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Intraoperative cytological examination of bone medullary. A useful technique to predict the extension of bone invasion in segmental mandibulectomy☆

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ABSTRACT

Aim: The main of the present report is to evaluate the utility of intraoperative cytological analysis of medullary bone to predict the extension of bone infiltration in segmental mandibulectomy.

Materials and method: Between the years 2016 and 2018, a total of 17 previously untreated patients with squamous cell carcinoma of the oral cavity underwent a segmental mandibular resection and intraoperative cytological analysis of the bone medullary at Virgen de las Nieves University Hospital (HUVN). The results of the intraoperative cytological analysis were compared with the result of the postoperative histopathological examination and sensitivity, specificity, positive predictive value, and negative predictive value of the test were calculated.

Results: Cytological analysis was positive in three patients and the bone resection was consequently extended. All the extensions of these bone margins were clean following the postoperative histological examination. However, two other patients previously classified as clean with intraoperative cytological analysis of bone medullary presented infiltration of bone margins postoperatively. The protocol demonstrated a high negative predictive value (85,7%). The positive predictive value, sensitivity, and specificity were 33,3%, 33,3%, and 85,7% respectively.

Conclusion: Intraoperative cytological analysis of bone medullary could represent an easy, fast, reliable and inexpensive method to reduce the rate of r1 surgeries attributable to the infiltration of the bone margin. This may have a positive impact on overall survival without increasing the duration and the iatrogenicity of surgery.

1. Introduction

Free surgical margins are essential to maximize the overall survival of oral squamous cell carcinoma and affected margins are associated with an increased risk of local recurrence (100%) and death at 5 years (90%) in the absence of adjuvant treatment [1]. Hence, surgeons usually review the resection margin by using intraoperative frozen sections. This precaution allows for detecting the presence of infiltrated margins intraoperatively and, at the same time, to extend the resection. However, the intraoperative control of surgical margins is extremely difficult when the tumor infiltrates bone structures. Bone needs decalcification prior to histological analysis, and this represents a long process that cannot be carried out during surgery [2]. Consequently, predicting the extension of bone invasion represents one of the major challenges in oral oncology. In this regard, intraoperative clinical assessment (periosteal stripping) and preoperative imaging tests (Ct scan, MRI, OPT, PET, and SPECT) usually define the extension of the resection [3,4]. However, these strategies do not ensure a reliable evaluation of the extent of bone infiltration and the surgeon usually removes at least 1 tooth on either side of the tumor in a dentate patient when bone invasion is suspected [5]. The main aim of the present report is to present our protocol to predict the extent of bone invasion in tumors

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with clear bone invasion at the preoperative imaging tests and, therefore, candidates for segmental mandibulectomy. Our method is based on the intraoperative cytological study of medullary bone samples taken at the level of the tumor resection margin. This technique could help achieve higher percentages of free surgical margins and consequently improve overall survival. Moreover, it is an easy, fast, and cost-effective procedure.

2. Materials and method

Between the years 2016 and 2018, a total of 17 previously untreated patients with squamous cell carcinoma of the oral cavity underwent a segmental mandibular resection and intraoperative cytological analysis of the bone medullary at Virgen de las Nieves University Hospital (HUVN). All patients underwent an accurate clinical examination and a computed tomography (CT) scan of the cervicofacial area before surgery. Moreover, the diagnosis of oral squamous cell carcinoma was confirmed in all patients with a preoperative biopsy. The mandibulectomy was carried out due to clear clinical evidence of bone involvement at the preoperative tests (CT scan and OPT). After completing the mandibulectomy, the surgeon proceeded to take the maximum possible amount of bone medullary at the proximal and distal ends of the mandibulectomy (Fig. 1). The sample was taken with a standard surgical curette. The authors defined as positive margin the presence of malignant cells at the margin for both frozen and final specimens. Specimens for frozen section pathology were taken always from the surgical site and never from the tumorectomy piece. Frozen sections obtained were placed on Telfa non adherent dressing (Covidien), labelled and quickly sent as frozen section to the pathologist to perform a cytological analysis and verify the presence of cellular atypia, tumor cells or other pathological findings. The final pathology specimen was oriented with silks and staples, immersed in formalin and sent to pathologists.

