A proposal to revise the histopathologic grading system of early oral tongue cancer incorporating tumor budding

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Abstract

The World Health Organization (WHO) grading system has a low prognostic value for early-stage oral tongue squamous cell carcinoma (OTSCC); greater prognostic power has been shown with tumor budding analysis. In this study, we combined tumor budding analysis with histopathologic grading according to WHO 2017. In our proposal, a revised Grade I tumor is defined as a “well differentiated cohesive tumor”; revised Grade II as a “moderately differentiated and/or slightly dissociated tumor”; and revised Grade III as a “poorly differentiated and/or dissociated tumor”. We evaluated the prognostic value of this proposed grading system in a multicenter cohort of 311 cases of early OTSCC. The proposed grading system showed significant prognostic value in multivariable analysis for disease-specific survival (DSS) with a hazard ratio (HR) of 3.86 and a 95% confidence interval (CI) of 1.36 to 10.9 ($P=0.001$). For disease-free survival (DFS), the proposed grading system showed good predictive power in multivariable analysis (HR 2.07, 95% CI 1.00-4.27; $P=0.009$). The conventional WHO grading system showed a low prognostic value for DSS and DFS ($P>0.05$). In conclusion, the prognostic power of the WHO histopathologic grading improved significantly with incorporation of tumor budding. Our proposed grading system can be easily included in pathology reports.

Keywords: Oral tongue squamous cell carcinoma (OTSCC); Prognosis; WHO histopathologic grading, Tumor budding.

1 Introduction

2 Oral tongue squamous cell carcinoma (OTSCC) is one of the most common cancers in the oral
3 cavity. OTSCC is known for its unpredictable spread and metastasis. Although many biomarkers
4 have been introduced for prognostication of OTSCC, none of them can be approved for daily
5 practice ¹. Moreover, the previously published biomarkers are not routinely performed. The
6 traditional histopathologic features (e.g., mitotic activity) are usually evaluated in some epithelial
7 tumors ². But, however, it is known that such features do not have reliable prognostic power for
8 oral carcinomas especially at early stages ^{3, 4}. Findings on the prognostic values of other
9 histopathologic parameters such as perineural invasion (PNI) and lymphovascular invasion (LVI)
10 have been promising in some recent studies of OTSCC ⁵⁻⁷. Regrettably, PNI and LVI are
11 sometimes difficult to be identified using routine hematoxylin and eosin (HE) staining ⁸. In
12 addition, underreporting of PNI and LVI might occur in daily practice with time constraints.

13 The World Health Organization (WHO) histopathologic grading system ⁹ has been widely
14 criticized due to its low predictive power especially for early-stage OTSCC ^{10, 11}. Although many
15 other histopathologic grading systems were introduced ^{3, 12, 13}, the WHO grading system is still
16 usually included in routine diagnosis. Further, the recent WHO classification ¹⁴ of head and neck
17 tumors states that “*Grading alone does not correlate well with prognosis*”. According to the
18 classification of WHO ¹⁴, a well keratinized OTSCC is classified as a “well differentiated tumor”
19 that is presumed to have a good prognosis even if it shows high cellular dissociation (i.e., a non-
20 cohesive tumor) and active invasive growth.

21 Tumor budding refers to single cancer cells or small cancer clusters (less than 5 cells) and
22 has recently been reported as a reliable prognostic marker for many epithelial cancers ¹⁵⁻¹⁷,
23 including some subsites of head and neck tumors ¹⁸. Tumor budding can be evaluated using HE
24 staining ¹⁹ even in a preoperative diagnostic biopsy ²⁰. Moreover, our recent meta-analysis revealed

promising prognostic value for tumor budding in oral squamous cell carcinoma (OSCC) in general and specifically for early-stage OTSCC ²¹.

This study introduces a proposal to incorporate tumor budding into the current WHO histopathologic grading system to improve its prognostic performance in early OTSCC. We compared the prognostic value of the conventional WHO grading system with our proposed grading system using data from OTSCC series collected from six institutions.

Materials and Methods

Patient selection: This retrospective study was conducted with the approval of the Finnish National Supervisory Authority for Welfare and Health (VALVIRA), the Brazilian Human Research Ethics Committee, and the ethics committee of each university hospital included (Helsinki, Turku, Tampere, Oulu, and Kuopio). Eligibility criteria were OTSCC diagnosed at an early stage (cT1-2N0M0) with no previous treatment. Clinicopathologic data including age, gender, stage, perineural invasion, WHO grading, and survival outcome were collected. Cases diagnosed between 1979 and 2010 were staged according to the 7th edition of the American Joint Committee on Cancer ²².

