REVIEW

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Tumor Budding and Poor Prognosis in Oral Cancer: A Systematic Review and Meta-Analysis

Sthefane Gomes Feitosa[1], Rafael Vidal de Oliveira[2], Thâmara Manoela Marinho Bezerra[1], Filipe Nobre Chaves[3], Khalil Fernandes Viana[1], Denis Francisco Gonçalves de Oliveira[4], Karuza Maria Alves Pereira[1,5]*

Abstract

Background: Tumor budding (TB) has been investigated in several types of solid tumors. In oral cancer, studies show its association with survival. However, for its implementation in routine histological analyses, results with a high certainty of evidence are needed. Therefore, the aim of this systematic review is to explore the association between tumor budding and overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS) in oral cancer. **Methods:** A search was performed in Embase, PubMed, Scopus, Livivo, Web of Science, and Google Scholar. We adopted the following inclusion criteria: studies that evaluate tumor budding in oral cancer, that investigate survival, and presenting cohort design. We excluded reviews and studies without hazard-ratio (HR) data. **Results:** This systematic review included 22 studies and showed an association between TB and survival. High-grade TB is associated with a worse OS in univariate analysis (HR = 3.11; 95% CI: 2.06-4.69, p<0.01) and multivariate analysis (HR = 2.62; 95% CI: 1.64-4.20, p<0.01); with a poorer DSS in univariate (HR = 2.43; 95% CI: 1.94-3.03, p<0.01) and multivariate analysis (HR = 2.01; 95% CI: 1.43-2.83, p< 0.01); and with a worse DFS in univariate (HR = 1.94; 95% CI: 1.44-2.62, p<0.01) and multivariate analysis (HR = 2.15; 95% CI: 1.31-3.53, p< 0.01). Sensitivity analysis showed that the results are robust, and no significant publication bias was identified in univariate analysis for DFS (Egger's test: p = 0.94). The certainty of the evidence was graded as low or very low. **Conclusion:** Our findings indicate that TB is an independent prognostic factor of OS, DSS, and DFS in oral cancer. However, further studies are needed to increase the certainty of the evidence.

Keywords: Mouth neoplasms- prognosis- survival- tumor budding

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Introduction

Oral cancer is frequent between head and neck cancers, and oral squamous cell carcinoma (OSCC) is the most common subtype. This subtype counts for about 90% of oral cancers. Patients with this malignant neoplasm show high morbidity and poor prognosis, with 5-year survival of approximately 50% (Jensen et al., 2015; Bjerkli et al., 2020; Dourado et al., 2020; Sá et al., 2020). Although the oral cavity allows easy inspection, 60% of OSCCs are diagnosed on an advanced stage. These data show the necessity of researching tools, clinical and laboratorial, for the early diagnosis (Manjula et al., 2015; Ebihara et al., 2019).

The clinical OSCC staging may be predicted based on morphological findings (tumor grade, depth of invasion, perineural invasion, lymphovascular invasion, lymphocytic host response, and mitotic activity) present on histopathological examination of the hematoxylin & eosin (H&E) stained sections (Almangush et al., 2018). This is a valuable tool, uniting simplicity, good reproducibility, and low cost while providing important pathological information for the prognosis and clinical resolution of the case. In the 8th edition of the American Joint Committee on Cancer (in 2017), depth of invasion and extranodal extension were added as prognosis parameters (Sakata et al., 2018; Dourado et al., 2020; Dolens et al., 2021). However, it is important to be aware, since some of these histopathological parameters, such as tumor grade and lymphocytic response, have not been promising prognosticators, especially in early stage OSCC (Chen et al., 2013; Almangush et al., 2015).

*[1]Postgraduate Program in Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Brazil. [2]Department of Clinical Dentistry, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará, Fortaleza, Brazil. [3]School of Dentistry, Federal University of Ceará Campus Sobral, Sobral, Brazil. [4]Department of Pathology and Legal Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil. [5]Department of Morphology, Medical School, Federal University of Ceará, Fortaleza, Brazil. *For Correspondence: karuzaalves@ufc.br*

In recent years, researchers have suggested a prognostic role of tumor budding (TB) in cancer. It is defined as the presence of single cancer cell(s) or cluster(s) of less than five cancer cells at the invasive front. TB represents the dissociation of invasive cancer cells from the main tumor mass (Almagunsh et al., 2018). The importance of TB in cancer prognosis has been studied widely in colorectal cancer (Rogers et al., 2016; Lugli et al., 2017). It was included as an additional prognostic factor in the TNM and World Health Organization (WHO) classification schemes only for colorectal cancer (in 2017 and 2019, respectively) (Lugli et al., 2021). Since then, TB has been investigated in lung (Thakur et al., 2022), breast (Huang et al., 2022), endometrial (Ocal and Guzelis, 2022), esophageal (Almangush et al., 2016), gastric (Xue et al., 2023), pancreaticobiliary (Karamitopoulou et al., 2018), and head and neck cancers (Zhu et al., 2018). In addition, studies have shown the association between this parameter and survival in oral cancer (Wang et al., 2011; Sakata et al., 2018; Ebihara et al., 2019; Xie et al., 2019; Yu et al., 2019; Lugli et al., 2021).

