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Positive Fresh Frozen Section Margins as an Adverse Independent Prognostic Factor for Local Recurrence in Oral Cancer Patients

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Objectives: To access 1) the value of further surgical resection (completion surgery) in cases with a positive intraoperative margin analysis, and 2) whether cancers that undergo completion surgery following positive intraoperative margin analysis with subsequent negative margins should be considered true margin-negative (R0) resections in terms of adjuvant treatment planning.

Study Design: Retrospective analysis of patients with primary oral cancer.

Methods: One hundred and fifty-one patients underwent primary surgical resection of oral squamous cell carcinoma with intraoperative margin examination. In all cases for which frozen section margin analysis was positive, an extended resection was performed. Only patients with clear final margins were included in the study.

Results: The intraoperative analysis of surgical margins revealed that cancer-free margins were achieved in 123 cases (81.5%). In 28 specimens (18.5%), the surgical margins were positive. Local recurrence was observed in 28 (18.5%) patients, whereas regional recurrence developed in 30 (19.9%) patients. Factors significantly (P < 0.05) increased the risk of local recurrence: advanced stage of the disease (III/IV), node N-positive status, lymphovascular invasion and positive fresh frozen surgical margins. On multivariate analysis, only positive fresh frozen surgical margins remained significant independent adverse factors.

Conclusion: Our study demonstrates that positive fresh frozen margins, regardless of re-resection to R0 status, could be a powerful adverse factor that determines an aggressive nature of the tumor. This feature should be taken into consideration in adjuvant treatment planning. The greatest impact this could have is in borderline clinical situations for which the indication for adjuvant treatment may be questionable.

Key Words: Oral cancer, fresh frozen, intraoperative, local recurrence.

Level of Evidence: 4.

INTRODUCTION

Oral cancer is the sixth most common malignancy worldwide. The incidence is on the rise, with the National Cancer Institute predicting nearly 50 thousand new cases in the United States in 2016 (42,440 in 2014), with almost 10 thousand (8,300 in 2014) deaths. Although tobacco use and alcohol consumption remain the main risk factors for cancer development, human papillomavirus (HPV) infection was proven to play a role in oral cancer etiology. Contrary to oropharynx cancer, for which 40% to 60% of all cancer cases are HPV-positive, only about 5% to 15% of oral cavity tumors are HPV-positive—and the data available in literature is inconsistent. The p16 overexpression determined by immunohistochemistry is a well-established surrogate marker for the HPV etiology in oropharyngeal tumors. Some authors, however, indicated that the p16 detection in oral cancer does not seem to reliably correlate with HPV status. Despite the advances in modern diagnostics and therapeutic options, the survival rates remain around 50%, mainly due to the frequent development of local recurrence. The current treatment of choice comprises surgical resection alone for early, superficial lesions, whereas advanced cases usually require adjuvant treatment following surgery. The decision to proceed with adjuvant therapy is determined by the presence of factors proved to alter survival rates, including advanced stage of the disease, depth of invasion, regional lymph nodes involvement, extracapsular spread, perineural invasion, or lymphovascular invasion. These significantly diminish prognosis and, when present, are indications for radiotherapy alone or in combination with systemic treatment. One of the most significant factors influencing the outcome and prognosis is the status of surgical margins. In oral cancer, the presence of positive

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surgical margins (termed R1 or R2 pathologically) is 1.7 times more frequent than in any other subsite of the head and neck, with higher recurrence rates and lower survival. Conversely, a negative surgical margin (R0) is one of the strongest prognostic factors for disease-free survival and locoregional control. Recently, the introduction of intraoperative margin analysis has been heralded as a method of improving the rate of R0 resection; however, its value remains debatable.

In this study, we address two important questions: 1) What is the value of further surgical resection (completion surgery) in those cases with a positive intraoperative margin analysis; and 2) should cancers that undergo completion surgery following positive intraoperative margin analysis with subsequent negative margins be considered true R0 resections in terms of adjuvant treatment planning, and does this affect locoregional control. We also compare positive intraoperative margin status with other, well-established adverse prognostic factors.

MATERIALS AND METHODS

Patients

One hundred and fifty-one patients with oral squamous cell carcinoma were included in a retrospective study, of which 109 (72%) were males and 42 (28%) were females. Patients with lip cancer or previous oncological treatment were not included in the study. The mean age at the time of diagnosis was 61.3 years (34–88 years; standard deviation [SD] 9.3). The subsites of tumor were tongue (72 patients [48%]), floor of mouth (64 patients [42%]), and other (15 patients [10%]). All patients underwent primary surgical resection of at least a 1 cm tumor-free lateral and deep margin. The mean follow-up was 24 months (range 12–98 months, SD 18.5 months).