Histological evaluation and our proposed grading: The HE-stained diagnostic sections were retrieved for all cases. The invasive front area was scanned with low magnification (´4) and the area with the highest number of tumor budding was counted using high magnification (´20) as previously described ¹¹. We used a numerical system (Revised Grade I, II, and III) as that makes it simple for the pathologist than using our descriptive nomenclature (well differentiated cohesive,...etc). Tumor budding was incorporated into the WHO grading system as follows:

Well differentiated cohesive tumor (Revised Grade I): Tumor tissue (and individual tumor cells) is reminiscent of squamous epithelial cells in the normal oral mucosa. The tumor contains

foci of extra- or intracellular keratinization. The cells have few mitotic figures and rarely show nuclear pleomorphism or variation in cell size and shape. Atypical mitoses are rare or absent. Tumor budding is absent.

Moderately differentiated and/or slightly dissociated tumor (Revised Grade II): Tumor cells are less keratinized and there is more nuclear pleomorphism or variation of cell size and shape. A few atypical mitotic figures may be seen. Alternatively, the tumor may have a few tumor buds (i.e., 4 buds or less).

Poorly differentiated and/or dissociated tumor (Revised Grade III): Tumor cells are rarely keratinized and have frequent mitotic figures, including atypical ones. Nuclear pleomorphism and variation in cell size and shape are commonly seen. Regardless of histological features, tumors with 5 buds or more are classified as revised Grade III.

Statistical analysis: All statistical analyses were performed using IBM SPSS Statistics version 24 and MedCalc version 18 software. Associations between our proposed grade and patient age, gender, cTNM stage, perineural invasion, or WHO grade were analyzed using cross-tabulation and chi-square test. Kaplan-Meier curves were constructed for disease-specific survival (DSS) and disease-free survival (DFS) for WHO grade and our proposed grade. The log-rank test was used to assess differences in survival between groups. The Cox proportional-hazards model was used to conduct survival analyses without and with adjustment for confounders ²³. Hazard ratios (HR) with 95% confidence intervals (CI) were reported for all models.

Results

Demographic and clinical information for the 311 patients is shown in Table 1. There was a slight male preponderance (53%) and more T2 tumors (60.1%) than T1 tumors. There was no significant relationship between our proposed grade and patient age ($P=0.048$), gender ($P=0.707$), cTNM

stage ($P=0.365$), or perineural invasion ($P=0.053$). The WHO grading system had a significant relationship with our proposed grade ($P<0.001$). Of note, there was upgrading of 35 “well differentiated tumors” according to conventional WHO grading to “Revised Grade II” in our proposed system; and upgrading of 26 “well differentiated tumors” to “Revised Grade III” in our system. A total of 45 “moderately differentiated tumors” according to conventional WHO grading system were upgraded to “Revised Grade III” in our proposed grading system.

Analysis of cancer-related mortality (Table 2) showed low prognostic value for the WHO grade in both univariable (HR 1.58, 95% CI 0.79-3.16; $P=0.199$) and multivariable analysis (HR 1.93, 95% CI 0.95-3.92; $P=0.072$). On the other hand, our proposed grade showed a statistically significant increase in cancer-related mortality for tumours with revised Grade III in univariable (HR 3.42, 95% CI 1.23-9.56; $P=0.001$) and multivariable analysis (HR 3.86; 95% CI 1.36-10.9; $P=0.001$).

For DFS (Table 3), the WHO grade again showed insignificant prognostic value in univariable (HR 1.25, 95% CI 0.72-2.16; $P=0.739$) and multivariable analysis (HR 1.48, 95% CI 0.84-2.59; $P=0.405$). Our proposed grade showed a significant prognostic value for identification of early OTSCC that has poor DFS in univariable (HR 1.85, 95% CI 0.91-3.76; $P=0.024$) and multivariable analyses (HR 2.07, 95% CI 1.00-4.27; $P=0.009$).

