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REVIEW ARTICLE

The prognostic role of the pre-treatment neutrophil to lymphocyte ratio (NLR) and tumor depth of invasion (DOI) in early-stage squamous cell carcinomas of the oral tongue

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Abstract

The appropriate surgical management of early-stage oral tongue squamous cell carcinoma (OTSCC) remains a debated topic. The aim of this study is to investigate the role of the pre-treatment neutrophil to lymphocyte ratio (NLR) and tumor depth of invasion (DOI) in predicting the presence of occult neck metastases in early-stage OTSCC. A retrospective analysis of patients affected by early-stage (cT1-T2 cN0) OTSCC who were submitted to elective neck dissection (END) was performed. Tumors were classified retrospectively according to the 8th TNM classification, the DOI was assessed on the pre-operative magnetic resonance imaging, and the pre-treatment NLR was calculated for each patient. A logistic regression model to estimate the probability π (x) of cervical metastases by studying the NLR and DOI was carried out. Next, the correlation between the two variables, the NLR and DOI, was preliminarily studied. A cohort of 110 patients was analyzed (mean age, 62 years old; male to female ratio 1.2:1). The patients were staged as cT1 in 53 cases and cT2 in 57 cases. A DOI greater than 5.4 mm and a NLR greater than 2.93 are associated with an increased risk of presenting occult cervical metastases. Furthermore, the variables NLR and DOI are linearly associated with a positive correlation, proved by Spearman's rank correlation coefficient rho of 0.64, with a unitary increase in the DOI of 1 mm directly associated with an increase of 0.47 in the NLR. The DOI and NLR can be effectively used to predict the occurrence of occult neck metastasis and therefore to plan an END in early-stage OTSCC.

Keywords Oral tongue squamous cell carcinoma · Neutrophil to lymphocyte ratio (NLR) · Depth of invasion (DOI) · Occult neck metastasis

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Introduction

Different tumor markers might be useful to estimate the risk of occult metastases in early-stage oral tongue squamous cell carcinoma (OTSCC), such as the thickness of tumor infiltration, degree of differentiation, lymphovascular invasion, tumor budding, perineural invasion, depth of invasion (DOI), neutrophil to lymphocyte ratio (NLR), and E-cadherin expression [1]. However, despite these parameters, a proper identification of patients at a greater risk of cancer spreading from the neck nodes is still far from being elucidated [2–4].

The DOI of the primary tumor is currently considered the most reliable prognostic parameter [4]. Different cut points of the DOI ranging from 4 to 10 mm have been proposed in order to detect occult disease in the neck nodes, also

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associated with survival rates [5–10]. The eighth and most recent edition of the American Joint Commission on Cancer (AJCC) includes the DOI in the TNM classification criteria for oral cancer, emphasizing its prognostic relevance and reinforcing the need for a further definition of the optimal cut points in order to design appropriate treatment strategies [11–13].

Finally, an additional parameter has recently gained importance, namely the relationship between the pre-treatment neutrophils and lymphocytes in head and neck cancers, the NLR. Previous studies have demonstrated its prognostic value [14–17], especially in relation to cT1-T2 clinically negative neck (cN0) OTSCC. Abbate et al. have demonstrated how an increase in the NLR pre-operative value can be related to occult metastasis [18].

The aim of this study has been to investigate the role of the pre-treatment NLR and tumor DOI in predicting the presence of occult neck metastases in early-stage OTSCC with a cNO. The expected result has been to obtain additional parameters useful in the selection of the most appropriate strategy for the neck management in such cases.

Materials and methods

A retrospective analysis of patients affected by cT1-T2 cN0 OTSCC who were submitted to END between December 2004 and December 2016 at the Maxillofacial Surgery Department of the University Federico II of Naples was performed. The data were retrieved from the Institutional database. The study was conducted in accordance with the Helsinki Declaration and with policies approved by the local ethics council.

The inclusion criteria were organized as follows:

- Tumor characterization criteria: primary OTSCC histologically confirmed in an early stage (cT1-T2); lesion not previously treated surgically with END or by radiotherapy or chemotherapy; no previous tumors at other sites
- Diagnostic criteria: OTSCC without the presence of any regional (cN0) or distant (cM0) metastases assessed using neck ultrasound (US), head and neck contrast-enhanced CT and/or MRI, or a total body CT or PET scan
- No clinical distortions of the NLR: infections, serum virus markers, chronic inflammatory diseases, autoimmune hematological disorders, or simultaneous or long-term anti-inflammatory/steroidal treatments

