Lymphovascular invasion as a prognostic tool for oral squamous cell carcinoma: a comprehensive review

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Running title

Lymphovascular invasion in oral cancer

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Keywords

Oral squamous cell carcinoma; oral tongue squamous cell carcinoma; lymphovascular invasion; vascular invasion; oral cancer.

Abstract

Oral cancer is the most common malignancy of the head and neck region, characterised by poor prognosis. Novel prognostic markers are needed to better stratify these patients. Lymphovascular invasion (LVI) has been included in the 8th AJCC Cancer Staging Manual as an additional prognostic factor, but its influence on recurrence risk and lymph-node metastasis is relatively understudied. This is the first comprehensive review of literature regarding the clinical and prognostic role of LVI in oral cancer. LVI is an independent negative prognostic factor in oral cancer patients and appears associated to cervical lymph-node metastases and locoregional recurrence. Notably, in oral tongue cancer survival outcomes progressively worsen when LVI is associated with others adverse pathological features, especially in early stages. Therefore, these patients could benefit from elective neck dissection and/or adjuvant therapy. The highly variability of LVI prevalence hindering the comparison of literature results. Several methodological limitations are present on the collected articles: the lack of a rigorous definition for LVI, the difficult detection in routine histological section, the presence of potential confounders, the retrospective nature and the inadequate sample size used in most studies. Therefore, it is necessary to conduct prognostic studies using standardized methods to define and quantify LVI.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy, accounting for 2% of all cancers[1]. OSCC can develop in all oral cavity sites and is characterised by high invasive capacity, lymph node metastasis (LNM), and high recurrence rate. Even with improvement of treatment, the prognosis is still poor, showing a 5-year survival rate of 50-60%[2].

The 8th edition of the American Joint Committee on Cancer (AJCC) staging system, revised the rules on staging of OSCC, improving the prognostic accuracy[3]. Despite these advancements, some indications show that AJCC system still need to be improved, urging to find new prognostic markers in order to better stratify OSCC patients[2,4]. Immunohistochemistry and other molecular techniques are well-established methods to identify new prognostic markers, but the highly variable results influence their usefulness and the cost hinders their utility in daily clinical practice[5,6]. In the last years the attention has been focused to the morphological features of tumour tissue, with the aim to find new reliable markers for OSCC[7-10]. Among the morphological features, the prognostic role of lymphovascular invasion (LVI) is relatively understudied. LVI is defined as the presence of tumour cells within definite endothelial-lined spaces, either lymphatic or blood vessels, detected by haematoxylin & eosin (H&E) staining or immunohistochemistry[11,12]. Unequivocal LVI is present, if malignant cells are within an endothelial-lined space or focally adherent to the vessel wall. On the contrary, LVI is considered negative when findings are equivocal or are mere artifacts (e.g. tumour retraction or dislocation of tumour cells into empty spaces) (Figure 1)[13].

LVI has been included in the 8th AJCC staging system as an additional prognostic factor, but its ability to stratify OSCC patient risk for recurrence or survival is still discussed[3]. The exact role of LVI in OSCC is not yet elucidated, although this parameter seems to be mandatory for tumour spread through lymphatic vessels and for the development of LNM, which is one of the most important prognostic factors in patients with OSCC. Indeed, 40% of patients with OSCC are affected by LNM and the development of regional recurrences is one of the main causes of treatment failure[14]. The aim of this work is to conduct an extensive and critical review of literature regarding the clinical and prognostic role of LVI in OSCC.

LYMPHANGIOGENESIS AND LVI: TWO SIDES OF THE SAME COIN

The proliferation of new blood vessels (angiogenesis) and lymphatic vessels (lymphangiogenesis) is considered a hallmark of cancer[15]. Indeed, this process is considered essential for tumour growth, invasion, and metastasis. The lymphatic system plays multiple protective roles in human body but it is also the preferential diffusion pathway for cancer cells. This is especially true for OSCC because there are roughly 400 lymph nodes in the cervical region, connected to a branched network of lymphatic vessels[16]. Cancer cells mainly spread via the lymphatic system, eventually resulting in LNM. The role of blood vessels in metastasis formation in OSCC is less clear. It must be emphasised that angiogenesis permits, but does not guarantee, progressive spread of OSCC to distant organs and therefore could be considered as an additional pathway for distant metastases[17]. A possible explanation is that, unlike blood vessels, lymphatic capillaries are larger and lack a continuous basal membrane, making it easier for cancer

cells to invade lymphatic rather than blood vessels[16]. The presence of circulating cancer cells in the bloodstream of OSCC patients, even in the absence of distant metastases, could depend more on their indirect dissemination through the blood vessels of the lymph nodes or the lympho-venous communications rather than direct invasion of blood vessels[18].