For each patient, the following all clinical parameters were collected: age at the time of diagnosis, stage of the disease, tumor size, node status, perineural invasion, lymphovascular invasion, presence of ECS, and adjuvant therapy. Details of local, regional, or distant recurrence, as well as disease-free survival and overall survival, also were included.

Treatment

All patients were evaluated by the institutional multidisciplinary team (MDT), which made the decision as to whether adjuvant treatment would be offered. The standard radiotherapy protocol was 60 to 66 grays (Gy) (2.0 Gy/fraction) daily, Monday through Friday, over 6 to 7 weeks. Factors that qualified the patient for adjuvant radiotherapy were: pT3/4 tumor, close surgical margins (1–5 mm), positive nodes, and evidence of perineural/vascular invasion. The chemotherapy regimen consisted of concurrent single-agent cisplatin at 100 mg/m2 every 3 weeks; indications were positive surgical margins or extracapsular spread. Positive surgical margins in the frozen section specimen that were successfully re-resected were classified by MDT as an R0 resection. Patients who were unable to complete adjuvant treatment due to poor general status were not included in the study.

Histopathological Evaluation

In all cases, the tumor was resected with at least a 1 cm margin of healthy mucosa, as grossly evaluated by the operating surgeon. The tumor specimen was fixed to a tray and comprehensively described in terms of anatomical orientation.

At least five margin specimens (including 4 adjacent and 1 deep) were snap-frozen in liquid nitrogen for intraoperative evaluation. Distance from tumor was presented in millimeters. Margin specimens were classified as positive when distance from the tumor border was less than 1 mm (high-grade dysplasia or carcinoma in situ within the boarders also were counted as positive), close when it was 1 to 5 mm, and clear when it was > 5 mm. In all cases for which frozen section margin analysis was positive, an extended resection was performed unless further resection was limited by anatomical boundaries. Only patients with clear final margins were included in the study. All margins assessments were independently performed by two experienced pathologists.

Study approval was obtained from the Research Ethics Board at Poznan University of Medical Sciences, Poznan, Poland.

Statistical Analysis

Statistical analysis was performed using Statistica software (data analysis software system) version 12, StatSoft Inc (2014). Outcomes were evaluated as disease-free survival, being calculated from the time of surgery until the time of recurrence or last follow-up visit, and overall survival from the time of surgery until death or last follow-up visit. Kaplan–Meier methods were used to estimate the above outcomes, and the log-rank test was used to compare survival curves. The following factors were analyzed: age, sex, clinical stage, T stage, N stage, frozen section margin status, presence of perineural invasion, presence of lymphovascular invasion, presence of ECS, and adjuvant therapy. Chi-square test was used to evaluate the examined factors on locoregional control and survival, P < 0.5 being statistically significant.

RESULTS

Patients

The majority of patients presented with early staged T1 and T2 tumors (48 patients [31%] and 72 patients [47%], respectively); most were classified as intermediate (G2) with regard to tumor differentiation (92 patients [60%]). In 90 (59%) patients, there was no evidence of metastases to regional lymph nodes (N0). Perineural invasion was present in 21 patients (14%), vascular invasion in 17 patients (11%), and ECS in 27 patients (18%). All patients were followed for at least 12 months. Most patients (122, 80%) received postoperative radiotherapy, of which 41 (34%) received additional chemotherapy. During follow-up, recurrence developed locally in 19 patients (12.5%), locoregionally in nine patients (5.9%, of which 1 also developed distant disease), and regionally in 21 patients (14%). Distant metastasis was observed in eight patients (5%), and a second primary was observed in four (2.5%) patients.

Margins

The intraoperative analysis of surgical margins revealed that cancer-free margins were achieved in 123 cases (81.5%). In 28 specimens (18.5%), the surgical margins were positive; of these, no tumor was found in the completion specimen in 17 cases, with 11 cases containing tumor but with a free margin of healthy mucosa.

