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(Article begins on next page)

1 **Title**

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

4

5 **Running title**

6 Lymphovascular invasion in oral cancer

7

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32

33 **Keywords**

34 Oral squamous cell carcinoma; oral tongue squamous cell carcinoma; lymphovascular

35 invasion; vascular invasion; oral cancer.

36 **Abstract**

37

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

55

56

57 INTRODUCTION

58

59 Oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy,
60 accounting for 2% of all cancers¹. OSCC can develop in all oral cavity sites and is
61 characterised by high invasive capacity, lymph node metastasis (LNM), and high
62 recurrence rate. Even with improvement of treatment, the prognosis is still poor, showing
63 a 5-year survival rate of 50-60%².

64 The 8th edition of the American Joint Committee on Cancer (AJCC) staging system,
65 revised the rules on staging of OSCC, improving the prognostic accuracy³. Despite these
66 advancements, some indications show that AJCC system still need to be improved, urging
67 to find new prognostic markers in order to better stratify OSCC patients^{2,4}.

68 Immunohistochemistry and other molecular techniques are well-established methods to
69 identify new prognostic markers, but the highly variable results influence their usefulness
70 and the cost hinders their utility in daily clinical practice^{5,6}. In the last years the attention

71 has been focused to the morphological features of tumour tissue, with the aim to find new
72 reliable markers for OSCC⁷⁻¹⁰. Among the morphological features, the prognostic role of

73 lymphovascular invasion (LVI) is relatively understudied. LVI is defined as the presence
74 of tumour cells within definite endothelial-lined spaces, either lymphatic or blood vessels,
75 detected by haematoxylin & eosin (H&E) staining or immunohistochemistry^{11,12}.

76 Unequivocal LVI is present, if malignant cells are within an endothelial-lined space or
77 focally adherent to the vessel wall. On the contrary, LVI is considered negative when
78 findings are equivocal or are mere artifacts (e.g. tumour retraction or dislocation of
79 tumour cells into empty spaces) (Figure 1)¹³.

80 LVI has been included in the 8th AJCC staging system as an additional prognostic factor,
81 but its ability to stratify OSCC patient risk for recurrence or survival is still discussed³.
82 The exact role of LVI in OSCC is not yet elucidated, although this parameter seems to be
83 mandatory for tumour spread through lymphatic vessels and for the development of
84 LNM, which is one of the most important prognostic factors in patients with OSCC.
85 Indeed, 40% of patients with OSCC are affected by LNM and the development of regional
86 recurrences is one of the main causes of treatment failure¹⁴. The aim of this work is to
87 conduct an extensive and critical review of literature regarding the clinical and prognostic
88 role of LVI in OSCC.

89

90 **LYMPHANGIOGENESIS AND LVI: TWO SIDES OF THE SAME COIN**

91

92 The proliferation of new blood vessels (angiogenesis) and lymphatic vessels
93 (lymphangiogenesis) is considered a hallmark of cancer¹⁵. Indeed, this process is
94 considered essential for tumour growth, invasion, and metastasis. The lymphatic system
95 plays multiple protective roles in human body but it is also the preferential diffusion
96 pathway for cancer cells. This is especially true for OSCC because there are roughly 400
97 lymph nodes in the cervical region, connected to a branched network of lymphatic
98 vessels¹⁶. Cancer cells mainly spread via the lymphatic system, eventually resulting in
99 LNM. The role of blood vessels in metastasis formation in OSCC is less clear. It must be
100 emphasised that angiogenesis permits, but does not guarantee, progressive spread of
101 OSCC to distant organs and therefore could be considered as an additional pathway for
102 distant metastases¹⁷. A possible explanation is that, unlike blood vessels, lymphatic
103 capillaries are larger and lack a continuous basal membrane, making it easier for cancer

104 cells to invade lymphatic rather than blood vessels¹⁶. The presence of circulating cancer
105 cells in the bloodstream of OSCC patients, even in the absence of distant metastases,
106 could depend more on their indirect dissemination through the blood vessels of the lymph
107 nodes or the lympho-venous communications rather than direct invasion of blood
108 vessels¹⁸.

