



The prognostic role of histologic grade, worst pattern of invasion, and tumor budding in early oral tongue squamous cell carcinoma: a comparative study

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Abstract

Major pathology guidelines often mandate stating the histologic grade as a component of the pathology report for various types of cancer. However, the prognostic value of histologic grade in head and neck squamous cell carcinoma (HNSCC) is controversial at best, and there is a need for more reliable prognostic histologic factors to better stratify and manage patients with HNSCC. In this study, we compared three relevant histopathologic features (histologic grade, worst pattern of invasion (WPOI), and tumor budding) in a large single-center retrospective cohort of early oral tongue squamous cell carcinoma (OTSCC) with tumor greatest dimension ≤ 4 cm. Only histologic grade predicted distant metastasis free survival (DMFS) on univariate analysis. Tumor budding was associated with nodal metastasis, overall survival (OS), regional recurrence-free survival (RRFS), and DMFS and was a significant predictor for nodal metastasis on the multivariable logistic regression model. WPOI 5 was associated with high frequency of nodal metastasis and shortened OS and was an independent adverse prognostic factor for OS on multivariate analysis using the Cox proportional hazards model. WPOI and tumor budding were prognostically more relevant than histologic grade. Consideration should be given to include WPOI and tumor budding in the pathology reporting of OTSCC.

Keywords Oral tongue squamous cell carcinoma · Tumor budding · Histologic grade · Pattern of invasion · Prognosis

Introduction

Histologic grading is a crucial component of pathologic reporting and a known prognostic factor in many types of human cancer, e.g. Nottingham grade of breast cancer [1] and grade group of prostatic carcinoma [2]. Major pathology guidelines, e.g., World Health Organization (WHO) classification [3, 4], the College of American Pathologists (CAP) protocol [5], and the International Collaboration on Cancer Reporting (ICCR) dataset [6], advocate for a three-tiered grad-

ing system for head and neck squamous cell carcinomas (HNSCC). This grading system was first proposed by Broders in 1920 [7] and was later adopted by the WHO classification of head and neck tumors [3, 4]. In brief, a tumor is classified into well, moderately, and poorly differentiated based on a combination of features, i.e., degree of keratinization, cytonuclear atypia, and infiltrative pattern [3]. However, the prognostic value of the grading system is controversial at best, with the majority of studies showing that the grading system correlates poorly with patients' outcome [8–13]. Alternative grading schemes using a point system and/or other histologic features, especially when evaluated only at the invasive front have been advocated by several groups but failed to gain wide acceptance in the pathology community [8, 12].

In 1973, Jakobsson et al. [14] discussed the concept of pattern of invasion (POI) and included POI in a point-based grading system of HNSCC, which was later modified by Anneroth et al. in 1982 [15] and Bayne et al. in 1992 [8]. The four patterns of invasion described in their grading schemes were as follows: POI 1, pushing, well-delineated

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infiltrative borders; POI 2, infiltrative, solid cords, bands, and/or strands; POI 3, small groups or cords of > 15 cells; and POI 4, small group of < 15 tumor cells and/or single cells. In their cohorts, POI 1 and 2 were often associated with well-differentiated HNSCC while POI 3 and 4 were commonly seen in poorly differentiated tumors. In 2005, Brandwein-Gensler et al. modified and expanded the concept of POI, notably POI 2 was changed to “finger-like” pushing pattern, and a POI 5, defined as satellite tumor nodule(s) at least 1 mm away from the main tumor, was added [16]. These authors showed that the worst POI (WPOI) 5 was an independent adverse prognostic factor for recurrence and survival, whereas nuclear grade did not predict outcome in a large cohort of oral and oropharyngeal squamous cell carcinoma. Therefore, they advocated including WPOI (rather than grade) as a histologic component in the risk stratification model for HNSCC. Most but not all subsequent studies have confirmed the prognostic values of WPOI in HNSCC [11, 17–27]. In 2018, the ICCR dataset added WPOI as a mandatory pathology reporting element for oral cavity SCC [6].

Another relevant histologic concept is tumor budding. A tumor bud is defined as the presence of small clusters of < 5 tumor cells. Based on the guideline published by the International Tumor Budding Consensus Conference (ITBCC), tumor budding should be assessed using $\times 20$ objective (with an adjusted standard field size of 0.786 mm^2) within the hotspot at the invasive front, and graded as low (0–4 buds), intermediate (5–9 buds), and high (≥ 10 buds) for any given tumor [28]. In colorectal adenocarcinoma, high tumor budding is an important prognostic pathologic parameter to predict survival and recurrence, and is thought to reflect tumor dehiscence, motility and invasiveness and promote oncogenesis [28]. Similarly, in HNSCC, recent studies have shown that tumor budding is a novel prognostic factor, predicting risk of nodal metastasis at the time of the initial resection, overall survival (OS), disease free survival, and progression-free survival [29–32].

