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Prognostication for oral carcinomas based on two histological scoring systems (BD and iBD models)

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Oral squamous cell carcinomas (OSCC) display a very poor prognosis, partially because the majority of patients is diagnosed at an advanced stage, with locoregionally aggressive disease. Despite improvements in therapeutic options, the overall 5-year survival rate of patients with OSCC has stagnated for several decades at 40-50% (Chi et al., 2015). Currently the treatment and prognosis of OSCC rely mostly on clinical stage (TNM classification), which was recently revised by incorporating the depth of invasion and extracapsular infiltration into the T stage and N stage respectively (Ridge et al., 2017). Although several studies have shown better stratification of patients with this revised version of clinical staging (Mascitti et al., 2018; Moeckelmann et al., 2018), the accuracy and predictive value to support the clinical management and patient's prognosis need to be refined. Indeed, many clinical, morphological, biological and molecular characteristics displaying biomarker potential for early diagnosis, responsiveness to treatment, prognosis and post-therapeutic monitoring are constantly under investigation. Among them are histological features, that individually or in combination in a histological grading system, have shown predictive value in prognostication of patients with OSCC (Brandwein-Gensler et al., 2005; Speight and Farthing, 2018; Safi et al., 2019). The histological grading system adopted by the World Health Organization (WHO), which is based on cellular and nuclear pleomorphism, degree of keratinization and mitotic activity, is the most applied system for OSCC, however, its poor predictive value is widely recognized (Weijers et al., 2009; Lindenblatt et al., 2012; Sawazaki-Calone et al., 2015).

In 2015, Almangush et al. (2015) proposed a histological system (the BD model) for OSCC based on 2 histological features: tumor budding (B), the presence of single cells or clusters of up to 5 tumor cells in the invasive front, and depth of tumor invasion (D). The BD model has been significantly associated with poor disease-specific survival and high rates of recurrence in OSCC patients (Almangush et al., 2015, Sawazaki-Calone et al., 2015; Strieder et al., 2017 Hori et al., 2017). Recently, Yu and collaborators (2019) added the intensity of host inflammatory response in the invasive tumor front to the BD model, introducing the iBD model. In a sample composed of 246 patients with tongue squamous cell carcinoma, this prognostic model was able to improve the risk stratification of patients (Yu et al., 2019). This study was conducted to compare the performance of the BD model and its recently described variant, the iBD model, in the prognostication of patients with OSCC.

After ethics review board approval, 243 patients with OSCC treated in the UOPECCAN and CEONC Cancer Hospitals in Cascavel-Parana and Hospital Bom Pastor in Varginha-Minas Gerais were included in this study. A complete description of the demographic and clinical data has been previously reported (Dourado et al., 2020). Depth of invasion (Supplementary Fig. 1),

tumor budding (Supplementary Fig. 2) and inflammatory response (Supplementary Fig. 3) were individually assessed in the HE stained slides retrieved from the pathology archives and later grouped in risk scores (low, intermediate and high risk) according to the original articles of Almangush et al. (2015) and Yu et al. (2019) (Supplementary Table 1). The assessment of the histological parameters is detailed in Supplementary File 1. The number of available slides of the primary tumor for each case ranged from 2 to 16. Cancer-specific survival was assessed by univariable and multivariable Cox regression analyses, and survival curves for the BD and iBD models were constructed according to the Kaplan-Meier method and compared by the log-rank test. A receiver operating characteristic (ROC) curve with area under the curve (AUC) was applied to compare the discriminatory ability of the models.

According to the BD model, 49 (20.1%) cases were classified as low risk, 107 (44.1%) as intermediate risk and 87 (35.8%) as high risk. Applying the iBD classification, 35 cases classified as high risk in the BD model were classified as intermediate risk in the iBD model due to the presence of an intense inflammatory reaction forming a band at the invasive front. Thus, 49 (20.1%), 142 (58.5%) and 52 (21.4%) cases were classified as low, intermediate and high risk with the iBD model, respectively.

