando López[1,2], 0000-0001-7019-9746, Contributions: study concept and design and critical revision

Dra. Juana M. García-Pedrero[1,2], 0000-0002-5891-9488, Contributions: study concept and design and critical revision.

Prof. Dr. Juan P. Rodrigo[1,2]. 0000-0003-3063-0890, Contributions: study concept and design, analysis and interpretation of the data and critical revision.

[1]Department of Otolaryngology-Head and Neck Surgery. Hospital Universitario Central de Asturias, Instituto de Investigación Sanitaria del Principado de Asturias, Instituto Universitario de Oncología del Principado de Asturias, University of Oviedo. Oviedo, Spain.

[2] Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain.

Corresponding author:

Luis Juesas Iglesias

luisjuesas94@gmail.com

Telf: +34 649818542

Department of Otolaryngology-Head and Neck Surgery

Hospital Universitario Central de Asturias

Av. Roma SN, 33011 Oviedo, Spain

This article was presented at:

- The 73th National Congress of the Spanish Society of Otorhinolaryngology and Head and Neck Surgery, Las Palmas de Gran Canaria, Spain, October 13-15, 2022.
- The 10th European Congress of Head and Neck Oncology, Lisbon, Portugal, 8-11 March 2023 as a e-poster.
- The 6th Spring Meeting of the Head and Neck Oncology Committee of the Spanish Society of Otorhinolaryngology and Head and Neck Surgery, Oviedo, Spain, 13-14 April 2023.

Short running title: Inflammatory ratios in early glottic cancer.

Key words: prognostic score; glottic cancer; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; systemic immune-inflammatory index.

Conflicts of interest: none

Sponsor name or funding source: This study was supported by grants from the Instituto de Salud Carlos III [ISCIII (PI19/00560 and PI22/00167 to JMGP), and CIBERONC (CB16/12/00390 to JPR)], the Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Ayudas a Grupos PCTI Principado de Asturias (IDI/2021/000079 to JPR), the Fundación Bancaria Cajastur, and the FEDER Funding Program from the European Union.

Institutional Review Boards (IRB) approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Hospital Universitario Central de Asturias (date of approval May 14th, 2019; approval number: 141/19, for the project PI19/00560)

Abstract

Background: There is growing evidence regarding the prognostic utility of ratios such as Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammatory index (SIII) in head and neck squamous cell carcinoma (HNSCC). However, most studies to date include heterogeneous series with different treatments or tumor subsites.

Methods: We collected data of 201 patients with stage I-II glottic squamous cell carcinoma treated with transoral laser surgery. NLR, PLR and SIII were calculated from preoperative cell blood count, cut-off points were obtained by ROC curve analysis, and survival rates were calculated.

Results: High NLR (p=0.012) and SIII (p=0.037), but not PLR (p=0.48), were associated with worse disease-specific survival (DSS). Similar trend was observed with overall survival (OS), although did not reached statistical significance. On multivariable analyses, both high NLR (HR=3.8, 95%CI=1.5-9.9, p = 0.006) and high SIII (HR=2.77, 95%CI=1.1-6.9, p = 0.03) were significantly associated with shortened DSS.

Conclusions: Preoperative NLR and SIII emerge as independent prognostic biomarkers for early-stage surgically treated glottic tumors and could guide individualized follow-up.

Introduction

Laryngeal cancer currently has an incidence almost reaching 200,000 (184,615) new cases in 2020 and almost 100,000 (99,840) related deaths[1]. Laryngeal squamous cell carcinoma (LSCC) represents the predominant histological type, and the prognosis depends on several clinical and anatomical features showing varying differences in mortality risk among patients[2].

In the last years an increasing consideration has been given to the immune tumor microenvironment and related immunologic characteristics. This fact is supported by new evidence on the role of immunological factors in the prognosis and survival rates of cancer patients[3,4]. A wide variety of immune cell types infiltrate the tumor microenvironment regulating the balance between pro-tumorigenic and anti-tumorigenic signals. Several studies have reported better outcomes in LSCC patients harboring high density of CD8+ tumor-infiltrating lymphocytes (TIL)[5]. By contrast, host inflammatory responses to cancer have been associated with a worse outcome[6,7]. The tumor progression process is associated with immune evasion mechanisms while triggering an inflammatory response that has pro-tumorigenic effects. This local inflammatory state also triggers systemic inflammation[8]. The induced inflammatory response can be observed by an increased neutrophil, platelets and monocytes counts.

Prognostic markers that can be easily obtained from peripheral blood counts constitute an affordable and easily measurable tool that could be useful in day-to-day clinical practice and decision making[6]. Various systemic inflammation ratios such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and the systemic immune-inflammatory index (SIII) among others have

shown prognostic implications in several cancer types, including head and neck squamous cell carcinoma (HNSCC)[6,8-12]. Specifically, in HNSCC, high pretreatment values of NLR, PLR and SIII were found to be associated with poor prognosis[13-15]. Studies focused on LSCC led to analogous results[7,16-20]. Nevertheless, most studies to date include heterogeneous series of patients who received different treatments and mixed tumors from various locations and stages. Therefore, further validation of these ratios is needed using independent patient cohorts with well-defined tumor locations and stages, and homogeneously treated.

It is well known that early stage LSCC is associated with good survival outcomes when treated appropriately with a single modality in most cases. However, although the overall prognosis for these patients is good, there is still marked variation in the risk of death among patients, and some patients fare poorly and die from tumor recurrence and/or metastases.

Growing evidence suggests that systemic inflammation ratios may emerge as valuable prognostic biomarkers in different cancer patients. This prompted us to perform a retrospective cohort analysis to evaluate the predictive potential of several blood-based biomarkers on a large homogeneous cohort of LSCC patients with early stage glottic tumors resected by transoral laser surgery (TLS). We aim to establish a cut-off value for these ratios in our cohort that will allow improved risk stratification of patients and discuss its clinical implications when considering risk-adapted follow-up or treatment.