109 Despite the importance of lymphogenic metastases in OSCC, little is known about
110 biological processes underlying the lymphatic-tumour crosstalk and several aspects have
111 yet to be clarified. The first aspect is to what extent OSCC induces neo-
112 lymphangiogenesis and whether these new lymphatic vessels are mainly located at intra-
113 tumoral or peritumoral positions. Tumour-induced lymphangiogenesis has been found in
114 several OSCC samples¹⁹, although this process seems to be very slow¹⁸. Furthermore,
115 these tiny ill-defined vessels seem to be frequently connected to non-functional and
116 incomplete lymphatic networks¹⁶.

117 Another aspect is if cancer cells mainly disseminate through the peritumoral or intra-
118 tumoral lymphatic system. Some evidence suggests that cancer cells primarily spread
119 through peritumoral lymphatic vessels, while intra-tumoral lymphatics should be
120 regarded as an additional pathway for LNM²⁰. Another key aspect is how the vessel
121 invasion by oral cancer cells occurs. The most accepted theory is that the tumour growth
122 leads both to the incorporation of the lymphatic vessels already present within sheets of
123 tumour cells and to the production of lymphangiogenic growth factors (mainly VEGF-
124 family members) which result in formation of new vessels²¹. Then, cancer cells detach
125 themselves from the growing tumour mass and move toward lymphatic vessels, following
126 a path of the least resistance. After attaching to lymphatic endothelium, cancer cells cross
127 the endothelial barrier and enter into the lymphatic lumen^{16,21}.

128 Related to this topic, some studies in head and neck cancers have found a certain
129 relationship between deeper invaded tumours and higher LVI^{12,22}. Although this aspect
130 has not yet been adequately investigated, it is possible that as tumour spread into
131 surrounding host tissues, it encounters larger lymphatic vessels. Therefore, the increase
132 of the depth of invasion (DOI) could increase the prognostic value of LVI, especially in
133 structures like the oral tongue¹². This is likely to be the consequence of the three-
134 dimensional architecture of the lymphatic network, in which the lymphatic capillaries,
135 smaller and more superficial, converge into deeper and larger collecting vessels²³.
136 However, neither lymphangiogenesis nor angiogenesis are equivalent to LVI despite their
137 strong associations. Indeed, although the development of new vessels increases the
138 probability of LVI, it does not equate to LVI itself²⁴.

139

140 **SEARCH STRATEGY AND STUDY RESULTS**

141

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

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

152 investigated through histopathological examination; (c) only H&E-based studies; and (d)
153 minimum number of 50 cases. The exclusion criteria were: (a) reviews, meeting abstracts,
154 short reports, communications, letters; (b) studies based only on the evaluation of medical
155 and pathological records; (c) studies that are not strictly focused on OSCC (i.e.
156 oropharyngeal cancers, head and neck cancers); (d) articles not published in English.
157 We initially identified 105 studies on PubMed, 62 on Scopus, and 136 on Web of Science.
158 Of these articles, 29 were assessed for eligibility.

159

160 **PROGNOSTIC ROLE OF LVI IN OSCC**

161

162 The first investigation of LVI involving OSCC were conducted more than 40 years ago,
163 although the aim of these studies was to evaluate the prognostic role of this parameter in
164 head and neck cancers²⁵⁻²⁷. However, the first evaluation of LVI focused on OSCC was
165 conducted in 1993, with the aim to investigate the role of several clinicopathological
166 parameters in predicting LNM. In a cohort of 90 OSCC patients, LVI was histologically
167 proved in 20 cases, 2 of which developed LNM²⁸.