Individually, tumor budding (HR: 1.58, 95% CI: 1.01-2.46, p=0.04) and depth of invasion (HR: 2.44, 95% CI: 1.53-3.89, p=0.001), but not inflammatory response (HR: 1.43, 95% CI: 0.91-2.25, p=0.12), influenced significantly cancer-specific survival (Table 1). Kaplan-Meier curves revealed that the BD model (p=0.002) and, less intensely, the iBD model (p=0.05) had a significant impact on the cancer-related survival of OSCC patients (Fig. 1A and 1B). In comparison with patients classified as low risk, patients classified at high risk with the BD model (HR: 2.56, 95% CI: 1.47-4.46, p=0.0009) and intermediate risk with the iBD model (HR: 1.94, 95% CI: 1.13-3.31, p=0.02) had significantly shortened cancer-specific survival (Table 1). Patients with tumors classified as intermediate risk with the BD model showed a strong tendency to have shortened cancer-specific survival (HR: 1.63, 95% CI: 0.86-3.06, p=0.13) (Table 1).

Multivariate analyses, adjusted by age, gender, location of tumor, clinical stage (based on the 7th edition), type of treatment and surgical margins, confirmed that tumor budding, depth of invasion and the BD model were independently associated with disease-specific survival (Table 2). To further characterize the BD and iBD models, the AUC of ROC curves were compared. As depicted in Fig. 1C, the BD model showed a significantly superior discrimination with an AUC of 0.616 (95% CI: 0.552-0.678) compared to 0.536 (95% CI: 0.471-0.600) for the iBD model (p=0.007).

Next, we investigated whether the BD and iBD models show differential prognostic significance for early-stage tumors (clinical stages I and II) and advanced-stage tumors (clinical stages III and IV). For early-stage tumors, tumor budding, depth of invasion and the BD model were significantly associated with outcome in both univariate (Table 1) and multivariate (Table 2) analyses, whereas no association was detected for these parameters in advanced-stage tumors. The inflammatory response and the iBD model were associated with neither early-stage nor advanced-stage tumors.

For a long period, histological features have been recognized as informative for the prognosis of OSCC. However, there are still some concerns regarding incorporation of the histological grade as an adjuvant in the therapeutic decision-making and prognosis of OSCC, because the system recommended by the WHO, which is based on subjective parameters resulting in high intra- and inter-examiner variability, shows traditionally an inappropriate association with patient outcomes. The results of this study confirmed that the BD model and, less intensely, the iBD model are associated with patient survival. The difference between the BD and iBD models is the host inflammatory response incorporated into the iBD model, which was not associated individually with patient's survival and did not contribute in combination with tumor budding and depth of invasion. Indeed, the association of the iBD model with the death of patients was driven by tumor budding and depth of invasion.

Given the complexity of the inflammatory response in the tumor microenvironment, immune cells may release growth factors, cytokines and chemokines creating a pro-tumorigenic milieu or an anti-tumorigenic environment (Comen, Bowman and Kleppe, 2018). A recent systematic review with meta-analysis showed that infiltration of $CD8^+$, $CD45RO^+$ and $CD57^+$ lymphocytes in the OSCC microenvironment is related to better overall survival, whereas the presence of $CD68^+$ and $CD163^+$ macrophages was associated with poor prognosis (Huang et al., 2019). Although quantification of the inflammatory response has been associated with the outcomes of OSCC patients in some studies, more important than the amount of cells, which is measured in histological scoring models, is the composition of the inflammatory infiltrate in the tumor microenvironment. Thus, the analysis should not concentrate on the magnitude of the inflammatory response, but rather on its composition in the tumor microenvironment.

Individually, both parameters within the BD model have been shown to be of prognostic value for OSCC (Almangush et al., 2018; Caldeira et al., 2019), which is in line with our findings. Moreover, the last AJCC staging system (8th edition) incorporated depth of invasion into the T stages of OSCCs, and the same AJCC adopted tumor budding as a tumor-related prognostic

factor for colorectal cancers. In OSCC, the incorporation of tumor budding into the WHO histological grade improved the stratification and prediction of outcomes compared to the WHO histological grade alone (Elseragy et al., 2019). Although these parameters are individually associated with survival, the combination of them in the BD model improved discrimination between low- and high-risk tumors, with a higher score being associated with a worse outcome regarding 5-year cancer-specific survival. Indeed, there is consensus that the combination of several independent markers is a better predictor of prognosis than individual parameters alone (Hussein et al., 2018). In the limitations of this study, the clinical value of the BD model for OSCC was well validated.