TB can be analyzed through H&E staining, which makes this tool promising and completely applicable in daily histopathological practice. However, for a histopathological parameter to be included in routine histological analyses, studies must show that this parameter is a good prognostic factor with a high certainty of evidence. Therefore, this systematic review explores the association between tumor budding and survival in oral cancer.

Materials and Methods

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021), and the protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42021275388.

Eligibility criteria

The focused question was: Is there an association between tumor budding and the prognosis of oral cancer patients? For the construction of the question, we used the PECOS strategy:

1. Population (P): oral cancer patients.
2. Exposure (E): high-grade tumor budding.
3. Control (C): low-grade tumor budding.
4. Outcome (O): overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS).
5. Studies (S): cohort.

We established the following inclusion criteria: studies published in the Roman alphabet; that evaluate tumor budding in oral cancer patients; that investigate OS, DSS, or DFS; and cohort studies. There was no restriction regarding the time of publication. The following exclusion criteria were used: reviews, series and case reports, case-control, cross-sectional studies, in vitro and animal models, and studies that did not report hazard ratio (HR).

Information sources

The searches were performed in the following databases: PubMed, Embase, Web of Science, Scopus, and Livivo. An additional gray literature search was conducted on Google Scholar (the first 100 records were selected). The software EndNote Web was used to collect references and exclude duplicates. We conducted database searches on August 25, 2021; and updated it on November 16, 2022.

Search strategy

The following search strategy was used: ("Mouth Neoplasms" OR "Mouth Neoplasm" OR "Oral Neoplasm" OR "Oral Neoplasms" OR "Cancer of Mouth" OR "Mouth Cancers" OR "Oral Cancer" OR "Oral Cancers" OR "Cancer of the Mouth" OR "Mouth Cancer" OR "Oral Tongue Squamous Cell Carcinoma" OR "Oral Squamous Cell Carcinoma" OR "Oral Cavity Squamous Cell Carcinoma" OR "Oral Squamous Cell Carcinomas" OR "Squamous Cell Carcinoma of the Mouth") AND ("tumor budding" OR "tumor-budding" OR "tumour budding" OR budding) AND (Survival OR Prognosis OR Prognoses OR "Prognostic Factors" OR "Prognostic Factor" OR "overall survival" OR "hazard ratio" OR "disease-free survival" OR "Lymphatic Metastasis" OR "Lymphatic Metastases" OR "Lymph Node Metastasis" OR "Lymph Node Metastases"). Adaptations were performed in the present search strategy according to the characteristics of each database (Supplementary Table 1).

Study selection

Study selection was performed in two phases, using Rayyan software (Ouzzani et al., 2016). In the first phase, two independent reviewers (DFGO and RVO) screened titles and abstracts of articles. In the second phase, full-text articles were reviewed. The conflicts were solved with a third reviewer (SGF).

Data collection process

Two reviewers (DFGO and RVO) performed the data collection in an independent way. The conflicts were solved with a third reviewer (SGF). We collect data about author, publication year, country, staining method, gender, age, sample size, tumor site, histologic type, differentiation, TNM, clinical stage, follow-up, the cutoff point for high-grade tumor budding, HR, and confidence interval (OS, DSS, and DFS).

Risk of bias

The risk of bias assessment was conducted by two independent reviewers (DFGO and RVO), using the Joanna Briggs Institute critical appraisal checklist for cohort studies (Moola et al., 2020). A third examiner (SGO) resolved any disagreement. The overall risk of bias was judged according to the following: "high risk" when the study reached up to 49% score "yes"; "moderate" when the study reached 50%-69% score "yes"; and "low risk" if "yes" scores were more than 70% (Polmann et al., 2020). The risk of bias figure was generated using the statistical software R version 4.0.5.

Summary measures and synthesis of the results

We evaluated OS, DSS, and DFS in this systematic review. Therefore, HR was the effect measure adopted. For this purpose, we performed a meta-analysis of the pre-calculated data. We used the statistical software R version 4.0.5 (meta package) (Balduzzi et al., 2019). We adopted the random model as the included studies were from different populations. To pool the data, we used the inverse variance method and Restricted maximum-likelihood to estimate the tau² value. I^2, Q test, and tau² were investigated to evaluate the statistical heterogeneity. To explore the heterogeneity sources, subgroup analysis was performed based on the continent, country, cut-off, oral subsite, staining method, and clinical stage. In addition, sensitivity analysis was conducted by omitting one study at a time to evaluate the robustness of the pooled HR. We also investigated publication bias in the meta-analysis for DFS (univariate analysis) because the number of studies was more than 10. So, we used the funnel plot and Egger test. All the statistical tests were two-sided with a significance level of 5% ($p<0.05$).