The diagnostic workup for all the patients involved a complete physical examination, routine blood count, liver function test, neck US, and full-body CT and/or MRI with contrast. The DOI measurements on contrast-enhanced MRI were performed in patients without any contraindications, with a cutting thickness of 1 mm. The scanning protocol included T1 (repeat time (TR) 912 ms, echo time (TE) 17 ms) and T2 (TR 5000 ms, TE 99 ms) axial, coronal, and sagittal sequences together with axial and coronal T2 sequences with fat suppression (FS) (TR 5830 ms, TE 99 ms) (Fig. 1). For patients where it was not possible to perform a pre-operative MRI, DOI measurements were performed on CT images. All the tumors were classified retrospectively according to the eighth edition of the TNM classification staging system [12]. From a histological point of view, OTSCC has been classified according to the World Health Organization classification system, 4th edition, which is recognized and used worldwide [13]. The NLR was obtained as the absolute neutrophil count divided by the absolute lymphocyte count. After surgical treatment, based on the final histology report, the patients were divided into two groups according to the presence (pN + group) or absence (pN0 group) of occult neck node metastases. Informed written consent was obtained from all the patients before any diagnostic or therapeutic procedure.

Statistical analysis

A logistic regression model with the statistical software R 3.3.0 (R Development Core Team, Auckland, 2017) was used to estimate the probability π (x) of cervical metastases by studying the NLR level in blood tests [19]. The analysis was performed using Newton–Raphson's iterative algorithm.

$$\pi(x) = \frac{\exp(\text{Intercept} + \beta x)}{1 + \exp(\text{Intercepy} + \beta x)}$$

The intercept and β parameters were estimated using the statistical software R3.3.0. The procedure used is based on the maximum likelihood estimation (MLE) of the parameters, in accordance with Newton–Raphson's iterative algorithm. The same logistic regression model was used to study the relationship between the thickness in millimeters of the DOI and the presence of occult laterocervical metastases. By setting "X" as the variable that represents the length of the DOI resulting from the clinical analyses, we aimed to evidence the probability of the patient proceeding to present occult metastases. In this context, the variable under study can be represented through a binomial variable (presence/absence of metastasis), thereby providing a logistic regression model to study the relationship between the probability of having metastasis in the neck and the thickness in mm of the DOI. Finally, the correlation between the two variables, NLR and DOI, was preliminarily studied. A regression analysis was therefore performed, using the NLR as a dependent variable and the DOI as an independent variable.