Clearly, all three parameters (histologic grade, WPOI, and tumor budding) take into account invasive patterns and tumor cell dehiscence (i.e., small clusters of tumor cells) and show a certain degree of overlapping. For example, a tumor with high tumor budding (i.e., ≥ 10 tumor buds of < 5 cells) by definition has a WPOI of 4 (small tumor clusters of < 15 cells) or above and are often poorly differentiated by the Broders’ grading scheme. Conversely, tumors with a WPOI 1 to 3 contain no tumor bud and are frequently graded as well differentiated. The major differences among the three parameters are as follows: grade is a multifactorial subjective qualitative measurement, WPOI is objective and qualitative, whereas tumor budding is objective and semi-quantitative. The current CAP checklist for HNSCC [5] includes grade as a mandatory reporting element, WPOI as an optional element, whereas tumor budding is excluded.

In this study, we aimed to investigate the prognostic role of histologic grade, WPOI and tumor budding in predicting nodal metastasis, survival, and recurrence in a large single-center retrospective cohort of 329 patients with early oral tongue squamous cell carcinoma (OTSCC).

Materials and methods

Study cohort

After obtaining Institutional Review Board approval, the surgical departmental database was searched for patients who fulfilled the following inclusion criteria: (1) patients who underwent primary resection at Memorial Sloan Kettering Cancer Center (New York, NY, USA) from 2000 to 2012, (2) a diagnosis of OTSCC with a tumor greatest dimension of 4 cm or less, and (3) the slides of primary resection were available for review. Exclusion criteria were (1) synchronous HNSCC, (2) prior treatment of the reference carcinoma, (3) distant metastasis at presentation, and (4) prior history of non-endocrine head and neck cancer. A total of 329 patients were included in this retrospective study.

Pathologic review

A detailed pathologic review was conducted by 3 head and neck attending pathologists (RG, NK, or BX).

The worst pattern of invasion (WPOI) was defined as follows: WPOI 1, broad pushing tumor front; WPOI 2, finger-like pushing invasion; WPOI 3, large tumor islands > 15 cells; WPOI 4, small tumor island ≤ 15 cells or tumor strands; and POI 5, satellite tumor nodule at least 1 mm away from the main tumor (Fig. 1 and Supplementary Fig. 1) [16, 33].

The number of tumor buds (defined as a tumor cluster of < 5 tumor cells) was counted using $\times 20$ objective with an adjusted standard field size of 0.785 mm^2 at the site with the highest number of buds within the tumor (i.e., hotspot). Tumor budding was then classified as low (0–4 buds), intermediate (5–9 buds), and high (≥ 10 buds) [28].

The histologic grade of the tumor was classified using the Broders’ definition [7] into three tiers, being well, moderately, or poorly differentiated based on a combination of cytonuclear atypia, keratinization, and infiltrative pattern [3, 4]. In brief, a well-differentiated OTSCC had no to minimal cytonuclear atypia, abundant keratinization, and keratin pearl, and often with a WPOI of 1 to 3. A moderately differentiated tumor showed notable cytonuclear atypia, less numerous areas of keratinization, and infiltrative growth. A poorly differentiated OTSCC showed marked cytonuclear atypia, no or minimal keratinization, and often an infiltrating pattern of single cells and small tumor clusters.

