Frozen sections and complete resection in oral cancer surgery

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Abstract

Objectives: Although the reliability of frozen sections for the intraoperative assessment of complete tumour excision has been established, the best location for collection and the impact of the type of sampling are still debated. We retrospectively investigated the reliability of frozen sections when collected from the surgical bed as tissue strips representative of the whole superficial margin and as a bowl of tissue underlying the resection site for deep margin, and the possibility of relying on frozen section negativity to consider resections complete.

Materials and Methods: Frozen section reliability was calculated by comparing histology before and after formalin embedding and then categorised by sampling type, in 182 patients undergoing transoral resection of oral cancer.

Results: Comparing frozen and permanent histology, sensitivity, specificity and accuracy were 69%, 98% and 96%, respectively; categorisation by sampling type failed to produce statistically significant differences. Based on frozen section negativity after formalin embedding, complete resections were obtained in 91.7% of patients with multiple-strip and bowl frozen sections.

Conclusion: Frozen sections collected as tissue strips and bowl are as reliable as point sampling in the intraoperative guidance of surgical resections. They effectively provide for margin enlargement, thereby increasing the surgeon's confidence that negative margins are clear.

KEYWORDS
biopsy, frozen section, oral cancer, resection margins, shrinkage, transoral surgery

1 | INTRODUCTION

Clear surgical margins with oral squamous cell carcinoma (OSCC) are of primary importance as positive surgical margins are related to local recurrence risk and survival (Weijers, Snow, Bezemer, van der Wal, & van der Waal, 2002) and represent one of the adverse features requiring postoperative adjuvant treatments (NCCN, 2018), which increase costs and toxicity. The issue of complete tumour resection is even more important during transoral surgery (TOS), a surgical approach completely performed through the mouth and minimising injury to healthy tissue (Tirelli, Boscolo Nata, Gatto, et al., 2018). The surgical field in TOS is narrower compared to the classical open approaches (transmandibular, pull through), and complete tumour exposure and margin control may

become challenging. In the recent literature, two different approaches to help the surgeon reduce the rate of positive margins have been proposed and validated: narrow-band imaging (NBI) and piecemeal resection. By enhancing mucosal and submucosal vessels and the vascular pattern alterations indicative of malignant transformation (Tirelli, Marcuzzo, & Boscolo Nata, 2018), intraoperative NBI reduces the rate of positive superficial margins (Tirelli, Marcuzzo, et al., 2018; Tirelli et al., 2015; Tirelli, Piovesana, Gatto, Torelli, & Boscolo Nata, 2016; Tirelli, Piovesana, et al., 2017). On the other hand, piecemeal resection, introduced to allow laryngeal tumour removal through the small diameter of laryngoscopes (Steiner & Ambrosch, 2000), has been used in TOS for oropharyngeal (Hinni, Zarka, & Hoxworth, 2013) and oral cancer (Choi et al., 2017; Tirelli, Piovesana, et al., 2018) because it allows one to identify the transition from cancer to healthy tissue thereby improving deep margin control. Despite the help given by these methods, the surgeon needs real-time information about completeness of the resection, as provided by frozen sections (FS). However, while FS have been used since the early 1900s, controversy still exists regarding collection and submission of the tissue (Songra, Ng, Farthing, Hutchinson, & Bradley, 2006).

In the classical open approaches, where a large amount of healthy tissue is resected, margin clearance is determined by the microscopic distance between the invasive tumour front and the edge of the resected specimen. In contrast, during TOS the surgeon tries to tailor the resection to each patient to minimise the amount of resected surrounding healthy tissue and minimally impact functionality. In this FS-driven approach, the surgery stops only when all FS have been intraoperatively defined as tumour-free by the pathologist. Consequently, it is essential for FS collection to be both focused and representative of the whole margin because tumour clearance will ultimately be defined by FS histologic negativity after formalin embedding.

The aim of this paper was to assess the best way to harvest FS by critically reviewing almost 18 years of experience with FS during transoral oral cancer resection at our Department and to analyse open questions and possible solutions in FS. Specifically, for the first time in the literature, we investigated the reliability of FS collected as tissue strips representative of the whole superficial margin and as a bowl of tissue underlying the site of the resected tumour in providing a complete analysis of both superficial and deep margins and reducing the risk of underestimation.

