INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world, with 890,000 new cases and 450,000 deaths in 2018 worldwide.\(^1\) Despite improvements in research and therapy made in the last decades, survival has not significantly improved and the 5 years overall survival (OS) rate is still less than 50%.\(^2\) Classic prognostic factors are not sufficient to predict patients' prognosis, due to the heterogeneity of molecular mechanisms and tumor behaviors related to HNSCC. For these reasons, there has been an intensified interest in biomarkers' discovery for early diagnosis, prognosis and personalized treatment.

In this scenario, inflammatory biomarkers became a reliable and accessible source of information to investigate and correlate to...
Evidence suggests that inflammation contributes to tumour development and metastasis. A high number of neutrophils and macrophages infiltrating the tumour microenvironment seems to be associated with worse outcomes. The most commonly reported inflammatory parameters are C-reactive protein, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR). Among these, NLR represents the most studied and promising clinical biomarker. Neutrophils might help carcinogenesis in different ways, as the release of prostaglandin E2 and proteases, the destruction of the extracellular matrix, inhibition of T-cells and the modulation of macrophage activity, facilitating tumour growth and its progression.

The purpose of this systematic review and meta-analysis was to evaluate the prognostic role of the pre-treatment NLR, in terms of OS and disease-free survival (DFS), in patients with primary HNSCC treated using surgery followed or not by adjuvant therapies. Additionally, Trial Sequential Analysis (TSA) for the time-to-event outcomes was performed aiming to investigate the statistical power of the reported meta-analytic findings.

2 | MATERIALS AND METHODS

2.1 | Protocol

This systematic review was performed according to the Cochrane Handbook, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and recommendations from the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. The protocol was designed a priori and registered on the online database PROSPERO (CRD42020216751).

2.2 | Search strategy

The literature search was performed in the PubMed, Web of Science and Embase databases up to December 2020. The following search string has been used on PubMed: (HNSCC OR head and neck squamous cell OR oral OR larynx OR pharynx OR tongue OR oropharynx OR hypopharynx OR buccal OR mouth OR SCCHN OR OSCC OR oral cancer) AND (NLR OR neutrophil lymphocyte ratio OR neutrophil-to-lymphocyte ratio OR neutrophil-lymphocyte ratio OR Systemic inflammatory markers OR hematologic markers OR neutrophil-to-lymphocyte ratio). The string was modified to adapt it for each search engine used. Moreover, bibliographies of systematic reviews and grey literature were hand-revised to gather additional studies.

2.3 | Eligibility criteria

To be included, studies had to fulfil the following criteria: (i) prospective and retrospective cohort studies analysing prognostic impact of peripheral blood NLR in HNSCC; (ii) patients with histologically confirmed diagnosis of primary HNSCC undergoing surgery as first treatment with or without adjuvant therapy; (iii) studies which evaluated the association between the pre-treatment NLR values and survival outcomes, calculating at least one of the following parameters: OS and Disease Free Survival (DFS) (including the articles evaluating Recurrence Free Survival (RFS), which fell under the definition of DFS); (iv) a minimum number of 30 patients; (v) studies which used a single cut-off value of the NLR to stratify patients; (vi) studies which directly reported either hazard ratio (HR) with its 95% Confidence Interval (CI) or the Kaplan–Meier graph (in this case, the HR of survival analysis and its 95% CI were estimated applying the method by Tierney et al.) and (vii) full-text articles published in English.

The exclusion criteria were: (i) reviews, letters, or case reports; (ii) animal studies; (iii) studies with patients affected by HNSCC HPV+; (iv) patients with concurrent tumours.

2.4 | Study selection, data collection and data items

The process of study selection was divided into multiple steps. First, authors screened for articles by reading only title and abstract. Full texts of publications, meeting the initial inclusion criteria, were analysed in the second round. At the end of the second phase, two reviewers (PM and DR) provided independently a final judgement of inclusion for the selected articles and notified such recommendations to a third author (GT). This author calculated a value of k-statistic to ascertain the level of reviewers’ agreement. In cases of disagreement, the same author (GT) took a final decision. At the end of the selection process, papers fulfilling all inclusion criteria were included.

Data extraction was performed independently by two authors (PM and MM) using a specific extraction sheet; subsequently, data were double-checked in a joint session with a third author (GT). The following parameters were extracted from each included study: name of the first author, year of publication, nation where the study was performed, type of study, age (mean or median) of cohort, head and neck tumour sub-localization, staging, treatment, cut-off methods, cut-off values, outcomes, HRs and 95% CI for OS and DFS.