Materials and methods

Patients.

We reviewed the clinical records of 201 patients with early stage (stages I-II) glottic squamous cell carcinoma, who were diagnosed and treated in our department, between 1998 and 2019. All patients were treated with TLS for a single primary tumor and received no treatment prior to surgery nor adjuvant treatment. No patient had distant metastasis at the time of diagnosis. All experimental protocols were approved by the Institutional Ethics Committee of the Hospital Universitario Central de Asturias and by the Regional CEIm from Principado de Asturias (date of approval May 14th, 2019; approval number: 141/19, for the project PI19/00560). Informed consent was obtained from all patients.

Clinical, demographic, hematological, pathological (histological grade, surgical margins) and follow-up data were collected from the medical records. Patient evaluation included a complete physical examination by an experienced otorhinolaryngologist at the office, in which cervical palpation, oral cavity and oropharynx examination and fibro-laryngoscopy were systematically performed. Patients with lesions involving the anterior commissure, ventricle, arytenoids, subglottis, and those with abnormal vocal fold mobility underwent contrast-enhanced computed tomography (CT) of the larynx and neck to rule out thyroid cartilage involvement or invasion of the paraglottic space. The UICC/AJCC TNM classification (8th edition) was used for clinical tumor staging.

TLS was indicated in T1-T2 glottic tumors with adequate tumor exposure, irrespective of the age and comorbidities of the patient. All the patients underwent a TLS with curative intent, following Steiner's recommendations[21]. The surgical procedures were performed under general anesthesia after oro-tracheal intubation with Sharplan 30C CO2 Laser System and AcuPulse CO2 Laser System. Endoscopic resection was performed using en-bloc or piecemeal techniques according to several variables, including tumor localization and size, as well as the laryngeal exposure.

Hematological parameters

Pretreatment blood cell counts were obtained from preoperative blood tests required to proceed to preanesthetic consultation. Complete blood cell counts were registered including hemoglobin (g/dL), absolute platelet (APC), neutrophil (ANC) and lymphocyte counts (ALC) ($10^3/\mu L$). NLR was calculated dividing the ANC by ALC. PLR was obtained dividing the APC by ALC. Finally, SIII was calculated by multiplying the APC and the ANC and then dividing by the ALC.

Statistical analysis

Receiver Operated Characteristics (ROC) curves were calculated to determine the optimal cut-off point for NLR, PRL and SIII using the death by tumor as a variable. The Youden Index cut-off points were calculated by determining the area under the ROC curve expressed with 95% confidence intervals and *p* values. Kaplan-Meier analyses were plotted according to these cut-off values, and the survival curves compared using the log-rank test. Finally, univariable and multivariable analyses were performed using Cox proportional hazard models with 95% confidence interval and *p*-values considered statistically significant when ≤ 0.05.

Results

Patients' characteristics

The clinicopathological variables are summarized in Table 1. A total of 201 patients were included in the study, 191 male (95%) and 10 (5%) female. The mean age was 65 years (range 35-90). Most tumors (89.6%) were classified as stage I and 10.4% as stage II. The mean hospitalization time was 2.8 days (range 1-25).

The mean follow-up time was 54.6 months (range 24-195 months). Recurrent disease (considered either as local, regional, distant location or a combination of them) developed in 65 patients (32.3%), and a second primary tumor was observed in 24 patients (11.9%).

Hematological parameters

The results of the analyzed hematological parameters are shown in Table 2. NLR, PLR and SIII values were calculated, and the ROC curves generated (Figure 1). The NLR was found to have the greatest area under the curve (AUC) at 0.631, followed by SIII at 0.561, and PLR at 0.49. The maximal Youden index was calculated to determine the optimal cut-off value to maximize sensitivity and specificity. Cut-off points were set at 2.44 for NLR, with a sensitivity of 53% and a specificity of 66%; 99.7 for PLR, with a sensitivity of 68% and a specificity of 44%; and 533 for SIII, with a sensitivity of 53% and a specificity of 69%.

Relationship between hematological parameters and clinicopathological variables.

Using the optimal ROC cut-off points, we observed that patients with high NLR values (above a cut-off of 2.44) had a significantly higher percentage of deaths due to the tumor (10/60, 16.6%) compared to those below the cut-off point (9/141, 6%) (Fisher's test p = 0.033). No significant associations were found between NLR and other variables studied (Table 3).

Regarding the PLR, a significant association with tumor size was observed. Thus, cases with a high PLR were correlated with larger tumor sizes: 11/59 (18.6%) cases with high PRL were classified as T2 compared to 10/139 (7.2%)cases with a low PLR (Fisher's test p = 0.023). No further significant associations with other clinicopathological variables were observed (Table 3).

Patients with high SIII values (above a cut-off of 533) exhibited significantly higher rates of tumor recurrence (29/64, 45.3%) compared to those patients with low SIII values (36/133, 27%) (Fisher's test p = 0.01). Similarly, high SIII values were also found to significantly associate with a higher percentage of deaths due to tumor (15.6% vs 7.2% in those with low SIII, p = 0.047). No other associations between SIII and clinicopathological parameters were observed (Table 3).

Impact of hematological parameters on patient survival

Recurrence-free survival (RFS) was higher in patients with low NLR, low PRL and low SIII, although differences only reached statistical significance for the SIII (p = 0.003; Figure 2A-C). Disease-specific survival (DSS) was significantly higher in patients with low NLR and low SIII (p = 0.012 and p = 0.037, respectively), whereas PLR showed no association with DSS (p = 0.485) (Figure 2D-F).

Moreover, there was a trend towards higher overall survival (OS) in those patients with low NLR and low SIII; however, these differences did not reach statistical significance (Figure 2G-I).