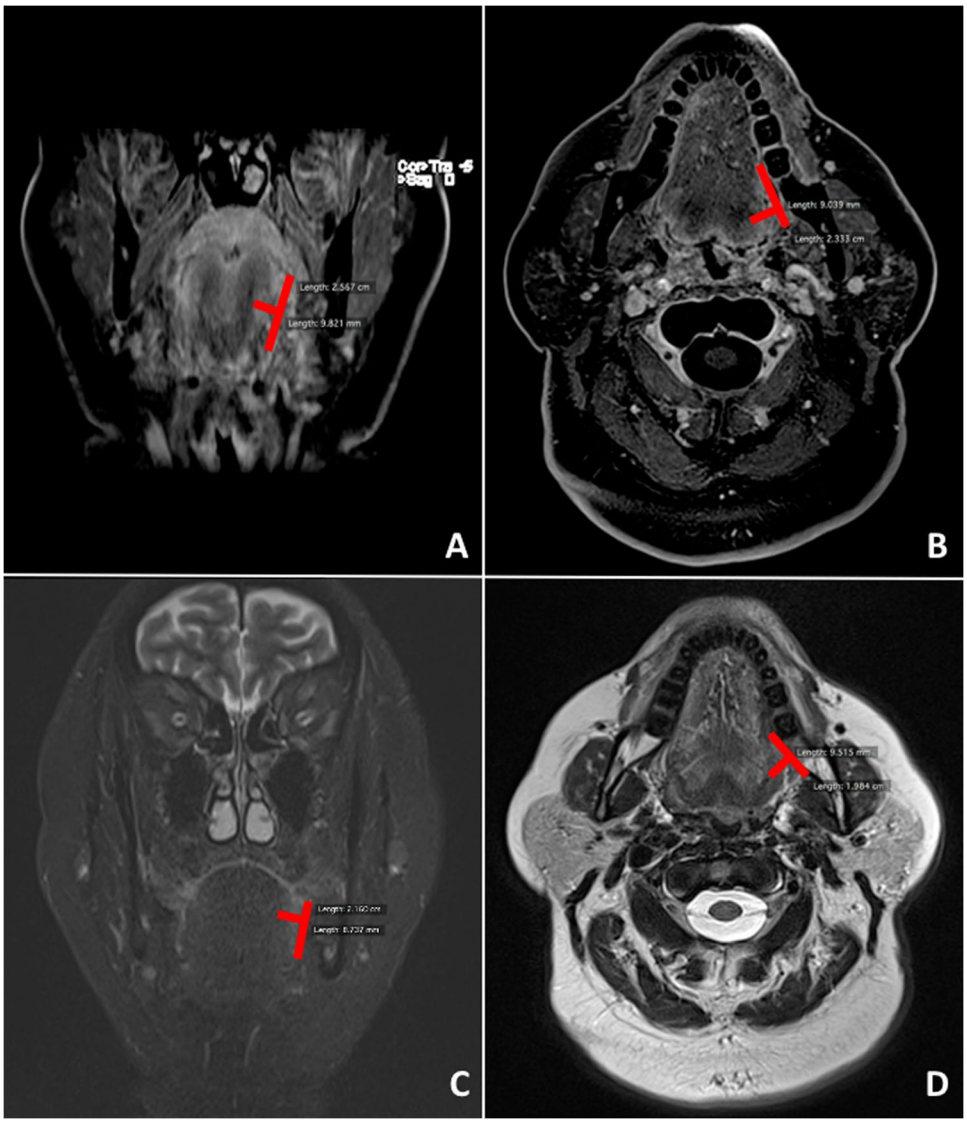

Fig. 1 The DOI measurement on MRI: coronal MRI t1 (**A**), axial MRI t1 (**B**), coronal MRI t2 (**C**), Axial MRI t2 (**D**)

Results

Of the 338 patients with OTSCC treated in our institution during the study period, 228 were excluded from this study because they did not meet the inclusion criteria:

- Two hundred five patients were excluded by reason of a non-applicability of the NLR due to the lack of any surgical indications:
  - One hundred fourteen patients were excluded on account of the advanced stage of the OTSCC (over cT1-T2);
  - Twenty-seven patients were affected by a recurrence of the OTSCC;
  - Eleven patients presented with distant metastases;
  - Eighteen patients had previously had other neoplasms (prostate, breast, lung, or colorectal cancer);
  - Thirteen patients had received transoral surgery for a removal of the primary tumor in which cases a "watchful waiting" was adopted to manage the cNO;
- Twenty-three patients more were excluded on account of comorbidities that could potentially influence and misrepresent the NLR value.

Finally, 110/338 (32.5%) met the inclusion criteria. Their mean age was 62 years (range, 19–88 years).

In all cases, the treatment modality was a complete removal of the primary OTSCC with free margins (> 5 mm) associated with END (levels from I to IV). In 4 cases, where the primary OTSCC transpassed the midline, a bilateral

END was performed. On the final histology report, 39/110 (35%) patients resulted positive for lymph node metastases (pN + group) while 71/110 (65%) patients tested negative (pN0 group). The clinical and pathological data of the two groups have been summarized in Table 1.

Of note, a perineural invasion was discovered by histological evaluation in 20% of the samples, 69% of which derived from patients who were declared smokers (Table 1).

To study the probability of developing occult laterocervical metastases considering the thickness in millimeter of the DOI, a logistic regression model was performed, as follows:

$$\pi(x) = \frac{\exp(-6.4618 + 1.1918x)}{1 + \exp(-6.4618 + 1.1918x)}$$

The odds ratio (OR), equal to exp (1.1918) = 3.29, represented the relationship and showed that a unit increase (1 mm) in the thickness in millimeter of the DOI corresponded to an increase of 3.29 in the propensity to present occult neck metastases, not evident by clinical and radiological examinations.

To obtain a 90% probability level of occult neck metastasis in T1-T2 cN0 OTSCC patients, the DOI length should be at least equal to:

$$\text{DOI} = \frac{\log\left(\frac{p}{(1-p)*\exp(\text{Intercept})}\right)}{\beta_{\text{DOI}}} = 7.26$$

The estimated level was illustrated graphically in a Cartesian model, with the abscissa axis representing the probability and the ordinate axis the thickness in millimeters of the DOI. In this graphical function, it is crucial to define the inflection point, which is the point where the straight line tangent to the curve crosses the curve itself, changing its concavity. This point is determined by canceling the second derivative of the curve. It corresponds to a DOI of 5.42857 mm and a 50.2% probability of presenting occult neck metastases (Fig. 2).

The mathematical relationship between the NLR and the probability of developing occult cervical metastases has been obtained with the same model:

$$\pi(x) = \frac{\exp(-3.3178 + 1.1280\text{x})}{1 + \exp(-3.3178 + 1.1280\text{x})}$$

With every 10 units representing an increase in the probability of the occurrence of occult cervical metastases, corresponding to a NLR value ranging from 0.99 (10%) to 7.63 (100%) with a non-linear distribution, a NLR value of 2.93 for a 49.91% probability of finding occult metastatic nodes was calculated in our model. This point corresponds to the inflection point of our distribution curve (Fig. 3).