Despite the importance of lymphogenic metastases in OSCC, little is known about biological processes underlying the lymphatic-tumour crosstalk and several aspects have yet to be clarified. The first aspect is to what extent OSCC induces neo-lymphangiogenesis and whether these new lymphatic vessels are mainly located at intra-tumoral or peritumoral positions. Tumour-induced lymphangiogenesis has been found in several OSCC samples[19], although this process seems to be very slow[18]. Furthermore, these tiny ill-defined vessels seem to be frequently connected to non-functional and incomplete lymphatic networks[16].

Another aspect is if cancer cells mainly disseminate through the peritumoral or intra-tumoral lymphatic system. Some evidence suggests that cancer cells primarily spread through peritumoral lymphatic vessels, while intra-tumoral lymphatics should be regarded as an additional pathway for LNM[20]. Another key aspect is how the vessel invasion by oral cancer cells occurs. The most accepted theory is that the tumour growth leads both to the incorporation of the lymphatic vessels already present within sheets of tumour cells and to the production of lymphangiogenic growth factors (mainly VEGF-family members) which result in formation of new vessels[21]. Then, cancer cells detach themselves from the growing tumour mass and move toward lymphatic vessels, following a path of the least resistance. After attaching to lymphatic endothelium, cancer cells cross the endothelial barrier and enter into the lymphatic lumen[16,21].

Related to this topic, some studies in head and neck cancers have found a certain relationship between deeper invaded tumours and higher LVI[12,22]. Although this aspect has not yet been adequately investigated, it is possible that as tumour spread into surrounding host tissues, it encounters larger lymphatic vessels. Therefore, the increase of the depth of invasion (DOI) could increase the prognostic value of LVI, especially in structures like the oral tongue[12]. This is likely to be the consequence of the three-dimensional architecture of the lymphatic network, in which the lymphatic capillaries, smaller and more superficial, converge into deeper and larger collecting vessels[23].

However, neither lymphangiogenesis nor angiogenesis are equivalent to LVI despite their strong associations. Indeed, although the development of new vessels increases the probability of LVI, it does not equate to LVI itself[24].

SEARCH STRATEGY AND STUDY RESULTS

Structured research was performed on the major electronic databases for studies published until 31st July 2020: PubMed, Scopus, and Web of Science databases. The following keywords were used in order to perform database searches: “lymphovascular invasion”, "oral squamous", "cancer", and "carcinoma", in combination with the Boolean operators “AND” and “OR”. The literature search was conducted by two independent investigators (M.M. and L.T.). In the case of any disagreements, the investigators reached consensus through discussion. The articles found in the databases were screened by title and abstract and in the final stage the full texts were read carefully.

The inclusion criteria were: (a) articles that evaluated the prognostic potential of LVI in patients affected by OSCC; (b) retrospective studies in which OSCC and LVI were

investigated through histopathological examination; (c) only H&E-based studies; and (d) minimum number of 50 cases. The exclusion criteria were: (a) reviews, meeting abstracts, short reports, communications, letters; (b) studies based only on the evaluation of medical and pathological records; (c) studies that are not strictly focused on OSCC (i.e. oropharyngeal cancers, head and neck cancers); (d) articles not published in English.

We initially identified 105 studies on PubMed, 62 on Scopus, and 136 on Web of Science. Of these articles, 29 were assessed for eligibility.