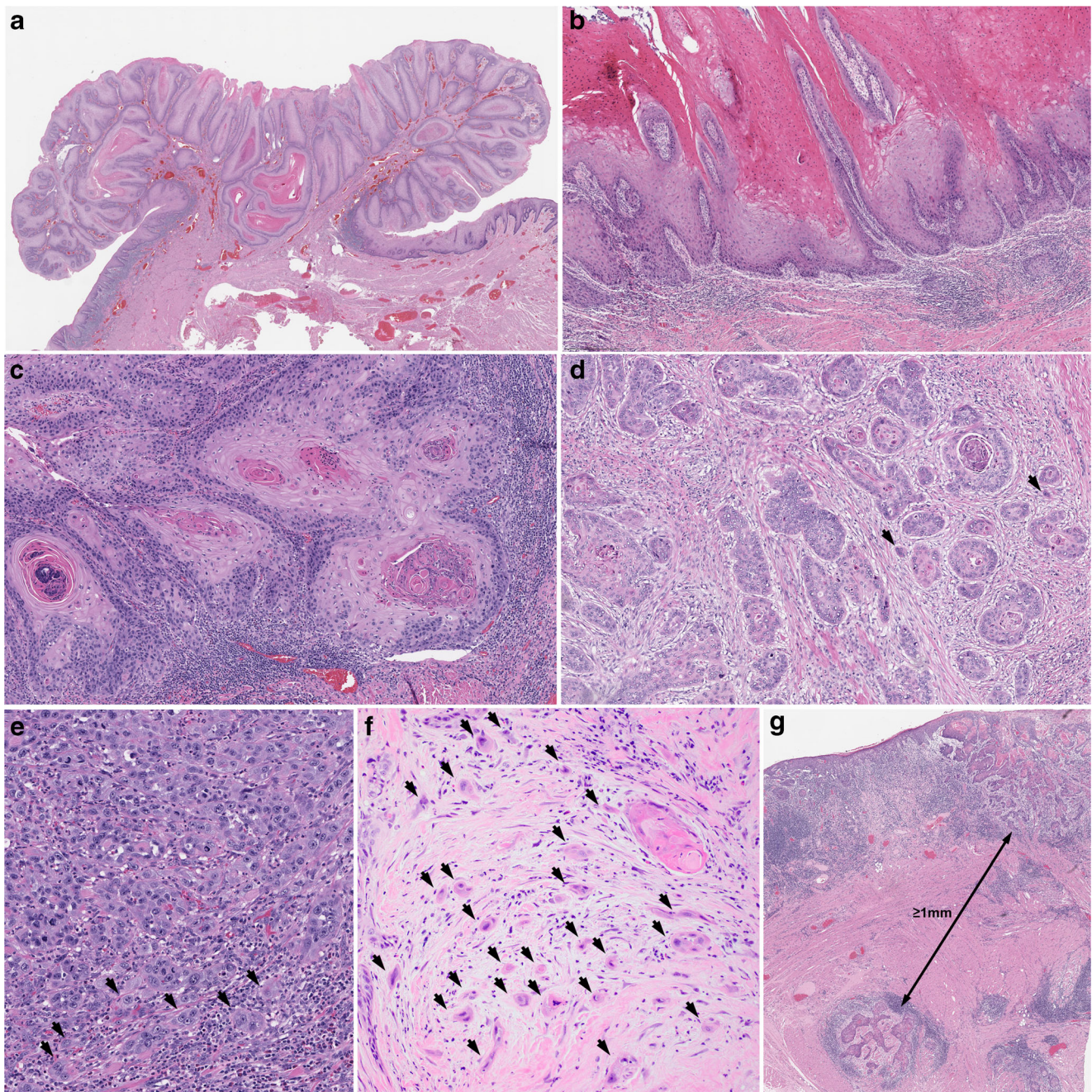


Fig. 1 Worst pattern of invasion (POI), tumor budding, and histologic grade in oral tongue squamous cell carcinoma (OTSCC). **a** WPOI 1: broad pushing invasion. **b** WPOI 2: finger-like tumor front. **c** WPOI 3: large tumor islands > 15 cells. **d–f** WPOI 4: small tumor islands ≤ 15 cells. **g** POI 5: satellite nodule(s) at least 1 mm away from the main tumor. A tumor bud (black arrows) is defined as a tumor cluster of < 5 tumor cells or single tumor cells. Tumor budding is assessed at × 20 objective (field size 0.785 mm²) and is classified as low (0–4 buds, **d**), intermediate (5–9 buds, **e**), and high (≥ 10 buds, **f**). By definition, a tumor with WPOI 1–3 lacks tumor buds (i.e., low tumor budding), whereas a tumor with

WPOI 4–5 may show any degree of budding. Histologic grade is classified using a combination of keratinization, cytonuclear atypia, and infiltration pattern. Well-differentiated OTSCCs (**a–c**) show minimal cytologic atypia and abundant keratinization. Moderately differentiated tumors (**d** and **g**) are infiltrative, have notable cytologic atypia, and show a certain degree of differentiation/keratinization. Poorly differentiated OTSCCs (**e** and **f**) frequently lack clear evidence of differentiation/keratinization and demonstrate marked nuclear pleomorphism and a growth pattern of single cells/small tumor clusters

Other histologic features collected included tumor greatest dimension, depth of invasion, perineural invasion, lymphovascular invasion, margin status (classified as

positive—tumor present at the inked margin or negative), AJCC pT, and pN stage.

Outcomes and statistical analysis

All statistics were performed using SPSS software v25.0 (IBM Corporation, Armonk, NY, USA) or SAS software (SAS Institute, Cary, NC, USA). The endpoints studied included OS, LRFS, regional recurrence-free survival (RRFS), distant metastasis free survival (DMFS), and the risk of nodal metastasis at the time of initial resection.

Univariable logistic regression model was performed to calculate the risk of nodal metastasis in the primary resection according to the grade, tumor budding status, and WPOI. Parameters that were significant on univariable analysis were subjected to the multivariable logistic regression model adjusted with AJCC 8th pT stage, perineural invasion, lymphovascular invasion, and margin status. The odds ratio (OR) and its 95% confidence interval (CI) were calculated.