2 | MATERIALS AND METHODS

We retrospectively analysed the oncologic database of the ENT Department of Trieste searching for patients who underwent surgery for OSCC from January 2000 to April 2018. We included in the study 182 patients treated with a transoral approach for OSCC, so as to avoid possible bias given the different difficulty levels of collecting FS in TOS compared to open approaches. This study was approved by the University of Trieste Ethics Committee (n.89/2018) and followed the principles stated in the Declaration of Helsinki (1964).

Demographic information, as well as pathological data (FS type, FS histology before and after formalin embedding, final margin status on the resected specimen and pathological staging), was collected.

Surgical interventions were always performed by the same experienced head and neck cancer surgeon using different magnification systems (surgical loops, microscope or endoscope) according to lesion site and exposure. FS were always collected from the tumour bed from both superficial and deep margins, a defect-driven approach. Until August 2015, the surgeon took, from both the superficial and deep margins, multiple “point sample” FS focused on the most suspicious areas and submitted them to the pathologist indicating the site of collection (Figure 1a). From September 2015, following the “margin mapping” method proposed by Hinni, Zarka, et al., 2013, FS were taken as 3- to 4-mm-thick strips of tissue around the tumour for the superficial margins and as one or two bowls of tissue underlying the site of the resected tumour for the deep margins (Figure 1b). The surgeon stained with ink the most lateral surface of each surgical sample and presented them to the pathologist for FS examination. Using the cryostat, two or three slices were obtained from each sample and coloured with rapid staining for FS using Harris haematoxylin and eosin. FS presenting intraoperatively with moderate- and high-grade dysplasia or cancer were considered positive and, if possible, a surgical enlargement was immediately performed and FS repeated until a negative result for dysplasia or cancer was obtained; by contrast, when all the samples were negative, the surgeon closed the defect. Given that our hospital's pathology unit does not have a single pathologist dedicated to FS analysis, our samples could be analysed by any one of four experienced head and neck pathologists. All the samples evaluated on FS were revised after formalin embedding and haematoxylin and eosin staining by a dedicated pathologist (R.B.) who also analysed the main surgical specimen. In accordance with the NCCN guidelines (NCCN, 2018), preoperative imaging and intraoperative observation guided the extension of mandibular resection. When preoperative imaging showed contact between tumour and the bone without cortical erosion, we evaluated intraoperatively if the periosteum was detachable from the underlying bone (periosteal stripping). If detachable, we sent the rectangular piece of periosteum directly below the tumour for FS analysis: if confirmed negative, we would perform a light cortical bone drilling to obtain a safety resection enlargement; otherwise, we performed a partial (marginal or sagittal) resection of the mandible or an inferior maxillectomy for tumours located in the hard palate. Conversely, if a cortical infiltration was evident on the CT scan, a partial mandibulectomy or an inferior maxillectomy with piezosurgery was immediately carried out.

2.1 | Reliability of frozen section analysis

Reliability was calculated by comparing FS histology before and after formalin embedding. Only patients in whom FS were collected from both the superficial and deep margins were considered. We defined all FS with dysplasia or cancer confirmed after formalin embedding as true positive (TP) and all FS without histologic alteration before