Certainty of evidence

The certainty of evidence was evaluated through the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Guyatt et al., 2011). The GRADE categorizes evidence into 4 levels: high, moderate, low, and very low. Cohort studies are initially classified in low evidence, and some factors can downgrade the certainty of evidence (studies limitations, inconsistencies of the results, imprecision, indirect evidence, and publication bias) and others can upgrade the certainty of evidence (large effects, dose-response gradient, and effect of plausible residual confounding). A summary of the findings table was produced using the GRADEpro (McMaster University and Evidence Prime Inc.).

Results

Selection of the Studies

A total of 559 registers were retrieved from databases and gray literature. After excluding duplicates, 373 studies were screened, including 75 records. Of these articles, 13 studies were conference abstracts (reports not retrieved). Therefore, 62 full-text articles were reviewed, including a total of 22 studies in this systematic review (Figure 1).

Studies Characteristics

This systematic review included 22 studies, conducted in China (5), Japan (5), Brazil (2), Denmark (2), Norway (1), USA (2), India (1), South Korea (2), Italy (1), and one with samples from two countries (Brazil and Finland). These were published between 2011 and 2022, including a total of 3504 patients with a mean age of 58.94 years (Table 1, Table 2, and Supplementary Table 2). The mean follow-up was 60.14 months, and 61.81% of patients were male. Most tumors were found in the tongue and mainly classified in T2, N0, and M0. Concerning the histologic type, one study analyzed salivary duct carcinoma, and 21 studies evaluated squamous cell carcinoma (SCC) (95.45%). Most SCC was moderately differentiated (53.37%). Regarding tumor budding, 59.12% of patients had a low grade.

Risk of Bias

The principal methodological difficulty identified was related to the follow-up. However, overall, all studies were classified as low risk of bias (Supplementary Figure 1).

Synthesis of results

Overall survival

Meta-analyses for OS are presented in Figure 2. High-grade TB was associated with a worse OS in univariate analysis (HR = 3.11; 95% CI: 2.06-4.69, $p<0.01$) and multivariate analysis (HR = 2.62; 95% CI: 1.64-4.20, $p<0.01$). The test for heterogeneity finds significant heterogeneity ($I^2 = 73\%$, $p < 0.01$). In addition, we performed subgroup analyzes which showed reduced statistical heterogeneity when the HR was pooled by continent, country, cut-off, oral subsite, and staining method (Supplementary Table 3). To evaluate the stability of this meta-analysis, a sensitivity analysis was performed. The results demonstrated no significant changes in HR values (Supplementary Figure 2). Publication bias was not assessed, as less than ten studies were included in each meta-analysis.

Disease-specific survival

In Figure 3, we showed the meta-analyses for DSS. The pooled HR demonstrated high-grade TB correlated with poorer survival in univariate (HR = 2.43; 95% CI: 1.94-3.03, $p<0.01$) and multivariate analyses (HR = 2.01; 95% CI: 1.43-2.83, $p < 0.01$). The test for heterogeneity did not find significant heterogeneity in univariate analysis ($I^2 = 0\%$, $p = 0.60$) or multivariate analysis ($I^2 = 32\%$, $p = 0.20$). In addition, the pooled effect estimate for early-stage patients was 3.14 in multivariate analysis (95% CI: 1.84-5.36, $p< 0.01$; heterogeneity: $I^2 = 0\%$; p=0,82). We also performed a sensitivity analysis, which showed that the results were robust (Supplementary Figure 3). Publication bias was not evaluated, as less than ten studies were included in each meta-analysis.

Disease-free survival

Forest plots for DFS are presented in Figure 4. The pooled effect size estimate indicated that high-grade TB was significantly associated with a worse DFS in univariate (HR = 1.94; 95% CI: 1.44-2.62, $p < 0.01$) and multivariate analyses (HR = 2.15; 95% CI: 1.31-3.53, $p<0.01$). Sensitivity analysis showed that the results were stable (Supplementary Figure 4) and there was no significant publication bias in univariate analysis (Supplementary Figure 5, Egger's test: $p = 0.94$). However, the results had high heterogeneity. We also performed subgroup analyzes which demonstrated reduced statistical heterogeneity when the HR was pooled by continent, staining method, cut-off, oral subsite, country, and clinical stage (Supplementary Table 4).

