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CLINICAL REVIEW

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Emerging histopathologic markers in early-stage oral tongue cancer: A systematic review and meta-analysis

Amr Elseragy BDS, MSc[1,2,3] | **Ibrahim O. Bello BDS, PhD**[3,4] | **Awais Wahab DDS**[2,3] | **Ricardo D. Coletta DDS, PhD**[5,6] | **Antti A. Mäkitie MD, PhD**[7,8,9] | **Ilmo Leivo MD, PhD**[10,11] | **Alhadi Almangush DDS, PhD**[3,9,10,12] | **Tuula Salo DDS, PhD**[1,2,3]

[1]Cancer and Translational Medicine Research Unit, Medical Research Center Oulu, University of Oulu and Oulu University Hospital, Oulu, Finland

[2]Department of Oral and Maxillofacial Diseases, University of Helsinki, Helsinki, Finland

[3]Department of Pathology, University of Helsinki, Helsinki, Finland

[4]Department of Oral Medicine and Diagnostic Sciences, King Saud University College of Dentistry, Riyadh, Saudi Arabia

[5]Department of Oral Diagnosis, School of Dentistry, University of Campinas, Piracicaba, São Paulo, Brazil

[6]Graduate Program in Oral Biology, School of Dentistry, University of Campinas, Piracicaba, São Paulo, Brazil

[7]Department of Otorhinolaryngology – Head and Neck Surgery, University of Helsinki and HUS Helsinki University Hospital, Helsinki, Finland

[8]Division of Ear, Nose and Throat Diseases, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

[9]Research Program in Systems Oncology, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[10]Institute of Biomedicine, Pathology, University of Turku, Turku, Finland

[11]Turku University Central Hospital, Turku, Finland

[12]Faculty of Dentistry, Misurata University, Misurata, Libya

Correspondence
Amr Elseragy, Cancer and Translational Medicine Research Unit, Medical Research Center Oulu, University of Oulu and Oulu University Hospital, Oulu, Finland.
Email: amr.elseragy@oulu.fi

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Abstract

Although there are many histopathologic prognosticators, grading of early oral tongue squamous cell carcinoma (OTSCC) is still based on morphological cell differentiation which has low prognostic value. Here we summarize the emerging histopathological markers showing powerful prognostic value, but are not included in pathology reports. Using PubMed, Scopus, Ovid Medline, and Web of Science databases, a systematic literature search was preformed to identify early OTSCC studies that investigated the prognostic significance of hematoxylin–eosin-based histopathologic markers. Our meta-analysis showed that tumor budding was associated with overall survival (hazard ratio [HR] 2.32; 95% CI 1.40–3.84; $p < 0.01$) and disease-specific survival (DSS) (1.89; 95% CI 1.13–3.15; $p = 0.02$). Worst pattern of invasion was associated with disease-free survival (DFS) (1.95; 95% CI 1.04–3.64; $p = 0.04$). Tumor–

Alhadi Almangush and Tuula Salo jointly supervised this work.

stroma ratio was also associated with DFS (1.75, 95% CI 1.24–2.48; $p < 0.01$) and DSS (1.69; 95% CI 1.19–2.42; $p < 0.01$). Tumor budding, worst pattern of invasion, and tumor–stroma ratio have a promising prognostic value in early OTSCC. The evaluation and reporting of these markers is cost-effective and can be incorporated in daily practice.

KEYWORDS

early stage, oral tongue cancer, tumor budding, tumor stroma ratio, worst pattern of invasion

1 | INTRODUCTION

Oral tongue squamous cell carcinoma (OTSCC) is the most common cancer occurring in the oral cavity.[1] OTSCC is considered an aggressive malignancy with a poorer prognosis than SCC of the other locations of the oral cavity.[2] The incidence of OTSCC increases with age; however, the incidence in young patients under the age of 40 has reported to be increasing.[3] Staging of OTSCC is a critical step in the diagnosis process, with various objectives such as treatment planning, prognosis assessment, and treatment evaluation. The 8th edition of TNM classification (AJCC 8)[4] addresses tumor size and tumor depth of invasion (T), lymph node status and extranodal extension (N), and the presence of distant metastasis (M). These are important in both clinical (cTNM) and pathological (pTNM) staging.[5]