2.5 | Quality of evidence and risk of bias assessment

The risk of bias (ROB) of the included studies was performed using parameters derived from the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK). The scale consists of six parameters evaluating: samples, clinical data of the cohort, marker quantification, prognosis, statistics and classical prognostic factors. Based on the REMARK guidelines each factor was considered: adequate (A), inadequate (I) or not evaluable. Furthermore, an analysis of the ROB across studies was performed using Q and I² tests.
| Study | Year | Country | Study design | Sample size | Age (mean) | Tumour site | Tumour stage | Treatment | NLR (mean) | Cut-off values | Outcomes |
|-------|------|---------|--------------|-------------|------------|-------------|-------------|-----------|------------|----------------|----------|
| Bobdey et al. | 2016 | India | Retrospective | 471 | 50 (25– 85) | OC | | | 2.38 | NA | OS |
| Chen et al. | 2018 | China | Retrospective | 473 | 63.48 ± 10.21 | L | | | 2.20 ± 1.45 | 1.96 | 1.16 | OS, DFS |
| Chen et al. | 2016 | China | Retrospective | 306 | median | L | | | 2.61 | ± 2.0 | 2.45 | OS, DFS |
| de Almeida et al. | 2019 | Canada | Retrospective | 551 | 61 ± 13.7 | OC, HP | | | 2.79 | ± 2.5 | 2.56 | OS, DFS |
| Fang et al. | 2013 | Taiwan/ China | Retrospective | 226 | 52.47 (27– 84) | OC | | | 2.88 | ± 2.05 | 2.44 | DFS |
| Fu et al. | 2016 | China | Retrospective | 420 | 60 (33– 84) | median | | | 2.95 | ± 2.9 | 2.81 | OS, DFS |
| Hasegawa et al. | 2020 | Japan | Retrospective | 433 | 63.3 ± 13.5 | OC | | | 2.51 | ± 1.73 | 2.22 | OS, DFS |
| Ikeguchi | 2016 | Japan | Retrospective | 59 | 68.7 ± 9.5 | OC | | | 2.51 | ± 1.73 | 2.22 | OS, DFS |
| Kao et al. | 2018 | Taiwan | Retrospective | 613 | 53.0 ± 11.33 | OC | | | 2.78 | ± 1.36 | 2.44 | OS, DFS |
| Lee et al. | 2020 | Rep. of Korea | Retrospective | 291 | 63.1 ± 13.5 | OC | | | 2.79 | ± 1.73 | 2.44 | OS, DFS |
| Lo et al. | 2017 | Taiwan | Retrospective | 105 | 55 (22– 84) | median | | | 2.95 | ± 2.9 | 2.81 | OS, DFS |
| Lu et al. | 2020 | China | Retrospective | 120 | 55 (22– 84) | median | | | 2.95 | ± 2.9 | 2.81 | OS, DFS |
| Song et al. | 2014 | China | Retrospective | 146 | 57.5 (34– 89) | OC | | | 2.68 | (0.71– 8.75) | 2.39 | OS |
| Szilasi et al. | 2020 | Hungary | Retrospective | 156 | 58.1 ± 8.7 | OC, OP, HP | | | 2.78 | ± 0.72 | 2.44 | OS, DFS |
| Tazeen et al. | 2020 | India | Retrospective | 112 | 58.3 (34– 89) | OC, OP, HP | | | 2.78 | ± 0.72 | 2.44 | OS, DFS |
| Wang et al. | 2016 | Taiwan | Retrospective | 120 | 55 (22– 84) | OC | | | 2.79 | ± 2.32 | 2.81 | OS, DFS |
| Xun et al. | 2019 | China | Retrospective | 151 | 65 (44– 84) | median | | | 2.25 | ± 2.25 | 2.36 | OS, DFS |
| Yang et al. | 2018 | China | Retrospective | 197 | 61 (36– 87) | median | | | 2.78 | ± 2.25 | 2.36 | OS, DFS |
| Ye et al. | 2020 | China | Retrospective | 103 | <40 and >60 | OC | | | 2.56 | ± 2.56 | 2.39 | OS, DFS |
| Zhang et al. | 2019 | China | Retrospective | 103 | <40 and >60 | OC | | | 2.56 | ± 2.56 | 2.39 | OS, DFS |

(Continues)
The quality of the evidence was evaluated via the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) for each comparison between the study groups at the outcome level. The evaluation was performed utilizing the GRADEpro platform (McMaster University, Hamilton, Canada - https://gradepro.org).