Table 1. Study Characteristics

Author	Country	Laboratory method	Tumor site	Histologic type	Sample	Cut-off
Wang et al., 2011	China	H&E/IHQ	Tongue	SCC	230	5 buds
Manjula et al., 2015	India	H&E	Gingivo-bucal complex	SCC	33	10 buds
Jensen et al., 2015	Denmark	IHQ	Tongue (105); floor of the mouth (94)	SCC	199	median of counting
Pedersen et al., 2017	Denmark	IHQ	Floor of the mouth (103); tongue (94); others (25)	SCC	222	Digital Tumor Bud Count
Sakata et al., 2018	Japan	H&E/IHQ	Tongue	SCC	97	4 buds
Shimizu et al., 2018	Japan	IHQ	NI	SCC	91	10 buds
Ebihara et al., 2019	Japan	IHQ	Tongue	SCC	64	10 buds
Elseragy et al., 2019	Brazil; Finland	H&E	Tongue	SCC	311	5 buds
Nakaguro et al., 2019	Japan	H&E	salivary duct: parotid gland (117); submandibular gland (30); others (4)	salivary duct carcinoma	151	10 buds
Xie et al., 2019	China	H&E	Tongue	SCC	255	10 buds
Yu et al., 2019	China	H&E	Tongue	SCC	246	5 buds
Zhang et al., 2019	China	H&E/IHQ	NI	SCC	80	5 buds
Sá et al., 2020	Brazil	H&E	Tongue	SCC	82	5 buds
Dourado et al., 2020	Brazil	H&E	Tongue (170); floor of the mouth (67); others (17)	SCC	254	5 buds
Bjerkli et al., 2020	Norway	H&E	Tongue	SCC	150	5 buds
Xu et al., 2021	USA	H&E	Tongue	SCC	329	10 buds
Kang et al., 2021	China	H&E	Tongue	SCC	103	5 buds
Sung et al., 2021	South Korea	IHQ	NI	SCC	256	4,26 buds/mm^2
Mascitti et al., 2022	Italy	H&E	Tongue	SCC	211	5 buds
Kligerman et al., 2022	USA	H&E	NI	SCC	34	No cut-off
Sakai et al., 2022	Japan	H&E/IHQ	Tongue	SCC	70	5 buds
Cho et al., 2022	South Korea	H&E	Tongue	SCC	36	5 buds

H&E, Hematoxylin & Eosin; IHQ, immunohistochemical; NI, not informed; SCC, squamous cell carcinoma