Finally, the correlation between the two variables NLR and DOI was studied. The NLR and DOI variables are linearly associated with a positive correlation resulting in a Spearman rho correlation coefficient equal to 0.64. Given this connection between the two variables of interest, it was considered appropriate to investigate the linear relationship that may link them. Therefore, a regression analysis was implemented, considering the NLR as the dependent variable and the DOI as the independent variable. The estimated regression coefficient was set at 0.47 obtaining statistically significant values with a 95% confidence level. In other words, a unit increase (1 mm) of the DOI variable resulted in an increase of 0.47 in the NLR variable. The estimated model is robust having passed the *F* test, which measures the

Table 1 The clinicopathological parameters of our sample

		pN0 group (71 cases)	pN + group (39 cases)
Gender	Male	38	22
	Female	33	17
Age	Range	19–88	24–87
	Mean	58	64
Smokers	Yes	47	29
	No	24	10
Alcohol	Yes	19	24
	No	52	15
pT classification	pT1	38	15
	pT2	33	24
DOI	Range (mm)	2.4–6.5 mm	3.7–9.81 mm
	Mean (mm)	3.36 mm	7.28 mm
NLR	Range	0.27–5.17	1.21–8.21
	Average	2.11	2.88
Perineural invasion	Present	6	16
	Absent	65	23

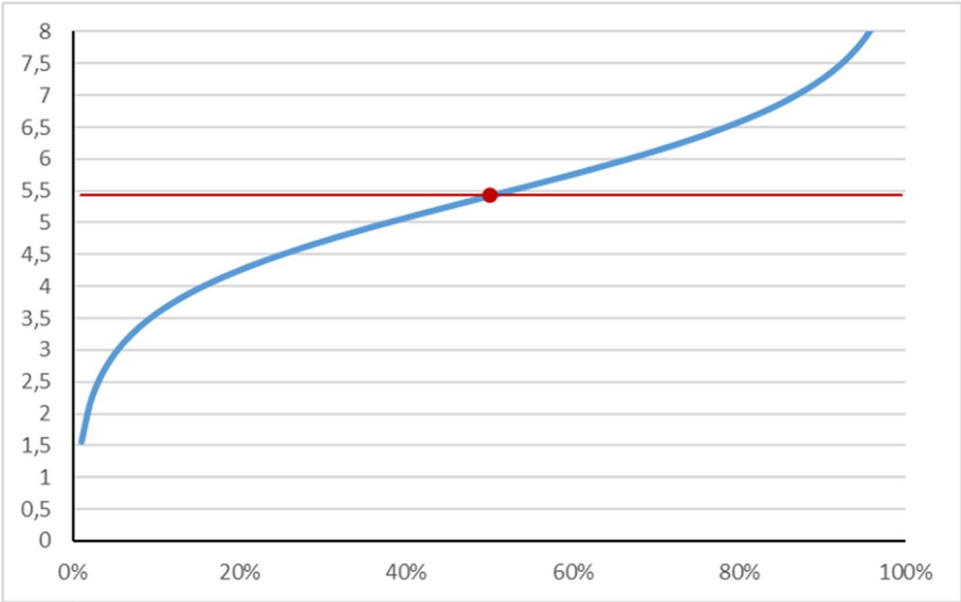

Fig. 2 The graph represents the estimated model, showing the probabilities on the abscissa axis and the DOI length on the ordinate axis. The red dot indicates the inflection point. The point corresponds to a length of 5.42857 and a probability of 50.20%