PROGNOSTIC ROLE OF LVI IN OSCC

The first investigation of LVI involving OSCC were conducted more than 40 years ago, although the aim of these studies was to evaluate the prognostic role of this parameter in head and neck cancers[25-27]. However, the first evaluation of LVI focused on OSCC was conducted in 1993, with the aim to investigate the role of several clinicopathological parameters in predicting LNM. In a cohort of 90 OSCC patients, LVI was histologically proved in 20 cases, 2 of which developed LNM[28].

Although the existence of LVI had long been known, most of the studies on this histological feature in OSCC have been conducted in the last decades. In a large study conducted on 571 cases, Adel et al. tried to discriminate lymphatic invasion from vascular invasion. In particular, they found a higher incidence of lymphatic invasion (4.9%) than vascular invasion (2.8%). Although lymphatic and vascular invasion were associated with several adverse pathological features, these two parameters failed to demonstrate a significant impact on survival outcomes[34]. In another study, 88 OSCCs were investigated with the aim to find new prognostic factors related to locoregional recurrence. LVI was

observed in 6.8% of patients and was the only parameter associated with locoregional recurrence in multivariate analysis[35].

In addition to causing an increased risk of developing metastases, LVI seems to be an independent negative prognostic factor in OSCC. The role of LVI was also examined in a study on 163 OSCC patients, showing an association between this parameter and smoking (29.6% in smokers versus 10.9% in non-smokers). In addition, LVI was found to be an independent prognostic factor for worse overall survival (OS) and disease-free survival (DFS)[36].

Recently, several studies focused on the impact of LVI in OSCC. In a large cohort study on 341 patients, Chang et al. found a higher prevalence of LVI in patients with high T status, advanced Stage, and metastases. Furthermore, LVI was found to have a negative impact on 5-year OS in patients without LNM[37]. Similar results were obtained by Chatterjee, showing an association between LVI and higher risk of LNM, both in tumours located on the tongue and on the buccal mucosa[38]. On the contrary, another study failed to demonstrate the prognostic role of LVI in 149 cases of OSCC, although a quarter of the cases were classified as "unknown" regarding the presence of LVI[39].

Interestingly, some studies highlighted the association between LVI and other features like PNI and tumor budding, suggesting the presence of a cluster of histological risk factors for more aggressive tumours[40,41].

The prognostic impact of LVI in young patients affected by OSCC was recently, suggesting a higher prevalence of this parameter in young patients[42]. Nevertheless, the prognostic role of LVI in this group of patients is still debated. Indeed, a recent study conducted on 66 young subjects failed to demonstrate LVI as an independent prognostic marker in multivariate analysis[7].

Focusing on early stage OSCC, several Authors suggested a role of LVI in this subgroup of patients. In a cohort of 212 T1-T2 OSCC patients, LVI was an independent predictor of the true nodal status in multivariate analysis, strongly associated with LNM with an odd ratio of 3.8[43]. In another study, early-stage OSCC showed more favorable histological features, such as lower rate of LVI (9.6% in T1 versus 31.1% in T2). Furthermore, a trend of association between LVI and the presence of LNM was found, although without any association with a reduced disease-specific survival (DSS)[44]. In a large study conducted on 463 cases of early OSCC, the multivariate analysis showed that LVI was an independent variable for predicting LNM, detecting metastases with a sensitivity of 80% and a specificity of 74%[45]. According to a recent study conducted on 150 subjects, LVI was observed in 23.3% of patients and was significantly associated with the presence of LNM (45.8% in metastatic patients versus 8.8% in non-metastatic group)[46]. Similar results were reported by Wei et al., although they failed to demonstrate a significant impact on survival outcomes[47].

Lastly, Lin et al. evaluated the prognostic role of several histological parameters, including LVI, in 123 advanced (T4a) OSCC patients. In particular, multivariate analysis demonstrated that LVI was associated to locoregional recurrence[48].

Therefore, it would seem reasonable to suppose that the presence of LVI at the primary site of OSCC would predict for cervical metastases, since invasion of the lymphatic vessels is the first step in the development of LNM.