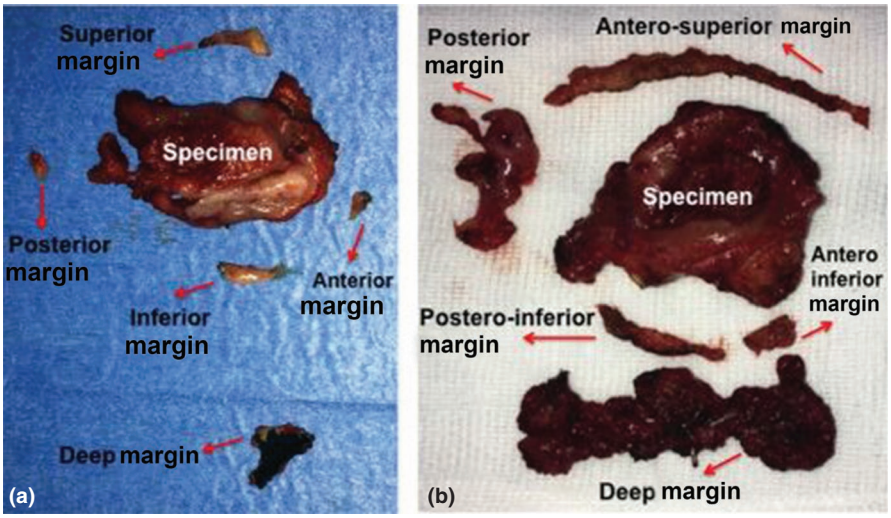

FIGURE 1 Frozen sections can be sampled with two different techniques. (a) The surgeon takes multiple point samples from both the superficial and deep margins and sends them to the pathologist only indicating the site of collection. (b) The surgeon takes 3- to 4-mm-thick strips of tissue all around the tumour for the superficial margins and a bowl of tissue underlying the site of the resected tumour for the deep margins; the second surgeon will then stain with ink the most lateral surface of each surgical sample and will present them to the pathologist for frozen section examination [Colour figure can be viewed at wileyonlinelibrary.com]

and after formalin embedding as true negative (TN); FS with dysplasia or cancer at intraoperative analysis and histologically clear at definitive histology were defined as false positive (FP), whereas negative FS presenting histologic positivity after formalin fixation were considered false negative (FN). Data were then stratified according to the type of sample ("point sample" vs. strips and bowl).

2.2 | Definition of "complete resection"

The definition of "complete resection" changed according to the FS collection method. When FS were collected as point samples, the definition was based on final margin status in the resected specimen, which was entirely analysed en face by the pathologist after formalin fixation: the resection was considered complete when margins were at least 3 mm from the tumour, and incomplete in the case of close (1–3 mm) or positive (<1 mm or clearly infiltrated by the cancer) margins. Conversely, when using "margin mapping," according to TOS philosophy, completeness was based on FS histology after formalin embedding: negative FS at definitive histology defined a "complete resection," even with positive or close margins in the resected specimen. As regards the bone, when periosteal stripping and cortical bone drilling were performed we based our judgement on the histology of the periosteum after formalin embedding, while in the case of partial mandibulectomy or inferior maxillectomy, the resection was finally considered complete if the deep bone margin was defined as negative at definitive histology.

We relied on histology after formalin embedding because it still represents the gold standard to assess radicality while molecular analysis is still being studied (Clark & Mao, 2017).

The resection is referred to as "complete" rather than "radical" because the latter implies an aggressive operation, whereas in TOS the surgeon removes the least amount of normal tissue necessary to assure a clear margin.

Disease-free survival (DFS) was defined as time from the date of surgery to the occurrence of local recurrences (LR) defined as appearance of a carcinoma at the same site as the previous tumour. Patients undergoing the margin mapping system were followed up until the date of LR or when censored at the date 25 January 2019 in order to calculate the reliability of FS negativity after formalin embedding to define a resection as complete. To avoid possible bias, we decided to focus DFS analysis only on patients treated exclusively with surgery.

2.3 | Statistical analysis

The patients' characteristics are summarised as descriptive analysis. Continuous variables are reported as mean ± standard deviation. Categorical variables are presented as absolute frequencies and percentages and compared using the chi-square test. The McNemar test and Cohen's kappa were used to evaluate the concordance between FS before and after formalin embedding (samples collected from superficial and deep margins were considered as a whole because the sampling and analysis technique is the same). The analysis was repeated after stratifying by sampling type ("point samples" vs. strips and bowl). Cohen's kappa was used to describe the degree of concordance as follows: 0.01–0.20, "weak"; 0.21–0.40, "fair"; 0.41–0.60, "moderate"; 0.61–0.80, "substantial"; 0.81–1.00, "almost perfect" (Landis & Koch, 1977). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated and reported with a confidence interval of 95%. Statistical analysis was performed with R software (version 3.3.2). Statistical significance was defined as p-value <0.05.

3 | RESULTS

Among the 182 patients, 103 were men, and the mean age was 68 years. Tumours were located in the gum (#5), cheek (#18), floor of mouth (#48), tongue (#78), hard palate (#12) and retromolar trigone (#21). There were 96 T1, 54 T2, 19 T3 and 13 T4 (TNM 7th edition).

Frozen sections were collected from both the superficial and deep margins in 121 patients, for a total of 785 samples, with an average of 6.5 per patient: in 85 patients (540 samples), they were collected as point samples focused on the most suspicious areas, while in 36 patients (245 samples), tissue strips were taken from the superficial margins and a tissue bowl from the deep margin. In this second group, we never found cases in which the surgical defect was too large to prevent FS collection as strips and bowl.

