Prognostic significance of the neural invasion in oral squamous cell carcinoma

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ABSTRACT

Background

Although nerve involvement can predict recurrence and prognosis in oral squamous cell carcinomas, there still have controversies and limitations regarding the standardization for its detection. In this study, we explore the impact of neural invasion in oral squamous cell carcinomas prognosis, comparing intraneural invasion (tumor cells inside nerve structure) and perineural invasion (cells involving the nerve, but not invading its sheath).

Methods

Surgical slides stained with hematoxylin and eosin from 235 patients with oral squamous cell carcinomas were carefully verified for the presence of intraneural invasion and perineural invasion. The location in the tumor (intratumoral vs. peritumoral) and number of foci (unifocal or multifocal) were also explored. Survival analyses for cancer-specific survival and disease-free survival were performed with Cox proportional model.

Results

Neural invasion was identified in 74 cases, 64.9% displayed intraneural invasion and 35.1% displayed perineural invasion. Univariate analysis revealed a significantly poorer cancer-specific survival, but not disease-free survival, in patients with intraneural invasion, in contrast to cases with perineural invasion that did not achieve significant association with both cancer-specific survival and disease-free survival. Further analyses revealed that the location in the tumor and number of foci had little impact on discriminatory ability of intraneural invasion. Multivariate analysis confirmed that intraneural invasion is significantly and independently associated with poor cancer-specific survival (hazard ratio: 2.50, 95% CI: 1.31–3.79, p = 0.003).

Conclusion

This study provides evidence that intraneural invasion, but not perineural invasion, is a relevant predictor of survival in patients with oral squamous cell carcinomas, suggesting that its association with other clinical and pathological prognostic factors should be consider in determining the optimal treatment protocol and prognosis of these patients.

KEYWORDS: histopathological features, oral cancer, perineural invasion, prognosis

1 INTRODUCTION

Oral squamous cell carcinoma (OSCC), the most common tumor in the head and neck region, shows a magnitude of ~400 000 new cases and 200 000 deaths every year.[1] OSCC traditionally displays an aggressive behavior, with many patients showing locoregionally advanced disease at the time of diagnosis.[2] Despite advances, the mortality rates associated with OSCC are still very high, with only half of the patients surviving for more than 5 years after diagnosis.[3] In general, a meticulous evaluation of the clinical, radiographic and pathological features, such as the depth of invasion and extranodal extension,[4] is performed to properly stage the patients with OSCC in order to select the optimal therapeutic protocol. However, there is no universal definition of high-risk characteristics for OSCC, and in many situations, the clinical staging is not able of differentiating tumors with more aggressive behavior, that would benefit from specific therapeutic approaches.[5-7] Some histopathological features, besides depth of invasion and extranodal extension, have been associated with poor prognosis and in those cases, adjuvant treatment is frequently recommended.[8, 9] Among those features are the infiltration of the neural structures. Although the invasion of neural structures by tumor cells is proposed for over a century as an indicator of poor prognosis, only in the last decades several lines of evidence demonstrated that the neural structures may contribute to the growth and progression of several neoplasms, including OSCCs.[10, 11]

After the article by Liebig et al.,[12] many studies call the invasion of the nerve structures by tumor cells as perineural invasion, representing the presence of tumor cells inside the nerve sheath (intraneural invasion [INI]) and/or in close proximity to the nerve involving more than one-third of its circumference (perineural invasion [PNI]). Even though the definition is clear, low interobserver agreement is an issue raised in the literature,[13] contributing for the conclusion of some studies that the prognostic value of PNI is meaningless in OSCC.[14, 15] In a recent comprehensive review and meta-analysis, a positive association of PNI with poor outcomes of patients with OSCC was reported, though the certainty of evidence was severely influenced by situations of risk of bias, inconsistency, and imprecision, weakening the magnitude of the conclusion.[8] This meta-analysis also suggested promising results for the association of quantitative and qualitative features of PNI, including number of foci and its localization in the tumor (intratumoral vs. peritumoral).

As there is still controversy arising from conflicting results about the clinical impact of neural invasion for OSCC and a recent study revealed improved interobserver agreement in the classification of INI compared with PNI,[16] we aimed in this study to explore the impact of invasion of neural structures by cancer cells in OSCC prognosis, comparing INI (tumor cells inside nerve sheath) and PNI (cells at least involving one-third of the nerve circumference, but not invading the nerve). Furthermore, we examined whether the location of the involved nerve in the tumor (intratumoral vs. peritumoral) or the number of foci could improve risk stratification of OSCC.