Thus, the surgeon decided not to extend the resection in the cases in which the intraoperative cytological analysis did not show the presence of atypical cells in medullary bone. However, the resection was extended by approximately 1 cm when the cytological examination was positive. In these cases, a new cytological examination was performed in the area (proximal or distal bone) where tumorectomy was extended. Importantly, it is worth emphasizing that all mandibulectomy and cytological analyzes were carried out by the same surgeon and the same pathologist.

3. Results

The sample was composed of 12 males (70.5%) and 5 females (29.4%). Patient ages ranged between 53 and 76 years old, with a mean of 68.7 years. The clinical stage of the primary tumor was established following the recommendations of the International Union Against Cancer (UICC) [6]. The location of the primary tumor was the tongue in 9 cases, the floor of the mouth in 4 cases, and the retromolar zone in another 4 cases. Mandibular invasion was observed in all cases following postoperative pathological examination. Hence, 16 patients were classified as Stage IVa (8 T4aN0M0, 5 T4aN1M0, and 3 T4aN2M0) and 1 as Stage IVb (T4aN3M0) postoperative (Table 1). Ct scan showed mandibular erosion or invasion of the cortex in 11 patients, and in two of these there were clear signs of involvement of the medullary bone. In the other 6 patients, the tumor was located very close to the mandibular cortex without showing clear signs of bone invasion. Cytological analysis revealed malignant cells in the medullary bone of 3 patients. In these cases, the mandibulectomy was extended by approximatively 1 cm in the direction of the end where the atypical cells were observed and a new cytological examination was performed in the area where tumorectomy was extended. No carcinogenic cells were evidenced in this second cytological test. Interestingly, postoperative pathological examination of the bone margins was tumor-free in these three patients and only one of them presented an infiltrated margin after surgery. However, it was attributed to the infiltration of the mucosal margin rather than the bone margin. Moreover, only 1 of these bone margins diagnosed as positives with intraoperative cytological examination was effectively affected by the tumor at postoperative histological examination (true positive). Fortunately, no tumor cells were evidenced on the extension of this bone margin. The other two cases that were positive at the intraoperative cytological examination could be attributed to the accidental contamination of the bone margin with tumor tissue (false positives, $n = 2$).

According with postoperative pathological analysis, two patients in whom the intraoperative cytological analysis did not show presence of tumor cells in the margins of bone resection presented infiltrated bone margins after postoperative pathological study. Consequently, these patients were classified as false negative ($n = 2$).

In total, we observed a total of eight patients with close ($n = 4$) or infiltrate margins ($n = 4$) following postoperative histological analysis (8/17; 47%) but 6 of these cases were attributed to the infiltration of the mucosal margin and only 2 to the involvement of the bone margins. Hence, this protocol demonstrated a high negative predictive value (85,7%) considering that only two cases classified as negative by the intraoperative analysis resulted positive following the postoperative examination (true negatives, $n = 12$). In contrast, the positive predictive value was 33.3%. In fact, only 1 of the 3 bone margins diagnosed as positives intraoperative was effectively affected by the tumor at postoperative (true positive, $n = 1$). As stated before, the intraoperative extension of this bone margin was negative following postoperative pathological analysis. The sensitivity and specificity of the cytological frozen section analysis were 33,3% and 85,7%, respectively (Table 2). Moreover, this test does not extend surgical time. Indeed, no more than 35 min were needed to obtain the results of the cytological analysis.

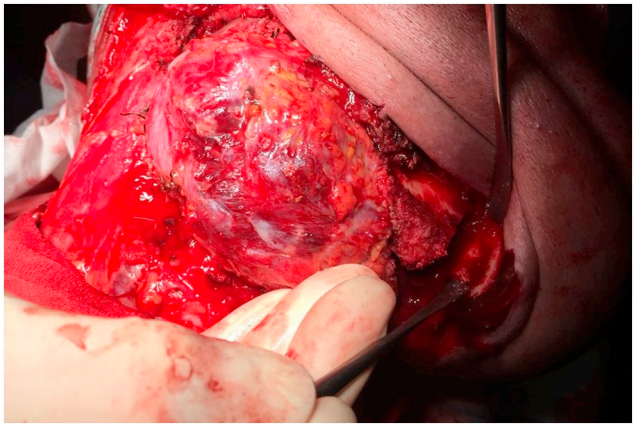

Fig. 1. A standard surgical curette is used to take the maximum possible amount of bone medullary at the proximal and distal ends of the mandibulectomy.