Table 4 shows the results of univariable analyses for RFS, DSS and OS. In these analyses, the variables found to be significantly associated with a lower RFS were alcohol consumption of more than two standard drink units (SDU) per day (HR = 1.98, 95%CI = 1.1-3.6, p = 0.023), stage II disease (HR = 2.46, 95%CI = 1.3-4.7, p = 0.007), surgical margin involvement (HR = 3.63, 95%CI = 1.8-7.2, p = <0.001), and a high SIII (HR = 2.03, 95%CI = 1.2-3.3, p = 0.005). The variables significantly associated with a lower DSS were stage II disease (HR = 2.49, 95%CI = 1.1-5.4, p = 0.02), poor histological differentiation (HR = 1.92, 95%CI = 1.03-3.6, p = 0.038), involvement of surgical margins (HR = 4.1, 95%CI = 1.3-12.5, p = 0.013), a high NLR (HR = 3.23, 95%CI = 1.3-7.8. p = 0.009), and a high SIII (HR = 2.74, 95%CI = 1.1-6.6, p = 0.025). Finally, the variables associated with a lower OS were age over 65 years (HR = 3.06, 95%CI = 1.6-5.8, p = 0.001), poor histological differentiation (HR = 1.83, 95%CI = 1.1-2.9, p = 0.012), and surgical margin involvement (HR = 4.96, 95%CI = 2.1-11.6, p = 0.001).

Subsequently, multivariable Cox regression analyses were performed by including those hematological parameters (NLR and SIII) and clinicopathological variables found significant or near significant ($p < 0.1$) in the univariable analyses, as follows: age (dichotomized at 65 years), alcohol consumption (dichotomized at 2 SDU), stage, histological grade (dichotomized as G1-G2 vs G3), and surgical specimen resection border involvement (R). When the NLR was included in the models (Table 5), the variables independently associated with a lower RFS were

alcohol consumption of more than 2 SDU (HR = 2.05, 95%CI = 1.1-3.7, *p* = 0.019), stage II disease (HR = 2.48, 95%CI = 1.3-4.8, *p* = 0.008), and surgical margin involvement (HR = 3.48, 95%CI = 1.5-7.8, *p* = 0.002); the variables associated with a lower DSS were poor histological differentiation (HR = 2.7, 95%CI = 1.4-5.4, *p* = 0.003) and a high NLR (HR = 3.8, 95%CI = 1.5-9.9, *p* = 0.006); and the variables associated with a lower OS were age over 65 years (HR = 3.04, 95%CI = 1.5-6.1, *p* = 0.002), poor histological differentiation (HR = 2.22, 95%CI = 1.3-3.8, *p* = 0.004), and a high NLR (HR = 2.05, 95%CI = 1.01-4.1, *p* = 0.047). When SIII was included in the analysis (Table 6), the variables that showed an independent association with worse RFS were alcohol consumption of more than 2 SDU (HR = 2.17, 95%CI = 1.2-3.9 *p* = 0.012), stage II disease (HR = 2.33, 95%CI = 1.2-4.6, *p* = 0.014), and surgical margins involvement (HR = 3.26, 95%CI = 1.4-7.4, *p* = 0.005); the variables associated with lower DSS were poor histological differentiation (HR = 2.44, 95%CI = 1.3-4.6, *p* = 0.007) and a high SIII (HR = 2.77, 95%CI = 1.1-6.9, *p* = 0.03); and the variables independently associated with a worse OS were age over 65 years (HR = 3.01, 95%CI = 1.5-6.2, *p* = 0.003) and poor histological differentiation (HR = 2.05, 95%CI = 1.3-2.5, *p* = 0.009).

Discussion

Currently there are no validated biomarkers to guide the clinical management and decision making of LSCC patients. In recent years many retrospective studies have been conducted to determine the value of systemic inflammatory parameters as potential prognostic biomarkers in HNSCC[13-15], including LSCC[7,16-20]. However, most of these studies were designed and performed on

heterogeneous series of patients, comprising different tumor locations and treatment regimens. Hence, this work was focused on a specific location (the glottis) and stages (early stages I and II), as well as a single treatment (TLS) to assess the prognostic relevance of preoperative hematological parameters specifically in this subgroup of patients.

Our results showed that both NLR and SIII were consistently and specifically associated with a lower DSS in both univariable and multivariable analyses. In contrast, none of these hematological parameters were found to significantly associated with OS in our cohort, although a similar trend was observed. This is probably due to the fact that this series consists of early stage glottic tumors, which generally have a good prognosis. Therefore, the main causes of death in this population affecting the OS rates are different from the index tumor, and likely not influenced by the systemic inflammatory state.

The role of host immunity in the natural history of cancer is nowadays well recognized. Immune responses against cancer are manifested by the infiltration of lympho-mononuclear immune cells into the surrounding tumor microenvironment to protect against tumor growth[5]. In contrast, there is evidence associating systemic inflammation with cancer[22]. Cancer cells recruit and activate neutrophils, the so-called tumor-associated neutrophils (TAN). Although their precise role within the tumor microenvironment remains controversial, they appear to facilitate tumor progression[23,24]. Studies on LSCC have shown that increased TAN is associated with decreased CD4+/CD8+ T cell infiltration, which inhibits TNF-α and IFN-γ production, ultimately resulting in an immunosuppressive environment[25]. In animal models, chronic inflammation also led to immunosuppression and lymphopenia in the tumor microenvironment[26].

Thus, it seems that an inflammatory response to the tumor (with predominance of neutrophils) could be harmful, while an immune response (with predominance of lymphocytes) could be more beneficial to the patient.