2.6 Summary measures and planned methods for statistical analyses

2.6.1 Meta-analysis

The pooled analyses were performed using the software Review Manager version 5.2.8 (Cochrane Collaboration, Copenhagen, Denmark; 2014). Only studies reporting HR and CI for the multivariate analysis were included. For survival analysis, the impact of NLR on OS and DFS, the natural logarithmic of the HR and its standard error (SE) were calculated using the Calculator function of Review Manager. Heterogeneity among studies was evaluated though Higgins Index ($I^2$) and the chi-square test and classified as follows: low heterogeneity ($<30\%$), medium heterogeneity ($30\%–60\%$) and high heterogeneity ($>60\%$). Overall effects were compared using the inverse of variance test, setting a threshold of significance of $p < 0.05$. Only data assessed with multivariate analysis in the included studies were included in the meta-analysis.

2.6.2 Trial sequential analysis (TSA)

TSA was carried out to assess the power of the meta-analytic findings and adjust results to avoid type I and II errors. Data were analysed using metacumbounds command in statistical Stata Statistical Software version 13.0 (StataCorp, College Station, TX). We used the O’Brien–Fleming spending function to calculate the monitoring boundaries because of its conservative behaviour. Each cumulative z-value was determined by a random-effect model because the meta-analysis showed high heterogeneity ($I^2 > 60\%$). The calculation of the information size is based on an a priori anticipated intervention effect (a priori information size, API), and setting a 20% relative risk reduction (RRR), 5% type I error and 20% type II error.

3 RESULTS

3.1 Study selection process and study features

A total of 3071 records were retrieved by the initial search. After the first screening process, 99 articles meet the inclusion criteria and were full-text assessed. Subsequently, only 27 articles were considered eligible (Table 1). The flowchart with details of
the study selection process and reasons for exclusion is shown in Figure 1 and Supplementary materials (Table S1). Agreement between reviewers was excellent with a Cohen’s kappa coefficient of 0.905.

All the selected studies were included in the qualitative analysis, consisting of 28 cohorts (Lu et al. evaluated training and validation cohorts) with a total of 7525 patients. Of these, only 19-20,23-30,32,36,37,39,42-44 were included in the quantitative synthesis with a total of 4881 patients (Table 2). All the included studies were published between 2013 and 2020 with sample sizes ranging from 59 to 708. Twenty-five studies 18-32,34-40,42-44 were conducted in Asia, one in North America and one 41 in Europe (Table 1). Twenty-three 18-24,27-31,33,34,36-44 studies included patients with TNM stages I-II and III-IV, three studies 25,26,32 included only patients in the stages III-IV and one study 24 included exclusively patients in stages I-II.

The NLR cut-off values for the OS ranged from 1.96 to 5, while for the DFS ranged from 1.96 to 4.25. Ten studies 18,20,22,27,30,36,37,41 reported the association between the NLR and OS, three 21,34,39 between NLR and DFS and fourteen 19,21,24,28,29,31-33,35,38,40,43,44 studies reported the effect of NLR on both outcomes (OS and DFS/RFS). Cut-offs values were obtained from ROC curves, median value, X-tile software, or with Contal and O’Quigley method or using values previously reported in the literature.

### 3.2 | Risk of bias within studies

Seven studies 18,24,26,28,29,40,44 fully complied with REMARKS guidelines, while the remaining twenty 18,20-23,25,27,30-39,41-43 showed weakness in some of the parameters (Table S2).

All the studies included were adequate in at least half of the parameters analysed; hence, the ROB of these studies can be considered medium overall (Figure 2).

The GRADE ratings about the outcome-centred quality of the evidence and pooled summary estimates, where applicable, have been outlined in the summary of findings table (Table S3). The overall quality of evidence was rated as moderate for both OS and DFS. Results of the GRADE analysis indicated that there is moderate evidence to support that high pre-treatment NLR values are associated with a worse prognosis in patients with HNSCC in terms of OS and DFS (Table S3); these results are mainly related to
### 3.3 NLR and prognosis in HNSCC

The meta-analysis assessing the association between NLR and OS included 17 studies25–30,32,33,34–37,39,41–47 with a total of 4597 patients. Meta-analysis (Figure 3A) revealed that a higher value of pre-treatment NLR correlates with a statistically significant decrease of OS in HNSCC patients (HR, 1.56; 95% CI: [1.35, 1.80]; p < 0.0001). A random-effect model was used by the presence of high heterogeneity (I²=61%). These results were also confirmed by the TSA. The graphical evaluation shows that the z-curve crossed the monitoring boundary and APIS, revealing a significant statistical power (Figure 3b).