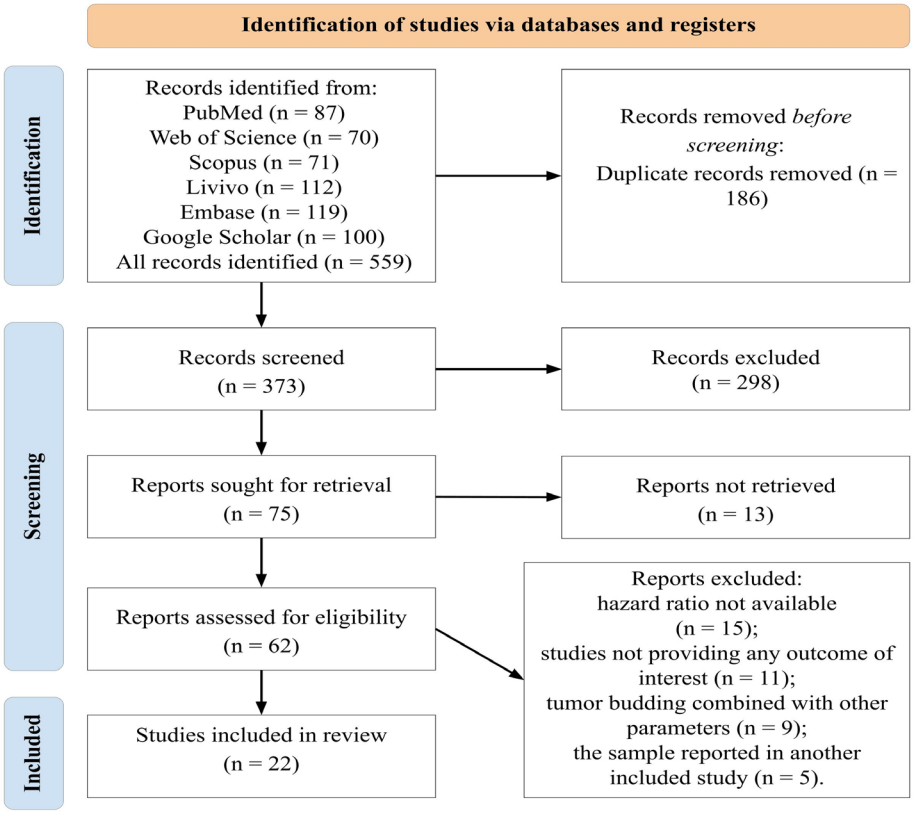

Figure 1. Selection of Articles for the Systematic Review

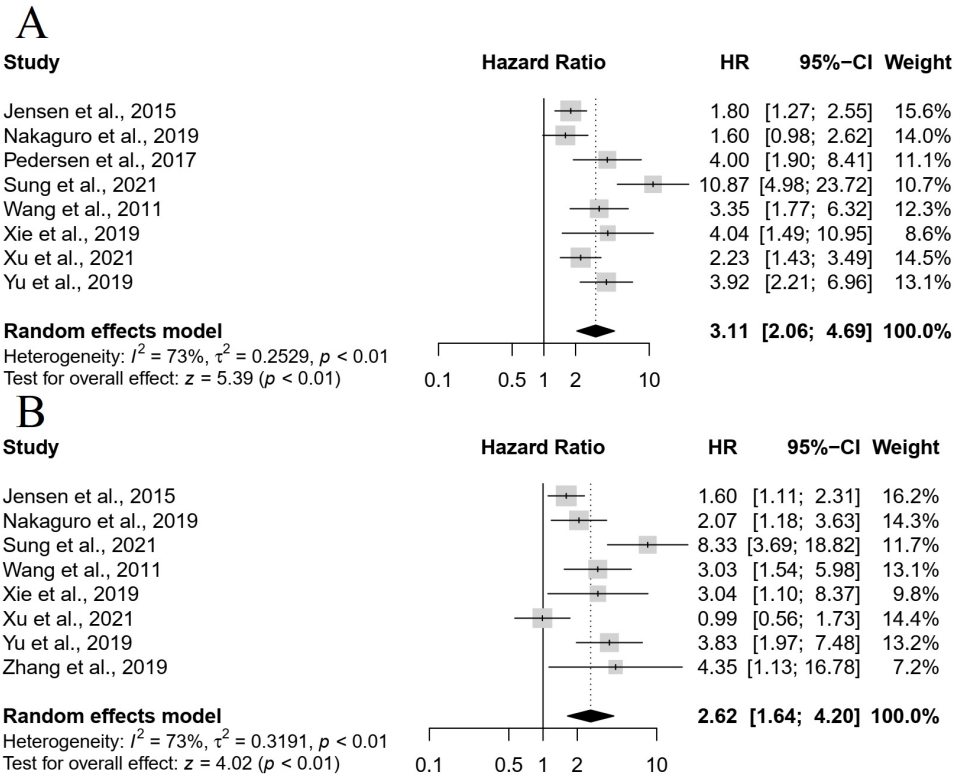

Figure 2. Meta-Analysis for Overall Survival. (A) univariate analysis, (B) multivariate analysis

Table 2. Individual Results of the Studies

Author	Main findings (association between TB and survival)
Wang et al., 2011	OS: HR (UA) = 3.350 (95% CI 1.774 - 6.323), p = 0.0002; HR (MA) = 3.029 (95% CI 1.535 - 5.977), p = 0.0014.
Manjula et al., 2015	DFS: HR (UA) = 1.32 (95% CI 0.59-2.95), p= 0.49.
Jensen et al., 2015	OS: HR (UA) = 1.8 (95% CI 1.3-2.6), p < 0.001; HR (MA) = 1.6 (95% CI 1.1-2.3), p= 0.01; DFS: HR (UA) = 2.1 (95% CI 1.2–3.6), p <0.01.
Pedersen et al., 2017	OS: HR (UA) = 4.0 (95% CI 1.9-8.4), p <0.001.
Sakata et al., 2018	DSS: HR (UA) = 9.605 (95% CI 2.473-63.023), p <0.001; HR (MA) = 3.114 (95% CI 0.520-32.052) p = 0.232.
Shimizu et al., 2018	DFS: HR (MA) = 2.19 (95% CI 1.51-3.18), p<0.01.
Ebihara et al., 2019	DSS: HR (UA or MA) = 4.41 (95% CI 1.34-14.5), p= 0.02.
Elseragy et al., 2019	DSS: HR (UA) = 2.69 (95% CI 1.46-4.96), p=0.001; HR (MA) = 2.86 (95% CI 1.53-5.35), p = 0.001; DFS: HR (UA) = 1.85 (1.13-3.06), p = 0.016; HR (MA) = 2.03 (95% CI 1.20-3.43), p = 0.010.
Nakaguro et al., 2019	OS: HR (UA) = 1.60 (95% CI 0.98-2.63), p = 0.062; HR (MA) = 2.07 (95% CI 1.18-3.62), p = 0.011.
Xie et al., 2019	OS: HR (UA) = 4.043 (95% CI 1.493-10.950), p = 0.001; HR (MA) = 3.039 (95% CI 1.103-8.370), p = 0.008.
Yu et al., 2019	OS: HR (UA) = 3.921 (95% CI 2.210-6.956), p<0.001; HR (MA) = 3.833 (95% CI 1.965-7.476), p<0.001; DFS: HR (UA) = 1.759 (95% CI 1.127-2.744), p=0.013; HR (MA) = 1.767 (95% CI 1.088-2.871), p = 0.021
Zhang et al., 2019	OS: HR (MA) = 4.347 (95% CI 1.126-16.776), p = 0.033.
Sá et al., 2020	DSS: HR (UA) = 1.94 (95% CI 0.86-4.38), p = 0.11; DFS: HR (UA) = 1.43 (95% CI 0.69-2.97), p = 0.33.
Dourado et al., 2020	DSS: HR (UA) = 1.89 (95% CI 1.01-2.49), p = 0.04; HR (MA) = 1.47 (95% CI 1.05-2.05), p = 0.02; DFS: HR (UA) = 1.29 (0.78-2.14), p = 0.31.
Bjerkli et al., 2020	DSS: HR (UA) = 2.269 (95% CI 1.182-4.356), p = 0.016.
Xu et al., 2021	OS: HR (UA) = 2.235 (95% CI 1.433–3.488), p < 0.001; HR (MA) = 0.987 (95% CI 0.564 – 1.726), p = 0.963; LRFS: HR (UA) =1.359 (95% CI 0.68-2.699), p = 0.382.
Kang et al., 2021	DSS: HR (UA) = 3.677 (95% CI 1.58-8.54), p = 0.002; DFS: HR (UA) = 1.84 (95% CI 1.0-3.4), p = 0.049.
Sung et al., 2021	OS: HR (UA) = 10.87 (95% CI 5.0-23.81); HR (MA) = 8.33 (95% CI 3.846-19.608); DFS: HR (UA) = 8.4 (95% CI 4.66-15.19); HR (MA) = 7.34 (95% CI 2.96-13.59)
Mascitti et al., 2022	DSS: HR (UA) = 2.37 (95% CI 1.57-3.56), p=0.00; HR (MA) = 2.21 (95% CI 1.41-3.45), p = 0.00; DFS: HR (UA) = 1.79 (1.09-2.95), p = 0.02.
Kligerman et al., 2022	RFS: HR (MA) = 1.17 (95% CI 1.05-1.3), p = 0.006.
Sakai et al., 2022	RFS: HR (UA) = 2.28 (95% CI 1.13-4.59), p = 0.017.
Cho et al., 2022	DSS: HR (UA) = 3.86 (95% CI 0.37 - 40.66), p = 0.26; HR (MA) = 1.18 (95% CI 0.43-3.26), p=0.75; RFS: HR (UA) = 1.65 (95% CI 0.28 – 9.86), p = 0.58; HR (MA) = 2.06 (95% CI 0.41-10.47), p = 0.38.