In OTSCC patients, the common cause for failure of treatment is the regional recurrence after surgery.[6] An abundant vascular and lymphatic supply of the tongue apparently facilitates cancer cell invasion and metastasis.[7] Large tumor size, significant depth of invasion, insufficient resection margins and metastasis to cervical lymph nodes are considered unfavorable prognostic factors.[8] It is of a great clinical significance to predict biological behavior of OTSCC in its early stage rather than in the advanced stage, while the latter usually receives multimodality treatment. Patients with an early-stage tumor typically receive treatment based on the clinical judgment and established institutional practice. Thus, in most cases, early-stage OTSCC receive multimodality treatment only if they are deemed highly aggressive. In order to ensure better results with treatment of early OTSCC, it is important to identify histological markers that can accurately predict the aggressiveness of the tumor.

Although research on molecular biomarkers has reported hundreds of biomarkers, none have found use in clinical management of OTSCC patients.[9] Therefore, pathologists still routinely consider mainly the classic histopathological features/parameters recognized with standard hematoxylin and eosin (HE) staining.

Histological characteristics of the tumor and its surrounding tissues play an important role in the diagnosis of tumor biopsies, and are becoming increasingly important in prognostication. Such features include depth of invasion and perineural invasion, which are currently included in the pathology report[10] and have been recently reviewed in studies of heterogenous subsites of oral squamous cell carcinoma.[10–12] Furthermore, many HE-related prognostic markers have been introduced in recent studies. Although these have shown good prognostic value for early-stage OTSCC, they are not included in clinical implementation. Examples of such markers include tumor budding,[13–15] worst pattern of invasion (WPOI),[13,16] tumor stroma ratio (TSR),[17] tumor-infiltrating lymphocytes (TILs),[18] and cell-in-cell phenomenon,[19] which has been recently studied in many cancers including early OTSCC.[19]

To avoid heterogeneity among subsites of the oral cavity and to identify newly introduced histologic markers that can identify high-risk early OTSCC, this systematic review aims to provide a critical summary of promising histopathologic features identified by HE staining that are currently not yet included in the daily practice of pathologists.

2 | METHODS

2.1 | Search strategy

A systematic search of databases for scientific articles related to early-stage OTSCC was undertaken. Using advanced search function, the following terms were included in the search fields: title, abstract, subject heading word and keyword heading word (tongue OR lingual) AND (cancer* OR squamous cell carcinoma* OR neoplasm* OR tumor*) AND (early stage OR low stage OR small OR stage I–II OR T1-T2 OR T1 OR T2 OR cT1/2N0 OR N0) AND (prognosis* OR predict* OR survival* OR recurrence* OR mortality* OR metastasis*). These search terms were entered into PubMed, Scopus, Ovid Medline, and Web of Science databases (up to and including December 2020).

The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) were followed.[20] This systematic review is registered in PROSPERO (an international database of prospectively registered systematic reviews) with a registration number: CRD42018109527.

2.2 | Screening

The titles and abstracts retrieved by the electronic data searches were screened by two independent reviewers (AE, IOB) to remove any unrelated studies. If a reviewer was uncertain about whether a study was related, it was initially retained, then separately re-checked by both reviewers.

2.3 | Data extraction

From the relevant articles, we retrieved the first author's name, publication year, country, total number of patients, stage at the time of diagnosis and the histopathological marker/s that were analyzed. For those emerging markers (i.e., not included in the pathology report), additional data such as univariate and multivariate analyses, survival outcome (overall survival, disease-specific survival [DSS], and disease-free survival) were retrieved. In addition, statistical values (hazard ratio [HR], 95% confidence interval, and *p* value) were also retrieved.

2.4 | Inclusion criteria

Studies that used HE-stained cancer slides to evaluate any histopathologic prognostic parameters, from which we further focused on markers that are not assessed in routine diagnostics. In addition, other inclusion criteria included cohorts of early-stage OTSCC, original publications, and publications in English language.

2.5 | Exclusion criteria

We excluded studies of advanced stages of cancer, studies that included other subsites of the oral cavity, publications in languages other than English, conference abstracts, animal sample studies, and studies related to cancers other than OTSCC.