According to the intraoperative findings, the cortical bone was drilled to obtain a safety margin in 16 patients, 14 patients underwent a marginal mandibulectomy, and in 5 patients we performed an inferior maxillectomy.

3.1 | Reliability of frozen sections analysis

When FS were considered globally, comparing histology before and after formalin embedding we obtained 31 TP, 726 TN, 14 FP and 14 FN (McNemar test, $p = 0.99$), with a consequent sensitivity, specificity and accuracy of 69% (53%–82%), 98% (97%–99%) and 96% (95%–98%), respectively (Table 1). Cohen's kappa was 0.67 (0.60–0.74), indicating a substantial degree of concordance. Categorising by sampling type did not yield any statistically significant differences (Table 1).

3.2 | Definition of "complete resection"

A "complete resection," as defined in the Materials and Methods section, was obtained in 90 patients overall (74.4%). Specifically, in 33/36 (91.7%) patients undergoing intraoperative margin mapping, multiple-strip and bowl FS were negative before and after formalin embedding, and the surgical resection was consequently considered complete. Among these 33 patients, 25 were treated with surgery alone: after a median follow-up of 13 months, none experienced a local relapse.

4 | DISCUSSION

Intraoperatively, FS represent an instrument providing a real-time indication on the adequacy of resection margins. The value of FS in guiding oncologic surgery has been widely demonstrated (Gandour-Edwards, Donald, & Wiese, 1993; Layfield, Schmidt, Esebua, & Layfield, 2018). However, some uncertainties remain: the inability to analyse resection margins in their entirety and to demonstrate the distance from the cancer, selection of the best collection site (surgical specimen or surgical bed), and the high costs and the time spent for analysis are all open issues (Songra et al., 2006). The NCCN guidelines do not provide clear recommendations on the intraoperative evaluation of resection margins, leaving the decision to the surgeon (NCCN, 2018). In a survey carried out by the American Head and Neck Society (AHNS), more than 90% of the surgeons interviewed stated they used FS (Meier, Oliver, & Varvares, 2005). In our Department, during head and neck cancer surgery, we performed FS because they guide the tumour removal and the surgeon's decision to continue or stop the resection. Considering this key role, it is crucial that FS be accurate to ensure that intraoperative histology will be confirmed after formalin embedding (Sharma, Prasad, Pushparaj, & Poojary, 2009). Indeed, FP results would lead to unnecessary additional surgery, with a negative impact on functionality, or a need for otherwise avoidable reconstruction flaps; on the other hand, FN samples would result in closure of the surgical defect burying residual cancer. The analysis of our results demonstrated a substantial concordance between the FS histology before and after formalin embedding, with an accuracy of 96%. Certainly, this result represents the experience of a well-established collaboration between surgeon and pathologists. However, we hope that these results could be reproducible in other head and neck cancer centres considering that nowadays there is often a multidisciplinary management of patients and each case is collegially discussed by the different specialists. This is demonstrated by the fact that our results are consistent with the recent available literature (Abbas, Ikram, Tarig, Raheem, & Saeed, 2017; Di Nardo, Lin, Karageorge, & Powers, 2000;

TABLE 1 Reliability of frozen sections: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of frozen sections (FS), considered as a whole and stratified according to the type of sampling, obtained by comparing histology before and after formalin embedding

Indicator	Frozen sections as a whole	Point sample focused on suspicious areas	Multiple-strip and bowl sample	*p*-value[a]
Sensitivity (95% CI)	69% (53%–82%)	70% (51%–85%)	67% (38%–88%)	0.82
Specificity (95% CI)	98% (97%–99%)	98% (96%–99%)	98% (96%–100%)	0.84
PPV (95% CI)	69% (53%–82%)	68% (49%–83%)	71% (42%–92%)	0.99
NPV (95% CI)	98% (97%–99%)	98% (97%–99%)	98% (95%–99%)	0.94
Accuracy (95% CI)	96% (95%–98%)	96% (95%–98%)	96% (93%–98%)	0.99

Note. Abbreviation(s): 95% CI, 95% confidence interval.

*p values refer to the comparison of the diagnostic indicators between point sample focused on suspicious areas and multiple-strip and bowl sample.