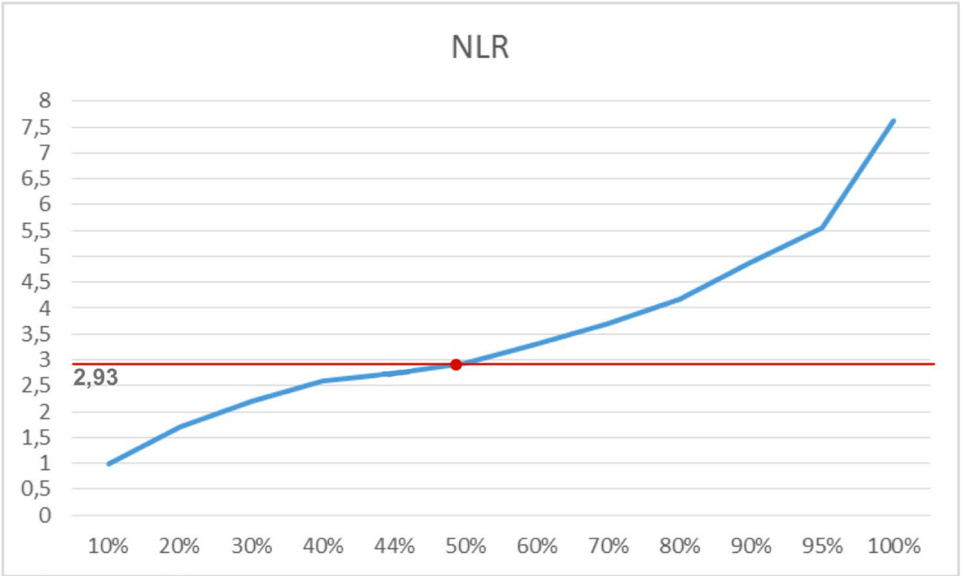

Fig. 3 Curve showing the mathematical relationship between the NLR and the probability of developing occult cervical metastases. The bold dot corresponds to the curve's inflection point. The corresponding NLR value is 2.93 with a probability of developing occult metastasis of 49.91%

goodness of the estimated model by assessing whether the group of independent variables considered is appropriate to explain the variance of the model, and h having an *R* square close to 1 or equal to 0.88. In fact, the higher this coefficient is, the better the DOI dependent variable can predict the value of the independent variable NLR.

Discussion

OTSCCs are the neoplasms of the oral cavity that can more easily develop lymph node metastases [19]. This propensity is also present in early-stage OTSCC, with a cumulative incidence described in the literature ranging from 8 to 46% [19]. Although the risk of occult neck metastases can be high, the appropriate management of the cN0 neck represents a debated topic.

Nowadays, the three main therapeutic strategies used for the management of the cN0 neck in this subset of patients are sentinel lymph node biopsy (SLNB), END, and watchful waiting. SLNB has been proposed as an alternative to neck lymph node dissection in early-stage OTSCC, with encouraging but very preliminary results [20]. In 2015, the Sentinel European Node Trial (SENT) reported a total sensitivity and a negative predictive value in the controlled National Bank of 86% and 95%, respectively, in a sample of 415 patients [20]. Regarding the watchful waiting approach, D'Cruz et al. described a prospective study of 500 patients treated with

END as compared with watchful waiting, reporting higher rates of overall and disease-free survival in the group submitted to neck dissection [21]. However, systematic END, although ensuring greater oncological safety as regards the control of occult neck metastases, is an invasive surgical treatment that can result in potentially serious post-operative complications in a variable percentage of cases, ranging from 20–40%. These may include skin scars with a cervical asymmetry, a possible paresis of the lower lip, pain and a loss of shoulder function (shoulder syndrome) in 29–39% of cases. Moreover, an overtreatment may result in 70–80% of patients having negative neck lymph nodes on the final histology report [22–24].

Therefore, the identification of factors associated with an increased risk of occult neck metastasis in early-stage OTSCC would be paramount to better distinguish patients requiring END from those who need only to be monitored according to a clinical and radiological watchful waiting policy.

Several variables have been described in the literature as being able to estimate the risk of occult metastasis in cNO early-stage OTSCC, including tumor grading, tumor thickness, tumor budding, perineural invasion, lympho-vascular invasion, the expression of E-Cadherin, the DOI, and the NLR.

To the best of our knowledge, this is the first study analyzing the association between two predictive markers of occult neck lymph node metastasis, namely the DOI and NLR.

The most recent edition of the AJCC staging system incorporated the DOI in the TNM classification of oral cavity cancers, emphasizing its prognostic importance. In detail, a DOI > 5 mm classifies the neoplasm as T2 even in tumors with a dimension < 2 cm. The DOI can be measured on pre-operative imaging and confirmed by an analysis of the intra-operative and post-operative pathological sections. Over the last few years, several studies have analyzed the tumor thickness and DOI to evaluate the prognosis of patients affected by OTSCC [4, 25–36]. However, there are several problems that make the interpretation of these results difficult. In particular, there has been some confusion about the definition of the DOI and tumor thickness [6–9]. Nowadays, it is well established that the DOI is obtained by drawing a line perpendicular to that passing through the basal membrane of the normal squamous epithelium, contiguous to the neoplasm, up to the deepest tumor invasion point. Conversely, "the tumor thickness" is the perpendicular distance between the highest point of the tumor surface and the deepest point of the infiltrative front of the tumor, taking into consideration both the exophytic and the infiltrative components of the tumor [10, 11].

The suggested cut points for the DOI value have varied significantly from 2 to 10 mm according to different studies. In this regard, Brockhoff II et al. in their study of 286 patients with a diagnosis of oral squamous cell carcinoma showed that, for a DOI of 1 mm or less, there were no patients with a positive node. For a DOI from 1.1 to 2 mm, 1 out of 11 patients (9%) had at least 1 positive node. At 2.1 mm to 3 mm, 5 out of 25 patients (20%) had at least 1 positive node [37]. However, the majority of the cut points have been proposed a priori and only a few studies have analyzed the prognostic impact of the DOI considering it as a continuous variable. Table 2 summarizes the most relevant studies addressing the role of the DOI in defining appropriate treatment strategies for OTSCC.