The prognostic values of grading, tumor budding, and POI were determined using the univariate Cox proportional hazards model. Kaplan–Meier curves were plotted. Hazard ratio (HR) and its 95% CI were calculated. Subsequent multivariate analysis using Cox proportional hazards model was performed for OS to adjust with other known prognostic factors, e.g., AJCC 8th pT stage, pN stage, extranodal extension, perineural invasion, lymphovascular invasion, and margin status. Insufficient events were observed in our cohort to conduct multivariate analysis for local recurrence-free survival (LRFS), regional recurrence-free survival (RRFS), and distant metastasis-free survival (DMFS). *p* Values less than 0.05 were considered significant.

Results

Clinicopathologic characteristics of the study cohort

The clinicopathologic features of the study cohort are shown in Table 1.

The majority of the OTSCC ($n = 262$, 79.6%) were classified as moderately differentiated. The histologic grade of the remaining tumors was well differentiated in 43 (13.1%) and poorly differentiated in 24 (7.2%).

Tumor budding was graded as low (0–4 buds per $\times 20$ objective, field size 0.785 mm²) in 200 (60.8%), intermediate (5–9 buds) in 64 (19.5%), and high (≥ 10 buds) in 65 (19.8%).

The distribution of WPOI among our cohort was as follows: WPOI 1 in 4 (1.2%), WPOI 2 in 25 (7.6%), WPOI 3 in 76 (23.1%), WPOI 4 in 190 (57.8%), and WPOI 5 in 34 patients (10.3%).

A significant association was detected among any two of these three histologic parameters (Supplementary Table 1, Fisher's exact test, $p < 0.001$). All four OTSCCs with WPOI 1 were classified as well-differentiated and low tumor budding. Conversely, WPOI 5 was not seen in well-differentiated

OTSCC. Tumor classified as poorly differentiated or high tumor budding only showed WPOI 4 and 5. There was also an association of poorly differentiated histologic grade with high tumor budding. The frequency of high tumor budding in well, moderately, and poorly differentiated OTSCC was 4.7% (2/43), 18.3% (48/262), and 62.5% (15/24), respectively.

The pT stage defined by AJCC 8th edition was pT1 in 179 (54.4%), pT2 in 114 (34.7%), and pT3 in 36 (10.9%). The greatest dimension of the tumor was 2 cm or less in 259 patients (78.7%) and 2.1 to 4 cm in the remaining 70 (21.3%). Perineural invasion, lymphovascular invasion, and positive resection margin were identified in 76 (23.1%), 31 (9.4%), and 7 (2.3%) patients, respectively.

Risk of nodal metastasis in the primary resection according to histologic grade, tumor budding, and WPOI

At the time of primary resection, 54 patients (16.4%) harbored pathology confirmed lymph node metastasis (pN1/N2/N3). Univariable logistic regression analysis shows that nodal metastasis was significantly associated with tumor budding and WPOI, but not histologic grading. The OR, its 95% CI, and *p* values are shown in Table 2. When substratified for histologic grade, the association between nodal metastasis and tumor budding/WPOI was only significant in moderately differentiated OTSCC (Supplementary Table 2).

On multivariable logistic regression when adjusted for AJCC pT stage, margin status, perineural invasion, and lymphovascular invasion, only high tumor budding was independently associated with a high risk of nodal metastasis (compared with low tumor budding, OR = 5.203, 95% CI 2.249–12.037, $p < 0.001$).

The prognostic values of histologic grade, tumor budding, and WPOI

The median follow-up of our cohort was 73 months (range 0.03–224 months). At the time of last follow-up, there were 100 deaths, 54 local recurrences, 43 regional recurrences, and 12 distant recurrences. The number of events for LRFS, RRFS, and DMFS was insufficient for multivariate survival analysis. Therefore, multivariate analysis was only conducted for OS.

The results of univariate and multivariate survival analysis using the Cox proportional hazards model are shown in Table 3, and the Kaplan–Meier curves are provided in Fig. 2. On univariate survival analysis, the histologic grade was a significant prognostic factor for DMFS (HR = 4.388, 95% CI 1.176–16.369, $p = 0.001$), but not OS, LRFS, or RRFS. High tumor budding was associated with decreased OS (HR = 2.235, 95% CI 1.433–3.488, $p < 0.001$), RRFS (HR = 4.651, 95% CI 2.388–9.058, $p < 0.001$), and DMFS (HR =