It appears that the inflammatory indices derived from hematological parameters (NLR, PLR and SIII) can provide valuable information on the relationship between the systemic inflammatory response and the anti-tumor immune response in a simple way. The interest in these indices lies in the advantages they could offer as blood-based biomarkers, being minimally invasive, cost-effective, and easy to implement and interpret. Nevertheless, there are no studies that correlate these systemic indices with the relative predominance of TAN or lymphocytes locally in the tumor microenvironment. Noteworthy, a recent publication has revealed that the NLR and SIII were both inversely and significantly correlated with the local tumor infiltration of CD8+, CD4+, CD20+ TILs in Oral Squamous Cell Carcinoma (OSCC)[27]

The prognostic value of these indices (in particular the NLR) has been extensively demonstrated in multiple tumor types[6]. The NLR has been studied in several series of LSCC. A recent meta-analysis of 12 studies including a total of 3710 LSCC patients demonstrated the association between an elevated NLR and a lower survival, which was consistently observed in all 12 studies[7]. Although the heterogeneity among studies was low, all the studies in this meta-analysis included tumors from different laryngeal subsites and all disease stages, and 10 studies were conducted in Chinese populations. In a more recent study performed in Scotland[28] that included 220 LSCC (from all subsites and stages, and different treatment regimens), the NLR (and also the PLR and SIII) was independently associated to prognosis, confirming the results from the above-

mentioned meta-analysis by Yang et al.[7] in a different patient population. Our study further confirms the prognostic value of NLR in a homogeneous and minutely selected cohort of patients with stage I-II glottic cancer, thereby revealing NLR as a clinically relevant feature and independent predictor of DSS in the early stages of the disease.

The prognostic value of PLR and SIII is still under study, but both indices seem less powerful than the NLR. A meta-analysis of the prognostic value of PLR including all head and neck locations has shown a significant pooled effect (HR = 1.461, 95% CI 1.329–1.674; p = 0.0001), although there was substantial heterogeneity among the studies, and PLR was not prognostic in 7 out of 25 studies included[15]. Studies focused on all stages of LSCC also demonstrated an association between high PLR and a poorer prognosis[28-30]. On the other hand, we did not find a significant association of PLR with the prognosis of early-stage glottic tumors treated with TLS. Given that PLR was significantly associated with tumor size in our series, it is possible that the prognostic significance of PLR will become more apparent in other series with larger tumor sizes than ours.

Overall, the SIII has been less studied in HNSCC. In a recent meta-analysis of 12 studies including 4369 patients with HNSCC (all but one from Chinese populations), in the pooled results, a high pretreatment SIII was correlated with a poorer OS (HR = 2.09, 95% CI = 1.62-2.70, p < 0.001) and DSS (HR = 2.79, 95% CI = 1.99-3.89, p < 0.001)[33]. A stratified analysis indicated that the association of SIII with OS was independent of tumor site, treatment modality, and stage[33]. In three previous studies that included LSCC patients from all stages, SIII was also associated with prognosis[19,28,32]. Our study further and

significantly extends these data suggesting that SIII could also be a valuable prognostic biomarker even in early LSCC stages.

The present study demonstrates an association between a high NLR and a high SIII prior to treatment and shorter DSS in early-staged glottic cancer treated with TLS. Nevertheless, there are still several limitations. Firstly, this is a retrospective study with a non-randomized design, which might enhance the influence of confounding factors. Hence, our findings will require independent validation to further confirm the clinical implications. Second, since there are no established cut-off values for NLR, SIII and PLR, the cut-off points used in our study could not be applied to other subsites, stages, or treatments. Furthermore, hematological cell counts are known to vary among different ethnic groups. Using a similar methodology (ROC curve analysis) and location (larynx) the optimal cut-off points for NLR and SIII in our sample were 2.44 and 533, respectively. In the study carried out by Li et al.[32] the cut-off points calculated in 147 chinese patients were 1.88 for NLR and 517 for SIII, whereas in the study by Woodley et al.[28] in 220 british patients the cut-off points were 2.41 for NLR and 1144.46 for SIII. Despite these limitations, our results suggest that these parameters could increase our prognostic ability in patients with early stage glottic cancer.

We propose that the clinical utility of these findings could be to improve risk stratification in early-stage glottic cancers, identifying and classifying those patients with a high-risk profile by jointly combining these blood-based biomarkers with other clinicopathological criteria. This will allow to perform a closer and more intensive follow-up to promptly detect any recurrence in the high risk subgroup as well as to consider to be more radical in the treatment of these patients, while being more conservative in the subset of low risk tumors. The

minimal cost associated to this blood-based tests and its feasibility makes this field worthy of further research so they can be easily implemented in clinical practice, after establishing the optimal cut-off points to be used.

Further investigation it´s needed to evaluate the strength of the systemic inflammatory indexes and its clinical applications in a prospective design study or in clinical trials.

Conclusions

Our findings show that a high NLR and a high SIII, but not PLR, are associated with a shorter DSS in a selected population of early glottic cancers treated with TLS. More importantly, preoperative NLR and SIII emerge as independent prognostic factors for DSS, making this simple, easily quantifiable peripheral blood test a valuable biomarker-based stratification tool to refine prognosis and improve the management of LSCC patients.

Acknowledgments

None

Conflicts of interest

The Authors declare that there is no conflict of interest.

References

1- Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209-249.

2- López Álvarez F, Rodrigo Tapia JP, Boffetta P, Hainaut P. Laryngeal Cancer: Diagnosis and Treatment. In: Encyclopedia of Cancer. 3rd ed. Academic Press; 2019:332-345.

3- Chew V, Toh HC, Abastado JP. Immune microenvironment in tumor progression: characteristics and challenges for therapy. *J Oncol*. 2012;2012:608406.

4- Ruffin AT, Li H, Vujanovic L, Zandberg DP, Ferris RL, Bruno TC. Improving head and neck cancer therapies by immunomodulation of the tumour microenvironment [published online ahead of print, 2022 Dec 1]. *Nat Rev Cancer*. 2022;10.1038/s41568-022-00531-9.