When analyzing the literature on this topic, multiple confounding factors should be considered. First, the small sample size of the cohort analyzed may undermine the significance of the results obtained. Secondly, the inclusion of oropharyngeal cancers together with oral cavity cancers can be confusing. Thirdly, the DOI and tumor thickness were considered alternatively in different studies, and their definition as well as their measurement methods are not always clear, obscuring even more the data described. In this regard, any DOI measurement based on the T1 sequence can overestimate the values due to inflammation or a bloating of local tissues after the biopsy, while the T2 sequences are preferred for DOI measurement [34, 35].

Compared to other studies, our research has been conducted on a well-selected cohort of 110 patients affected by OTSCC, excluding other oral subsites and patients with confounding factors. In our study, the MRI procedures were performed with 1-mm cuts to obtain the maximum precision of the DOI assessment, and all the measurements were based on T2 sequences to ensure a consistency between the measured data. An average DOI of 3.36 mm was detected in the pN0 group while 7.28 mm in the pN + group. A limitation of this paper, related to the retropective nature of the study, is that only the DOI on the pre-operative MRI was analyzed, without considering its value also on histopathological sections. Future prospective studies are required to investigate the correlation between the DOI assessments on the pre-operative MRI and post-operative final histology report.

A further challenge, in our experience, could be measuring the DOI by replacing MRI with US. In this regard, rather encouraging results have been obtained by Valentini et al. in a recent 2020 study based on 32 patients with biopsy-proven OSCC who had undergone pre-operative intraoral US and were retrospectively reviewed. They verified a US sensitivity of 93.1%, a specificity of 100%, a PPV of 100%, and a NPV of 60% in the assessment of the infiltration of the tumor beyond the lamina propria into the submucosa. A significant correlation was found between the measurements of the US DOI and histological DOI ($r = 0.907$) [38]. Tarabichi et al. in their review considered 19 out of 104 articles analyzed (six hundred seventy-eight patients), ascertaining that US

Table 2 The most relevant studies addressing the role of the DOI in defining appropriate treatment strategies for OTSCC

Author	Study design	TOT. Patients	Primary lesion	DOI & thickness evaluation	Results	Limits
Akira B. [31]	Retrospective cohort study	53	Tongue squamous cell carcinoma (SCC)	Cutoff for END: 4 mm (radiological analysis: MRI)	Pathological DOI in UL was significantly smaller than that of DL (average 1.7 vs 4.6 mm, $p < 0.001$). Cutoff value between UL group and DL group was 3.5 mm (sensitivity 96%, specificity 75%). 96% of ULs had pathological DOI smaller than 4 mm, the recommended cutoff value for neck dissection	It was retrospective, included only a small number of patients, and single-center study. Larger studies would be needed to confirm results
Arora A. (2017) [29]	Retrospective cohort study	336	Oral cavity squamous cell carcinoma (OSCC)	4 mm to detect lymph node metastasis sensitivity 92%, specificity of 91% (histopathological analysis)	Survival score in LN metastasis: from 7 to 11: 6.4%, 12 to 16: 22.8%, > 17: 77.1%	It included every secondary site of the oral cavity: not all the 336 patients had undergone END: the patients were predominantly of one nationality Indian
Brockhoff H.C. [39]	Retrospective cohort study	286	Oral cavity squamous cell carcinoma (OSCC)	For a depth of invasion of 1 mm or less, there were no patients with a positive node. From 1.1 to 2 mm of the depth of invasion, there was 1 of 11 patients (9%) who had at least 1positive node. At 2.1 mm to 3 mm, 5 of 25 patients (20%) had at least 1positive node	At a 2-mm depth of invasion, there were 12 patients with negative node necks and 3 with positive node necks for an overall percentage of 20%. Increasing DOI beyond 2 mm demonstrated a greater than 20% node positivity rate reaching a rate higher than 50% at 10 mm. The DOI was significantly associated with node positivity (odds ratio, 1.3; p value 5.002)	It was retrospective, included only a small number of patients
Dirven R. (2017) [28]	Retrospective cohort study	456	Oral cavity squamous cell carcinoma (OSCC)	Mean DOI 9.5 mm vs mean thickness 10 mm (histopathological analysis)	Median difference thickness-DOI was 0 mm and the mean 0.7 mm (thickness > DOI) 26/456 (5.7%) classified in a different AJCC 8 T category	The variability over the extended period of the study in terms of pathology protocols used to assess DOI and inter-observer variability among pathologists over the last 30 years