Du et al., 2016; Gandour-Edwards et al., 1993; Ord & Aisner, 1997; Sharma et al., 2009). For the first time, we evaluated FS accuracy following stratification by sampling type ("point sample" vs. strips and bowl). The absence of a statistically significant difference demonstrates that the type of sampling does not impact FS reliability. Since no previous papers have addressed this specific issue, no comparison with the literature is possible.

Our results underscore that large specimens can be reliably analysed by the pathologist. As well as confirming the value of FS, in our opinion these results could represent the answer to one of the issues raised by previous studies regarding traditionally performed FS, that is the possibility of evaluating only a small part (0.1%–1%) of the resection margins (Smith et al., 2016). Conversely, if FS are collected as tissue strips and bowl for analysis of the superficial and deep margins, respectively, the surgical margins are examined in their entirety. By doing so, the risk of misjudging surgical radicality may be avoided (Figure 2). We should, however, underline that while it is quite fast to obtain strips from the superficial margins, collecting the tissue bowl from the deep margin can prove demanding.

A second criticism of FS is the high costs and time needed. However, as described by Di Nardo et al., an intelligent use is mandatory (Di Nardo et al., 2000): in our operating room, we start the operation with an NBI-guided tattoo (Tirelli, Marcuzzo, et al., 2018) followed by tumour removal and FS sampling, and then, while waiting for the response (40–60 min depending on the number), we perform neck dissection so as not to waste time; moreover, we continuously work together with the pathologist to minimise sampling and reading errors.

A recent review highlighted that negative FS do not guarantee negative margins on the surgical specimen (Shapiro & Salama, 2017). Both FP and FN results can be encountered. The loss of dysplastic or neoplastic tissue due to the width of the blades of the cutting tool and the presence of field cancerisation or skip lesions could explain FN results (Mannelli et al., 2014). On the other hand, shrinkage of the surgical specimen, the loss of healthy tissue induced by the blades of the surgical instruments or thermal damage decreasing the readable distance between the margin and the tumour (Mannelli et al., 2014), and freezing (Black, Marotti Zarovnaya, & Paydarfar, 2006) or thermal artefacts mimicking histologic alterations could justify FP results. The shrinkage phenomenon starts and is highest as soon as the specimen is removed, and it is more evident on the mucosa than on the deep musculature (Johnson, Sigman, Funk, Robinson, & Hoffman, 1997). A margin that was adequate in vivo could become close or even positive after removal, and FS collected from the specimen could prove to be FP (Figure 3). We think this problem could be minimised by collecting FS from the surgical bed. Moreover, the amount of shrinkage may differ according to lesion site (Thomas Robbins et al., 2019), but the paucity of lesions in certain sites prevented an analysis of this issue, which will be addressed in a future study.

When FS are collected as point samples (Figure 1), the surgeon has to rely on margin status in the surgical specimen to assess complete tumour resection. Conversely, if "margin mapping" is adopted and FS are collected as strips and bowl, a complete analysis of the superficial and deep margins is obtained because they are fully representative of the resection margins. As a result, analysis of the margins in the surgical specimen may no longer be necessary. FS effectively represent surgical defect enlargements so that, if they prove negative after formalin embedding, the resection can be considered complete even if the final margins on the specimen are positive. According to TOS philosophy, oncologic "complete resections" no longer rely merely on the metric evaluation of margins in the resected specimen: negative FS could result in a similar control rate independently from their width (Tirelli, Zacchigna, et al., 2017). This approach contrasts with the classic rule imposing a microscopic margin of at least 5 mm to define a clear resection (NCCN, 2018),