168 Although the existence of LVI had long been known, most of the studies on this
169 histological feature in OSCC have been conducted in the last decades. **In a large study**
170 **conducted on 571 cases, Adel et al. tried to discriminate lymphatic invasion from vascular**
171 **invasion. In particular, they found a higher incidence of lymphatic invasion (4.9%) than**
172 **vascular invasion (2.8%). Although lymphatic and vascular invasion were associated with**
173 **several adverse pathological features, these two parameters failed to demonstrate a**
174 **significant impact on survival outcomes³⁴. In another study, 88 OSCCs were investigated**
175 **with the aim to find new prognostic factors related to locoregional recurrence. LVI was**

176 observed in 6.8% of patients and was the only parameter associated with locoregional
177 recurrence in multivariate analysis³⁵.

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

184 Recently, several studies focused on the impact of LVI in OSCC. In a large cohort study
185 on 341 patients, Chang et al. found a higher prevalence of LVI in patients with high T
186 status, advanced Stage, and metastases. Furthermore, LVI was found to have a negative
187 impact on 5-year OS in patients without LNM³⁷. Similar results were obtained by
188 Chatterjee, showing an association between LVI and higher risk of LNM, both in tumours
189 located on the tongue and on the buccal mucosa³⁸. On the contrary, another study failed
190 to demonstrate the prognostic role of LVI in 149 cases of OSCC, although a quarter of
191 the cases were classified as “unknown” regarding the presence of LVI³⁹.

192 Interestingly, some studies highlighted the association between LVI and other features
193 like PNI and tumor budding, suggesting the presence of a cluster of histological risk
194 factors for more aggressive tumours^{40,41}.

195 The prognostic impact of LVI in young patients affected by OSCC was recently,
196 suggesting a higher prevalence of this parameter in young patients⁴². Nevertheless, the
197 prognostic role of LVI in this group of patients is still debated. Indeed, a recent study
198 conducted on 66 young subjects failed to demonstrate LVI as an independent prognostic
199 marker in multivariate analysis⁷.

200 Focusing on early stage OSCC, several Authors suggested a role of LVI in this subgroup
201 of patients. In a cohort of 212 T1-T2 OSCC patients, LVI was an independent predictor
202 of the true nodal status in multivariate analysis, strongly associated with LNM with an
203 odd ratio of 3.8⁴³. In another study, early-stage OSCC showed more favorable histological
204 features, such as lower rate of LVI (9.6% in T1 versus 31.1% in T2). Furthermore, a trend
205 of association between LVI and the presence of LNM was found, although without any
206 association with a reduced disease-specific survival (DSS)⁴⁴. In a large study conducted
207 on 463 cases of early OSCC, the multivariate analysis showed that LVI was an
208 independent variable for predicting LNM, detecting metastases with a sensitivity of 80%
209 and a specificity of 74%⁴⁵. According to a recent study conducted on 150 subjects, LVI
210 was observed in 23.3% of patients and was significantly associated with the presence of
211 LNM (45.8% in metastatic patients versus 8.8% in non-metastatic group)⁴⁶. Similar
212 results were reported by Wei et al., although they failed to demonstrate a significant
213 impact on survival outcomes⁴⁷.

214 Lastly, Lin et al. evaluated the prognostic role of several histological parameters,
215 including LVI, in 123 advanced (T4a) OSCC patients. In particular, multivariate analysis
216 demonstrated that LVI was associated to locoregional recurrence⁴⁸.

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

220

221 *LVI in oral tongue squamous cell carcinoma (OTSCC)*

222

223 Oral tongue squamous cell carcinoma (OTSCC) is the most common type of OSCC,
224 accounting for about 50% of the cases. This tumour is characterised by an aggressive
225 clinical behavior and a poor prognosis². Several studies showed that OTSCC exhibits
226 peculiar molecular and clinical behavior compared to OSCC from other oral cavity
227 subsites⁴⁹. These data suggest the presence of a potential “anatomical bias” that may
228 hinder the interpretation and clinical translation of OSCC data⁵⁰. Therefore, numerous
229 studies have focused on the role of several histological features, including LVI, in
230 OTSCC.