2.6 | Statistical method

We used the statistical software RStudio (version 1.4.1717) to run the "meta" package (version 4.13-0) for the meta-analyses. One inverse variance weighted fixed-effect analysis was carried out for each meta-analysis. In addition to the meta-analyzed effect sizes, "test for overall effect" was reported to estimate the pooled effect of statistical significance ($p < 0.05$). We considered the random-effect model analysis as the main result to assess any possible heterogeneity among the studies.[21] We included the estimated proportion of variation in effect sizes due to heterogeneity (I^2).

3 | RESULTS

Our search strategy retrieved a total of 5223 hits. After removal of duplicates, 2690 records were included for the eligibility stage (removing studies unrelated to the topic). A total of 116 studies were considered to be initially eligible and were fully screened by the two reviewers according to inclusion and exclusion criteria. Figure 1 illustrates the flowchart of the identification and selection of the eligible studies. For the study eligibility there was no disagreement between the two reviewers; by checking the citations and the references of the selected papers no additional studies required inclusion. The selected studies were published between 1986[22] and 2020.[19] Cohort sizes for the included studies ranged from 18 cases[23] to 616 cases.[24] The included studies were conducted in many countries including Japan, Finland, United States, India, China, Italy, Taiwan, South Korea, Sweden, Australia, Brazil, Israel, Norway, Pakistan, Spain, United Kingdom, Morocco, Netherland, New Zealand, and Saudi Arabia (countries are listed here according to the number of published studies). A total of 15 histopathologic markers and eight multiparameter grading and scoring systems were examined in these studies. Of these, the histopathologic prognostic markers that are routinely evaluated in pathology reports were reported more often than others. These include depth of invasion, lympho-vascular invasion, perineural invasion, grade of differentiation, and tumor thickness.

Regarding the emerging prognostic markers that are not included in the pathology report, the published studies revealed a promising prognostic value for cancer-related histopathologic markers including cell-in-cell structures (one study),[19] tumor budding (seven studies),[13,15,25–29] and pattern of invasion (15 studies) which were evaluated either as mode of invasion,[22,30] pattern of invasion,[31–34] invasive pattern,[28] or WPOI.[13,16,25,35–39] At the same time, some of the relevant studies analyzed stromal-related histopathologic markers including stromal infiltrating lymphocytes (one study)[18] and tumor–stroma ratio (TSR; three studies)[17,40,41] which were significantly associated with prognosis. However,