A total of 10 studies,19,23,24,28,29,32,39,40,42,44 including 2020 patients, evaluated the prognostic role of NLR for DFS. Meta-analysis (Figure 4A) assessed that a higher value of NLR was associated with worse survival in HNSCC patients (HR, 1.64; 95% CI: [1.30, 2.07]; p < 0.0001). The analysis was performed at random-effect model due to the high rate of heterogeneity (I²=69%). The graphical evaluation of TSA revealed a high statistical power as the z-curve crossed the trial sequential monitoring boundary and the API.
### TABLE 2
Synthesis of data extracted from the included studies related to outcomes pooled in the systematic review and meta-analysis

| Study                          | Category | HR 95% C.I       | p-value | HR 95% C.I       | p-value | HR 95% C.I       | p-value |
|-------------------------------|----------|------------------|---------|------------------|---------|------------------|---------|
| Zhong et al.                  |          | 1.392 1.045-1.855| 0.024   |                  |         |                  |         |
| Yang et al.                   |          | 1.64 1.06-2.54   | 0.026   |                  |         |                  |         |
| Xun et al.                    |          | 1.39 1.01-1.9    |         |                  |         |                  |         |
| Wang et al.                   |          | 1.181 1.046-1.333| 0.007   |                  |         |                  |         |
| Szilasi et al.                |          | 1.31 1.00-1.71   | 0.046   | 2.3 1.42-3.72    | <0.001  |                  |         |
| Lu et al. (validation)        |          | 5.586 1.169-2.682| 0.031   |                  |         |                  |         |
| Lu et al.                     |          | 1.78 1.01-3.14   | 0.045   |                  |         |                  |         |
| Kao et al.                    |          | 2.53 1.48-4.30   | 0.001   |                  |         |                  |         |
| Chen° et al.                  |          | 2.417 1.195-4.891| 0.014   |                  |         |                  |         |
| de Almeida et al.             |          | 1.612 0.807-3.22 | 0.176   |                  |         |                  |         |
| Chen et al.                   |          | 1.87 1.088-3.215 | 0.024   |                  |         | 1.33-4.18        | 0.003   |
| Lee et al.                    |          | 0.449-3.053      | 0.747   |                  |         |                  |         |
| MARIANI et al.                |          | 1.208-3.924      | 0.01    |                  | 1.85    | Estimated^1       | 0.021   |
|                              |          | 1.326-3.962      | 0.003   |                  | 1.91    | Estimated^1       | 0.004   |
|                              |          | 3.02 1.28-7.10   | 0.011   |                  |         |                  |         |
|                              |          | 0.95 0.63-1.43   | 0.796   |                  |         |                  |         |
|                              |          | 0.67 1.0-1.1     | 0.001   |                  |         |                  |         |
|                              |          | 1.579 1.217-3.092| 0.002   |                  |         |                  |         |
|                              |          | 0.692 0.329-1.456| 0.332   |                  | 3.371   | 2.490-4.563      | <0.001  |

**DISCUSSION**

Several inflammatory markers, such as NLR, PLR and LMR have been reported to be associated with clinical outcomes in patients with various types of cancer, including lung cancer, colorectal cancer, breast cancer, thyroid and so forth. Furthermore, a novel systemic immune-inflammation index (SII) based on peripheral neutrophil, platelet and lymphocyte count further enhanced the validity to predict the tumour prognosis.

Various potential mechanisms that may justify the prognostic role of NLR and the role of systemic inflammation in cancer biology have been hypothesized. The adaptive immune system carries out immune surveillance against cancer cells, but effective adaptive immune responses are always suppressed through several pathways.
Neutrophilia may inhibit the immune system by regulating the activation and the infiltration of regulatory T cells\textsuperscript{45} and suppressing the cytolytic activity of lymphocytes and natural killer (NK) cells.\textsuperscript{45,50}

Neutrophils and other cells such as macrophages have been reported to secrete tumour growth-promoting factors contributing to create a favouring microenvironment for extracellular matrix remodelling, endothelial cell migration and tumour dissociation.\textsuperscript{7}

**FIGURE 3** (A) Meta-analysis and (B) TSA related to the association between NLR and Overall Survival
Moreover, an elevated NLR has been associated with an increase in the peritumoural infiltration of macrophages and an increasing in IL-17. The effects of inflammatory cytokines and chemokines secreted by tumour, stromal and associated host cells in the tumour microenvironment are important in sustaining chronic inflammation (e.g. promoting and increasing the differentiation and the release of bone-marrows neutrophils).

Otherwise, lymphopenia could impair the role of cell-mediated immunity and its function of host cancer cell suppression. It has been reported that increasing infiltration of lymphocytes in the tumour microenvironment is associated with a better response to cytotoxic treatment and prognosis in cancer patients. Recent studies showed that the NLR may be useful in identifying patients at highest risk for neck nodal occult metastasis in tongue OSCC and orient the decision-making for neck dissection.