5- Rodrigo JP, Sánchez-Canteli M, López F, et al. Tumor-Infiltrating Lymphocytes in the Tumor Microenvironment of Laryngeal Squamous Cell Carcinoma: Systematic Review and Meta-Analysis. *Biomedicines*. 2021;9(5):486.

6- Mei Z, Shi L, Wang B, et al. Prognostic role of pretreatment blood neutrophil-to-lymphocyte ratio in advanced cancer survivors: A systematic review and meta-analysis of 66 cohort studies. *Cancer Treat Rev*. 2017;58:1-13.

7- Yang F, Huang Q, Guan Z, Diao Q. Prognostic significance of pretreatment neutrophil-to-lymphocyte ratio in patients with laryngeal cancer: a systematic review and meta-analysis. *Eur Arch Otorhinolaryngol*. 2021;278(2):417-425.

8- Aguilar-Cazares D, Chavez-Dominguez R, Marroquin-Muciño M, et al. The systemic-level repercussions of cancer-associated inflammation mediators produced in the tumor microenvironment. *Front Endocrinol (Lausanne)*. 2022;13:929572.

9- Mikoshiba T, Ozawa H, Watanabe Y, et al. Prognostic Value of the Lymphocyte-to-Monocyte Ratio in Patients with Parotid Gland Carcinoma. *Laryngoscope*. 2021;131(3):E864-E869.

10- Lucca I, de Martino M, Hofbauer SL, Zamani N, Shariat SF, Klatte T. Comparison of the prognostic value of pretreatment measurements of systemic inflammatory response in patients undergoing curative resection of clear cell renal cell carcinoma. *World J Urol*. 2015;33(12):2045-2052.

11- Stotz M, Pichler M, Absenger G, et al. The preoperative lymphocyte to monocyte ratio predicts clinical outcome in patients with stage III colon cancer. *Br J Cancer*. 2014;110(2):435-440.

12- Kinoshita A, Onoda H, Imai N, et al. The Glasgow Prognostic Score, an inflammation based prognostic score, predicts survival in patients with hepatocellular carcinoma. *BMC Cancer*. 2013;13:52.

13- Takenaka Y, Oya R, Kitamiura T, et al. Prognostic role of neutrophil-to-lymphocyte ratio in head and neck cancer: A meta-analysis. *Head Neck*. 2018;40(3):647-655.

14- Valero C, Pardo L, Sansa A, et al. Prognostic capacity of Systemic Inflammation Response Index (SIRI) in patients with head and neck squamous cell carcinoma. *Head Neck*. 2020;42(2):336-343.

15- Kumarasamy C, Tiwary V, Sunil K, et al. Prognostic Utility of Platelet-Lymphocyte Ratio, Neutrophil-Lymphocyte Ratio and Monocyte-Lymphocyte Ratio in Head and Neck Cancers: A Detailed PRISMA Compliant Systematic Review and Meta-Analysis. *Cancers (Basel)*. 2021;13(16):4166.

16- Sánchez-Canteli M, Juesas L, Redin E, et al. Immune Cell Infiltrates and Neutrophil-to-Lymphocyte Ratio in Relation to Response to Chemotherapy and Prognosis in Laryngeal and Hypopharyngeal Squamous Cell Carcinomas. *Cancers (Basel)*. 2021;13(9):2079.

17- Hu X, Tian T, Sun Q, Jiang W. Prognostic value of the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in laryngeal cancer:

What should we expect from a meta-analysis?. *Front Oncol*. 2022;12:945820.

18- Du J, Liu J, Zhang X, et al. Pre-treatment neutrophil-to-lymphocyte ratio predicts survival in patients with laryngeal cancer. *Oncol Lett*. 2018;15(2):1664-1672.

19- Zhao Y, Qin J, Qiu Z, Guo J, Chang W. Prognostic role of neutrophil-to-lymphocyte ratio to laryngeal squamous cell carcinoma: a meta-analysis. *Braz J Otorhinolaryngol*. 2022;88(5):717-724.

20- Shen LF, Wang QY, Yu Q. The Systemic Immune-Inflammation Index and Albumin as Prognostic Predictors in Laryngeal Carcinoma. *Nutr Cancer*. 2021;73(10):1916-1923.

21-Weiss BG, Ihler F, Pilavakis Y, et al. Transoral laser microsurgery for T1b glottic cancer: review of 51 cases. *Eur Arch Otorhinolaryngol*. 2017;274(4):1997-2004.

22- Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860-867.

23- Szczerba BM, Castro-Giner F, Vetter M, et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature*. 2019;566(7745):553-557.

24- Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunol Immunother*. 2012;61(8):1155-1167.

25- Zhang D, Zhou J, Tang D, et al. Neutrophil infiltration mediated by CXCL5 accumulation in the laryngeal squamous cell carcinoma microenvironment: A mechanism by which tumour cells escape immune surveillance. *Clin Immunol*. 2017;175:34-40.

26- Murata M. Inflammation and cancer. *Environ Health Prev Med*. 2018;23(1):50.

27- Ruiz-Ranz M, Lequerica-Fernández P, Rodríguez-Santamarta T, et al. Prognostic implications of preoperative systemic inflammatory markers in oral squamous cell carcinoma, and correlations with the local immune tumor microenvironment. *Front Immunol*. 2022;13:941351.

28- Woodley N, Rogers ADG, Turnbull K, et al. Prognostic scores in laryngeal cancer. *Eur Arch Otorhinolaryngol*. 2022;279(7):3705-3715.

29- Xun Y, Wang M, Sun H, Shi S, Guan B, Yu C. Prognostic Analysis of Preoperative Inflammatory Biomarkers in Patients With Laryngeal Squamous Cell Carcinoma. *Ear Nose Throat J*. 2020;99(6):371-378.