Table 1 Histologic features of the study cohort divided by nodal status at the initial resection

| | Total (<i>n</i> = 329) | pN0/Nx (<i>n</i> = 275) | pN1/N2/N3 (<i>n</i> = 54) |
|----------------------------------|-------------------------|--------------------------|----------------------------|
| Histologic grade | | | |
| Well differentiated | 43 (13.1%) | 43 (15.6%) | 0 (0%) |
| Moderately differentiated | 262 (79.6%) | 215 (78.2%) | 47 (87.0%) |
| Poorly differentiated | 24 (7.3%) | 17 (6.2%) | 7 (13.0%) |
| Tumor budding | | | |
| Low | 200 (60.8%) | 186 (67.6%) | 14 (25.9%) |
| Intermediate | 64 (19.5%) | 54 (19.6%) | 10 (18.5%) |
| High | 65 (19.8%) | 35 (12.7%) | 30 (55.6%) |
| Worst pattern of invasion (WPOI) | | | |
| 1 | 4 (1.2%) | 4 (1.5%) | 0 (0%) |
| 2 | 25 (7.6%) | 24 (8.7%) | 1 (1.9%) |
| 3 | 76 (23.1%) | 72 (26.2%) | 4 (7.4%) |
| 4 | 190 (57.8%) | 152 (55.3%) | 30 (70.4%) |
| 5 | 34 (10.3%) | 23 (8.4%) | 11 (20.4%) |
| Other histologic features | | | |
| Tumor greatest dimension (cm) | | | |
| 0.1–2 cm | 259 (78.7%) | 234 (85.1%) | 25 (46.3%) |
| 2.1–4 cm | 70 (21.3%) | 41 (14.9%) | 29 (53.7%) |
| Tumor thickness (mm) | | | |
| 0.1–5 mm | 167 (50.8%) | 162 (58.9%) | 5 (9.3%) |
| 5.1–10 mm | 99 (30.1%) | 78 (28.4%) | 21 (38.9%) |
| > 10 mm | 63 (19.1%) | 35 (12.7%) | 28 (51.9%) |
| Depth of invasion (mm) | | | |
| 0.1–5 mm | 181 (55.9%) | 174 (64.4%) | 7 (13.0%) |
| 5.1–10 mm | 85 (26.5%) | 66 (24.4%) | 20 (37.0%) |
| > 10 mm | 57 (17.6%) | 30 (11.1%) | 27 (50.0%) |
| AJCC 7th pT stage | | | |
| pT1 | 259 (78.7%) | 234 (85.1%) | 25 (46.3%) |
| pT2 | 70 (21.3%) | 41 (14.9%) | 29 (53.7%) |
| AJCC 8th pT stage | | | |
| pT1 | 179 (54.4%) | 173 (62.9%) | 6 (11.1%) |
| pT2 | 114 (34.7%) | 87 (31.6%) | 27 (50.0%) |
| pT3 | 36 (10.9%) | 15 (5.5%) | 21 (38.9%) |
| Perineural invasion | | | |
| Absent | 253 (76.9%) | 228 (82.9%) | 25 (46.3%) |
| Present | 76 (23.1%) | 47 (17.1%) | 29 (53.7%) |
| Lymphovascular invasion | | | |
| Absent | 298 (90.6%) | 258 (93.8%) | 40 (74.1%) |
| Present | 31 (9.4%) | 17 (6.2%) | 14 (25.9%) |
| Margin status (<i>n</i> = 306) | | | |
| Negative | 299 (97.7%) | 246 (97.6%) | 53 (98.1%) |
| Positive | 7 (2.3%) | 6 (2.4%) | 1 (1.9%) |

6.121, 95% CI 1.785–20.986, $p = 0.004$). WPOI 5 was an adverse prognostic factor for OS (HR = 2.774, 95% CI 1.659–4.638, $p < 0.001$), but not LRFS, RRFS, or DMFS.

When substratified for histologic grade, tumor budding, and WPOI predicted OS only in moderately differentiated

OTSCC on univariate survival analysis (Supplementary Table 2).

On multivariate analysis using the Cox proportional hazards model for OS, WPOI remained an independent prognostic factor for OS (HR = 1.994, 95% CI 1.142–3.481, $p =$

Table 2 Univariable and multivariable logistic regression model to predict risk of nodal metastasis at initial resection according to the status. Multivariable logistic regression was performed adjusted for AJCC pT stage, margin status, perineural invasion, and lymphovascular invasion. OR odd ratio, CI confidence interval

| | Univariable | | Multivariable | |
|--------------------------------|-----------------------|----------------|----------------------|----------------|
| | OR (95%CI) | <i>p</i> value | OR (95%CI) | <i>p</i> value |
| Histologic grade | | | | |
| Well/moderately differentiated | Reference | | | |
| Poorly differentiated | 1.503 (0.943–2.398) | 0.087 | Not done | |
| Tumor budding | | | | |
| Low | Reference | | | |
| Intermediate | 2.460 (1.035–5.850) | <i>0.042</i> | 1.493 (0.563–3.958) | 0.421 |
| High | 11.388 (5.488–23.630) | < <i>0.001</i> | 5.203 (2.249–12.037) | < <i>0.001</i> |
| Worst pattern of invasion | | | | |
| 1 to 4 | Reference | | | |
| 5 | 1.294 (1.063–1.576) | <i>0.010</i> | 1.053 (0.838–1.325) | 0.656 |

Italic *p* values: significant *p* values

0.015). The 3-year, 5-year, and 10-year OS for patients with WPOI 5 was 55%, 55%, and 37%, respectively, compared with 88%, 81%, and 67% for patients with WPOI of 1 to 4. Tumor budding failed to reach significance on multivariate analyses.