FIGURE 4 (A) Meta-analysis and (B) TSA related to the association between NLR and Disease-free Survival
clinicians to the elective neck dissection,\textsuperscript{52} as it was also demonstrated in patients with melanoma.\textsuperscript{53}

Although other systematic reviews evaluated the prognostic role of NLR in HNSCC have been performed,\textsuperscript{54–59} the present meta-analysis is more comprehensive, including the most recent findings and being the first to perform TSA for time-to-event outcomes, to adjust for type I and type II errors and to quantify the power of the published evidence. This is the first systematic review to include only studies with patients undergoing surgery as first treatment with or without adjuvant therapy, as differences in the treatment plan could affect both the immune system response and the prognosis, and to include only studies with primary HNSCC and, in addition we also excluded HPV +tumours to further standardize the analysed cohort. Moreover this study is the only one to assess the certainty of evidence applying the GRADE approach.

Such analysis\textsuperscript{14} was performed to evaluate the quality of evidence and to observe the strength of recommendation obtained from the results. The quality of the evidence was determined to be moderate for the findings associated with OS and DFS. Based on our focused question (Is high NLR associated with a worse prognosis in HNSCC?) and the studies assessed, inconsistency was evaluated according to Guyatt et al.\textsuperscript{60} Although the $I^2$ values were high, due to different follow-up and cut-off values, (61% and 69% for OS and DFS respectively), only 2 studies (OS) and 1 study (DFS) have a value of HR below 1. Furthermore, only the CIs of 3 out of 17 studies show significant survival differences, the identification of a single cut-off value may reduce heterogeneity between studies obtaining even more precise results in the meta-analytic statistical analysis. The eligible studies identified NLR cut-off values using different methods, with a wide range (from 1.96 to 4.81) and it was not possible to identify the most effective cut-off as complete data for each study are not available, but summary data for each analysis were reported. Additionally, a non-uniform methodology was also identified in tumour staging, for this reason no subgroup analysis was performed.

This study presents some limitations. First, all the included studies were retrospective and observational, this could lead to a potential selection bias. Furthermore, although Cho et al.\textsuperscript{57} stated that absolute NLR cut-off values did not seem to matter instead of that groups below and above NLR cut-offs did show significant survival differences, the identification of a single cut-off value could improve the prognostic performance of this biomarker and may reduce heterogeneity between studies obtaining even more precise results in the meta-analytic statistical analysis. The eligible studies identified NLR cut-off values using different methods, with a wide range (from 1.96 to 4.81) and it was not possible to identify the most effective cut-off as complete data for each study are not available, but summary data for each analysis were reported. Additionally, a non-uniform methodology was also identified in tumour staging, for this reason no subgroup analysis was performed.

5 | CONCLUSION

This study reports the most recent data about the prognostic role of the NLR in HNSCC patients, confirming that a high pre-treatment NLR is associated with a worse prognosis in terms of OS and DFS. Considering the limitations highlighted during the elaboration of this work, the high heterogeneity founded and that the results are supported by a moderate quality of scientific evidence, it is strongly recommended that future studies on this topic should be developed prospectively to make better use of human and economic resources and to confirm the true-positive result that NLR is a prognostic factor for HNSCC.

ACKNOWLEDGEMENTS

Open Access Funding provided by Universita degli Studi di Foggia within the CRUI-CARE Agreement.

CONFLICT OF INTEREST

None.
AUTHOR CONTRIBUTION
Pierluigi Mariani: Conceptualization; Data curation; Formal analysis; Software; Writing – original draft. Diana Russo: Formal analysis; Investigation; Software. Marco Maisto: Data curation; Investigation; Visualization. Giuseppe Troiano: Conceptualization; Data curation; Software; Writing – review & editing. Vito Carlo Alberto Caponio: Investigation; Methodology; Resources. Marco Annunziata: Formal analysis; Methodology. luigi laino: Conceptualization; Funding acquisition; Project administration; Validation; Writing – review & editing.

ETHICAL APPROVAL
Given that this is a systematic review, no ethical approval was required.

PEER REVIEW
The peer review history for this article is available at https://publon ns.com/publon/10.1111/jop.13264.