30- Kara M, Uysal S, Altinişik U, Cevizci S, Güçlü O, Dereköy FS. The pre-treatment neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and red cell distribution width predict prognosis in patients with laryngeal carcinoma. *Eur Arch Otorhinolaryngol*. 2017;274(1):535-542.

31- Chen L, Zeng H, Yang J, et al. Survival and prognostic analysis of preoperative inflammatory markers in patients undergoing surgical resection for laryngeal squamous cell carcinoma. *BMC Cancer*. 2018;18(1):816.

32- Li Z, Qu Y, Yang Y, et al. Prognostic value of the neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index in patients with laryngeal squamous cell carcinoma. *Clin Otolaryngol*. 2021;46(2):395-405.

33- Wang YT, Kuo LT, Weng HH, et al. Systemic Immune-Inflammation Index as a Predictor for Head and Neck Cancer Prognosis: A Meta-Analysis. *Front Oncol*. 2022;12:899518.

Figure legends

Figure 1. ROC curves for NLR (A), PLR (B), and SIII (C). Cut-off points were set by selecting the highest sensitivity and specificity point of each curve. (NLR: 2.44; PLR: 99.7, SII: 533).

Figure 2. Kaplan-Meier recurrence-free survival curves (A-C), disease-specific survival curves (D-F) and overall survival curves (G-I) according to NLR values (A,D,G), PLR values (B,E,H), and SIII values (C,F,I).

Tables

Table 1. Clinicopathological characteristics of the selected cohort of 201 LSCC patients.

Characteristic	Number of cases (%)
Mean age in years (range)	65 (35-90)
Sex:	
- Male	191 (95%)
- Female	10 (5%)
Tobacco consumption	
- Missed	20 (9%)
- Never	12 (6%)
- Less than 10 pack-year	5 (2.5%)
- 10-40 pack-year	66 (32.8%)
- More than 40 pack-year	98 (48.8%)
Alcohol consumption	
- Missed	30 (15%)
- Never/occasionally	60 (29.9%)
- Mild (<2 SD)	8 (4%)
- Moderate (2-4 SD)	52 (25.9%)
- Severe (>4 SD)	51 (25.4%)
Location	
- No anterior commissure invasion	167 (83.1%)
- Anterior commissure invasion	34 (16.9%)
pT classification	
- T1	180 (89.6%)
- T2	21 (10.4%)
Histological Grade	
- G1	88 (43.8%)
- G2	70 (34.8%)
- G3	12 (6%)
Surgical margins (R)	
- Not evaluable	10 (4.9%)
- R0	176 (87.6%)
- R1	15 (7.5%)
Stage	
- I	180 (89,6%)
- II	21 (10,4%)
Recurrence	
- No	136 (67.7%)
- Local	53 (26.4%)
- Regional	2 (1%)
- Loco-regional	6 (3%)
- Local and/or regional + distant	5 (2%)

Second primary tumor	
- No	177 (88%)
- Head and neck	2 (1%)
- Lung	11 (5.5%)
- Other	11 (5.5%)

SD: Standard Drink (10 g)

Table 2. Values of hematological parameters including mean, median, standard deviation, and range.

	Leucocytes (cells/mm^3)	**Neutrophils (cells/mm^3)**	**Lymphocytes (cells/mm^3)**	**Eosinophils (cells/mm^3)**	**Platelets (cells/mm^3)**	**Hemoglobin (g/dL)**
Mean	7.31	4.28	2.14	0.19	229.26	15.35
Median	7.02	4	2	0.16	222	14.9
Standard deviation	2	1.52	0.77	0.13	70.38	8.83
Minimum	3.27	1.75	0.51	0	100	9.8
Maximum	15.8	10	5.25	0.8	520	138

Table 3. Correlations between NLR, PLR and SIII values dichotomized according to the respective ROC optimal cut-off point and the different clinicopathological variables studied.

Characteristic	NLR			PLR			SIII		
	Low <2.44	**High >2.44**	***P***	**Low <99.7**	**High >99.7**	***p***	**Low <533**	**High >533**	***p***
Sex									
- **Male**	133	58		133	55		127	60	
- **Female**	8	2	0.726	6	4	0.488	6	4	0.731
Tobacco consumption									
- **<10 P-Y**	12	5		8	9		11	6	
- **>10 P-Y**	113	51	1	115	46	0.053	105	55	1
Alcohol consumption									
- **<2 SD**	51	17		46	22		48	20	
- **>2 SD**	70	33	0.391	68	32	1	64	35	0.503
pT classification									
- **T1**	128	52		129	48		123	54	
- **T2**	13	8	0.451	10	11	**0.023**	10	10	0.084
Histological Grade									
- **G1**	56	32		60	27		51	36	
- **G2**	54	16		47	22		50	18	
- **G3**	8	4	0.183	9	3	0.893	8	4	0.153
Surgical margins (R)									
- **0**	124	52		124	49		118	54	
- **1**	9	6	0.394	10	5	0.767	8	7	0.256
Recurrence									
- **No**	99	37		97	36		98	35	
- **Yes**	44	23	0.252	42	23	0.249	35	29	**0.009**
Second primary tumor									
- **No**	124	53		125	49		117	56	
- **Yes**	17	7	1	14	10	0.233	16	8	1
Death by tumor									
- **No**	132	50		127	52		124	54	
- **Yes**	9	10	**0.033**	12	7	0.598	9	10	0.069
All deaths									
- **No**	113	45		110	46		107	48	
- **Yes**	28	15	0.454	29	13	0.851	26	16	0.458

NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SIII: Systemin immune-inflammatory index. P-Y: Package per year, SD: Standard Drink

Table 4: Results from univariable Cox proportional hazard analysis for recurrence-free survival (RFS), disease-specific survival (DSS) and overall survival (OS).