Discussion

The prognostic value of the histologic grade in HNSCC has not been clearly established in the literature. In the 3rd edition of WHO classification [3], it is stated that “histologic grade correlates poorly with patient outcome. The value of grading improves when only the deeply invasive

margins of the tumor are evaluated.” Indeed, numerous studies have shown that histologic grade did not correlate with the risk of nodal metastasis [10], disease-specific survival [8, 12, 13, 20], disease-free survival [16, 20], LRFS [11], or RRFS [11, 24] in HNSCC from various subsites, e.g., floor of mouth [8], oral cavity [10, 12, 20], oral cavity and oropharynx [16], and oral tongue [11, 13, 24]. The only study which found a significant correlation between grade and outcome is by Lin et al. [34]. The authors reported that poorly differentiated tumors were associated with higher risk of nodal metastasis at the initial resection (being 45.9% compared with 29.3% in moderately differentiated and 6.1% in well-differentiated tumors) and decreased recurrence free survival (hazard ratio = 1.973, 95% CI 1.167–

Table 3 Univariate and multivariate survival analysis using Cox proportional hazards model. OS overall survival, LRFS local recurrence-free survival, RRFS regional recurrence-free survival, DMFS distant metastasis-free survival, HR hazard ratio, CI confidence interval

| | OS (univariate) | | OS (multivariate) | | LRFS | | RRFS | | DMFS | |
|--------------------------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|----------------|----------------------|----------------|
| | HR (95%CI) | <i>p</i> value | HR (95%CI) | <i>p</i> value | HR (95%CI) | <i>p</i> value | HR (95% CI) | <i>p</i> value | HR (95% CI) | <i>p</i> value |
| Histologic grade | | | | | | | | | | |
| Well/moderately differentiated | Reference | | | | | | | | | |
| Poorly differentiated | 1.005 (0.486–2.075) | 0.990 | Not done | | 2.000 (0.901–4.442) | 0.089 | 1.434 (0.451–2.845) | 0.792 | 4.388 (1.176–16.369) | <i>0.028</i> |
| Tumor budding | | | | | | | | | | |
| Low | Reference | | | | | | | | | |
| Intermediate | 0.959 (0.548–1.679) | 0.884 | 0.569 (0.304–1.063) | 0.077 | 1.047 (0.527–2.082) | 0.895 | 1.649 (0.706–3.854) | 0.248 | 0.808(0.090–7.229) | 0.849 |
| High | 2.235 (1.433–3.488) | < <i>0.001</i> | 0.987 (0.564–1.726) | 0.963 | 1.359 (0.68–2.699) | 0.382 | 4.651 (2.388–9.058) | < <i>0.001</i> | 6.121 (1.785–20.986) | <i>0.004</i> |
| Worst pattern of invasion | | | | | | | | | | |
| 1 to 4 | Reference | | | | | | | | | |
| 5 | 2.774 (1.659–4.638) | < <i>0.001</i> | 1.994 (1.142–3.481) | <i>0.015</i> | 1.132 (0.337–3.800) | 0.792 | 1.616 (0.681–3.834) | 0.276 | 2.156 (0.470–9.895) | 0.323 |