LVI in oral tongue squamous cell carcinoma (OTSCC)

Oral tongue squamous cell carcinoma (OTSCC) is the most common type of OSCC, accounting for about 50% of the cases. This tumour is characterised by an aggressive clinical behavior and a poor prognosis[2]. Several studies showed that OTSCC exhibits peculiar molecular and clinical behavior compared to OSCC from other oral cavity subsites[49]. These data suggest the presence of a potential "anatomical bias" that may hinder the interpretation and clinical translation of OSCC data[50]. Therefore, numerous studies have focused on the role of several histological features, including LVI, in OTSCC.

The first investigation conducted on 66 OTSCC patients suggested an association between LVI and locoregional recurrence, although the multivariate analysis failed to demonstrate its independent prognostic significance[51]. An association between LNM and LVI was observed in 94 OTSCCs, indicating this histologic feature as a marker for more aggressive and invasive tumours[52]. However, these findings have not been confirmed in other studies on OTSCC, although the small number of cases may have influenced the results[56,57]. The role of LVI was also investigated in 120 never-smokers OTSCC patients, showing an association between LVI and disease recurrence in never-smokers [58]. Interestingly, the percentage of cases with LVI reported was the highest in literature (90%).

Some studies focused on the impact of LVI in early stage OTSCC. The prognostic role of several histologic markers was investigated in 190 patients affected by T1-T2 OTSCC, reporting a significant association between LVI and LNM. However, the prognostic effect of LVI appeared to be less marked than perineural invasion (PNI)[60] Similar results were obtained in 164 Stage I-II OTSCC patients who underwent glossectomy and elective neck dissection without adjuvant radiation therapy[61]. LVI was evaluated in 176 OTSCC

patients without lymph node involvement, confirming the association between LVI and poor prognosis. In particular, OTSCC patients with LVI had higher risk of regional recurrence and worse locoregional control[63] Although the prognostic role of LVI alone in early stage OTSCC is of uncertain value, the association with other histologic adverse features (e.g. PNI) suggests poor prognostic outcome.

Finally, some studies evaluated the prognostic role of several histologic parameters, including LVI, in young OTSCC patients. In 397 OTSCCs, LVI was found to be significantly more common in young patients (<45 years-old) than older patients[64]. However, these findings have not been confirmed by others, although such results may have been influenced by the small number of cases reported[65]. Nevertheless, the prognostic role of LVI in this group of patients is still debated. Indeed, a recent study conducted on 66 young subjects failed to demonstrate LVI as an independent prognostic marker in multivariate analysis[7].

LVI in other oral subsites

The floor of the mouth is the second most common site for OSCC, accounting for about 20-25% of the cases. Floor of the mouth is characterised by poorly defined borders and close proximity to numerous structures, such as muscles, sublingual glands, and submandibular lymph nodes. For these reasons, the floor of the mouth carcinoma (FOMC) may presents with early involvement of deep tissues, higher risk of positive margins after surgical resection, and early LNM.[66] Some Authors have focused their attention on the prognostic role of LVI in patients with FOMC. Fives et al. investigated the role of several parameters in 54 FOMC, reporting that LVI was an independent

prognostic factor for OS[66]. Furthermore, postoperative radiation therapy improved survival in patients with LVI, suggesting its possible predictive role in postoperative adjuvant treatment of FOMC. Recently, Beggan et al. investigated the ability of pathologists to reproducibly identify LVI in a cohort of 58 cases of FOMC[68]. Based on review of the pathological reports, the interobserver agreement for LVI was substantial (Cohen κ=0.64), suggesting that the main contributory factor to discrepant diagnoses was the interpretative variation, because of all three pathologists were allowed use their own criteria to define LVI. Therefore, due to the paucity of studies, the role of LVI alone in FOMC is of uncertain value, although the association with other features like PNI or histological pattern of invasion may reflect the presence of more aggressive tumours.

Buccal mucosal carcinoma (BMC) is a subtype of OSCC characterised by a low incidence and an aggressive course. Recently, in a retrospective study conducted on 120 BMC, LVI was observed in only 3.3% of patients, without any association with locoregional recurrence[69].