4. Discussion

The intraoperative control of surgical margins with frozen sections is widely used to improve the rate of R0 surgery. This is a key point in oral oncology because R status seems to be one of the most reliable predictors for overall survival [1,7]. Moreover, it is the only prognostic factor that may be controlled by the surgeon. In this regard, several studies have demonstrated that frozen section might detect R1 margins in approximately 23% of patients [2,8]. Unfortunately, Intraoperative analysis of surgical margins is usually performed only for soft tissue samples. The bone tissue needs to be decalcified prior to any histological examination and this process may take several weeks. Hence, numerous techniques have been proposed to eliminate this problem and

Table 1
Clinical pathological findings.

Stage	TNM	Site	Intraoperative cytological analysis	Bone margins after postoperative decalcification	Postoperative surgical margins (including soft tissue margins)
IVa	T4N0M0	Tongue	Negative	Negative	Infiltrated
IVa	T4N0M0	Tongue	Negative	Negative	Clean
IVa	T4N0M0	Floor of the mouth	Negative	Negative	Clean
IVa	T4N0M0	Floor of the mouth	Negative	Negative	Clean
IVa	T4N0M0	Retromolar zone	Positive	Negative	Clean
IVa	T4N0M0	Retromolar zone	Negative	Negative	Close
IVa	T4N0M0	Floor of the mouth	Positive	Negative	Close
IVa	T4N1M0	Tongue	Negative	Negative	Close
IVa	T4N1M0	Tongue	Negative	Negative	Clean
IVa	T4N1M0	Tongue	Negative	Negative	Clean
IVa	T4N1M0	Floor of the mouth	Negative	Negative	Clean
IVa	T4N2M0	Tongue	Positive	Positive (the intraoperative extension of this margins was negative after postoperative decalcification)	Clean
IVa	T4N2M0	Tongue	Negative	Negative	Close
IVa	T4N2M0	Retromolar zone	Negative	Negative	Infiltrated
IVa	T4N0M0	Tongue	Negative	Positive	Infiltrated
IVa	T4N1M0	Tongue	Negative	Positive	Infiltrated
IVb	T4N3M0	Retromolar zone	Negative	Negative	Clean

to allow the intraoperative examination of the bone margins [9]. However, most of these techniques presented certain limitations in terms of efficiency, simplicity, and cost effectiveness and were consequently abandoned [10,11]. In particular, Mahmood et al. reported that the intraoperative cytologic analysis of mandibular medullary bone might be extremely useful for predicting the real status of bone margins in oral oncology. These authors reported that the results of the intraoperative histological analysis perfectly correlated with the postoperative histological findings in 7 patients affected by oral squamous cell carcinoma and who underwent segmental mandibulectomy [12]. Nieberler et al. also evaluated the bone resection margin intraoperatively by using cytological techniques and they concluded that intraoperative cytological assessment of bone resection margins (ICAB) might be extremely helpful for reducing R1 disease after primary surgery. More specifically, ICAB showed a sensitivity and specificity of 94% and 97% respectively. In the same vein, Forrest and et al. analyzed the cancellous bone obtained at the end of mandibulectomy to predict the state of bone margins intraoperatively. According to these authors, the intraoperative cytological analysis of bone margins showed a correlation of 97% with the postoperative pathological findings [2].

