Grading of oral squamous cell carcinomas – Intra and interrater agreeability: Simpler is better?

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Abstract

Background: Numerous studies have been presented on histological grading of oral squamous cell carcinomas (OSCC) for predicting survival, but uncertainty of their usefulness rises due to discordances of results. A scoring system should be robust and well validated, and intra- and interrater agreement can be used as a tool to visualize the strength of reproducibility.

Methods: Here, we present an intra- and inter-observer study on evaluation of OSCC using some of the most common histopathological parameters. The observers were from different Norwegian university hospitals, and calibration to ensure accuracy was first performed. Percentage of the agreement was calculated for the score made by the individual observer at different times, as well as between pairs of observers.

Results: The evaluation made by the same observer at two different time points (intra-rater) correlated better than observations made by different participants (inter-rater). In an attempt to increase the rate of agreement, many of the parameters were either dichotomized into simply low- and high grade, or to a three-tier system when more than three options in the original design. This increased the concurrence with 15.4% for the intrarater and with 23% for the interrater comparisons.

Conclusion: High agreement for histopathological parameters can be difficult to obtain on hematoxylin and eosin staining in scoring systems with many options. A simpler system might be more advantageous to achieve higher degree of reproducibility.

KEYWORDS
histopathological parameters, intrarater and interrater agreement, oral cancer, squamous cell carcinoma
Oral cavity cancer originates almost exclusively from squamous cells (SC), and the histopathological evaluation of these tumors is the basis for their classification and further treatment.

The prediction of outcome and the selection of treatment for patients with oral squamous cell carcinomas (OSCC) are today based on the clinical tumor, nodes, and metastasis (TNM) staging. Tumor thickness, as measured during microscopic evaluation, was recently implemented in the T (size) variable. Further, according to the WHO classification of head and neck (HN) tumors, the tumor differentiation should also be reported in order to predict prognosis. The histological grading does not take into account the tumor-host interactions that modulate tumor progression and aggressiveness although several factors such as inflammation are likely to influence on prognosis.

During the last decades, several histopathological grading systems for SC carcinomas in the HN region have been suggested and tested. The first grading systems only considered the morphological characteristics of the tumor, but later on, the tumor-host relationship also came into consideration. For evaluation of tumor differentiation, nuclear polymorphism and keratinization have been important variables. The characteristics of tumor invasion in the surrounding tissue have been implemented when evaluating the tumor-host relationship, as well as immune response (plasma-lymphocytic infiltration), vascular invasion, and perineural infiltration. In particular, tumor budding (invading clusters of four or less tumor cells at the invasive front) has been proposed to be a simple and reliable prognostic marker for OSCC.

Reproducibility in the scoring of histopathological parameters is essential if they are to be used as prognostic markers. The purpose

| Variable                                      | Score                                      |
|-----------------------------------------------|--------------------------------------------|
| Differentiation, WHO whole tumor              | Well                                       |
| Differentiation, WHO worst pattern            | Moderate                                   |
| Degree, keratinization, whole tumor           | Poor                                       |
| Degree of keratinization, tumor front         | Highly keratinized (>50% of the cells)    |
| Nuclear polymorphism, whole tumor             | Moderately keratinized (20%-50% of the cells) |
| Nuclear polymorphism tumor front              | Minimal keratinization (5%-20% of the cells) |
| Nuclear polymorphism                           | No keratinization (0%-5%) of the cells      |
| Perineural infiltration                        | None                                       |
| Lymphocyte infiltration                       | Marked/continuous band                     |
| Worst pattern of invasion                     | Pushing, well-delineated infiltrating borders |
| Vascular infiltration                         | Subepithelial tissue (submucosa/lamina propria) |
| Infiltration                                  | Muscle                                     |
|                                              | Bone                                       |
of this study was to test the intra- and interrater agreement of a broad spectrum of parameters previously suggested as prognostic markers for OSCC.

2 | MATERIALS AND METHODS

2.1 | Observers and calibration

The observers were experienced pathologists/oral pathologist (TMS, EN, HL, ACJ, LUH, and SES), and two oral pathologist under training (DEC and EHO) from three university hospitals in Norway. Prior to the scoring, all the participants had taken part in two calibration workshops to agree on how to interpret the parameters.

One of the observers performed only one round of scoring, and one observer scored thickness and depth only once. The interrater observations were all calculated on the first set of scoring allowing all eight observers to participate.

2.2 | Slides for evaluation

Hematoxylin and eosin (HE) stained sections of 31 randomly selected formalin-fixed OSCC cases, representing various intraoral locations and different tumor stages, were distributed to each hospital. The participants reported each case with the assumption that the single slide available was representative of the whole lesion. No special stains were provided. The scoring was done independently by the observers for each variable at two different time points (3-6 month interval) permitting calculation of inter- and intrarater reliability. Thickness and tumor depth were measured in millimeter, but for statistical analyses, measurements were divided into three-triers; size ≤5 mm, 5.1-10.0 mm and >10 mm. This is according to The International Consortium for Outcome Research (ICOR) in Head and neck Cancer and TNM Classification of Malignant tumors. Tumor budding was divided according to recommendations by Almangush et al; <5 buds as low grade, 5-9 as intermediate grade, and ≥10 as high grade. The measurement of all other variables was on a nominal scale with three to six categories with no overlaps (mutually exclusive) as shown in Table 1.