IMPACT OF LVI ON CLINICAL MANAGEMENT

The 8th AJCC staging system recommend to report several histopathologic features in OSCC, including LVI. However, it is unclear whether the presence of LVI had an impact on the therapeutic decision-making algorithm, especially for early stage OSCC. Data reported in literature raise the question of whether patients with evidence of LVI following primary surgical resection should be selected to aggressive adjuvant therapy. Furthermore, according to some Authors, the presence of LVI should be considered an indication for elective neck dissection in early stage OSCCs[12,30,59]. Indeed, it would seem

reasonable that the presence of LVI at the primary site should predict LNM since the invasion of the lymphatic vessels is the first step in the metastasis development. However, the prognostic role of LVI in early-stage OSCC has not been yet elucidated. In the National Comprehensive Cancer Network guidelines for the treatment of oral cancers, the postoperative radiotherapy is suggested for all OSCC patients with PNI and/or LVI[70]. The application of postoperative radiotherapy in advanced-stage OSCC patients with positive-LVI is not a difficult decision for clinicians, because most of these subjects frequently present other significant risk factors. However, it is troublesome to decide whether to apply adjuvant therapy in early stages OSCCs with PNI and/or LVI as the only risk factors. Some Authors reported the association between the presence of LVI and adverse survival outcome in oral cancer, in particular OTSCC, suggesting the use of neck dissection in those cases. In OTSCCs cN0, the elective neck dissection seems to be associated with improved locoregional control but not with OS. Therefore, its management could require an appropriate adjuvant therapy.[54] This seems to be further supported by the improved OS for OSCC patients with LVI treated with postoperative radiotherapy compared with those treated by surgery alone[66]. Therefore, LVI could be a useful marker to better define the therapeutic strategies in OSCC patients, although larger multicentre prospective studies are needed to corroborate this hypothesis.

CURRENT LIMITATIONS AND FUTURE PERSPECTIVES

Overall, the results of the studies regarding the presence of LVI in OSCC are highly heterogeneous, describing an uncertain role for this parameter in the development of oral cancer metastases (Table 1). Many studies agree in showing that the presence of LVI

increases the risk to develop LNM, both at the time of diagnosis or as sites of regional recurrence[29-31,55,66]. These data are in agreement with the hypothesis about the role of lymphogenic spread of cancer cells in the development of LNM. LNMs are considered the most important prognostic factor in OSCC patients, helping to explain the prognostic role of LVI and justifying the use of neck dissection or postoperative radiotherapy[12,59,66]. However, several reports failed to demonstrate a prognostic significance of LVI, suggesting that its presence alone is not sufficient to stratify OSCC patients[61,62,67].

The heterogeneous results may be due to several methodological inconsistencies; in particular, the lack of a rigorous definition for LVI and the consequent different approaches used by pathologists to classify it. Interobserver agreement in the assessment of LVI is hindered by varying definitions and criteria used by pathologists in its evaluation[68]. Only recently, the 8th AJCC staging system reported that LVI should be classified according to the position (intra-tumoral and/or extra-tumoral) and the number of foci (focal/multifocal)[3]. Therefore, the detection of LVI has always been considered difficult in routine histological section, which led to it being excluded from grading and staging systems. Another aspect is the presence of potential confounders that could influence the histological evaluation. The identification of LVI in OSCC is influenced by several variables including the number of tumour sections submitted, the number of slides examined from each tissue block, and the use of H&E or immunohistochemistry. All these aspects could also explain the wide variability in the prevalence of LVI in OSCC, ranging from 3 to 90% (Table 1). Taken together, all these limitations prevent the possibility of conducting a metanalysis of LVI in OSCC. Finally, there are several methodological limitations in the studies reported, including the retrospective nature and the inadequate sample size. Furthermore, LVI has been almost always studied with other histological

features (e.g. PNI and DOI) underrepresenting this marker and hindering to draw any firm conclusion about its prognostic value[53].

In conclusion, although the presence of LVI has long been recognised in OSCC, not much attention has been paid to it so far. Therefore, the correct reporting of prognostic marker research is encouraged, with larger sample size and standardised methods to define and quantify LVI, in order to ensure the reproducibility and the comparability of the results from different studies.

Declarations

Funding: Authors certify that there is no institutional, private or corporate financial support for this work.

Competing Interests: The authors declare that there is no conflict of interest.