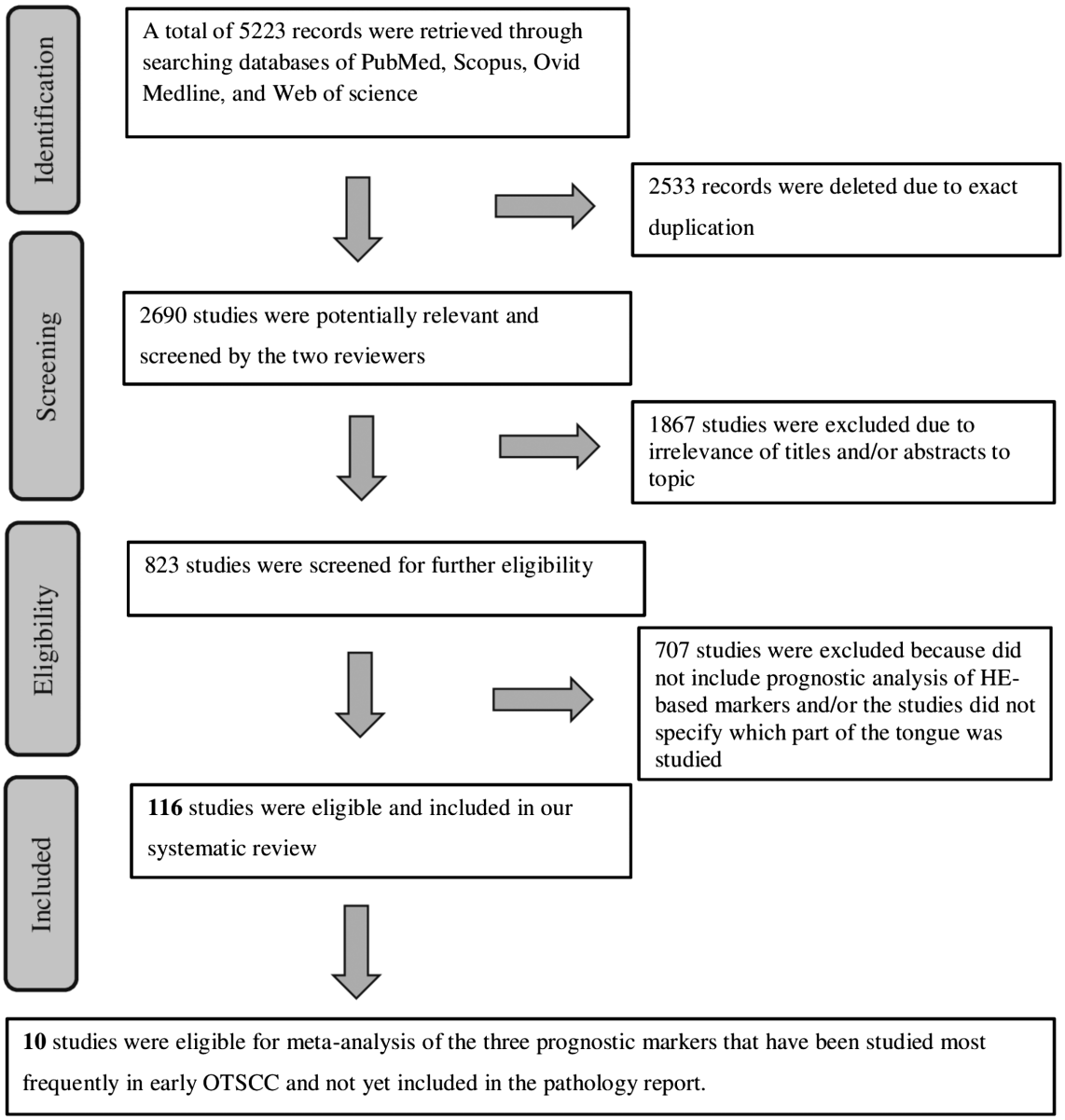


FIGURE 1 PRISMA flowchart showing the selection process of the studies included and excluded from this review

other stromal markers (including desmoplastic reaction in one study,[2] lymphocytic host response in two studies,[13,42] and muscular invasion in three studies[43–45]) were reported in the relevant studies and the findings were not suggestive of prognostic relevance. Among these emerging histologic markers, two cancer-related markers (tumor budding and WPOI) in addition to only one stromal-related marker (TSR) were repeatedly reported (Table 1). The other emerging histologic markers (cell-in-cell, stromal infiltrating lymphocytes, etc.) were summarized in Table S1.

Furthermore, histopathologic grading systems that are not included in the routine pathology report were summarized in Table S2. These include Anneroth Malignancy score (two studies),[46,47] BD score (three studies),[25,38,48] Brandwein-Gensler score (two studies),[13,49] Bryne score (two studies),[46,47] Martinez-Gimeno scoring system (two studies),[46,47] and a revised histological grading system (one study)[26] which suggested incorporating tumor budding into the current WHO histopathologic grading system to improve its prognostic function in early OTSCC.

3.1 | Meta-analyses

There were three emerging histologic markers that were repeatedly reported and therefore, considered for meta-analyses. These include tumor budding, WPOI, and TSR (summarized in Table 1).

TABLE 1 Histopathologic prognostic markers included in the meta-analysis and *not included* in the pathology report of early OTSCC (T1-2 N0)

Marker	Cancer-related or stroma-related	First author et al. (reference)	Number of cases	Endpoint	HR (95% CI)	*p* value
Worst pattern of invasion	Cancer-related	Almangush et al.[16]	479	DFS	1.46 (0.95–2.25)	**NA**
		Miguelañez et al.[36]	26	DFS	2.44 (0.36–16.55)	**NA**
		Hori et al.[38]	62	DFS	**3.84 (1.30–11.34)**	**<0.05**
Tumor budding	Cancer-related	Xie et al.[28]	195	OS	**5.582 (1.227–25.381)**	**0.026**
		Almangush et al.[25]	311	DSS	**1.76 (1.01–3.06)**	**0.044**
				OS	**1.62 (1.17–2.25)**	**0.004**
		Yamakawa et al.[29]	337	OS	**2.22 (1.15–4.30)**	**0.017**
		Hamada et al.[27]	99	OS	4.71 (1.47–15.1)	0.009
		Bjerkli et al.[15]	150	DSS	2.872 (0.742–11.121)[a]	0.089
Tumor–stroma ratio	Stroma-related	Almangush et al.[17]	311	DFS	**1.81 (1.17–2.79)**	**0.008**
				DSS	**1.71 (1.02–2.86)**	**0.03**
		Mascitti et al.[41]	211	DFS	1.65 (0.92–2.96)	0.111
				DSS	1.68 (1.03–2.75)	0.036

Notes: Bold values indicate multivariate analysis.