For multivariable analyses, we also included age, gender, T-stage, and perineural invasion; prognostic values are summarized in Tables 2 and 3. Kaplan-Meier analysis showed a low prognostic value for the conventional WHO grading system in prognostication of cancer-related mortality ($P=0.215$) and DFS ($P=0.733$); this low prognostic performance is replicated in the survival curves (Fig 2A, B). Kaplan-Meier curves (Fig 2C, D) revealed a significantly high risk of cancer-related mortality ($P=0.002$) and poor DFS ($P=0.026$) for revised Grade III tumors in our proposed grading system.

Discussion

The histological grading is the cornerstone for classification of SCC by the pathologist independent of any clinical measurement; and evaluating mainly the degree of anaplasia. Many histologic grading systems were proposed including, for example, those introduced by Broders²⁴, Jakobsson et al.²⁵, Anneroth et al.¹², and Bryne et al.^{3, 26}. The WHO histological grading system (tumor grade) is based on the Broders classification. Albert Broders was behind the concept of tumor grading in his article published in 1920 that evaluated 537 cases of squamous cell carcinoma of the lip²⁴. Subsequently, this concept was applied by Broders for grading of SCC of the skin²⁷, the genitourinary tract²⁸ and the head and neck²⁹.

WHO grading system evaluates the histological variations between the normal epithelial tissue and tumor tissue. This system is still widely applied for different SCCs. This classification focuses on the degree of cellular differentiation and divides OTSCC into “well differentiated”, “moderately differentiated”, and “poorly differentiated” tumors. In pathology reports, this system is generally applied for histopathologic classification of OTSCC specimens (both in preoperative biopsies and operative resection samples). Although WHO grading correlates with the expression of several molecular biomarkers^{1, 30}, it has low prognostic value in oral carcinomas³¹. Because of its wide use in daily practice, it would be desirable to revise this grading system and thus to improve its prognostic performance.

In this study, we introduced a proposal to incorporate tumor budding into the WHO grading scheme. Our findings showed a high prognostic value for early OTSCC. Of note, the recent WHO classification of head and neck tumors¹⁴ indicates that a non-cohesive pattern of invasion is associated with poor prognosis of OSCC. Recent studies have validated the clinical significance of tumor budding³²⁻³⁴ and our recent meta-analysis confirmed this significance for OTSCC²¹. Importantly, tumor budding was recently listed by the Union for International Cancer Control's

1 TNM classification as a prognostic marker for colorectal cancer ^{35, 36}. The assessment of tumor
2 budding using routine HE-stained sections has been successfully reported in many cancers ^{11, 16, 19}.
3 Moreover, HE staining was recommended in the recent guidelines introduced by the International
4 Tumor Budding Consensus Conference (2016) for evaluation of tumor budding in colorectal
5 cancer ¹⁹. We have discussed in detail the method of evaluating tumor budding in our recent meta-
6 analysis ²¹.

7 The latest WHO classification ¹⁴ states that “*well differentiated tumors tend to invade in*
8 *large islands*”. However, this is not always the case as small clusters of cancer cells may also be
9 present in such well differentiated tumors. Indeed, poorly differentiated tumors are more often
10 associated with invasive growth of small tumor clusters. The fact that the conventional WHO
11 grading system focuses only on tumor differentiation may perhaps be the reason for its low
12 prognostic value. This could be addressed by taking into consideration both tumor differentiation
13 and dissociation.

14 Akin to many previous studies ^{10, 13, 31, 37}, we did not find a statistically significant
15 association between the WHO grade and survival (Table 2 and 3). Moreover, most of our cases
16 comprised well differentiated (Grade I) or moderately differentiated (Grade II) tumors, while
17 poorly differentiated tumors (Grade III) are not common ¹⁴. We also noted that many of the well-
18 or moderately differentiated tumors displayed a high number of tumor budding, and these tumors
19 were classified as “Revised Grade III” in our proposed system. Notably, the incorporation of tumor
20 budding significantly improved the predictive power and upgrading of tumors (from Grade I to
21 Revised Grade II or III and also from Grade II to Revised Grade III) was frequently noted. In
22 multivariable analysis, the hazard ratio for cancer-related mortality in the group of patients with
23 “Revised Grade III” tumors in our proposed system was higher (3.86) than those with high tumor

budding alone (2.86) or those with poorly differentiated tumors (1.93) alone. It is important to explain that we used the numerical grading (i.e. I, II and III) to simplify it for daily practice.