Ethical Approval: Not applicable

Patient Consent: Not applicable

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574 **Table 1. LVI prevalence in OSCC.**

575

Authors (year)[Ref]	Site	T Stage	Treatment	n. of cases	LVI
Kim et al. (1993)[28]	Oral cavity	Any	Surgery, ND	90	22%
Hosal et al. (1998)[51]	Tongue	Any	Glossectomy, ND	60	15%
Chen et al. (2008)[52]	Tongue	Any	Glossectomy, ND	94	5%
An et al. (2008)[56]	Tongue	T1-T2	Glossectomy, END	63	15.9%
Iseli et al. (2012)[57]	Tongue	T1-T2	Glossectomy, END, RT, CHT	78	7.7%
Melchers et al. (2012)[43]	Oral cavity	T1-T2	Surgery, ND	212	N.A.
Tai et al. (2012)[60]	Tongue	T1-T2	Glossectomy, END, CHRT	190	21.6%
Durr et al. (2013)[58]	Tongue	Any	Glossectomy, ND, RT, CHT	120	90%
Ganly et al. (2013)[61]	Tongue	T1-T2	Glossectomy, END	164	3%
Tai et al. (2013)[44]	Oral cavity	T1-T2	Surgery, TND, END, RT	307	20.8%
Fives et al. (2015)[66]	FOM	Any	Surgery, ND, RT	54	18.5%
Adel et al (2015)[34]	Oral cavity	Any	Surgery, ND, CHRT	571	4.9%
Beggan et al. (2016)[68]	FOM	Any	Surgery, ND, RT	58	19%
Hakeem et al. (2016)[63]	Tongue	T1-T2	Surgery, ND	176	22.6%
Arora et al. (2017)[45]	Oral cavity	T1-T2	Surgery, ND	336	41.7
Lin et al. (2017)[48]	Oral cavity	T4	Surgery, ND, CHRT	123	73.1%

Abbas et al. (2018)[35]	Oral cavity	Any	Surgery	88	6.8%
Al Feghali et al. (2019)[36]	Oral cavity	Any	Surgery, ND, CHRT	163	23.3%
Chang et al. (2019)[37]	Oral cavity	Any	Surgery, END, CHRT	341	13.7%
Chatterjee et al. (2019)[38]	Buccal mucosa Tongue	Any	Surgery, ND	147	15.6%
Larson et al. (2019)[12]	Tongue	T1-T2, ≤2 cm	Surgery, END or TND, RT	100	19%
Ding et al. (2019)[39]	Oral cavity	Any	Surgery, ND, CHRT	149	36.9%
Ho et al. (2019)[41]	Oral cavity	Any	Surgery, TND, RT	200	35%
Manjula et al. (2019)[40]	Oral cavity	N.A.	Surgery, TND	105	24.7%
Wei et al. (2019)[47]	Oral cavity	T1-T2	Surgery, END, CHRT	314	20.7%
Sahoo et al. (2020)[46]	Oral cavity	T1-T2	Surgery, END	150	23.3%
Sowmya et al. (2020)[42]	Oral cavity	Any	Surgery, ND	117	12.0%
Riju et al. (2020)[69]	Buccal mucosa	Any	Surgery, ND, RT	120	3.3%
Mascitti et al. (2020)[7]	Oral cavity	Any	Surgery, RT, CHRT	66	30.3%

578

579 FOM = floor of mouth; N.A. = not available; ND = neck dissection (not specified if
580 elective or therapeutic); END = elective neck dissection; TND = therapeutic neck
581 dissection; RT = radiation therapy; CHT = chemotherapy; CHRT = chemoradiotherapy.

582

583 **Figure 1.** Schematic representation of LVI in a H&E slide of OSCC. Unequivocal LVI
584 is present if cancer cells are within a definite endothelial lining or focally adherent to the
585 vessel wall, both in peritumoral (A-B) and intra-tumoral position (C). LVI is considered
586 negative when findings are equivocal, such as when tumour cells are near a vessel without
587 reaching it (D). LVI is negative also in cases of retraction artifact during histological
588 processing, detected by the absence of endothelial lining around cancer cells (E-F).
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