Italic *p* values: significant *p* values

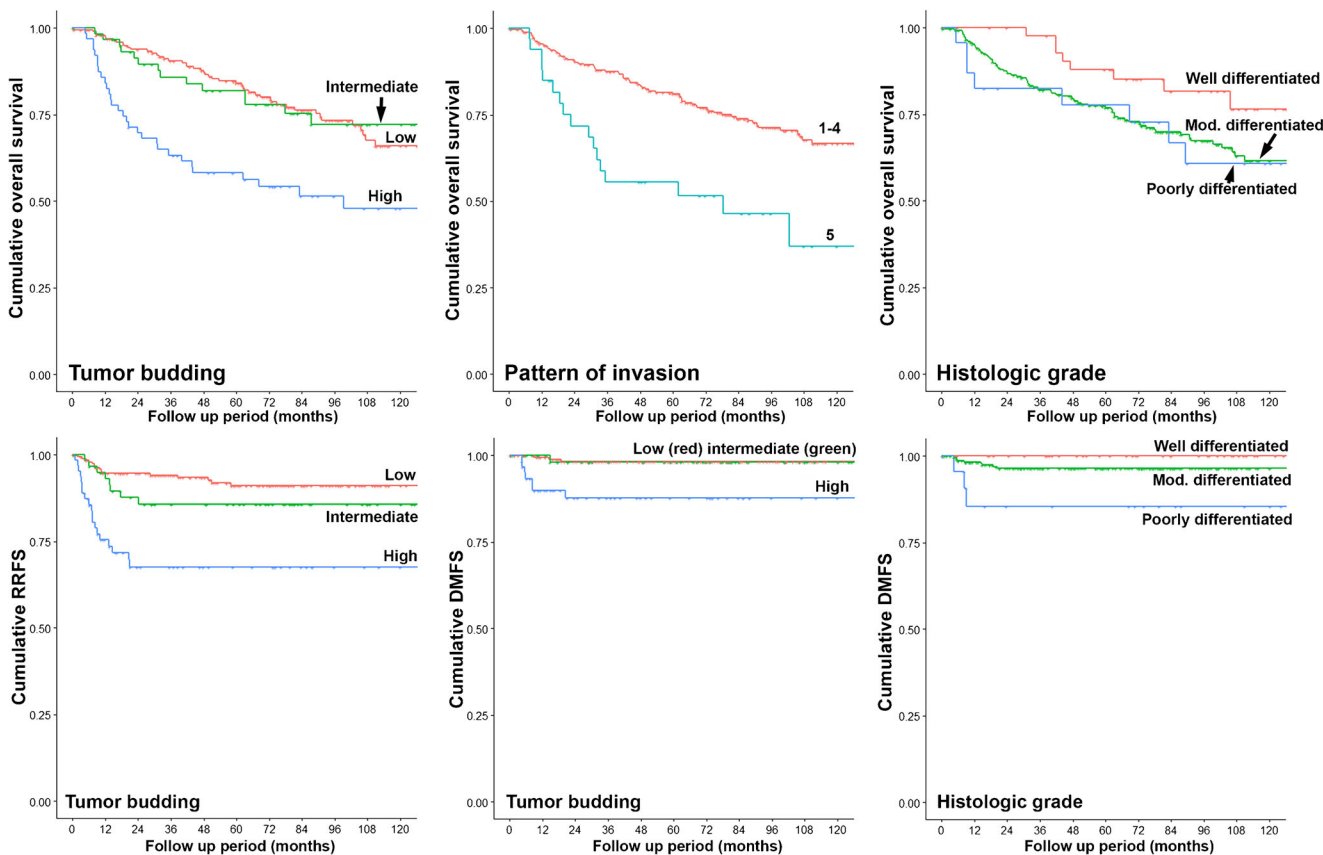


Fig. 2 Kaplan–Meier curves for overall survival (top rows), regional recurrence free survival (RRFS, bottom left), and distant metastasis-free survival (DMFS, bottom middle and right). On univariate survival analysis, high tumor budding was associated with decreased overall survival,

RRFS, and DMFS. Pattern of invasion 5 was associated with adverse overall survival. Poorly differentiated carcinoma predicted decreased DMFS but not overall survival

3.336) compared with well-differentiated tumor in a large cohort of 2535 patients with oral squamous cell carcinoma from a Taiwanese medical center. There was no significant difference between well-differentiated and moderately differentiated tumors.

Similar to the majority of previous studies, in our cohort of early OTSCC, we found that grade only predicted DMFS on univariate analysis but correlated poorly with the risk of nodal metastasis, OS, LRFS, and RRFs. The frequency of well-differentiated, moderately differentiated, and poorly differentiated OTSCC in the current study was 13.1%, 79.6%, and 7.3%, respectively, similar to what was reported by Lin et al. (17%, 78%, and 5%, respectively). The observed prognostic difference between our cohort and Lin et al. might have been a result of the different cohort size (329 vs. 2535), different stage of the analyzed tumors (early OTSCC vs. oral cavity squamous cell carcinoma of any stage), and the ethnic composition of the study population (single US center vs. single Taiwanese center) [34].

It is noteworthy that alternative grading schemes exist for HNSCC. For example, a point-based grading system (Bayan's grading) evaluated only at the invasive tumor front seemed to provide improved prognostication information [8]. Such

grading method has been shown to be an independent prognostic factor for survival in 79 patients with floor of mouth squamous cell carcinoma [8] and an independent predictor for disease specific survival in 85 patients with oral cavity squamous cell carcinoma [12].

Compared with histologic grade, the literature on WPOI and tumor budding in HNSCC appeared to be more consistent. Most studies performed univariate analysis and showed that WPOI 4 and 5 were associated with the risk of bone invasion [13] and nodal metastasis [20, 21, 23], worse disease-specific survival [20, 21, 35], and RRFs [25], whereas WPOI 5 was linked with high frequency of locoregional recurrence [26] and decreased disease-free survival [16, 22]. Only three studies did not report a significant association between WPOI and nodal metastasis (in 49 cases of oral squamous cell carcinoma [10] and 88 cases of OTSCC [17]), or progression free survival in 49 cases of OTSCC [19]), possibly due to small sample size.