CI, confidence interval; DFS, disease-free survival; DSS, disease-specific survival; HR, hazard-ratio; LRFS, local recurrence-free survival; MA, multivariate analysis; OS, overall survival; RFS, recurrence-free survival; UA, univariate analysis; TB, tumor budding

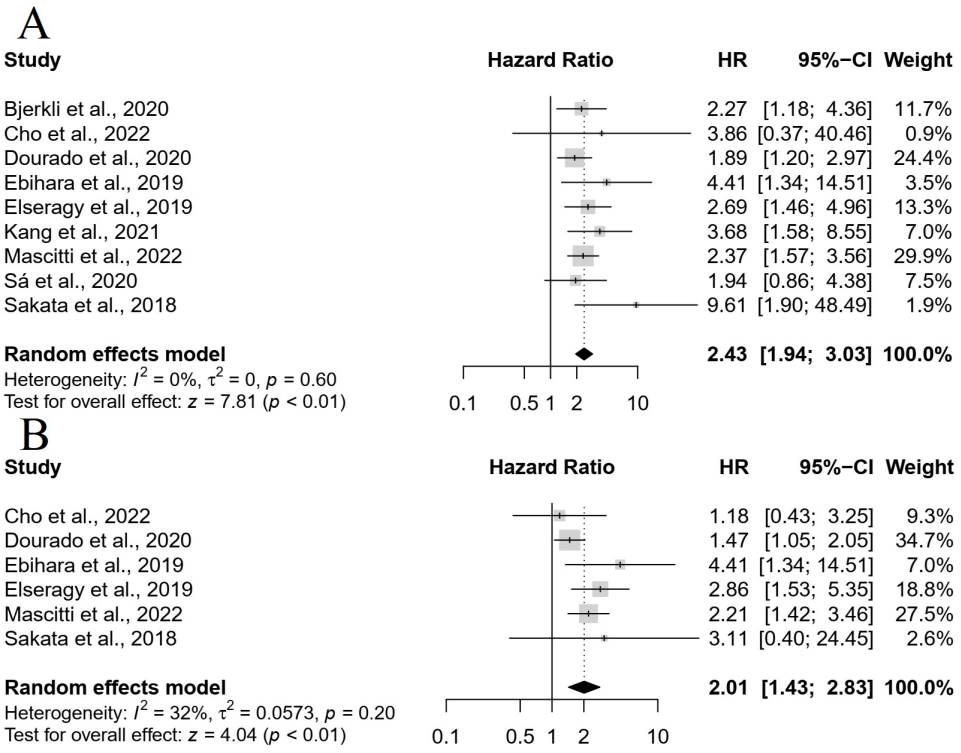

Figure 3. Meta-Analysis for Disease-Specific Survival. (A) univariate analysis, (B) multivariate analysis

Certainty of the Evidence

Table 3 presents the GRADE evaluation. The certainty of the evidence for overall survival and disease-specific survival was graded "very low" due to publication bias. While for disease-free survival the certainty was graded as "low".