2 MATERIALS AND METHODS

Patients

A total of 235 patients with OSCC, treated in the UOPECCAN Cancer Hospital (Cascavel-Parana) and in the Hospital Bom Pastor (Varginha-Minas Gerais) between 1998 and 2014, were included in this retrospective study. All patients met the following criteria: OSCC diagnosis, no therapy before surgery, and availability of surgical slides, and clinicopathological and follow-up data. Clinical staging and histopathological grading were defined according to the 7th edition of the American Joint

Committee on Cancer and the World Health Organization system, respectively. Patients were followed up from six to 178 months, and cases of recurrence were histologically confirmed. The study evaluated two primary outcomes: cancer-specific survival (CSS) and disease-free survival (DFS). This study was approved by the Human Research Ethics Committee of the School of Dentistry, University of Campinas (CAAE: 55927322.0.0000.5418).

Assessment of INI and PNI

Histological slides stained with hematoxylin and eosin (H&E) from the surgical resections were retrieved for evaluation of INI and PNI. The number of available slides of the primary tumors ranged from 1 to 19 (median of 4), excluding slides of the surgical margins and lymph nodes.

INI was classified as the presence of tumor cells inside nerve sheath, whereas PNI was applied if tumor cells were surrounding at least one-third of the nerve circumference. In situations of multifocal invasion with both INI and PNI, the sample was classified as INI. Further classifications included the location in the tumor, categorized as inside tumor (intratumoral) or in the stroma at the invasive front (peritumoral), and the number of foci, categorized as unifocal or multifocal (two or more involved nerves).

Statistical analysis

The semiparametric survival analyses were performed applying Cox proportional hazards regression model (stepwise method). A p-value ≤ 0.05 was considered to be statistically significant. Statistical analyses were performed using the MedCalc, version 20 (Belgium).

3 RESULTS

The clinicopathological characteristics of patients with OSCC are described in Table 1. Briefly, 184 (78.3%) were men, and the age ranged from 17 to 88 years with a mean of 58.1 ± 11.5 years. The majority of the patients was diagnosed at advanced clinical stages (III and IV, totaling 53.6%), the most frequent site was tongue (66%), and the WHO histopathological grading classified the tumors in well differentiated in 94 (40%) cases, moderately differentiated in 114 (48.5%), and poorly differentiated in 27 (11.5%) cases. All patients underwent curative surgery, and 128 patients received postoperative radiotherapy and 20 patients had chemoradiotherapy. In cases where chemotherapy was given, this was cisplatin-based monotherapy. At the end of the follow-up, 150 (63.8%) patients were alive, 71 (30.2%) patients developed recurrence, and 85 (36.2%) had died due to cancer.

Neural invasion was identified in 74 cases (31.5%), with 48 cases (64.9%) displaying INI and 26 (35.1%) cases with the cells only surrounding the nerve (PNI; Figure 1). The cases with INI were mainly characterized by cancer cells partially covering the perineurium, and rarely distributing into the endoneurium. Regarding location in the tumor, 44 (59.5%) cases were located inside the tumor area (intratumoral), with 26 showing INI and 18 PNI, and 30 (40.5%) cases were found at the invasive front (INI, n = 22 cases and PNI, n = 8 cases). The counting of foci classified 51 (68.9%) cases as multifocal and 23 (31.1%) as unifocal (only one focus). Among multifocal samples, 30 samples showed INI and 21 showed PNI, and among the samples displaying only 1 nerve with invasion neural, 18 showed INI, and 5 showed PNI (Table 2).

INI but not PNI was significantly associated with CSS (Table 3). Univariate survival analysis revealed a significantly poorer CSS in patients with INI, yielding an hazard ratio (HR) of 2.05 (95% CI: 1.20–3.52, p = 0.002). Stratified analyses, excluding the samples classified as PNI, revealed that the location in the tumor and number of foci had little impact on discriminatory ability of INI. Both clinical stage (HR: 1.58, 95% CI: 1.02–2.45, p = 0.03) and lymphovascular invasion (LVI; HR: 1.90, 95% CI: 1.11–3.23, p = 0.01) were also significantly associated with shortened CSS. No association with DFS was observed.

Cox regression analysis under a stepwise algorithm was conducted to confirm the importance of INI as a predictor of CSS. After adjusting for age, sex, clinical stage, location, treatment, margin status, LVI, WHO histopathological grading, INI was significantly and independently associated with poor CSS (HR: 2.50, 95% CI: 1.31–3.79, p = 0.003). In the multivariate analysis, clinical stage and LVI were no longer statistically significant.