On the other hand, Lubek et al. referred a sensitivity of 40% for intraoperative frozen section analysis in patients with total/extended maxillectomy. This data is comparable with our outcomes in term of sensitivity. However, some important details should be analyzed. In fact, Lubek et al. observed a high rate of false negative (9/25, 36%). In contrast, the rate of false negative was 11,7% in our series. Lubek et al., do not analyze the negative predictive value of the test in their article but it is reasonable to think that it is lower than what has been observed in our study if we consider the marked difference in the value of false negatives reported in the two series. The main reasons for these differences may be related to the heterogeneity of tumors analyzed by Lubek et al. and to the location of the tumor itself. In fact, these authors analyzed several types of head and neck tumors and not only oral squamous cell carcinoma. Particularly, of the 9 patients with false negative frozen section margins 3 had adenoid cystic carcinoma (ACC), 2 had osteosarcoma, and 1 had melanoma. Interestingly, only 3 of the 9 false negative results observed by Lubek et al. were attributed to the infiltration of the tumor margin by oral squamous cell carcinoma (33,3%). Another important issue is the anatomical area interested by the tumor. Lubek al. only analyze patient underwent total or partial maxillectomy. In contrast, only patients underwent segmental mandibulectomy was included in our study. In this sense, the shape of the maxilla and its relation to nearby structures may explain the higher rate of false negative observed by Lubek et al. According with the authors, the most common location identified for differences in error in margin analysis involved the posterior margin and the medial margin. However, these disadvantages are less frequent with the jaw and the intraoperative analysis of the bone medullary could be more predictable in diagnosing negative margins. The small amount of medullary bone took at the margin of mandibulectomy and/or the irregular invasion of the medullary bone could explain the two cases of false negatives observed in our sample.

Table 2
Sensitivity, specificity, positive predictive value and negative predictive value of intraoperative cytological analysis of bone medullary.

Sensitivity	Specificity
33,3%	85,7%

Positive predictive	Negative predictive
33,3%	85,7%

The advantages of cytopathologic techniques include the ability to analyze cells directly obtained from the bone resection margins. However, the intraoperative evaluation of medullary bone also presents some limitations. For instance, the cytologic evaluation of medullary bone cell might be particularly difficult in pre-irradiated tissues. In addition, false positive results can be caused by contamination of medullary bone with cancer cells present in the tumorectomy [2,4]. In our study, all patients were previously untreated and consequently there were no technical problems related to the radiation therapy. On the other hand, 2 of the 3 cases classified as positive with the intraoperative cytological analysis were finally defined as negative after the decalcification of the bone tissue. This data must be analyzed with caution because the high false-positive rate could lead to over-treatment. Nowadays, the majority of post mandibulectomy defects are

reconstructed with free bone flaps. Thus, the extra bone that would be removed in the case of false positive results might easily be replaced by increasing the size of the flap. Moreover, the infiltration of the bone margin following a micro-surgical reconstruction could represent a problem that is difficult to resolve. There is no justification for performing a second surgery to extend the margins in these cases and the patient will need more aggressive adjuvant treatments with poorer outcomes in terms of local recurrence and overall survival. In this complex scenario, the intraoperative cytological analysis of bone medullary may represent a useful tool for reducing the number of R1 surgeries secondary to the infiltration of the bone margins. In our opinion, the high negative predictive value of the test would justify its use. Moreover, the patients classified as false positive after the post-operative pathological examination did not show any complications related to the extension of the osteotomy. We are aware that this test has some limitations; however, it can help oncological surgeons to reduce the number of patients requiring R1 surgery due to the infiltration of the osteotomy margins. In addition, it is extremely inexpensive and does not delay surgery. Although a safe intraoperative assessment of bone margins still represents a difficult challenge, the intraoperative cytological analysis of bone medullary may be very helpful in cases where preoperative imaging tests and intraoperative clinical data do not allow a reliable assessment of the extent of bone infiltration.

5. Conclusions

Intraoperative cytological analysis of bone medullary could represent an easy, fast, reliable, and cost-effective method for reducing the rate of R1 surgeries attributable to the infiltration of the bone margin. This may have a positive impact on overall survival without increasing the duration and the iatrogenicity of surgery. This test may also represent an important complement to preoperative imaging tests (OPG, CT scan, MRI), intraoperative clinical evidence, and frozen section analysis. The high negative predictive value of the test offers acceptable oncological safety and may be very helpful in cases of microsurgical reconstruction of the defect.

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