ORCID
Giuseppe Troiano https://orcid.org/0000-0001-5647-4414

REFERENCES
1. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Prim. 2020;6. 10.1038/s41572-020-00224-3
2. Braakhuis BJM, Leemans CR, Visser O. Incidence and survival trends of head and neck squamous cell carcinoma in the Netherlands between 1989 and 2011. Oral Oncol. 2014;50:670-675. 10.1016/j.oraloncology.2014.03.008
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-674. 10.1016/j.cell.2011.02.013
4. Wu P, Wu D, Zhao L, et al. Inverse role of distinct subsets and distribution of macrophage in lung cancer prognosis: a meta-analysis. Oncotarget. 2016;7:40451-40460. 10.18632/oncotarget.9625
5. Shen M, Hu P, Donskov F, Wang G, Liu Q, Du J. Tumor-associated neutrophils as a new prognostic factor in cancer: a systematic review and meta-analysis. PLoS One. 2014;9:1-10. 10.1371/journal.pone.0098259
6. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014;106:1-11. 10.1093/jnci/dju124
7. Zhang X, Zhang W, Yuan X, Fu M, Qian H, Hu W. Neutrophils in cancer development and progression: roles, mechanisms, and implications (Review). Int J Oncol. 2016;49:857-867. 10.3892/ijo.2016.3616
8. Higgins J, Green S. Cochrane collaboration. cochrane handbook for systematic reviews of interventions. Cochrane Database Syst Rev. 2008;187-235.
9. Moher D, Liberati A, Tetzlaff J, Altman DG, Group TP. Linee guida per il reporting di revisioni sistematiche e meta-analisi: il PRISMA Statement. Evidence. 2015;7:1-36.
10. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting - meta-analysis of observational studies in epidemiology (MOOSE) group. JAMA Neurol. 2000;283:2008-2012.
11. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007;8:1-16. 10.1186/1745-6215-8-16

12. Altman DG, McShane LM, Sauerbrei W, Taube SE. Reporting recommendations for tumor marker prognostic studies (REMARK): explanation and elaboration. PLoS Med. 2012;9:e1001216. 10.1371/journal.pmed.1001216
13. Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting recommendations for tumor marker prognostic studies (REMARK): an abridged explanation and elaboration. J Natl Cancer Inst. 2018;110:803-811. 10.1093/jnci/djy088
14. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction - GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. 2011;64:383-394. 10.1016/j.jclinepi.2010.04.026
15. Miladinovic B, Hozo I, Djulbegovic B. Trial sequential boundaries for cumulative meta-analyses. Stat J. 2013;13:77-91. 10.1177/15365367135100106
16. Chen LM, Ibrahim JG, Chu H. Flexible stopping boundaries when changing primary endpoints after unblinded interim analyses. J Biopharm Stat. 2014;24:817-833. 10.1080/10543406.2014.901341
17. Wiggers J, Thorlund K, Brok J, Gluud C. Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. J Clin Epidemiol. 2008;61:64-75. 10.1016/j.jclinepi.2007.03.013
18. Bobdey S, Ganesh B, Mishra P, Jain A. Role of monocyt e count and neutrophil-to-lymphocyte ratio in survival of oral cancer patients. Int Arch Otorhinolaryngol. 2017;21:21-27. 10.1056/s-0036-1587318
19. Lee S, Kim DW, Kwon S, Kim HJ, Cha IH, Nam W. Prognostic value of systemic inflammatory markers for oral cancer patients based on the 8th edition of AJCC staging system. Sci Rep. 2020;10:1-9. 10.1038/s41598-020-6891-3
20. 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;99:371-378. 10.1177/0145561319876910
21. Zhang B, Du W, Gan K, Fang Q, Zhang X. Significance of the neutrophil-to-lymphocyte ratio in young patients with oral squamous cell carcinoma. Cancer Manag Res. 2019;11:7597-7603. 10.2147/CMAR.021847
22. Kao HK, Löstrand J, Loh CYY, et al. Nomogram based on albumin and neutrophil-to-lymphocyte ratio for predicting the prognosis of patients with oral cavity squamous cell carcinoma. Sci Rep. 2018;8:1-9. 10.1038/s41598-018-31498-z
23. Yang J, Hsueh CY, Cao W, Zhou L. Pretreatment lymphocyte-to-monocyte ratio as an independent prognostic factor for hypopharyngeal squamous cell carcinoma. Acta Otolaryngol. 2018;138:734-740. 10.1080/00016489.2018.1449965
24. Wu CN, Chung HC, Lin YT, Fang FM, Li SH, Chien CY. Prognosis of neutrophil-to-lymphocyte ratio in clinical early-stage tongue (cT1- T2N0) cancer. Onco Targets Ther. 2017;10:3917-3924. 10.2147/OTT.S140800
25. Ikeguchi M. Glasgow prognostic score and neutrophil-to-lymphocyte ratio are good prognostic indicators after radical neck dissection for advanced squamous cell carcinoma in the hypopharynx. Langenbeck’s Arch Surg. 2016;401:861-866. 10.1007/s00423-016-1453-9
26. Fu Y, Liu W, Ouyang D, Yang A, Zhang Q. Preoperative neutrophil-to-lymphocyte ratio predicts long-term survival in patients undergoing total laryngectomy with advanced laryngeal squamous cell carcinoma. Med (United States). 2016;95:1-6. 10.1097/MD.0000000000002689
27. Song Y, Liu H, Gao L, et al. Preoperative neutrophil-to-lymphocyte ratio as prognostic predictor for hypopharyngeal squamous cell carcinoma after radical resections. J Craniofac Surg. 2015;26:e137-e140. 10.1097/SCS.0000000000001235
28. Fang HY, Huang XY, Chien HT, et al. Refining the role of preoperative C-reactive protein by neutrophil/lymphocyte ratio in oral
cavity squamous cell carcinoma. Laryngoscope. 2013;123:2690-2699. 10.1002/lary.24105