4 DISCUSSION

Oral cancers are in the top mortality rates among malignant tumors, and due to lack of symptoms in the initial phases, many patients are diagnosed in an advanced stage.[2] The current standard treatments, including surgery, radiotherapy, and chemotherapy, alone or in combinations, result in severe and, in some situations, permanent side effects, and the 5-year overall survival rate does not exceed 50% of the treated patients.[3] In this context, prognostic parameters allowing risk-based stratification is critical to provide directions for optimal and individual therapeutic protocol. Despite the importance of the neural invasion with regard to the treatment and prognosis of OSCC, proposed definitions of PNI vary considerably.[13] The definition proposed by Liebig et al.[12] is the most frequently applied, where neural invasion (called as PNI) is classified as the presence of tumor cells inside nerve sheaths or surrounding at least one-third of nerve circumference. However, there is great disagreement among pathologists whether the tumor invading focally or touching the perineurium represents a real invasion of the nerve. In this context, a continuous challenge in this field is how to formulate a widely accepted and objective definition of neural invasion. In a recent study, the interobserver agreement for the diagnosis of neural invasion was better if the criteria of tumor cells invading the nerve sheath was applied compared with the definition involving tumor surrounding the nerve.[16] In this study, we conducted a retrospective cohort study that compared the impact of INI and PNI in 235 surgical specimens of OSCC. Our series indicates that the detection of tumor cells inside nerve sheath appears to be a critical prognostic feature for OSCC, promoting a significantly poorer CSS regardless of its location in the tumor and number of foci. In contrast, patients with tumor cells only surrounding the nerve (i.e., PNI) showed similar outcomes to those without neural invasion. If Liebig's criterion is applied (combination of INI and PNI), a significant association with shortened CSS is detected, but with lower hazards than INI alone (data not shown).

In this study, neither INI nor PNI was predictive of DFS and this result is probably related to the proposed management to the patients in the evidence of neural invasion. Neural invasion has long been considered a marker of aggressive tumor, and adjuvant treatment, particularly radiotherapy, is often advocated.[10, 17] All patients included in this study with involved nerves (either INI or PNI) were subjected to adjuvant radiotherapy at maximum safely possible intensity, independently of other adverse prognostic features including primary tumor location, surgical margins, lymph node metastasis, extranodal extension, and the presence of LVI. With a very similar finding, Chinn et al.[18] reported potential benefits of postoperative adjuvant radiotherapy for PNI-positive OSCC, with

improved locoregional control (DFS), but no effects on DSS or OS. According to a retrospective, multicenter cohort study recently published by Spoerl et al.,[19] adjuvant radiotherapy or chemoradiotherapy did not improve recurrence-free survival of patients with PNI-positive OSCC compared with patients without PNI. Together, those findings reinforce the prognostic impact of the neural invasion for CSS, whereas applying adjuvant radiotherapy can mitigate the detrimental effects in the risk of recurrence.

Although large neural invasion can be detected using magnetic resonance imaging,[15] the involvement of small nerves is a histological finding. Clinically, patients with neural invasion may report neurological symptoms, including paresthesia, numbness, and pain, which may represent a clue of neural invasion, which require a special attention of the clinicians to suspect of its diagnosis, but it should be confirmed through histopathological evaluation of the specimens. In this scenario, it still is the matter of debate as to whether immunohistochemistry should be used in association with H&E examination to improve the detection rate of neural invasion. It is also important to consider that a previous review found neural invasion rates in OSCC ranging from 5.2% to 90%,[20] which clearly reflect differences in the interpretation and identification between pathologists. Although previous studies have demonstrated that the combining H&E and immunostaining provides the identification of additional positive cases, the cost-effectiveness of this routine procedure was never evaluated.[21] In the current series, the histopathological assessment was performed by two pathologists using a large number of H&E-stained slides for each case. Furthermore, one previous study has demonstrated that careful analysis of the H&E-stained slides significantly improves the detection of neural invasion.[22]

Although neural invasion can be stratified based on size, number, and location of the involved nerves, relatively few studies have explored those aspects in OSCC. In this regard, recent studies showed no relationship between the location of the neural invasion and survival of OSCC patients.[23, 24] However, the presence of neural invasion at the invasive front has been associated with worse survival compared with intratumoral invasion in OSCC.[25] In this line, stratifications taking in consideration size and the number of nerves have generated controversial results.[24-28] Our findings demonstrate that INI, regardless its subclassifications, is a key determinant of poor survival in OSCC.