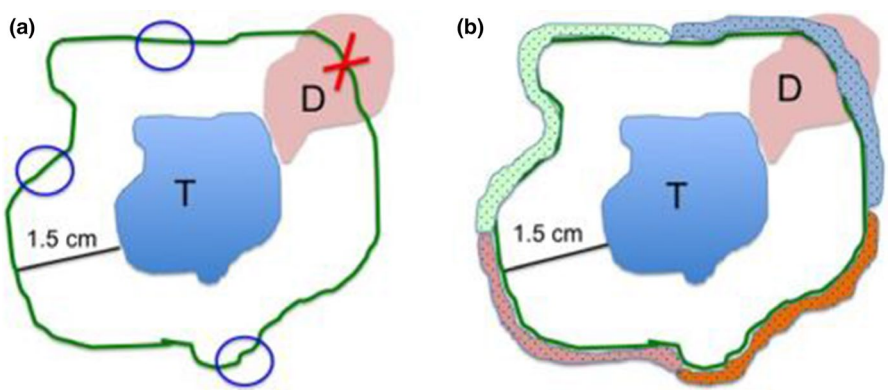

FIGURE 2 After tumour resection maintaining 1.5 cm of healthy tissue around the visible tumour, the surgical margins are evaluated with frozen sections. Two possible ways to evaluate surgical margins using frozen sections are presented (superficial margins are shown). (a) Frozen sections are collected as a point sample focused on the macroscopically most suspicious areas. If the sample is obtained in the place indicated with the red cross, the presence of dysplasia (D) is discovered and surgical enlargement can be immediately obtained; if the samples are taken where there are the blue circles, the surgeon has the false confidence of having performed a radical resection, while a positive margin will be discovered at definitive histology, with a need for further surgery or adjuvant radiotherapy. (b) Frozen sections are collected as 3- to 4-mm-thick strips of tissue around the tumour (coloured pointed strips), allowing the presence of dysplasia to be discovered in a superficial margin; this margin can be immediately enlarged ensuring a radical tumour resection. D, dysplasia; T, tumour [Colour figure can be viewed at wileyonlinelibrary.com]

and Baumeister, Baumüller, Harréus, Reiter, and Welz (2018) have recently underlined that even a few layers of normal cells separating the margins and cancer growth can technically be deemed clear. Moreover, as highlighted by Hinni, Zarka, et al., 2013, in transoral resections meticulous margin mapping and a constant dialogue between the surgeon and pathologist remove the need for a margin having a predetermined width, as long as it is free from cancer. This is consistent with the contemporary TOS philosophy whose aim is to resect the least amount of healthy tissue in order to reduce morbidity. In keeping with this approach, in our experience 33 of the 36 patients (91.7%) with FS sampled as strips and bowl, entirely representative of superficial and deep margins respectively, had a "complete resection" based on FS negativity after formalin embedding. Conversely, if the status of resection margins in the main specimen had been considered, only 13 of the 36 patients (32%) would have had a radical resection. This different approach to oncologic tumour resection could explain the different relationship between positive margins and local recurrence reported in previous papers (Brandwein-Gensler et al., 2005; Loree & Strong, 1990; McMahon et al., 2003; Woolgar et al., 1999). At the time of writing, with a median follow-up of 13 months, none of the patients in our cohort experienced local relapses. These preliminary data could appear still premature to draw prognostic conclusion, but the 99% of local control found by Hinni, Zarka, et al., 2013using the margin mapping approach makes us confident and, as soon as the follow-up allows, we will verify the finding.

Last but not least is the issue of the most appropriate site to collect FS: in the specimen-driven approach, FS are sampled from the excised specimen, whereas in the defect-driven approach, FS are assessed from the surgical bed after tumour removal (Amit et al., 2016) (Figure 4). Although recent studies recommend the first approach as more predictive for local control (Chang et al., 2013; Maxweel et al., 2015), additional prospective studies are necessary to confirm the results of previous retrospective experiences (Thomas Robbins et al., 2019). According to the AHNS survey (Meier et al., 2005), 76% of the respondents obtain FS from the surgical bed even if, according to the authors, this approach exposes to a greater risk of error in relocating the area to be enlarged in the case of positive FS. In our opinion, this is true if the traditional "point sample" technique is performed, that is, without any reference to the surgical specimen (Chang et al., 2013). In the present paper, no comparison of the two approaches is presented because at our Department we collect FS from the surgical defect. Even though this technique is mainly reported in studies on piecemeal resection (Hinni, Zarka, et al., 2013; Wilkie et al., 2016), a rigorously applied defect-driven approach might provide the most accurate tool to assist in tumour resection irrespective of the resection modality (en bloc or piecemeal), since the sampling site can be more easily relocated if additional surgery is needed. When removed, the surgical specimen starts to shrink leading to a mismatch between the specimen and the surgical bed, with the tumour bed often twofold to threefold larger than the resected specimen; consequently, if FS have been collected from the specimen, precise relocation of the positive margin can prove more difficult. It is critical that the surgeon collecting FS and the pathologist analysing them communicate effectively so that any positive margin results in additional surgery being performed on the exact location of the defect. Moreover, as previously stated, the shrinkage phenomenon poses the risk of false positive FS if a specimen-driven approach is used. The thermal damage induced by the cutting instrument on the specimen's margins can make reading the FS more challenging; the FS could prove FP because of tissue artefacts mimicking tumour (Black et al., 2006) and the loss of healthy tissue between the surgical blades (Mannelli et al., 2014) or, conversely, FN if small islands of neoplastic tissue are lost (Mannelli et al., 2014). On the other hand, the defect-driven approach allows FS sampling to be carried out with a cold knife ensuring the least possible thermal