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival; OS, overall survival.

[a]TB 2-tier system where 0 to 4 buds were indicated as low-Bd and ≥5 buds indicated as high-Bd (1, 2).

3.2 | Tumor budding

Four studies[25,27–29] on tumor budding reported statistical values for overall survival (OS) including HRs and 95% CI. These studies were included in the meta-analysis and are presented using a forest plot (Figure 2A) with results from multivariate analysis of the relevant studies. The meta-analysis showed that tumor budding is a significant predictor of OS (Figure 2A) with HR of 2.32 (95% CI 1.40–3.84; $p < 0.01$). The meta-analysis of OS revealed

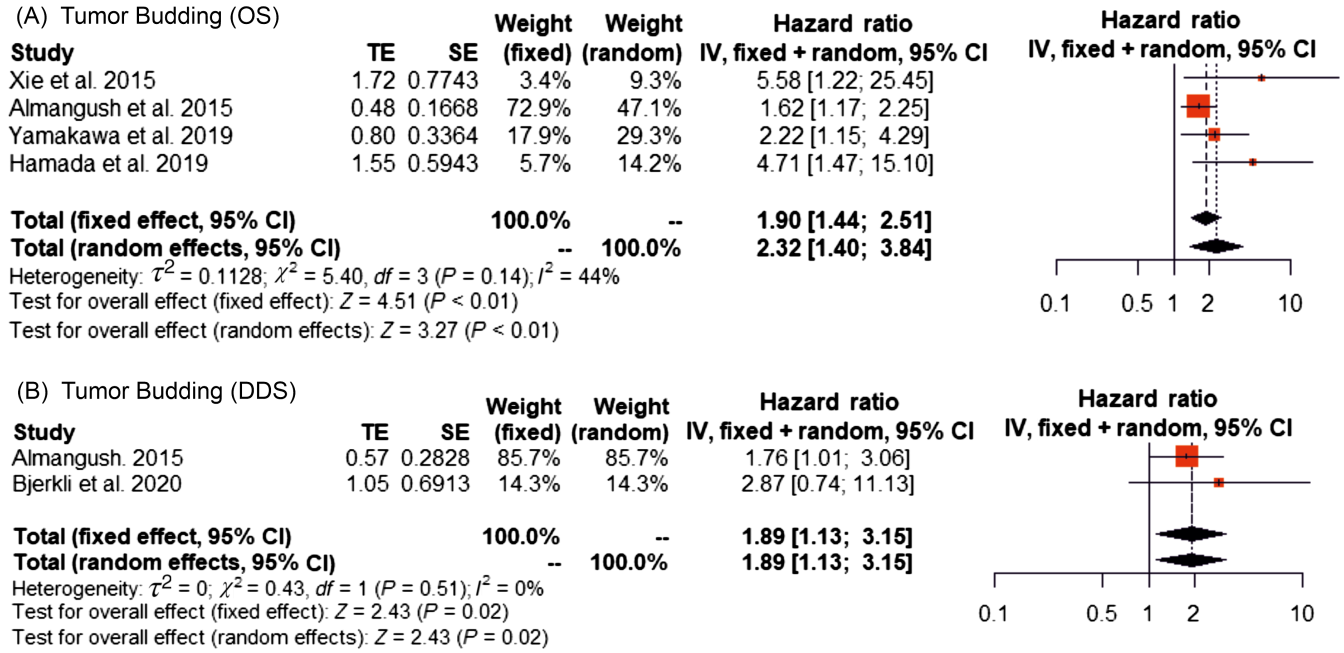


FIGURE 2 Forest plots for the pooled analyses of tumor budding in early OTSCC. (A) Tumor budding for multivariate overall survival; (B) Tumor budding for multivariate disease-specific survival [Color figure can be viewed at wileyonlinelibrary.com]

some heterogeneity ($I^2 = 44\%$). For DSS, two studies[15,25] were included in the meta-analysis and are visualized using a forest plot (Figure 2B). Again, tumor budding was indicated to be a predictor of DSS (HR 1.89, 95% CI 1.13–3.15; $p = 0.02$). The results of the meta-analysis on DSS did not present any heterogeneity ($I^2 = 0\%$).