Table 2 (continued)

Author	Study design	TOT. Patients	Primary lesion	DOI & thickness evaluation	Results	Limits
Ebrahimi A. (2014) [27]	International multicenter retrospective cohort study	3149	Oral cavity squamous cell carcinoma (OSCC)	5 mm for T1 tumors and at 10 mm for T2-4 mean DOI: 12.9 mm and 10.0 mm (histopathological analysis)	Statistically significant association between increasing DOI disease: pT category ($P < .001$) pN category ($P < .001$) extracapsular spread ($P < .001$), involved margins ($P < .001$)	The multicentric characteristic of the study did not permit a uniform definition of the DOI and several centers measured the tumor thickness rather than the DOI
Kwon M. (33)	Retrospective cohort study	53	Tongue squamous cell carcinoma (SCC)	The cutoff value of TT on the axial/coronal/sagittal MRI predicting occult LN metastasis was 6.7 mm, 7.2 mm, and 12.3 mm, respectively ($p < 0.05$) (radiological analysis: MRI)	TT on MRI in each plane showed relatively high concordance rates with the histological measurements. TT in all three planes was significantly correlated with lymph node (LN) metastasis	Relatively small study population and limited MRI values including image artifacts. In addition, relatively short time for clinical application of three-dimensional scanning including a sagittal view on MRI and the lack of follow-up data on the subjects
Lam P. (2004) [35]	Prospective study	18	Tongue squamous cell carcinoma (SCC)	The overall accuracy in the assessment of tumor thickness staging using contrast-enhanced T1-weighted and T2-weighted images was 83% and 56%, respectively (radiological analysis: MRI)	The tumoral thickness infiltration tested on the MRI in the T1 sequence was 0.8 mm greater than the pathological sections. The tumor thickness infiltration on the MRI at the T2 sequence was greater 2 mm greater than the average pathological section	It was retrospective, included only a small number of patients
Moore C. (1986) [36]	Retrospective cohort study	151	Upper aerodigestive tract cancer (UADT)	The thickness measurements yielded more even gradations of survival than did surface diameters (histopathological analysis)	Thickness was found to be more closely related to node metastasis and to survival than was surface diameter in middle-stage tumors, particularly in the tongue, floor of the mouth, buccal mucosa, gum, and soft palate sites	It was retrospective, included a relatively small number of patients
Park J.O. (2011) [34]	Retrospective cohort study	114	Oral/oropharyngeal carcinoma	Cutoff in determining positive nodes: 9.5 mm in oral cancer and 14.5 mm in tongue base cancer. (radiological analysis: MRI)	The mean histologic and MRI invasion depths were 13.57 ± 8.476 and 15.24 ± 10.700 mm, respectively	The relatively small number of cases for each subsite and errors caused by tissue shrinkage during preparation

Table 2 (continued)

Author	Study design	TOT. Patients	Primary lesion	DOI & thickness evaluation	Results	Limits
Tam S. (2019) [4]	Retrospective cohort study	212	Oral cavity squamous cell carcinoma (OSCC)	DOI cutoff for detection of occult nodal metastasis was 7.25 and 8 mm for OS and DSS (histopathological analysis)	Occult nodal disease was found in 55 (26%) of the 212 patients. DOI was an independent predictor of OS and DSS	The retrospective nature of cohort study. Secondly, patients who did not undergo elective neck dissection were not included in this study. This exclusion may bias calculated optimal DOI cutoff. The rate of locoregional recurrence was low in the study cohort, limiting the ability to investigate this end point
Xu C. (2020) [30]	Retrospective cohort study	151	Tongue squamous cell carcinoma (SCC)	Cutoff: 7.5 mm for neck lymph node metastasis sensitivity of 86.9% (radiological analysis: MRI)	END is suggested if DOI is > 7.5 mm in cT1N0 tongue SCC, and DOI ≥ 7.5 mm indicates additional risk for disease recurrence and cancer-related death	The statistical power was decreased by the inherent bias in a retrospective study, it had a small sample size, and additional large randomized control trials are needed
Yesuratnam A. (2014) [32]	Prospective study	88	Tongue squamous cell carcinoma (SCC)	Difference between the histology and T1 post-MRI contrast cut MRI moderate correlation with histological TT (radiological analysis: MRI)	The mean difference between TT histology and T1 post-contrast MRI was 2.99 mm (SD 4.41 mm) and between histology and T2-weighted MRI was 3.19 mm (SD 4.87 mm)	Limitations of study related to prospective observational analyses: patients in the study were managed based on clinical and radiological findings. This means that not all patients had resection of the primary lesion and nodal dissection. In addition, there was some variability in imaging obtained

tumor thickness measurements correlate well with histopathology and show promise as a predictor of cervical lymph node metastasis. The principal advantage of US consists in its safe use during surgery for an in-depth evaluation of the margins [39].