Variable	Univariable analysis RFS, HR (95% CI)	*p*	Univariable analysis DSS, HR (95% CI)	*p*	Univariable analysis OS, HR (95% CI)	*p*
Age						
≤65 years	1		1		1	
>65 years	0.98 (0.6-1.6)	0.94	2.41 (0.9-6.1)	0.06	**3.06 (1.6-5.8)**	**0.001**
Sex						
Male	1		1		1	
Female	1,23 (0.4-3.3)	0.69	1.01 (0.1-7.6)	0.98	0.93 (0.2-3.9)	0.93
Tobacco consumption						
<10 P-Y	1		1		1	
>10 P-Y	1.15 (0.5-2.9)	0.76	0.51 (0.1-1.7)	0.29	0.56 (0.2-1.3)	0.2
Alcohol consumption						
<2 SDU	1		1		1	
>2 SDU	**1.98 (1.1-3.6)**	**0.023**	1.52 (0.5-4.3)	0.42	1.5 (0.7-3.1)	0.28
Stage						
I	1		1		1	
II	**2.46 (1.3-4.7)**	**0.007**	**2.49 (1.1-5.4)**	**0.02**	1.87 (0.9-3.6)	0.069
Histological grade						
G1-G2	1		1		1	
G3	1.22 (0.8-1.9)	0.39	**1.92 (1.03-3.6)**	**0.038**	**1.83 (1.1-2.9)**	**0.012**
Surgical margins (R)						
R0	1		1		1	
R1	**3.63 (1.8-7.2)**	**<0.001**	**4.1 (1.3-12.5)**	**0.013**	**4.96 (2.1-11.6)**	**0.001**
NLR						
Low	1		1		1	
High	1.45 (0.9-2.4)	0.15	**3.23 (1.3-7.8)**	**0.009**	1.6 (0.9-3.06)	0.11
PLR						
Low	1		1		1	
High	1.43 (0.9-2.4)	0.16	1.57 (0.6-3.8)	0.32	1.34 (0.7-2.5)	0.37
SIII						
Low	1		1		1	
High	**2.03 (1.2-3.3)**	**0.005**	**2.74 (1.1-6.6)**	**0.025**	1.69 (0.9-3.1)	0.09

NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SIII: Systemic immune-inflammatory index.; HR, Hazard Ratio; 95% CI, 95% Confidence Interval; SDU: Standard Drink Unit

Table 5: Results from multivariable Cox proportional hazard analysis for recurrence-free survival (RFS), disease-specific survival (DSS) and overall survival (OS) introducing neutrophil-to-lymphocyte ratio (NLR) in the models.

Variable	Multivariable analysis RFS, HR (95% CI)	*p*	Multivariable analysis DSS, HR (95% CI)	*p*	Multivariable analysis OS, HR (95% CI)	*p*
Age						
≤65 years	NI	-	1		1	
>65 years			2.49 (0.97-6.4)	0.058	**3.04 (1.5-6.1)**	**0.002**
Alcohol consumption						
<2 SDU	1		NI	-	NI	-
>2 SDU	**2.05 (1.1-3.7)**	**0.019**				
Stage						
I	1		1		1	
II	**2.48 (1.3-4.8)**	**0.008**	2.17 (0.7-6.7)	0.17	1.53 (0.6-4.2)	0.35
Histological grade						
G1-G2	NI	-	1		1	
G3			**2.7 (1.4-5.4)**	**0.003**	**2.22 (1.3-3.8)**	**0.004**
Surgical margins (R)						
R0	1		1		1	
R1	**3.48 (1.5-7.8)**	**0.002**	1.12 (0.3-4.5)	0.86	2.04 (0.7-6.1)	0.2
NLR						
Low	1		1		1	
High	1.3 (0.7-2.3)	0.37	**3.8 (1.5-9.9)**	**0.006**	**2.05 (1.01-4.1)**	**0.047**

NI: Not included in the model

Table 6: Results from multivariable Cox proportional hazard analysis for recurrence-free survival (RFS), disease-specific survival (DSS) and overall survival (OS) introducing Systemic Immune Inflammatory Index (SIII) in the models.

Variable	Multivariable analysis RFS, HR (95% CI)	*p*	Multivariable analysis DSS, HR (95% CI)	*p*	Multivariable analysis OS, HR (95% CI)	*p*
Age						
≤65 years	NI	-	1		1	
>65 years			2.38 (0.93-6.1)	0.07	**3.01 (1.5-6.2)**	**0.003**
Alcohol consumption						
<2 SDU	1		NI	-	NI	-
>2 SDU	**2.17 (1.2-3.9)**	**0.012**				
Stage						
I	1		1		1	
II	**2.33 (1.2-4.6)**	**0.014**	1.73 (0.5-5.6)	0.36	1.47 (0.5-3.9)	0.44
Histological grade						
G1-G2	NI	-	1		1	
G3			**2.44 (1.3-4.6)**	**0.007**	**2.05 (1.3-2.5)**	**0.009**
Surgical margins (R)						
R0	1		1		1	
R1	**3.26 (1.4-7.4)**	**0.005**	1.48 (0.4-5.6)	0.56	2.36 (0.8-7.1)	0.12
SIII						
Low	1		1		1	
High	1.69 (0.98-2.9)	0.058	**2.77 (1.1-6.9)**	**0.03**	1.56 (0.8-3.2)	0.22