3.3 | Worst pattern of invasion

Three studies on WPOI reported statistical analyses for disease-free survival (DFS) including HRs and 95% CI.[16,36,38] These studies were included in the meta-analysis visualized using a forest plot (Figure 3). WPOI presented as a valuable prognosticator of DFS with HR of 1.95 (95% CI 1.04–3.64; $p = 0.04$) with some heterogeneity ($I^2 = 28\%$).

3.4 | Tumor–stroma ratio

Two studies[17,41] on TSR reported statistical analysis for meta-analysis of DFS. The forest plot (Figure 4A) showed TSR as a significant predictor of DFS (HR = 1.75, 95% CI 1.24–2.48; $p < 0.01$) and homogenous ($I^2 = 0\%$). Similarly, two studies on TSR reported statistical analysis for DSS. Forest plot (Figure 4B) was constructed with multivariate results of TSR as the predictor of interest for DSS which showed HR of 1.69 (95% CI 1.19–2.42; $p < 0.01$) and no heterogeneity ($I^2 = 0\%$).

4 | DISCUSSION

Identifying reliable histopathological prognostic markers for early-stage oral tongue cancer is of great importance when allocating suitable risk stratification to guide clinicians in making optimal decisions for subsequent treatment strategies.[50] The ability of early OTSCC to metastasize into lymph nodes is not always associated with the clinical TNM staging. It has become obvious that due to histopathological heterogeneity, there are dissimilarities in the biological behavior even with identical clinical stages of OTSCC. Hence, for appropriate treatment planning, validated histological prognostic markers are necessary for the identification of aggressive early-stage tumors.[51] To avoid heterogeneity among the subsites of the oral cavity, we included studies in which the cohorts were defined as OTSCC by the authors. In addition, the meta-analysis and conclusions are based on studies which included OTSCC in its early-stage (T1-T2N0M0). In addition, we focused on markers that are not currently included in pathology reports, as the ones that are included (e.g., depth of invasion, perineural invasion) were reviewed in other articles recently.[27,41,52,53] Of note, three newly introduced histologic features were of significant clinical relevance, namely, tumor budding, WPOI, and TSR.

Tumor budding has recently received significant attention in many solid tumors.[54] It is defined as the presence of isolated single cancer cell/s or a cluster/s of less than five cancer cells in the area of an invasive cancer front. The presence of five or more buds is an index of high-risk for poor prognosis, while less than five is considered low risk.[1355] These buds signify a more aggressive phenotype of cancer cells.[28] In addition, tumor budding was confirmed to be frequently associated with lymph node metastasis, clinical stage, differentiation, tumor size, and overall survival.[27–29,56] Tumor budding has been reported not only as a valuable prognosticator for different subsites of OSCC,[57] but also as a promising prognostic marker for many solid tumors such as esophageal squamous cell carcinoma,[58] nasopharyngeal carcinoma,[59] and colorectal cancer,[60] especially in early stage of these cancers. The identification of tumor budding is straightforward, as pathologists can identify the number of tumor buds on HE-stained sections. Wang et al. study is one of the few studies that examined the biological characteristics of tumor budding in OSCC and reported that