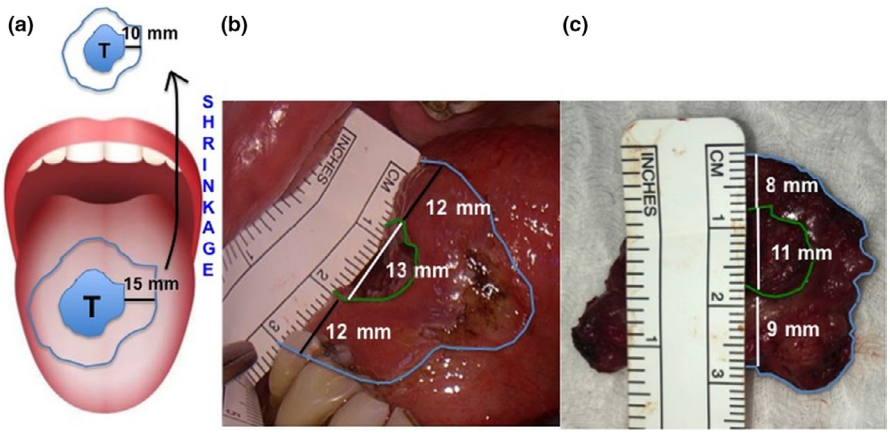

FIGURE 3 As soon as the specimen is removed, the shrinkage phenomenon starts. (a) Schematic representation of the risk of sampling frozen sections from the specimen: a margin that was adequate in vivo (15 mm) could become close or even positive after removal, and frozen sections collected from the specimen could prove false positive. Shrinkage is evident also in the tumour (T). (b) Intraoperative photograph of the distance between the tumour (13 mm width) and surgical margins in vivo in a right tongue cancer: the superior and inferior margins are both 12 mm wide. (c) Intraoperative photograph of the distance between the tumour and surgical margin as soon as the specimen has been removed; shrinkage is evident in both the superior (8 mm) and inferior (9 mm) margins, and in the tumour (11 mm) [Colour figure can be viewed at wileyonlinelibrary.com]