In conclusion, the conventional WHO grading system has low prognostic utility in early OTSCC but can be improved by incorporation of tumor budding. Our proposed system was easily applied and showed a good prognostic value. The proposed grading system considers both tumor differentiation and its dissociation and can be routinely included in pathology reports.

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Figure legend

Figure 1: Representative figures for our proposed grading system (hematoxylin and eosin staining). **A:** Our revised Grade I that indicates a well differentiated cohesive tumor. **B:** The tumor does not have any dissociation at the invasive front. **C:** Our revised Grade II shows slightly dissociated tumor. **D:** The tumor has few buds at the invasive front. **E:** Our revised Grade III in which tumor displays many tumor buds at the invasive front although the tumor is not poorly differentiated. **F:** High power view detailed the many buds in the invasive front.

A, C, E: small magnification of $\times 5$. B, D, F: higher magnification of $\times 10$.

Figure 2: DSS (**A**) and DFS (**B**) with the conventional WHO grading system in 311 cases treated for early OTSCC. There was no significant difference in the survival. With our proposed grade, there was an improvement in the prognostication for both of DSS (**C**) and DFS (**D**).

DSS: Disease-specific survival. DFS: Disease-free survival.

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Table 1: Relationship between our proposed grade and age of patient, gender, cTNM stage, perineural invasion (PNI), and the WHO histopathologic grading system for 311 cases treated for early OTSCC.

Variable	Total number of cases n = 311	Our Proposed Grade			
		Revised Grade I n = 44	Revised Grade II n = 121	Revised Grade III n = 146	P value of chi-square test
	Number (%)	Number (%)	Number (%)	Number (%)	
Age					0.048
≤60	129 (41.5)	13 (29.5)	45 (37.2)	71 (48.6)	
>60	182 (58.5)	31 (70.5)	76 (62.8)	75 (51.4)	
Gender					0.707
Male	165 (53)	23 (52.3)	61 (50.4)	81 (55.5)	
Female	146 (47)	21 (47.7)	60 (49.6)	65 (44.5)	
cTNM stage*					0.365
T1N0M0	124 (39.9)	21 (47.7)	50 (41.3)	53 (36.3)	
T2N0M0	187 (60.1)	23 (52.3)	71 (58.7)	93 (63.7)	
PNI					0.053
Absent	269 (86.5)	43 (97.7)	104 (86.0)	122 (83.6)	
Present	42 (13.5)	1 (2.3)	17 (14.0)	24 (16.4)	
Grade (WHO)					<0.001
Well differentiated	105 (33.8)	44 (100)	35 (28.9)	26 (17.8)	
Moderately differentiated	131 (42.1)	0 (0)	86 (71.1)	45 (30.8)	
Poorly differentiated	75 (24.1)	0 (0)	0 (0)	75 (51.4)	

* cTNM stage refers to the 7th edition of the American Joint Committee on Cancer as all cases were diagnosed before 2017.

Note: Revised Grade I refers to well differentiated cohesive tumor. Revised Grade II refers to moderately differentiated and/or slightly dissociated tumor. Revised Grade III refers to poorly differentiated and/or dissociated tumor.

Table 2: Univariable and multivariable analyses of disease-specific survival for WHO grade compared with our proposed grade and tumor budding in 311 cases treated for early OTSCC. In each model of multivariable analysis, we included age, gender, TNM stage and perineural invasion.