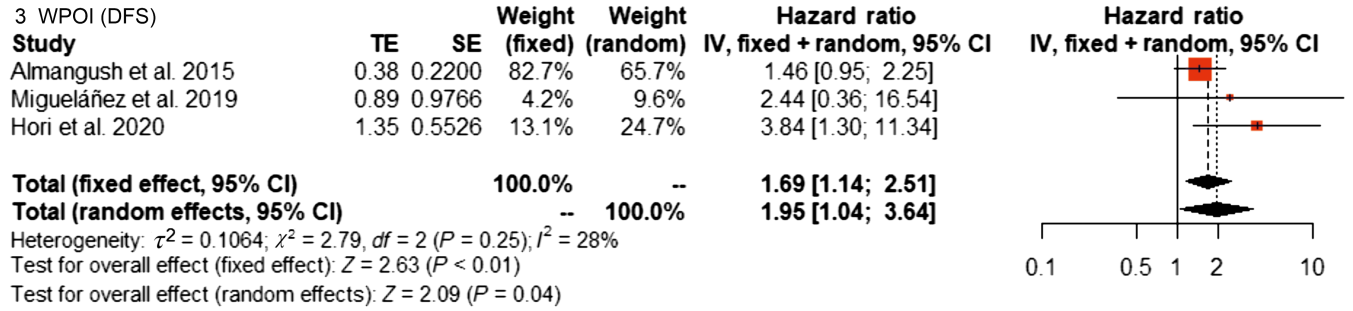
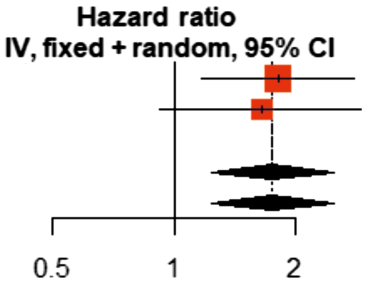


FIGURE 3 Forest plot for pooled analyses of worst pattern of invasion for disease-free survival in early OTSCC [Color figure can be viewed at wileyonlinelibrary.com]

(A) TSR (DFS)

Study	TE	SE	Weight (fixed)	Weight (random)	Hazard ratio IV, fixed + random, 95% CI
Almangush et al. 2018	0.59	0.2217	64.4%	64.4%	1.81 [1.17; 2.80]
Mascitti et al. 2020	0.50	0.2981	35.6%	35.6%	1.65 [0.92; 2.96]
Total (fixed effect, 95% CI)			**100.0%**	--	**1.75 [1.24; 2.48]**
Total (random effects, 95% CI)			--	**100.0%**	**1.75 [1.24; 2.48]**

Heterogeneity: $\tau^2 = 0$; $\chi^2 = 0.06$, $df = 1$ ($P = 0.80$); $I^2 = 0\%$
Test for overall effect (fixed effect): $Z = 3.15$ ($P < 0.01$)
Test for overall effect (random effects): $Z = 3.15$ ($P < 0.01$)



(B) TSR (DSS)

Study	TE	SE	Weight (fixed)	Weight (random)	Hazard ratio IV, fixed + random, 95% CI
Almangush et al. 2018	0.54	0.2630	47.6%	47.6%	1.71 [1.02; 2.86]
Mascitti et al. 2020	0.52	0.2505	52.4%	52.4%	1.68 [1.03; 2.75]
Total (fixed effect, 95% CI)			**100.0%**	--	**1.69 [1.19; 2.42]**
Total (random effects, 95% CI)			--	**100.0%**	**1.69 [1.19; 2.42]**

Heterogeneity: $\tau^2 = 0$; $\chi^2 = 0.00$, $df = 1$ ($P = 0.96$); $I^2 = 0\%$
Test for overall effect (fixed effect): $Z = 2.91$ ($P < 0.01$)
Test for overall effect (random effects): $Z = 2.91$ ($P < 0.01$)

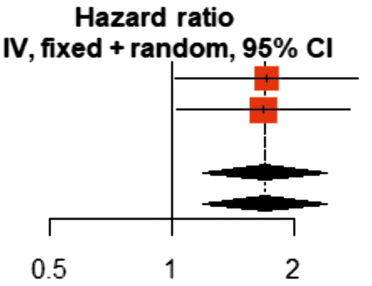


FIGURE 4 Forest plots for pooled analyses of tumor–stroma ratio in early OTSCC. (A) Tumor–stroma ratio for multivariate disease-free survival; (B) Tumor–stroma ratio for multivariate disease-specific survival [Color figure can be viewed at wileyonlinelibrary.com]

in immunohistochemical analysis it is associated with reduced expression of E-cadherin and overexpression of vimentin.[56] In addition, high-grade budding was associated with a higher expression of laminin-5 gamma 2 chain and a higher density of stromal myofibroblasts.[61] Moreover, tumor budding in cancer cells showed decreased expression of microRNAs miR-200a, miR-200b, and miR-200c.[62] All of these molecular features are associated with tumor aggressiveness. Despite the clear understanding of the biological background of tumor budding in some types of malignant cancers,[63] more details about the genetic background of cancer cells in tumor buds in early OTSCC needs to be revealed.