The reported results of multivariate survival analysis were more variable. While some studies showed that WPOI was an independent prognostic factor for RRFs [25], nodal metastasis [20, 36], and recurrence-free survival [18], others did not report WPOI to be significant on multivariate analysis for risk

of nodal metastasis [10], locoregional recurrence [26], progression-free survival [19], disease-free survival [22], LRFS [11], and RRFS [11]. The discrepant results in regard to multivariate analysis may be in part explained by the cohort size. Those studies which reported a significant result tended to have a larger cohort size, two of the four studies had a cohort size of 336 to 340 patients [18, 20], whereas those studies with no significant result on multivariate analysis had a sample size of 39 to 126 patients [10, 11, 19, 22, 26]. In the current study with a large sample of 329 patients, we reported that WPOI 5 was the only independent prognostic factor for OS on multivariate analysis among the other three parameters studied, further supporting the prognostic role of WPOI 5 in HNSCC. Meta-analysis to pool prognostic data from multiple studies or large-scale multicenter studies may be useful to confirm WPOI as an important histologic factor to risk stratify patients with HNSCC. The current ICCR dataset includes WPOI as a mandatory reporting element [6], whereas WPOI is an optional component in the CAP checklist [5].

A limitation of WPOI 5 in the clinical setting is that the frequency of WPOI 5 is quite low in HNSCC, being 10.3% (34 patients) in the current cohort. Therefore, WPOI5 alone will not be able to capture all HNSCCs with adverse outcomes (i.e., 43 patients with nodal metastases at the initial resection, 100 deaths, 54 local recurrences, and 43 regional recurrences in the current study). A multifactorial histologic stratification is needed to stratify patients with HNSCC.

In two recent meta-analyses by Almangush et al. in 2018 (which included 9 studies) and Zhu et al. in 2019 (which included 15 studies), high tumor budding was found to be associated with lymph node metastasis at the initial resection, OS, disease-free survival, and progression-free survival in HNSCC [29, 30]. Additionally, the authors have shown that high tumor budding was associated with OS in OTSCC [30] and clinical T1/T2 oral squamous cell carcinoma [29]. Several individual studies performed multivariate analysis, confirming an independent prognostic role of tumor budding in predicting nodal metastasis [20, 36], OS [37], disease-specific survival [18], progressive-free survival [37], and RRFS [24].

Most of the published individual studies adopted a two-tiered system (high budding vs. low budding) and the cutoff used varied from 3, 5, to 10 buds [18, 20, 22, 24, 29, 30, 36, 37]. The only study that used the three-tiered system proposed by the ITBCC consensus showed that tumor budding was an independent prognostic factor for disease free survival in a cohort of 91 patients with clinical T1/T2N0 oral squamous cell carcinoma (21). In contrast, WPOI failed to reach a significant level in the same study [22]. Data from our current study further confirm the role of tumor budding in early OTSCC. Among the three factors studied, high tumor budding was the only independent predictor for increased risk of nodal metastasis on multivariable

logistic regression model and was associated with poor OS, RRFS and DMFS on univariate survival analysis. Together, these data support the prognostic relevance of tumor budding in HNSCC, including OTSCC. Furthermore, several authors have suggested including tumor budding in the risk stratification model for HNSCC, e.g., budding-depth of invasion (BD) model [25, 38]. Therefore, it is relevant to score tumor budding in a standardized fashion as defined by ITBCC consensus and to include it as an element of pathology reporting for HNSCC to provide additional prognostication information.

When we substratified the study cohort by histologic grade, the impacts of WPOI and tumor budding on risk of nodal metastasis of OS appeared to be significant only in the moderately differentiated OTSCC. However, such results should be interpreted with caution as the number of cases in well-differentiated and poorly differentiated OTSCC was very small, being 43 and 24, respectively. Larger studies or meta-analyses are needed to determine the prognostic significance of WPOI and tumor budding in each histologic grade.

The current study is the only large-scale study that evaluated tumor budding using the standardized ITBCC consensus approach and also included a multivariate analysis. It is also the only study which correlated all three histologic parameters (histologic grade, tumor budding using ITBCC consensus guideline, and WPOI). The limitation of the study was that it only included OTSCC ≤ 4 cm in size. Therefore, the results might not be generalized to larger tumors or HNSCC originated from other sites.

In conclusion, we compared the performance of three related histologic parameters, the multifactorial descriptive histologic grade, the qualitative WPOI, and the semi-quantitative tumor budding, and found that WPOI and tumor budding were superior to histologic grade in OTSCC. While WPOI 5 was an independent adverse prognostic factor for OS, high tumor budding was associated with a high risk of nodal metastasis on multivariable logistic regression analysis. Therefore, we propose to include tumor budding and WPOI in the routine pathologic reports of patients with OTSCC.

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Declarations

Competing interests The authors declare that they have no competing interests.

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