In conclusion, our results demonstrate that the BD model shows a superior prognostic value to that of the iBD model, and the assessment of the histological grade of OSCC using the BD model holds a prognostic significance for predicting patient survival, especially in early stage tumors. With simple and objective parameters, the BD model can easily be incorporated in histopathology reports in daily clinical practice.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

AUTHOR'S CONTRIBUTIONS

C.B. Domingueti: Methodology, formal analysis, data curation, investigation and writing-original draft. K.Y.M. Miwa: Methodology, formal analysis, data curation, investigation and writing-original draft. M.R. Dourado: Methodology, formal analysis, data curation and writing-original draft. I. Sawazaki-Calone: Methodology, formal analysis, data curation and writing-original draft. T. Salo: Conceptualization and writing-original draft. L.M.R. Paranaiba: Conceptualization, methodology, validation, investigation, visualization, supervision, funding acquisition, writing-original draft and project administration. R.D. Coletta: Conceptualization, methodology, validation,

investigation, visualization, supervision, funding acquisition, writing-original draft and project administration. All authors have critically revised the manuscript.

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Figure Legend

Figure 1. Disease-specific survival curves of OSCC patients based on the BD and iBD models and receiver operating characteristic (ROC) curve. (A) Kaplan-Meier cumulative curve for disease-specific survival with OSCC patients divided into low, intermediate and high risk based on the BD model. Patients classified with intermediate and high risk corresponded to those with a significantly poorer outcome compared to low risk (p=0.002). (B) Kaplan-Meier cumulative curve based on the iBD model. Applying the iBD model, a stratification among 3 levels was not evident though patients classified with a high risk had worse survival (p=0.05). (C) ROC curve with area under the curve (AUC) comparing the BD and iBD models. The BD model showed a significantly superior discrimination compared to the iBD model (p=0.007).

Supplementary Fig. 1. The assessment of the depth of invasion in representative samples of OSCC. (A) Superficial tumor with < 4 mm of depth of invasion and (B) tumor with ≥ 4 mm of depth of invasion.

Supplementary Fig. 2. Tumor budding assessment in OSCC, with arrows to indicate budding foci. (A) Tumor with low budding activity (fewer than 5 buds per field), and (B) tumor with high budding activity (more than 5 buds per field).

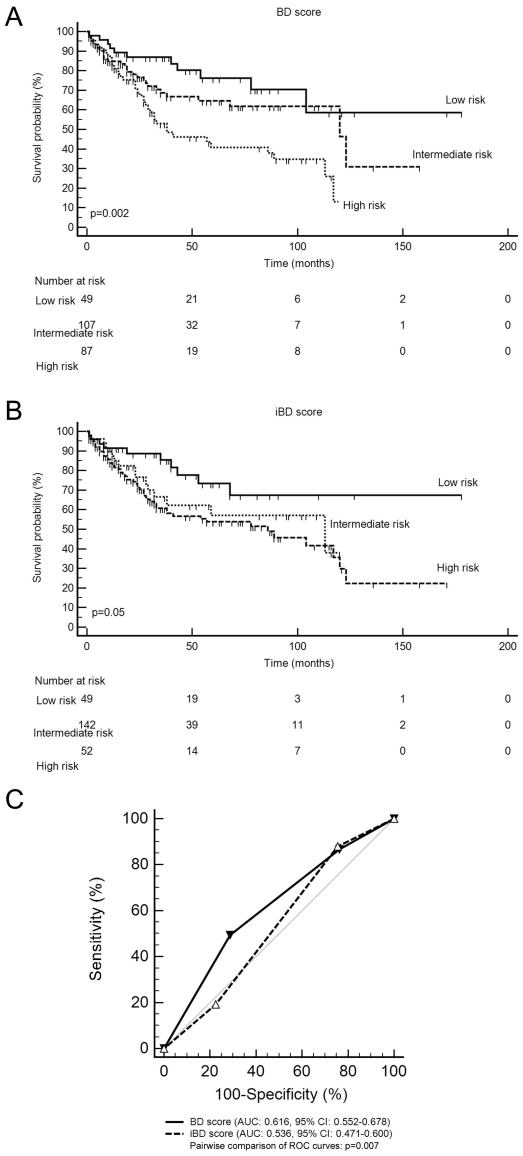
Supplementary Fig. 3. The assessment of the inflammatory response in OSCC. (A) A tumor with an intense inflammatory response at the invasive front, and (B) a tumor with a mild inflammatory reaction at the invasive front.