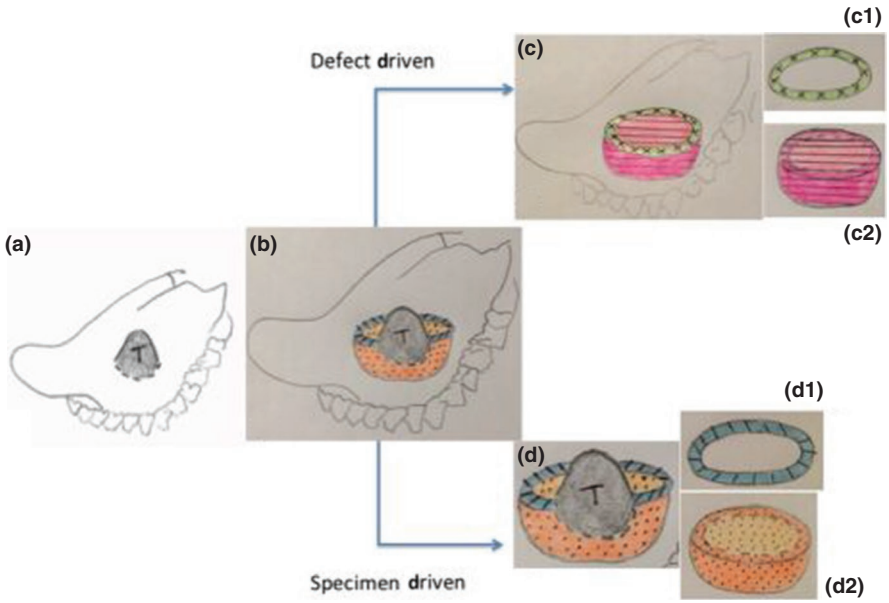

FIGURE 4 Two possible approaches to collect frozen sections are presented. (a) Tumour (T) of the tongue is drawn as a grey mass. (b) Tumour (T) of the tongue and healthy tissue to be resected all around, deeply (orange pointed area) and superficially (light blue area with oblique lines). (c) Defect in the surgical bed after tumour resection. In the defect-driven approach, frozen sections are collected from the surgical bed. Frozen sections are collected as 3- to 4-mm-thick strips of tissue (green crossed area) around the tumour for superficial margins (c1) and as one or two bowls of tissue (pink area with horizontal lines) underlying the site of the resected tumour for the deep margins (c2). (d) Surgical specimen comprised the tumour (T) and healthy tissue all around, deeply (orange pointed area) and superficially (light blue area with oblique lines). In the specimen-driven approach, frozen sections are collected from the surgical specimen. Frozen sections are collected as 3- to 4-mm-thick strips of tissue (light blue area with oblique lines) around the tumour for superficial margins (d1) and as one or two bowls of tissue (orange pointed area) underlying the site of the resected tumour for the deep margins (d2) [Colour figure can be viewed at wileyonlinelibrary.com]

damage (Liboon, Funkhouser, & Terris, 1997) and, by inking the margin to be analysed (the farthest from the tumour), it minimises the risk of interpretation errors. The need to perform haemostasis twice, after tumour removal and after FS collection, should not be overlooked. Specimen-driven supporters claim that if FS are collected en face during the defect-driven approach, margins can only be defined as positive or negative, but the distance from the tumour cannot be measured (Thomas Robbins et al., 2019; Williams, 2016). Nonetheless, as previously stated, meticulous intraoperative margin mapping may eliminate the need for a numerical distance from the tumour. As underlined by Chang et al. (2013), if the defect-driven approach is used, the surgical specimen must be accurately evaluated by the surgeon, margins should be thick enough and oriented to indicate the new true margins; moreover, re-approximating the FS to the main specimen to precisely understand the relation between them is useful (Chiosea, 2017). We are accustomed to collecting FS from the surgical bed, orienting them with ink and/or stitches, precisely describing the sampling site in the histologic request and also providing an explicative drawing. Indeed, only continuous communication between the surgeon and pathologist can ensure reliability of the definitive histologic report (Black et al., 2006). An exception to this behaviour is laryngeal cancer, especially if the glottis is affected, because removing additional tissue from the tumour bed could have a heavy negative impact on functionality (Hinni, Ferlito, et al., 2013): in this situation, we prefer to collect FS from the specimen.

5 | CONCLUSION

Frozen sections represent a valid instrument for a real-time assessment of oncological "complete resection" during transoral surgery for OSCC. The present study sought to shed light on the many variables involved in the FS method (sampling site and type and shrinkage phenomenon) that could justify the heterogeneity of results found in the medical literature. For the first time, we have demonstrated that the type of sampling does not impact the reliability of FS. Unlike the classical "point sample" technique, usually performed on the macroscopically most suspicious areas, the technique presented herein could ensure a more comprehensive evaluation of margins, limiting the risk of incomplete assessment. As FS are representative of margins in their entirety and effectively represent surgical defect enlargements, the surgeon could be confident that negative FS truly define a "complete resection" without having to consider final margin status in the resected specimen. By doing so, the least amount of healthy tissue can be resected and morbidity reduced. No doubt it is a delicate and complex process that requires continuous collaboration between the surgeon and pathologist, and creation of a dedicated team to optimise the procedure; moreover, the different difficulty in applying this technique according to the specific tumour site should not be overlooked. Starting with tumour removal and FS collection and proceeding with neck dissection while waiting for the response may be a possible strategy to avoid

idle time and reduce operating room costs. In this retrospective study, we specifically focused on the reliability of the technique, with only preliminary results on prognosis: future studies to verify its prognostic impact are needed. While we did not demonstrate a difference between our "strips and bowl" vs a "point sample" approach, the study may be underpowered to measure a difference that could exist.

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CONFLICT OF INTEREST

Authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Giancarlo Tirelli designed the study and critically revised the manuscript Michael L. Hinni critically revised the manuscript Mario M. Fernández-Fernández critically revised the manuscript Rossana Bussani interpreted data and critically revised the manuscript Annalisa Gatto collected data and drafted the manuscript Pierluigi Bonini designed the study and drafted the manuscript Fabiola Giudici interpreted data and drafted the manuscript Francesca Boscolo Nata collected and analyzed data and drafted the manuscript.

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