231 The first investigation conducted on 66 OTSCC patients suggested an association
232 between LVI and locoregional recurrence, although the multivariate analysis failed to
233 demonstrate its independent prognostic significance⁵¹. An association between LNM and
234 LVI was observed in 94 OTSCCs, indicating this histologic feature as a marker for more
235 aggressive and invasive tumours⁵². **However, these findings have not been confirmed in**
236 **other studies on OTSCC, although the small number of cases may have influenced the**
237 **results^{56,57}**. The role of LVI was also investigated in 120 never-smokers OTSCC patients,
238 showing an association **between LVI and disease recurrence in never-smokers**⁵⁸.
239 Interestingly, the percentage of cases with LVI reported was the highest in literature
240 (90%).

241 Some studies focused on the impact of LVI in early stage OTSCC. **The prognostic role**
242 **of several histologic markers was investigated in 190 patients affected by T1-T2 OTSCC,**
243 **reporting a significant association between LVI and LNM. However, the prognostic effect**
244 **of LVI appeared to be less marked than perineural invasion (PNI)⁶⁰** Similar results were
245 obtained in 164 Stage I-II OTSCC patients who underwent glossectomy and elective neck
246 dissection without adjuvant radiation therapy⁶¹. **LVI was evaluated in 176 OTSCC**

247 patients without lymph node involvement, confirming the association between LVI and
248 poor prognosis. In particular, OTSCC patients with LVI had higher risk of regional
249 recurrence and worse locoregional control⁶³ Although the prognostic role of LVI alone in
250 early stage OTSCC is of uncertain value, the association with other histologic adverse
251 features (e.g. PNI) suggests poor prognostic outcome.

252 Finally, some studies evaluated the prognostic role of several histologic parameters,
253 including LVI, in young OTSCC patients. In 397 OTSCCs, LVI was found to be
254 significantly more common in young patients (<45 years-old) than older patients⁶⁴.
255 However, these findings have not been confirmed by others, although such results may
256 have been influenced by the small number of cases reported⁶⁵. Nevertheless, the
257 prognostic role of LVI in this group of patients is still debated. Indeed, a recent study
258 conducted on 66 young subjects failed to demonstrate LVI as an independent prognostic
259 marker in multivariate analysis⁷.

260

261 *LVI in other oral subsites*

262

263 The floor of the mouth is the second most common site for OSCC, accounting for about
264 20-25% of the cases. Floor of the mouth is characterised by poorly defined borders and
265 close proximity to numerous structures, such as muscles, sublingual glands, and
266 submandibular lymph nodes. For these reasons, the floor of the mouth carcinoma
267 (FOMC) may presents with early involvement of deep tissues, higher risk of positive
268 margins after surgical resection, and early LNM.⁶⁶ Some Authors have focused their
269 attention on the prognostic role of LVI in patients with FOMC. Fives et al. investigated
270 the role of several parameters in 54 FOMC, reporting that LVI was an independent

271 prognostic factor for OS⁶⁶. Furthermore, postoperative radiation therapy improved
272 survival in patients with LVI, suggesting its possible predictive role in postoperative
273 adjuvant treatment of FOMC. Recently, Beggan et al. investigated the ability of
274 pathologists to reproducibly identify LVI in a cohort of 58 cases of FOMC⁶⁸. Based on
275 review of the pathological reports, the interobserver agreement for LVI was substantial
276 (Cohen $\kappa=0.64$), suggesting that the main contributory factor to discrepant diagnoses was
277 the interpretative variation, because of all three pathologists were allowed use their own
278 criteria to define LVI. Therefore, due to the paucity of studies, the role of LVI alone in
279 FOMC is of uncertain value, although the association with other features like PNI or
280 histological pattern of invasion may reflect the presence of more aggressive tumours.