Outcome

Local recurrence was observed in 28 patients (isolated local and locoregional [18.5%]), whereas regional
### TABLE I.
Univariate Analysis for Local Control.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Pattern of Comparison</th>
<th>Hazard Ratio (95%, CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Continuous variable</td>
<td>0.99 (0.39–1.03)</td>
<td>0.81</td>
</tr>
<tr>
<td>Sex</td>
<td>M vs. F</td>
<td>0.87 (0.39, 1.93)</td>
<td>0.73</td>
</tr>
<tr>
<td>Stage</td>
<td>I + II vs. III + IV</td>
<td>2.89 (1.27–6.57)</td>
<td>0.01</td>
</tr>
<tr>
<td>T-stage</td>
<td>1 + 2 vs. 3 + 4</td>
<td>1.37 (0.58–3.22)</td>
<td>0.47</td>
</tr>
<tr>
<td>N-stage</td>
<td>0 vs. 1 + 2</td>
<td>2.34 (1.11–4.93)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fresh frozen section</td>
<td>Positive vs. free</td>
<td>4.61 (2.18–9.72)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PNI</td>
<td>Present vs. absent</td>
<td>2.02 (0.67–5.90)</td>
<td>0.19</td>
</tr>
<tr>
<td>LVI</td>
<td>Present vs. absent</td>
<td>2.59 (1.04–6.44)</td>
<td>0.03</td>
</tr>
<tr>
<td>ECS</td>
<td>Present vs. absent</td>
<td>1.72 (0.72–4.08)</td>
<td>0.21</td>
</tr>
<tr>
<td>RT</td>
<td>Yes vs. no</td>
<td>1.54 (0.53–4.47)</td>
<td>0.42</td>
</tr>
<tr>
<td>CRT</td>
<td>Yes vs. no</td>
<td>1.25 (0.55–2.86)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Cl = confidence interval; CRT = chemotherapy; ECS = extracapsular spread; F = female; LVI = lymphovascular invasion; M = male; N = node; PNI = perineural invasion; RT = radiotherapy; T = tumor.

recurrence developed in 30 patients (isolated regional and locoregional [19.9%]) patients. The average times from completion of treatment to local and regional recurrence were 12.1 months (range 5–65 months; SD 18.4) and 10.5 months (range 4–39 months; SD 8.5), respectively.

Univariate analysis was performed to determine the effect of age, sex, stage of disease, T/N status, positive fresh frozen surgical margins, PNI, ECS, LVI, and adjuvant treatment with regard to the incidence of local recurrence (Table I). Four factors significantly increased the risk of recurrence: stage III/IV disease (P = 0.01; HR 95%, 2.89, 95% CI 1.27–6.57), N-positive status (P = 0.02; HR 2.34, 95% CI 1.11–4.93), LVI (P = 0.03; HR 2.59, 95% CI 1.04–6.44), and positive fresh frozen surgical margins (P < 0.0001; HR 4.61, 95% CI 2.18–9.72). On multivariate analysis, only positive fresh frozen surgical margins (P = 0.001) remained significant independent adverse factors for local recurrence formation (Table II). Additionally, disease-free survival was lower in patients with positive fresh frozen margins, regardless of stage of disease (Fig. 1).

The effect of these factors also was assessed in relation to regional recurrence analysis. As above, univariate analysis demonstrated that advanced stage of the disease (III/IV) (P = 0.04; HR 2.12, 95% CI 1.01–4.47), N-positive status (P = 0.004; HR 2.92, 95% CI 1.40–6.08), LVI (P = 0.02; HR 2.61, 95% CI 1.11–6.08), and positive fresh frozen surgical margins (P = 0.01; HR 2.57, 95% CI 1.17–5.63), as well as PNI (P < 0.001; HR 4.71, 95% CI 2.21–10.0) all significantly increased the risk of regional recurrence (Table III). On multivariate analysis, only PNI (P = 0.004) remained an independent risk factor for regional recurrence (Table IV).

For disease-free survival, the presence of PNI and positive fresh frozen margins was associated with worse outcome; however, the only statistically significant difference was observed when comparing those patients with positive fresh frozen margins, and PNI with negative fresh frozen margins and no evidence of PNI (P = 0.002) (Fig. 2).

Evaluation of overall survival shows that N-positive status (P = 0.002), advanced stage of disease (P = 0.01), NNI (P = 0.001), and ECS (P = 0.006) significantly compromise life expectancy. On multivariate analysis, however, only PNI had independent effect on OS (P = 0.01).