The WPOI is a recent modification of the pattern of tumor invasion. It can be categorized as either "cohesive" when the tumor has a pushing border, finger-like growths, and/or expands as large islands (>15 cells), or "infiltrative" when the tumor invades as small islands (≤15 cells) or tumor satellites that are at the distance of 1 mm or more from the main tumor.[16,64] Recently, it was reported that WPOI has a good prognostic value for patient survival in early OTSCC.[13,36,38] In addition, Brandwein-Gensler et al.[64] reported that their histologic risk assessment model comprising WPOI, perineural invasion and lymphocytic host response was significantly predictive of survival. Some investigators reported that the WPOI aggressive patterns (WPOI 4 and 5) were significantly associated with poorer overall survival and positive lymph nodes, in comparison with WPOI 1–3 in their cohort.[65] Moreover, it was clear in this systematic review that some authors such as Hori and Kubota[38] and Almangush et al. reported WPOI and the combined score of budding and depth (BD model) were identified as prognostic factors for DFS.[13] Although WPOI was recently reported as a strong pathological predictor for locoregional recurrence in OTSCC,[16,36–38] it has not been considered in treatment planning of early-stage OTSCC until now. Most of the recent studies have confirmed the prognostic significance of WPOI in head and neck SCC.[66]

Most of the studies on histopathological prognostic markers (e.g., depth of invasion, degree of differentiation, pattern of invasion, and mitotic activity) in conventional HE-stained samples have focused on cancer cells. However, tumor progression has also been reported to depend on the stroma surrounding the tumor.[17] TSR, defined as the proportion of tumor tissue relative to its surrounding stromal tissue has been shown to be a good prognosticator in head and neck tumors.[40] Tumor stroma generally consists of fibroblasts, basement membrane, immune cells, and extracellular matrix. Malignancy changes in the stroma may occur promoting tumor invasion, growth, and metastasis. When such stromal change occurs at the invasive front, the appearance of carcinoma-associated fibroblasts (CAFs) is usually noted. CAFs are considered an important part of the reactive tumor stroma and they play significant roles in tumor progression.[67]

Identification of TSR in sections stained with HE is fast and easy.[68] However, the prognostic value of TSR and its role in early-stage OTSCC has been studied only recently. TSR assessed in HE-stained sections was first reported by Mesker et al.[69] in colon cancer patients, it has since been used more recently in other cancer types.[70,71] Cancer patients were divided according to TSR into "stroma-poor" and "stroma-rich" groups which consistently showed discriminatory prognostic properties.[71] The stroma acts as a barrier in tumorigenesis by limiting cancer cells migrations into the healthy tissues.[72] However, the components of cancer-related stroma may enhance tumor differentiation, growth, and even locomotion of cancer cells.[71] Thus, the stroma has an important supportive and sustaining role and it could offer different strategies for biological intervention in the diagnosis/prognosis of different types of malignant tumors.[72,73] In two studies stroma-rich OTSCC was reported to have a higher risk of recurrence and poor DSS than stroma-poor tumors.[17,41] Importantly, TSR showed a remarkable prognostic value that was superior to the WHO histopathological grading system and the traditional cTNM staging system.[17] Further studies are recommended to confirm these promising findings and to elucidate the mechanisms behind the impact of TSR on the invasiveness of OTSCC cells. Some of the included studies are limited by the fact that they did not concentrate on the histopathological feature(s) as the main parameter(s) in the analyses. Consequently, such studies may not be used to detect the changes in the outcomes of early OTSCC. However, data from eligible studies that met the present inclusion criteria showed that there are significant emerging histopathological markers for early OTSCC. In conclusion, the present study reports that the newly described histopathological prognostic markers identified by HE staining include tumor budding, WPOI, and TSR, and they have a promising prognostic power in early OTSCC. Understanding the molecular background operative in these biomarkers will require further research. Introduction of these markers into routine pathology reports, requires large scale validation studies.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Amr Elseragy https://orcid.org/0000-0003-1009-1375
Ibrahim O. Bello https://orcid.org/0000-0003-2085-3185
Antti A. Mäkitie https://orcid.org/0000-0002-0451-2404
Alhadi Almangush https://orcid.org/0000-0003-4106-314X

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