Moreover, in this study, the association of the DOI with a pre-operative value of the NLR was analyzed for the first time in the literature. It is well established how the inflammatory response plays a strategic role in the development of the tumor, influencing also the survival outcomes of the patients. Several studies have demonstrated how the neutrophils can inhibit the immunity system by suppressing the lymphocytes, the active T cells, and the natural killer cells (NK cells) [40–42]. The leukocytes propagate in the transformed cells also, releasing cytokines that activate protumorigenic signals, such as Wnt signaling and NF-kB [43].

The prognostic role of the NLR in head and neck cancers is well recognized nowadays. Abbate et al. demonstrated also the relationship between NLR values and the probability of having occult neck lymph node metastasis in early-stage OTSCC [18]. The authors showed that an NLR value of 2.93 was associated with a probability of developing occult neck metastasis of 49.91%. The probability value for the identification of occult metastasis of 49.91%, which corresponds to the identified NLR cutoff point, is similar to the value of 44.4%, described by Weiss et al. and later by Okura et al. in 2009 [44, 45]. Similarly, Wu et al. [46] found an increased risk of neck metastasis with comparable values of the NLR (2.95). These authors reported also a positive correlation between an NLR > 2.95 and other neck lymph node metastasis predictors such as tumoral infiltration thickness and the presence of perineural invasion.

The present study is able to reinforce such preliminary findings, analyzing for the first time the correlation between the DOI and NLR in predicting the risk of occult neck metastasis in early-stage OTSCC. Of note, the statistical analysis used for the present study is grounded on a mathematical model, which reinforces the value of the results obtained. In our study, the odds ratio at exp (1.1918) = 3.29, which represents the desired relation, shows that a unit increase (1 mm) in the thickness of the DOI corresponds to an increase of 3.29 in the propensity to present occult metastases, not evident by clinical and radiological examination. As demonstrated in Fig. 1, the inflection point, where the straight line tangent to the curve crosses the curve itself and the curve changes its concavity, corresponds to a probability of 50.20% (very similar to the 49.91% of the NLR) to present occult cervical metastases and a DOI thickness of 5.42 mm. Finally, once the values of the NLR and DOI associated with a probability greater than 44.4% of developing occult lymph node metastases had been identified, we analyzed the correlation between the two variables. The present study demonstrated for the first time that the variables NLR and DOI are linearly associated with a positive correlation, proved by Spearman's rank correlation coefficient rho of 0.64.

Having demonstrated this, we investigated also the linear relation that connected the DOI and NLR by performing a regression analysis with the NLR as the dependent variable and the DOI as the independent variable. The estimated regression coefficient was set at 0.47 and it is significant with the confidence level at 95%. This implies that a unitary increase in the DOI of 1 mm results in an increase in the NLR of 0.47.

Conclusions

The appropriate management of the cNO in early-stage OTSCC still remains an open issue since no validated factors are available to decide whether END or radiological surveillance is recommended. To the best of our knowledge, this is the first study that correlates two predictive markers of occult lymph node metastases, namely the DOI and NLR. In detail, a pre-treatment NLR value of 2.93 and a DOI measurement of 5.43 mm are associated with a probability of 50% of developing occult neck metastases, not yet evident both clinically and using current imaging techniques. We have also found that the NLR and DOI variables are linearly associated with a positive correlation: a unit increase (1 mm) of the DOI variable results in an increase of 0.47 in the NLR variable.

These preliminary results can offer clinicians an easily obtainable method to stratify patients based on their risk of developing occult neck lymph node metastases and therefore can assist them in planning an END in suitable cases.

The limitations of this study include its retrospective design, the exclusion of a non-negligible proportion of patients not submitted to END, and the DOI measurement being based only on the pre-operative MRI without considering any histopathological findings. Considering the encouraging results, we have obtained, while admitting a small number of cases, we would like to recommend the use of the NLR and DOI as additional tools in predicting the occurrence of occult neck metastasis and therefore in planning an END in early-stage OTSCC (cT1-T2).

Further prospective studies on a larger cohort of patients will be necessary to confirm these preliminary results, to standardize the measurement techniques of the DOI, comparing the pre-operative radiological findings with the histopathological post-operative analysis, and to further refine the cutoff levels of the NLR and DOI. In this way, it should be possible to distinguish between patients affected by early-stage OTSCC at a high risk of developing occult cervical metastasis, who therefore require END, and those at low risk, who need only to be monitored with radiological

surveillance, in which cases the use of US pre- or intra-operatively would be advantageous.

Declarations

Ethical approval The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Federico II University of Naples.

Conflict of interest The authors declare no competing interests.

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