Discussion

Oral cancer is one of the most common malignancies worldwide (Karjol et al., 2020). Of these, 90% are squamous cell carcinomas, which shows a 5-year overall survival rate of 50% and less than 30% in advanced stages

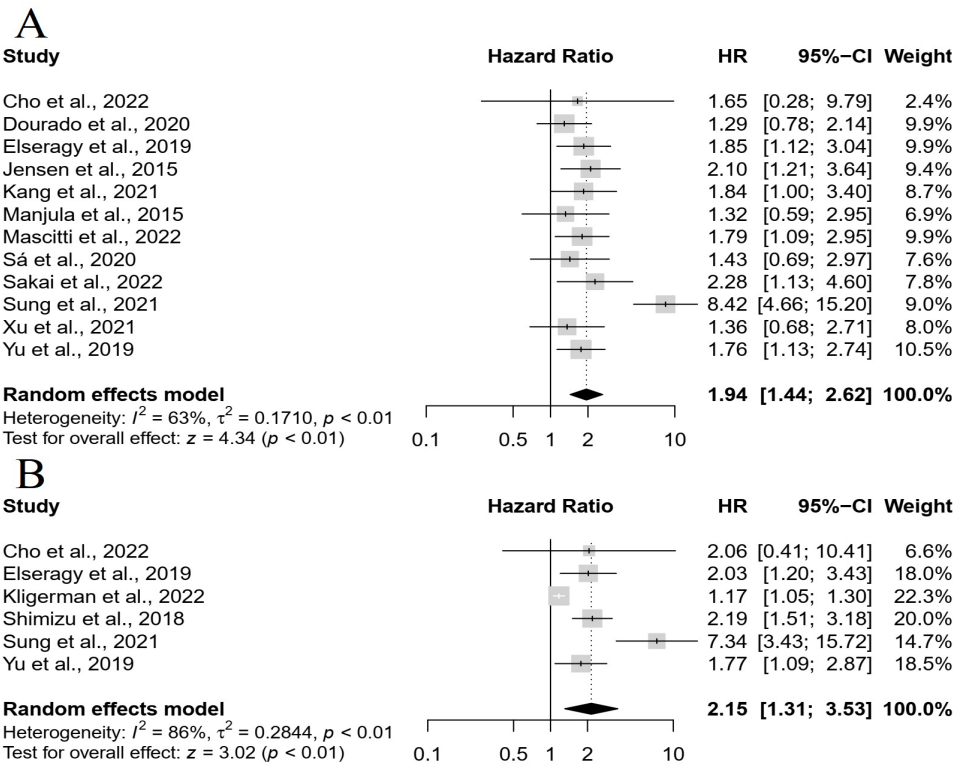

Figure 4. Meta-Analysis for Disease-Free Survival. (A) univariate analysis, (B) multivariate analysis

Table 3. GRADE Summary of Findings

Outcomes						
Overall survival						
Univariate analysis (HR = 3.11; 95% CI: 2.06-4.69, p < 0.01); multivariate analysis (HR = 2.62; 95% CI: 1.64-4.20, p < 0.01).						
Participants (studies)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Certainty
1,968 (9 cohorts)	not serious	not serious	not serious	not serious	suspected*	⊕OOO Very low
Disease-specific survival						
Univariate analysis (HR = 2.43; 95% CI: 1.94-3.03, p < 0.01); multivariate analysis (HR = 2.01; 95% CI: 1.43-2.83, p < 0.01).						
Participants (studies)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Certainty
1,308 (9 cohorts)	not serious	not serious	not serious	not serious	suspected*	⊕OOO Very low
Disease-free survival						
Univariate analysis (HR = 1.94; 95% CI: 1.44-2.62, p < 0.01); multivariate analysis (HR = 2.15; 95% CI: 1.31-3.53, p < 0.01).						
Participants (studies)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Certainty
2,255 (14 cohorts)	not serious	not serious	not serious	not serious	undetected	⊕⊕OO Low

* Publication bias was suspected as less than ten studies were included in each meta-analysis; HR: Hazard Ratio; CI: confidence interval

(Dolens et al., 2021). Regarding the staging system, the TNM system is the most used classification. Of note, studies show patients in similar stages may have different prognoses (Xie et al., 2019; Kang et al., 2021). Therefore, new biomarkers and histopathological parameters are needed to predict survival in oral cancer.

In 2017, two new histopathological parameters were included in the TNM system. The T category incorporated the depth of invasion, and the N category included the extranodal extension (Dourado et al., 2020; Dolens et al., 2021). Tumor budding is a new histopathological feature that has been investigated in recent years. It is defined as a single isolated cancer cell or small cluster composed of fewer than five cells at the invasive front, representing epithelial-mesenchymal transition (Xie et al., 2019; Dourado et al., 2020). It indicates the dissociation of invasive cancer cells from the tumor mass and has been reported in many cancers as a promising prognostic parameter (Almangush et al., 2018; Zhu et al., 2018; Karjol et al., 2020). Systematic reviews with meta-analyses show that high-grade TB is significantly associated with poor prognosis in patients with gastric cancer, gynecologic cancer, lung cancer, metastatic colorectal cancer, and head and neck squamous cell carcinoma (Zhu et al., 2018; Guo et al., 2019; Ailia et al., 2022; Thakur et al., 2022; Qu et al., 2023).