29. Tu XP, Qiu QH, Chen LS, et al. Preoperative neutrophil-to-lymphocyte ratio is an independent prognostic marker in patients with laryngeal squamous cell carcinoma. BMC Cancer. 2015;15:1-7. 10.1186/s12885-015-1727-6

30. Hasegawa T, Iga T, Takeda D, et al. Neutrophil-lymphocyte ratio as associated with poor prognosis in oral cancer: a retrospective study. BMC Cancer. 2020;20:1-9. 10.1186/s12885-020-07063-1

31. Wang J, Wang S, Song X, et al. The prognostic value of systemic and local inflammation in patients with laryngeal squamous cell carcinoma. Onco Targets Ther. 2016;9:7177-7185. 10.2147/OTT.5113307

32. Lo WC, Wu CT, Wang CP, et al. The pretreatment neutrophil-to-lymphocyte ratio is a prognostic determinant of T3–4 hypopharyngeal squamous cell carcinoma. Ann Surg Oncol. 2017;24:1980-1988. 10.1245/s10434-017-5865-8

33. de Almeida JR, Yao CMKL, Ziai H, et al. Postoperative wound infections, neutrophil-to-lymphocyte ratio, and cancer recurrence in patients with oral cavity cancer undergoing surgical resection. Oral Oncol. 2019;97:23-30. 10.1016/j.joraloncol.2019.07.023

34. Chen H, Song S, Zhang L, Dong W, Chen X, Zhou H. Preoperative platelet-lymphocyte ratio predicts recurrence of laryngeal squamous cell carcinoma. Futur Oncol. 2020;16:209-217. 10.2217/ fon-2019-0527

35. Chen S, Guo J, Feng C, Ke Z, Chen L, Pan Y. The preoperative platelet-lymphocyte ratio versus neutrophil-lymphocyte ratio: which is better as a prognostic factor in oral squamous cell carcinoma? Ther Adv Med Oncol. 2016;8:160-167. 10.1177/1758834016638019

36. 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;18:1-9. 10.1186/s12885-018-4730-x

37. Chen F, Lin L, Liu F, et al. Three prognostic indexes as predictors of response to adjuvant chemoradiotherapy in patients with oral squamous cell carcinoma after radical surgery: a large-scale prospective study. Head Neck. 2018;41(2):301-308. 10.1002/hed.25495

38. Lu Z, Yan W, Liang J, et al. Nomogram based on systemic immune-inflammation index to predict survival of tongue cancer patients who underwent cervical dissection. Front Oncol. 2020;10:1-11. 10.3389/fonc.2020.00341

39. Lu HJ, Tseng SW, Peng CY, et al. Predictors of early progression after curative resection followed by platinum-based adjuvant chemoradiotherapy in oral cavity squamous cell carcinoma. Postgrad Med. 2021;133:377-384. 10.1007/s00354-020-00986-9

40. Zhou S, Yuan H, Wang J, et al. Prognostic value of systemic inflammatory marker in patients with head and neck squamous cell carcinoma undergoing surgical resection. Futur Oncol. 2020;16:559-571. 10.2217/fon-2020-0010

41. Szlasi Z, Jósa V, Zrubka Z, 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;17:1742. 10.3390/ijerph17051742

42. Ye J, Liao B, Jiang X, et al. Prognosis value of platelet counts, albumin and neutrophil-lymphocyte ratio of locoregional recurrence in patients with operable head and neck squamous cell carcinoma. Cancer Manag Res. 2020;12:731-741. 10.2147/CMAR.5234618