Our study has some limitations, including its retrospective design with samples geographically restrictive from two hospitals for cancer treatment, and variability in the number of slides for each case, though the histopathological features were all reviewed to ensure homogeneity. The study also included tumors from different subsites in the oral cavity, and the restricted number of cases for some sites precludes stratified analyses. One strength is that complete clinicopathological information of the entire cohort was obtained, which allowed multivariate analysis taking in consideration important confounding variables that could individually influence the outcomes.

5 CONCLUSION

Our results advocate INI but not PNI as an independent risk factor for poor prognosis in patients with OSCC. However, it is still a challenge to delineate the individual contribution of neural invasion in OSCC prognosis. Guidelines with clear definitions on neural invasion and validation with large, prospective multicenter studies are mandated to apply this knowledge in a more efficient manner.

AUTHOR CONTRIBUTIONS

Eder Silva da Dolens: Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, and writing – review and editing. Everton Freitas de Morais: Data curation, formal analysis, and writing – review and editing. Lívia Máris Ribeiro Paranaíba: Data curation, formal analysis, and writing – review and editing. Ana Lúcia Carrinho Ayroza: Data curation, formal analysis, and writing – review and editing. Alhadi Amlangush: Conceptualization, formal analysis, and writing – review and editing. Tuula Salo: Conceptualization, formal analysis, and writing – review and editing. Peter A. Brennan: Formal analysis and writing – review and editing. Ricardo D. Coletta: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, writing – original draft, and writing – review and editing.

FUNDING INFORMATION

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; process no. 2018/16077-6 to Ricardo D. Coletta). Ricardo D. Coletta is a research fellow of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; process no. 303589/2019-1) and Everton Freitas de Morais (2022/00994-5) is a research fellow supported by FAPESP. Lívia Máris Ribeiro Paranaíba received a grant from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; process no. PPM-00179-18).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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FIGURE LEGENDS

Figure 1 Representative histopathology images of neural invasion. Neural invasion was categorized as intraneural (INI) when there was a clear presence of tumor cells inside the nerve structure, and perineural (PNI) when the tumor cells were adjacent, evolving at least one-third of the circumference of the nerve. (A) Low-power view of a large nerve (*) with multiple tumor cells inside the nerve structure (arrowhead), and (B) high-power image, corresponding the nerve in (A), revealing multiple tumor cells (arrowhead) inside the perineurium, in close contact with Schwann cells and axons. (C) Nerve (*) with both PNI (arrow) and INI (arrowhead). (D) Massive presence of the tumor cells (arrowhead) inside the nerve (*), characterizing an INI. (E) Two neural structures (*) displaying tumor cells (arrow) around but not invading (PNI). (F) Small nerve (*) completely surrounded by tumor cells (PNI). (A) magnification ×100 (×10 eyepiece and ×10 objective) and (B–F) magnification ×200 (×10 eyepiece and ×20 objective).

TABLES

TABLE 1. Clinicopathological features of 235 patients with oral squamous cell carcinoma included in this study.

	***n* (%)**
Age (years)	
Mean ± standard derivation	58.1 ± 11.5
Range	17–88
Sex	
Male	184 (78.3)
Female	51 (21.7)
Smoking	
Never smoker	68 (28.9)
Smoker or former smoker	128 (54.5)
Missing	39 (16.6)
Alcohol consumption	
Abstainers	57 (24.3)
Drinker or Former drinker	121 (51.4)
Missing	57 (24.3)
Clinical stage (7th ed)	
I	47 (20.0)
II	62 (26.4)
III	50 (21.3)
IV	76 (32.3)
Location	
Tongue	155 (66.0)
Floor of mouth	56 (23.8)
Retromolar area	15 (6.4)
Palate	5 (2.1)
Gingiva	4 (1.7)
Treatment	
Surgery	87 (37.0)
Surgery + radiotherapy	128 (54.5)
Surgery + radiotherapy + chemotherapy	20 (8.5)
Margin status	
≥5 mm	192 (81.7)
<5 mm	43 (18.3)
WHO histopathological grading	
Well differentiated	94 (40.0)
Moderately differentiated	114 (48.5)
Poorly differentiated	27 (11.5)
Lymphovascular invasion	

	***n* (%)**
No	199 (84.7)
Yes	36 (15.3)
Local recurrence	
No	194 (82.6)
Yes	41 (17.4)
Regional recurrence	
No	216 (91.9)
Yes	19 (8.1)
Distant recurrence	
No	224 (95.3)
Yes	11 (4.7)
Status	
Alive	150 (63.8)
Dead	85 (36.2)

1

2

TABLE 2. Distribution of the neural invasion and its association regarding location in the tumor and number of foci in the oral squamous cell carcinoma included in this study (n = 235).