Variable	Univariable analysis	Multivariable analysis		
		Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
WHO grade				
Well differentiated	1	1		
Moderately differentiated	1.68 (0.92-3.07)	1.89 (1.031-3.48)		
Poorly differentiated	1.58 (0.79-3.16)	1.93 (0.95-3.92)		
	<i>P</i> = 0.199	<i>P</i> = 0.072		
Our proposed grade				
Revised Grade I	1		1	
Revised Grade II	1.51 (0.51-4.47)		1.58 (0.53-4.73)	
Revised Grade III	3.42 (1.23-9.56)		3.86 (1.36-10.9)	
	<i>P</i> = 0.001		<i>P</i> = 0.001	
Tumor budding				
No budding	1			1
Low budding (<5)	1.04 (0.59-2.21)			1.05 (0.50-2.16)
High budding (≥5)	2.69 (1.46-4.96)			2.86 (1.53-5.35)
	<i>P</i> = 0.001			<i>P</i> = 0.001
Age				
≤60	1	1	1	1
>60	1.87 (1.09- 3.18)	2.03 (1.17-3.53)	2.18 (1.26-3.78)	2.09 (1.21 -3.63)
	<i>P</i> = 0.018	<i>P</i> = 0.009	<i>P</i> = 0.004	<i>P</i> = 0.006
Gender				
Men	1	1	1	1
Women	1.21 (0.74- 1.99)	1.21 (0.73-2.03)	1.17 (0.69 -1.94)	1.08 (0.65-1.79)
	<i>P</i> = 0.441	<i>P</i> = 0.458	<i>P</i> = 0.559	<i>P</i> = 0.770
TNM Stage				
I	1	1	1	1
II	1.48 (0.87- 2.54)	1.65 (0.94 -2.86)	1.49 (0.86 -2.59)	1.57 (0.91-2.72)
	<i>P</i> = 0.141	<i>P</i> = 0.071	<i>P</i> = 0.145	<i>P</i> = 0.100
Perineural invasion				
Absent	1	1	1	1
Present	1.27 (0.65- 2.5)	1.17 (0.59 -2.32)	0.97 (0.49 -1.94)	0.92 (0.46 -1.84)
	<i>P</i> = 0.502	<i>P</i> = 0.657	<i>P</i> = 0.937	<i>P</i> = 0.810

Note: Revised Grade I refers to well differentiated cohesive tumor. Revised Grade II refers to moderately differentiated and/or slightly dissociated tumor. Revised Grade III refers to poorly differentiated and/or dissociated tumor.

Table 3: Univariable and multivariable analyses of disease-free survival for WHO grade compared with our proposed grade and tumor budding in 311 cases treated for early OTSCC. In each model of multivariable analysis, we included age, gender, TNM stage and perineural invasion.

Variable	Univariable analysis	Multivariable analysis		
		Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
WHO grade				
Well differentiated	1	1		
Moderately differentiated	1.11 (0.68-1.82)	1.20 (0.73-1.97)		
Poorly differentiated	1.25 (0.72-2.16)	1.48 (0.84-2.59)		
	<i>P</i> = 0.739	<i>P</i> = 0.405		
Our proposed grade				
Revised Grade I	1		1	
Revised Grade II	1.04 (0.49-2.21)		1.07 (0.50-2.28)	
Revised Grade III	1.85 (0.91-3.76)		2.07 (1.00-4.27)	
	<i>P</i> = 0.024		<i>P</i> = 0.009	
Tumor budding				
No budding	1			1
Low budding (<5)	0.96 (0.55-1.68)			1.03 (0.58-1.82)
High budding (≥5)	1.85 (1.13-3.06)			2.03 (1.20-3.43)
	<i>P</i> = 0.016			<i>P</i> = 0.010
Age				
≤60	1	1	1	1
>60	1.79 (1.14-2.79)	1.87 (1.18-2.96)	1.98 (1.25-3.14)	1.95 (1.23-3.09)
	<i>P</i> = 0.011	<i>P</i> = 0.007	<i>P</i> = 0.003	<i>P</i> = 0.004
Gender				
Men	1	1	1	1
Women	1.08 (0.71-1.64)	0.96 (0.62-1.49)	0.91 (0.59-1.41)	0.87 (0.56-1.35)
	<i>P</i> = 0.715	<i>P</i> = 0.867	<i>P</i> = 0.684	<i>P</i> = 0.552
TNM Stage				
I	1	1	1	1
II	0.87 (0.57-1.33)	0.87 (0.56-1.35)	0.83 (0.54-1.29)	0.85 (0.55-1.33)
	<i>P</i> = 0.526	<i>P</i> = 0.524	<i>P</i> = 0.410	<i>P</i> = 0.488
Perineural invasion				
Absent	1	1	1	1
Present	1.46 (0.83-2.55)	1.53 (0.87-2.69)	1.34 (0.75-2.38)	1.26 (0.71-2.25)
	<i>P</i> = 0.180	<i>P</i> = 0.163	<i>P</i> = 0.328	<i>P</i> = 0.444

Note: Revised Grade I refers to well differentiated cohesive tumor. Revised Grade II refers to moderately differentiated and/or slightly dissociated tumor. Revised Grade III refers to poorly differentiated and/or dissociated tumor.