This systematic review and meta-analysis included 22 studies that investigated the prognostic role of TB in oral cancer. According to our analyses, this histopathological feature was significantly correlated with oral cancer. Therefore, high-grade tumor budding is an independent predictor of overall survival, disease-specific survival, and disease-free survival in this cancer. To the best of our knowledge, this is the first systematic review that included all subtypes of oral cancer in the meta-analyses and included the largest number of studies.

In our review, all studies were cohort types. This design is susceptible to confounding bias, which can be controlled by multivariate analysis. Thus, other strengths of this review are the pooled HRs from multivariate analyses. This effect estimate was mainly adjusted for age, gender, tumor size, lymph node invasion, and clinical stage, showing that tumor budding is an independent prognostic factor in oral cancer. In addition, we conducted a sensitivity analysis, suggesting the robustness of the findings.

This study has some limitations. Firstly, there is no common consensus on the assessment method. Most studies evaluated tumor buds using H&E staining. This parameter easily adapts to routine histopathologic examination without requiring additional expensive techniques (Wang et al., 2011). However, tumor buds may sometimes be hard to identify. In this case, a cytokeratin stain may be used to confirm the suspected tumor bud (Studer et al., 2021). Another limitation is the different cutoff points, which contributed to the detection of statistical heterogeneity. Subsequently, we removed the heterogeneity when grouping studies with the same cutoff point (5 buds).

There is consensus on the evaluation of tumor budding only in colorectal cancer. In 2016, a scoring method for tumor budding in colorectal cancer was proposed by the International Tumor Budding Consensus Conference (ITBCC), contributing to the implementation of the assessment of this histopathological parameter in routine practice, clinical trials, and meta-analyses (Studer et al., 2021). The main recommendations according to ITBCC were to count tumor buds in the hotspot (20x objective), using a three-tier system (0-4 buds, 5-9 buds, and 10 or more buds) (Lugli et al., 2017).

In this study, the certainty of the evidence was graded as very low for OS and DSS due to publication bias. Conversely, certainty was graded as low for DFS. Recently, the certainty was graded as very low in a systematic review that investigated the association between tumor budding and DFS in oral SCC (Dolens et al., 2021). Here, the researchers only included five studies in the meta-analysis. We included twelve studies, and no publication bias was identified. Therefore, there was no downgrade. We did not upgrade for moderate certainty because the HR did not greater than 2 (Guyatt et al., 2011).

Further studies are needed to assess the prognostic role of tumor budding and allow for an increase in evidence. These studies need to standardize tumor budding

assessment, present HR data for each subsite of the oral cavity and perform multivariate analysis. We recommend prospective cohorts as the design, and further systematic reviews with meta-analyses should be conducted. Thus, we will have certainty about the prognostic role of tumor budding in oral cancer, more precise risk stratification, and better clinical decision-making.

In conclusion, This systematic review and meta-analysis showed an association between tumor budding and the prognosis of oral cancer patients. We found that high-grade tumor budding is an independent prognostic parameter for overall survival, disease-specific survival, and disease-free survival. However, the certainty of the evidence was graded as low or very low. Another point identified in this research was the variability of evaluation methods for this histopathological parameter. Therefore, more studies with standardized tumor budding assessments are necessary to increase the level of evidence.

Abbreviations

AJCC, American Joint Committee on Cancer; DFS, disease-free survival; CI, confidence interval; DSS, disease-specific survival; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; H&E, Hematoxylin & Eosin; HR; hazard-ratio; IHQ, immunohistochemical; ITBCC, International Tumor Budding Consensus Conference; LRFS, local recurrence-free survival; MA, multivariate analysis; NI, not informed; OS, overall survival; OSCC, oral squamous cell carcinoma; PECOS, Population, Exposure, Control, Outcome, and Studies; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO, International Prospective Register of Systematic Reviews; RFS, recurrence-free survival; SCC, squamous cell carcinoma; TB, tumor budding; TNM, tumor, nodes, and metastasis; UA, univariate analysis; UICC, Union for International Cancer Control; WHO, World Health Organization.

Author Contribution Statement

SGF, DFGO, and RVO: Conceptualization, methodology, investigation, formal analysis, and writing - original draft. TMMB, FNC, and KFV: Writing - original draft, and writing - review & editing. KMP: conceptualization, methodology, writing - review & editing, supervision, and project administration.

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Ethics approval and consent to participate

Not applicable.

Data availability statement

The data that support the findings of this study are available in the supplementary file and on request from the corresponding author.

Study Registration

The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registry number CRD42021275388.

Conflict of Interest

The authors declare no conflict of interest.

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