281 **Buccal mucosal carcinoma (BMC) is a subtype of OSCC characterised by a low incidence**
282 **and an aggressive course. Recently, in a retrospective study conducted on 120 BMC, LVI**
283 **was observed in only 3.3% of patients, without any association with locoregional**
284 **recurrence⁶⁹.**

285

286 **IMPACT OF LVI ON CLINICAL MANAGEMENT**

287

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

295 reasonable that the presence of LVI at the primary site should predict LNM since the
296 invasion of the lymphatic vessels is the first step in the metastasis development. However,
297 the prognostic role of LVI in early-stage OSCC has not been yet elucidated. In the
298 National Comprehensive Cancer Network guidelines for the treatment of oral cancers, the
299 postoperative radiotherapy is suggested for all OSCC patients with PNI and/or LVI⁷⁰. The
300 application of postoperative radiotherapy in advanced-stage OSCC patients with positive-
301 LVI is not a difficult decision for clinicians, because most of these subjects frequently
302 present other significant risk factors. However, it is troublesome to decide whether to
303 apply adjuvant therapy in early stages OSCCs with PNI and/or LVI as the only risk
304 factors. Some Authors reported the association between the presence of LVI and adverse
305 survival outcome in oral cancer, in particular OTSCC, suggesting the use of neck
306 dissection in those cases. In OTSCCs cN0, the elective neck dissection seems to be
307 associated with improved locoregional control but not with OS. Therefore, its
308 management could require an appropriate adjuvant therapy.⁵⁴ This seems to be further
309 supported by the improved OS for OSCC patients with LVI treated with postoperative
310 radiotherapy compared with those treated by surgery alone⁶⁶. Therefore, LVI could be a
311 useful marker to better define the therapeutic strategies in OSCC patients, although larger
312 multicentre prospective studies are needed to corroborate this hypothesis.

313

314 **CURRENT LIMITATIONS AND FUTURE PERSPECTIVES**

315

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

319 increases the risk to develop LNM, both at the time of diagnosis or as sites of regional
320 recurrence^{29-31,55,66}. These data are in agreement with the hypothesis about the role of
321 lymphogenic spread of cancer cells in the development of LNM. LNMs are considered
322 the most important prognostic factor in OSCC patients, helping to explain the prognostic
323 role of LVI and justifying the use of neck dissection or postoperative radiotherapy^{12,59,66}.
324 However, several reports failed to demonstrate a prognostic significance of LVI,
325 suggesting that its presence alone is not sufficient to stratify OSCC patients^{61,62,67}.
326 The heterogeneous results may be due to several methodological inconsistencies; in
327 particular, the lack of a rigorous definition for LVI and the consequent different
328 approaches used by pathologists to classify it. Interobserver agreement in the assessment
329 of LVI is hindered by varying definitions and criteria used by pathologists in its
330 evaluation⁶⁸. Only recently, the 8th AJCC staging system reported that LVI should be
331 classified according to the position (intra-tumoral and/or extra-tumoral) and the number
332 of foci (focal/multifocal)³. Therefore, the detection of LVI has always been considered
333 difficult in routine histological section, which led to it being excluded from grading and
334 staging systems. Another aspect is the presence of potential confounders that could
335 influence the histological evaluation. The identification of LVI in OSCC is influenced by
336 several variables including the number of tumour sections submitted, the number of slides
337 examined from each tissue block, and the use of H&E or immunohistochemistry. All these
338 aspects could also explain the wide variability in the prevalence of LVI in OSCC, ranging
339 from 3 to 90% (Table 1). Taken together, all these limitations prevent the possibility of
340 conducting a meta-analysis of LVI in OSCC. Finally, there are several methodological
341 limitations in the studies reported, including the retrospective nature and the inadequate
342 sample size. Furthermore, LVI has been almost always studied with other histological

343 features (e.g. PNI and DOI) underrepresenting this marker and hindering to draw any firm
344 conclusion about its prognostic value⁵³.

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

350

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

355 **Ethical Approval:** Not applicable

356 **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%

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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.

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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