NI: Not included in the model

Figure 1

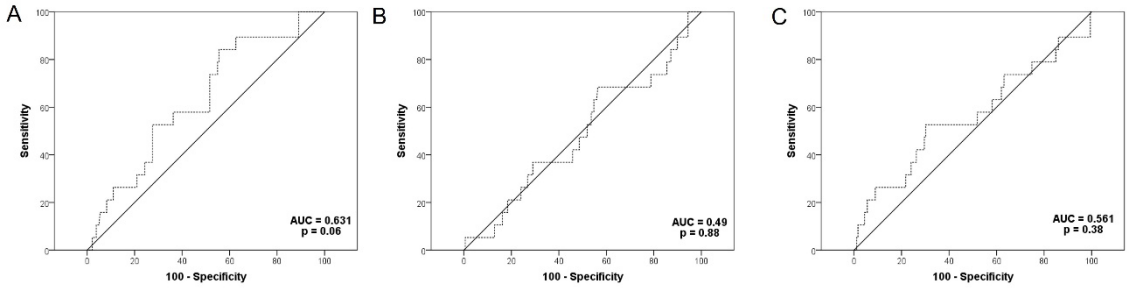
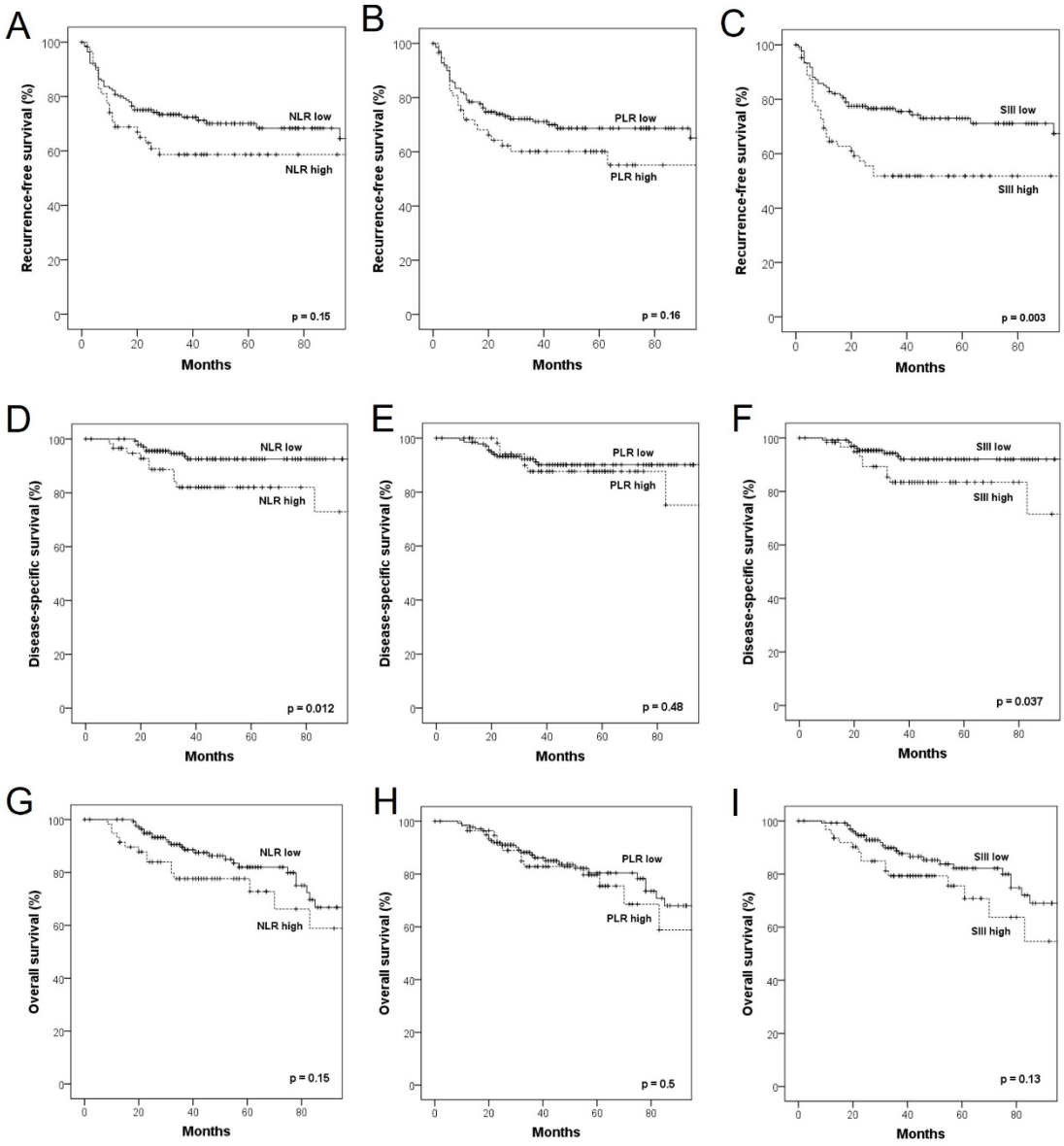

A
Sensitivity
100 - Specificity
AUC = 0.631
p = 0.06
B
Sensitivity
100 - Specificity
AUC = 0.49
p = 0.88
C
Sensitivity
100 - Specificity
AUC = 0.561
p = 0.38

Figure 2.


A
Recurrence-free survival (%)
NLR low
NLR high
p = 0.15
Months
B
Recurrence-free survival (%)
PLR low
PLR high
p = 0.16
Months
C
Recurrence-free survival (%)
SIII low
SIII high
p = 0.003
Months
D
Disease-specific survival (%)
NLR low
NLR high
p = 0.012
Months
E
Disease-specific survival (%)
PLR low
PLR high
p = 0.48
Months
F
Disease-specific survival (%)
SIII low
SIII high
p = 0.037
Months
G
Overall survival (%)
NLR low
NLR high
p = 0.15
Months
H
Overall survival (%)
PLR low
PLR high
p = 0.5
Months
I
Overall survival (%)
SIII low
SIII high
p = 0.13
Months