43. Tazeen S, Prasad K, Harish K, Sagar P, Kapali AS, Chandramouli S. Assessment of pretreatment neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in prognosis of oral squamous cell carcinoma. J Oral Maxillofac Surg. 2020;78:949-960. 10.1016/j.joms.2020.01.001

44. Zhong B, Deng D, Du JT, Chen F, Liu YF, Liu SX. Prognostic value of the preoperative neutrophil to lymphocyte ratio in patients with sinonasal squamous cell carcinoma. Cancer Manag Res. 2019;11:9733-9741. 10.2147/CMAR.5231085

45. Mandó P, Rizzo M, Roberti MP, et al. High neutrophil to lymphocyte ratio and decreased CD69+NK cells represent a phenotype of high risk in early-stage breast cancer patients. Onco Targets Ther. 2018;11:2901-2910. 10.2147/OTT.S160911

46. Fogar P, Sperti C, Basso D, et al. Decreased total lymphocyte counts in pancreatic cancer: an index of adverse outcome. Pancreas. 2006;32:22-28. 10.1097/01.mp.0000188305.90290.50

47. Loi S, Sirtaine N, Piette F, et al. Predictive and prognostic value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013;31:860-867. 10.1200/JCO.2011.41.0902

48. Lang BHH, Ng CPC, Au KB, Wong KP, Wong KKC, Wan KY. Does preoperative neutrophil lymphocyte ratio predict risk of recurrence and occult central nodal metastasis in papillary thyroid carcinoma? World J Surg. 2014;38:2605-2612. 10.1007/s00268-014-2630-z

49. Islam MM, Faruque MRI, Islam MT. A compact disc-shaped printed antenna using parasitic element on ground plane for super wide-band applications. Appl Comput Electromagn Soc J. 2016;31:960-969. 10.1038/ni.2703.Innate

50. Chen Y, Yan H, Wang Y, Shi Y, Dai G. Significance of baseline and pretreatment neutrophil-to-lymphocyte ratio in predicting prognosis: a retrospective analysis in advanced pancreatic ductal adenocarcinoma. Sci Rep. 2017;7:1-9. 10.1038/s41598-017-00859-5

51. Coffelt SB, Wellenstein MD, De Visser KE. Neutrophils in cancer: neutral no more. Nat Rev Cancer. 2016;16:431-446. 10.1038/nrc.2016.52

52. Abbate V, Dell’Aversana Orabona G, Salzano G, 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;27:503-507. 10.1016/j.suronc.2018.06.002

53. Robinson AV, Keeble C, Lo MCI, et al. The neutrophil–lymphocyte ratio and locoregional melanoma: a multicentre cohort study. Cancer Immunol Immunother. 2020;69:559-568. 10.1007/s00026-019-02478-7

54. 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. 2018;2019:1525-1535. 10.1002/hed.25583

55. Yu Y, Wang H, Yan A, et al. Pretreatment neutrophil to lymphocyte ratio in determining the prognosis of head and neck cancer: a meta-analysis. BMC Cancer. 2018;18:1-9. 10.1186/s12885-018-4230-z

56. 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;40:1091-1100. 10.1002/hed.25075

57. Cho JK, Kim MW, Choi IS, et al. Optimal cutoff of pretreatment neutrophil-to-lymphocyte ratio in head and neck cancer patients: a meta-analysis and validation study. BMC Cancer. 2018;18:1-9. 10.1186/s12885-018-4876-6

58. Takenaka Y, Oya R, Kitamura T, et al. Prognostic role of neutrophil-to-lymphocyte ratio in determining the quality of evidence - Inconsistency. J Clin Epidemiol. 2016;69:1294-1302. 10.1016/j.jclinepi.2015.06.017

59. Pogue JM, Yusuf S. Cumulating evidence from randomized trials: utilizing sequential monitoring boundaries for cumulative meta-analysis. Control Clin Trials. 1997;18:580-593. 10.1016/S0197-2456(97)00051-2
62. Miladinovic B, Kumar A, Hozo I, Mahony H, Djulbegovic B. Trial sequential analysis may be insufficient to draw firm conclusions regarding statistically significant treatment differences using observed intervention effects: a case study of meta-analyses of multiple myeloma trials. *Contemp Clin Trials*. 2013;34:257-261. 10.1016/j.cct.2012.12.006

**SUPPORTING INFORMATION**
Additional supporting information may be found in the online version of the article at the publisher’s website.

**How to cite this article:** Mariani P, Russo D, Maisto M, et al. Pre-treatment neutrophil-to-lymphocyte ratio is an independent prognostic factor in head and neck squamous cell carcinoma: Meta-analysis and trial sequential analysis. *J Oral Pathol Med*. 2022;51:39–51. doi:10.1111/jop.13264