### DISCUSSION

The surgical resection of oral cancer is the treatment of choice, providing the best oncologic outcome, although a number of factors have been described that affect locoregional control and therefore compromise prognosis, including the presence of cancer infiltration at the surgical margins. To provide an R0 resection in oral cancer, fresh frozen section examination has been implemented, and when performed by experienced pathologists has an accuracy in excess of 90%. However, there are conflicting data regarding the role of frozen section margins in oral oncology. As a result, no consensus has been reached on how patients with initially involved fresh frozen margins with cleared margins achieved upon completion of resection should be considered by the MDT in terms of further treatment planning. A survey conducted among U.S. head and neck surgeons found that 90% regarded this resection as complete, whereas a study by Patel et al revealed that such re-resected frozen margins still are associated with a higher incidence of local recurrence. However, those margins had no impact on regional control, and in patients without evidence of nodal involvement, survival was similar in groups with clear frozen section margins and in those who required re-resection. In an historical study by Byers et al., no significant difference in local control was observed between patients with negative frozen section margins and those intraoperatively re-resected. Similarly, a study by Kwok et al. found that tumors for which R0 status had been achieved upon intraoperative completion had a similar outcome to those with primarily R0 margins. In our study, however, positive intraoperative margins were significantly associated with local recurrence, regardless of completeness of re-excision. This finding may be due to the exceptionally malignant potential of tumors for which an initial clear margin could not be achieved by the surgeon. Furthermore, multivariate analysis proved positive intraoperative margins to be the only independent adverse factor.

### TABLE II.
Multivariate Analysis for Local Control.

<table>
<thead>
<tr>
<th>Hazard Ratio</th>
<th>CI 95% Lower</th>
<th>CI 95% Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced stage</td>
<td>0.097671</td>
<td>2.065742</td>
</tr>
<tr>
<td>LVI</td>
<td>0.488057</td>
<td>1.406699</td>
</tr>
<tr>
<td>Positive fresh frozen</td>
<td>0.002861</td>
<td>3.458950</td>
</tr>
</tbody>
</table>

*Only statistically significant values from univariate analysis included. N status excluded due to direct correlation with stage. Cl = confidence interval; LVI = lymphovascular invasion; N = node.
Similar findings were presented by Ettl et al. (42.2% developed local recurrence in comparison with 16.2% with negative fresh frozen surgical margins). However, unlike our cohort, their study consisted of a less homogenous group representing cancers from all head and neck subsites. Brandwein-Gensler et al. did not find a correlation between margin status and local recurrence and overall survival, but did report a link between PNI and limited lymphatic response in LR and OS.

With regard to regional failure, additional factors were proven to be statistically significant in our cohort. Advanced stage, positive N status, PNI, and LVI increased the risk of regional recurrence. All these factors have been well described as risk factors related to locoregional recurrence. In our study, similarly to local recurrence, fresh frozen margin status also influenced regional control. Multivariate analysis revealed that only PNI was independent risk factor for regional recurrence. The above study by Ettl et al. also described lymphangiosis, hemangiosis, lymph node metastasis, and adjuvant therapy as affecting regional control, but did not demonstrate a link between this and positive fresh frozen margins. Additionally, Binahmed et al. found no association between positive surgical margins and neck failure.

In overall survival evaluation, advanced stage, N-positive disease, PNI, and ECS had a significant impact; on multivariate analysis, PNI was the only independent factor influencing overall survival.

We believe that our results could provide some additional data to validate R1 to R0 margins status on intraoperative examination as a risk factor for highly aggressive tumors. This hypothesis could be supported by differences in the percentages of other risk factors of highly aggressive tumors in our subgroups (PNI 25%, LVI 25%, and ECS 39% in the group with R1 to R0...
margins; PNI 11%, LVI 8%, and ECS 22% in the group with primarily negative margins).

Our study has some limitations. Due to the high percentage of patients receiving adjuvant therapy, the direct impact of examined factors may be diminished. Additionally, tumor thickness was not examined in some of our patients and therefore was not included in the analysis. What is more, precise intraoperative localization of the site of the involved margin to be re-excised can be extremely challenging, which may explain the much lower reliability of final R0 status. Clinical situations when further resection is limited by anatomical boundaries, however unfavorable, eliminates the bias of false-positive R0 diagnosis. In Kerawala and Ong’s study, the mean variation in margin location was 9 mm, and the surgeons missed the correct location by more than 1 cm in 32% of cases.30 Another challenging situation is when there is no further tumor identified in the re-resected margin, which occurred in 17 out of 28 cases in our study. There are several possible reasons for this: a subtotal initial resection, wrong site of re-resection, or misinterpretation of the frozen section specimen.18,30 Further studies should focus on the impact of these factors in relation to intraoperative frozen section analysis.

CONCLUSION

Our study demonstrates that positive fresh frozen margins, regardless of re-resection to R0 status, could be a powerful adverse factor that determines an aggressive nature of a subset of tumors arising in oral cavity. Despite some limitations, this feature should be taken into consideration in adjuvant treatment planning. In our opinion, the greatest impact this could have is in borderline clinical situations when the indication for adjuvant treatment may be questionable. We believe that positive fresh frozen margins in these cases may be key to facilitating a decision regarding supplementary treatment.

BIBLIOGRAPHY