	***n* (%)**
Neural invasion	
No	161 (68.5)
Intraneural invasion	48 (20.4)
Perineural invasion	26 (11.1)
Intraneural invasion	
Location in the tumor	
Intratumoral	26 (54.2)
Peritumoral	22 (45.8)
Number of foci	
Unifocal	18 (37.5)
Multifocal	30 (62.5)
Perineural invasion	
Location in the tumor	
Intratumoral	18 (69.2)
Peritumoral	8 (30.8)
Number of foci	
Unifocal	5 (19.2)
Multifocal	21 (80.8)

TABLE 3. Univariate analysis for cancer-specific survival and disease-free survival of 235 patients with oral squamous cell carcinoma.

	Cancer-specific survival			Disease-free survival		
	% in 5 years	**HR (95% CI)**	***p*-Value**	**% in 5 years**	**HR (95% CI)**	***p*-Value**
Age (years)						
≤58 years	60.7	1		65.8	1	
>58 years	52.7	1.15 (0.75–1.76)	0.51	75.3	0.86 (0.51–1.43)	0.57
Sex						
Male	57.5	1		69.7	1	
Female	53.1	1.24 (0.73–2.09)	0.41	72.2	1.50 (0.80–2.79)	0.20
Clinical stage (7th ed.)						
Early (I + II)	63.6	1		70.5	1	
Advanced (III + IV)	51.0	1.58 (1.02–2.45)	0.03	70.5	1.00 (0.59–1.67)	0.99
Tumor site						
Tongue	59.9	1		72.0	1	
Floor of mouth	58.8	0.97 (0.58–1.64)	0.90	70.9	1.02 (0.55–1.89)	0.94
Others	37.1	1.75 (0.85–3.99)	0.06	60.4	1.46 (0.61–3.51)	0.33
Treatment						
Surgery	63.2	1		72.0	1	
Surgery + radiotherapy	54.2	1.14 (0.71–1.81)	0.57	74.2	0.90 (0.51–1.59)	0.72
Surgery + radiotherapy + chemotherapy	47.9	1.58 (0.65–3.87)	0.28	38.1	2.49 (0.87–7.09)	0.02
Margin status						
≥5 mm	55.6	1		69.2	1	
<5 mm	64.4	0.72 (0.39–1.38)	0.29	73.7	0.76 (0.36–1.61)	0.48
Lymphovascular invasion						
No	59.2	1		69.9	1	

	Cancer-specific survival			Disease-free survival		
	% in 5 years	**HR (95% CI)**	***p*-Value**	**% in 5 years**	**HR (95% CI)**	***p*-Value**
Yes	43.1	1.90 (1.11–3.23)	0.01	75.7	0.77 (0.33–1.79)	0.54
WHO histopathological grading						
Well differentiated	50.7	1		63.4	1	
Moderately differentiated	63.1	0.71 (0.45–1.12)	0.14	76.1	0.69 (0.40–1.20)	0.19
Poorly differentiated	58.3	0.95 (0.44–2.03)	0.89	76.5	0.84 (0.56–1.36)	0.55
Neural invasion						
Absent	66.5	1		69.5	1	
INI	29.4	2.05 (1.20–3.52)	0.002	66.9	1.02 (0.53–1.97)	0.93
PNI	65.8	1.01 (0.49–2.09)	0.99	79.8	0.61 (0.26–1.42)	0.35
INI: Location in the tumor						
Absent	66.5	1		69.5	1	
Intratumoral	29.7	2.00 (1.00–4.01)	0.01	57.5	1.31 (0.56–3.06)	0.58
Peritumoral	29.2	2.10 (1.01–4.35)	0.009	78.6	78.6 (0.71–1.71)	0.42
INI: Number of foci						
Absent	66.5	1		69.5	1	
Unifocal	24.7	2.05 (0.97–4.33)	0.006	57.0	1.43 (0.56–3.64)	0.49
Multifocal	34.1	2.05 (1.04–4.04)	0.006	72.2	0.74 (0.33–1.64)	0.39

FIGURES

Figure 1