Table 1. Univariate analysis for cancer-specific survival of patients with oral squamous cell carcinoma.

	Whole sample (n=243)			Early stage tumors (n=100)			Advanced stage tumors (n=143)		
	% in 5 years	HR (95% CI)	p value	% in 5 years	HR (95% CI)	p value	% in 5 years	HR (95% CI)	p value
Tumor budding									
< 5 buds	67.1	1		76.8	1		60.7	1	
≥ 5 buds	45.8	1.58 (1.01-2.46)	0.04	52.3	1.88 (1.23-4.25)	0.001	39.2	1.40 (0.82-2.39)	0.21
Depth of invasion									
< 4 mm	80.2	1		88.9	1		69.1	1	
≥ 4 mm	50.4	2.44 (1.53-3.89)	0.001	55.5	3.33 (1.51-7.32)	0.003	47.4	1.90 (1.03-3.50)	0.04
Inflammatory response									
High grade	63.2	1		76.7	1		54.7	1	
Low grade	51.1	1.43 (0.91-2.25)	0.12	57.3	1.51 (0.76-3.20)	0.11	45.6	1.41 (0.79-2.52)	0.24
BD score									
Low risk	76.2	1		86.9	1		61.6	1	
Intermediate risk	64.6	1.63 (0.86-3.06)	0.13	70.0	2.23 (0.71-7.03)	017	61.9	1.10 (0.48-2.53)	0.82
High risk	40.8	2.56 (1.47-4.46)	0.0009	50.9	3.05 (1.37-8.94)	0.009	34.6	1.83 (0.88-3.77)	0.10
iBD score									
Low risk	73.4	1		79.0	1		68.4	1	
Intermediate risk	53.8	1.94 (1.13-3.31)	0.02	60.5	1.87 (0.78-4.46)	0.16	49.8	1.77 (0.67-4.91)	0.13
High risk	57.1	1.75 (0.81-3.82)	0.15	76.3	1.19 (0.31-4.54)	0.79	45.7	1.81 (0.67-4.91)	0.24

Table 2. Multivariate analysis of cancer-specific survival for the patients with oral squamous cell carcinoma.

	Whole Sample		Early stage tumors		Advanced stage tumors	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Model 1						
Tumor budding (< 5 buds vs. ≥ 5 buds)	1.91 (1.18-3.09)	0.001	1.81 (1.14-4.77)	0.005	1.16 (0.61-2.21)	0.64
Depth of invasion (< 4 mm vs. ≥ 4 mm)	3.80 (1.88-7.69)	0.0002	2.78 (1.25-6.17)	0.01	1.20 (0.64-2.25)	0.55
Inflammatory response (High vs Low)	1.45 (0.88-2.39)	0.14	1.10 (0.44-2.77)	0.82	1.30 (0.59-2.84)	0.51
Model 2						
BD (Low vs. Intermediate vs. High)	1.84 (1.28-2.63)	0.001	2.34 (1.27-5.31)	0.01	1.44 (0.65-3.21)	0.36
Model 3						
iBD (Low vs. Intermediate vs. High)	1.16 (0.79-1.69)	0.43	1.43 (0.67-3.07	0.34	1.28 (0.74-2.23)	0.37



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