2.3 | Ethics

The study was approved by the Northern Norwegian Regional Committee for Medical Research Ethics (REK Nord; 2013/1786 and 2015/1381).
Statistics was performed by using IBM SPSS statistics 24. We did statistical calculations both in percent agreement and Cohen's kappa (κ). The variability (spread of scoring) was low, and therefore, Cohen's kappa was of no/little value; thus, all correlations are given in percent.

### RESULTS

#### Intrarater and interrater agreement

The first nine parameters in Table 1 had three to five different scoring options, and they were all categorized into new groups with fewer options (Table 2). The first seven were dichotomized, while the worst pattern of infiltration had two and three different scoring options (Table 2). Lymphocyte infiltration was dichotomized into two different groups according to different cut-offs. Dichotomizing variables increased the mean intrarater agreement from 68.3% (range 60.7%-77.0%) to 83.5% (range 77.1%-92.5%). Mean agreement for each variable prior to and after categorization is listed in Table 3. Prior to categorization, perineural infiltration showed the highest intrarater agreement, whereas differentiation was most agreed upon after categorization.

Some variables had predefined categories that were not changed (Table 4). These had a mean intrarater agreement of 85.4% (range 79.2%-93.3%), and vascular infiltration and infiltration into deeper tissues showed the highest intrarater agreement.

In order to evaluate interrater agreement, two observers from different hospitals were paired randomly. The average interrater agreement was lower than the intrarater agreement for all variables (Table 3). Dichotomizing variables increased the mean interrater agreement from 42.9% (range 26.7%-55.9%) to 70.6% (range 59.3%-84.4%). As for the intrarater agreement, perineural infiltration and differentiation had the highest agreement prior to and after categorization, respectively (Table 3). For the variables with predefined categories, the average interrater agreement was 72.7% (range 65.0%-78.9%), where vascular infiltration was the variable with the highest agreement (Table 4).

### DISCUSSION

This study was conducted to investigate how consistent an observer was at measuring the same histopathological variables at different times, as well as the consistency between different observers. We
In this study, re-categorizing differentiation compared to our results. In a study by Rodrigues et al,\textsuperscript{13} 50 random samples of OSCC were selected and examined for worst pattern of invasion twice with a 2-week interval by calibrated observers. The intra- and interobserver agreement was strong in both cases (κ = 0.77-0.89 and 0.84). In a study evaluating tumor budding, Wang et al\textsuperscript{14} found excellent intrarater and good interrater agreement (κ = 0.880/0.838 and 0.717). The high degree of agreement in these two studies might be due to the fact that they only had one parameter to score giving this full attention. Also, the time interval between first and second scoring in the first study was only 2 weeks with the possibility that the observer still could remember the previous scoring.

### 5 CONCLUSION

To be of value, a tumor prognostic marker must be both reproducible and significantly associated with disease progression or survival. In this study, we have evaluated the reproducibility of a number of proposed histopathological prognostic markers in OSCC. Our findings suggest that simpler uncomplicated scoring protocols will increase the reproducibility. However, we have not tested whether the new categorizations influence the prognostic value of the parameters. In our study, we included most of the previously proposed histopathological parameters and many observers, but a limited number of patient samples to avoid fatigue of the observers. We included tumors of different stages and from various intraoral locations; thus, the cohort was not suited for survival/prognostic analyses. The prognostic value of the revised categorization of the parameters should be tested in a larger, more homogenous cohort.

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**TABLE 4** Intra- and interobserver agreement in variables with predefined categories, or unchanged variables

| Tumor characteristics | Intrarater, mean agreement (%) | Interrater, mean agreement (%) |
|-----------------------|-------------------------------|-------------------------------|
| Number of buds, categorized |                                |                               |
| Low, intermediate, high grade | 79.2                          | 72.8                          |
| Tumor thickness, categorized |                                |                               |
| 0-5.0 mm, 5.1-10 mm, >10 mm | 80.7                          | 69.6                          |
| Depth of invasion, categorized |                                |                               |
| 0-5.0 mm, 5.1-10 mm, >10 mm | 80.7                          | 65.0                          |
| Vascular infiltration |                                |                               |
| Not found, present | 93.3                          | 78.9                          |
| Infiltration |                                |                               |
| Subepithelial, muscle, bone | 93.0                          | 77.1                          |

For the worst pattern of invasion, immunohistochemical staining for cytokeratin could ease the recognition of tumor cells among stromal cells, and thus, make scoring more reproducible. The low grade of the agreement for this variable in our study indicates that HE staining only is not sufficient. Likely, parameters such as number of buds, tumor thickness, depth of tumor, and infiltration into the underlying tissue could also benefit from special staining of epithelial cells. To our knowledge, no special staining has been promoted before scoring these variables in the different proposed scoring systems. The intrarater agreement for the budding, tumor thickness, and depth of infiltration was however 80% and 70% for the interrater groups, suggesting that HE stain is sufficient for categorized variables.

Other studies have shown a higher grade of interrater agreement compared to our results. In a study by Rodrigues et al,\textsuperscript{13} 50 random samples of OSCC were selected and examined for worst pattern of invasion twice with a 2-week interval by calibrated observers. The intra- and interobserver agreement was strong in both cases (κ = 0.77-0.89 and 0.84). In a study evaluating tumor budding, Wang et al\textsuperscript{14} found excellent intrarater and good interrater agreement (κ = 0.880/0.838 and 0.717). The high degree of agreement in these two studies might be due to the fact that they only had one parameter to score giving this full attention. Also, the time interval between first and second scoring in the first study was only 2 weeks with the possibility that the observer still could remember the previous scoring.
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