
Intraoperative Margin Control in Transoral Approach for Oral and Oropharyngeal Cancer

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Objectives: Piecemeal resection provides an innovative conceptual tool for margins surveillance because it entails the intraoperative evaluation of the whole resection margins and not just sample points, which should result in a better control of deep margins compared to en bloc resection. Although it is recognized that the intraoperative use of narrow band imaging (NBI) results in a better control of superficial margins, in this exploratory study we investigated whether NBI and piecemeal resection could be used in combination to improve margin control at both superficial and deep levels. Because piecemeal resection is based on frozen section analysis, we wanted to verify its reliability compared to definitive histological examination.

Methods: The status of resection margins in a group of patients with oral and oropharyngeal cancers treated with NBI and laser CO₂ piecemeal resection (group 1) was compared with that of an historical group of patients (group 2) treated with NBI and conventional en bloc resection. In group 1, sensitivity, specificity, and positive and negative predictive values were used to verify the rate of concordance between frozen section and definitive histology.

Results: The difference between deep positive margins in the two groups was statistically significant ($P = 0.042$). The high sensitivity and specificity (94.6% and 94.7%, respectively) of frozen section analysis also demonstrated its reliability in the examination of larger samples corresponding to the whole margin.

Conclusion: Even if our findings are limited by the small number of patients, we are confident that the combined use of NBI and piecemeal resection could represent an attractive surgical strategy to improve margin control.

Key Words: Piecemeal resection, transoral approach, narrow band imaging, intraoperative margin control, frozen section analysis.

Level of Evidence: 2b

INTRODUCTION

When performing surgical procedures to resect head and neck cancer, different factors could affect the clinical outcome, as tumor site, tumor-node-metastasis (TNM) classification, tumor histologic features, and the adequacy of resection margins. Among these factors, the achievement of adequate surgical margins, which means the complete removal of the tumor at the primary site, is the only one that is modifiable by the surgeon. In fact, an incomplete tumor resection increases the risk of local recurrence up to 66%^{1,2} and reduces survival.^{3,4} To achieve a better local control, the surgeon should try to obtain both superficial and deep clear margins. Considering that inadequate resection margins are reported

between 30% and 65%,⁵ this represents a difficult task for the clinician. Recently, the intraoperative use of narrow band imaging (NBI) emerged as a promising tool to help the surgeon in the assessment of the real superficial extension of oral, oropharyngeal, and laryngeal cancer.^{6–8} Narrow band imaging is a video endoscopic system with narrow band filters that enhance the microvascular abnormalities, commonly associated with preneoplastic and neoplastic lesions of the upper aerodigestive tract. It allows visualization of features of atypical lesions, invisible at standard endoscopy, which can be present around the visible tumor mass; therefore, tailored surgical resections, no longer based solely on ruler measurement at clinical observation, can be achieved. Its intraoperative use helps in the definition of the most appropriate superficial resection line.^{9,10}

On the other hand, the entrenched dogma of en bloc tumor removal to avoid tumoral cells seeding has been challenged by the so-called *piecemeal* resection. Indeed, the new wave of transoral approaches, which include surgical procedures performed through the mouth such as transoral laser microsurgery (TLM),^{11,12} transoral robotic surgery (TORS),¹³ and transoral ultrasonic surgery (TOUSS),¹⁴ can rely on piecemeal resection. In this way, the surgeon may deliberately cut through tumor to remove manageable sections and assess tumor extension.^{15,16} This multistep resection is carried out until the tumor mass is completely removed and all frozen sections

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f the margins are defined as tumor-free by the pathologist. This technique changes the paradigm of margin surveillance because the en bloc approach is replaced by the so-called *multiblock* approach.¹⁷ Indeed, following the rules of conventional en bloc surgery, tumors must be removed in one piece and the surgeons send small tissue fragments to the pathologist as a means of margin assessment. Differently in transoral piecemeal resection, the whole margin (superficial and deep) of each piece of tumor is carefully oriented by inking its external margin, which is carefully and entirely analyzed by focused intraoperative frozen sections.

In addition, it has been reported that CO2 laser piecemeal resection offers the chance to better characterize tumor extension in depth, which represents a major challenge in head and neck surgery thanks to a different interaction of the laser beam with tumoral and healthy tissue.^{12,17–21} Thus, on the one hand NBI allows to better define the superficial spread of the tumor,^{7,9,10} while on the other hand piecemeal resection better assesses deep margins.^{12,17–21}

In this exploratory study, we investigated whether the combination of NBI and the CO2 laser piecemeal resection could improve margin control of both superficial and deep margins. A cohort of patients with oral and oropharyngeal cancer was compared to an historic one treated with conventional en bloc resection. We decided to put together oral and oropharyngeal cancer because, even if there are significant differences regarding etiology and prognosis,^{3,22} no differences are reported following National Comprehensive Cancer Network (NCCN) guidelines²³ in terms of surgical approach, resection modalities, and margin adequacy. In the same way, no differences in NBI ability to better define superficial resection margins have been reported according to tumor site.¹⁰ Thus, our aim was specifically to merely investigate the feasibility of the combination of these two emerging techniques in achieving margins control without any considerations on prognosis or etiology. In parallel, considering that piecemeal resection is based on frozen section analysis,

our further aim was to prove its reliability comparing the results obtained on frozen sections to those obtained at definitive histological examination on paraffin samples.

MATERIALS AND METHODS

From April 2015 to November 2016, 42 treatment naive patients with oral and oropharyngeal cancer were enrolled in this prospective pilot study. In our series, transoral piecemeal resection was performed with CO2 Laser with flexible fiber (n = 15) and CO2 laser with micromanipulator (n = 27) following the standpoint of TLM.¹² The study was conducted at the ear, nose, and throat department of the University Hospital of Trieste, Italy, in accordance with the Declaration of Helsinki, and approved by the local ethics committee. Patient assessment was performed according to NCCN guidelines.²³ The first step was to perform the NBI high-definition television (HDTV) evaluation in order to underline superficial resection margins that we defined with a CO2 laser tattoo. If we did not observe any NBI positive area beyond the macroscopic tumor boundaries, we performed a tattoo at 1.5 cm from the macroscopic tumor margin. Differently, if NBI-positive areas^{24–26} were present in the mucosa around the tumor, these were comprised in the tattoo (Fig. 1A). As a second step, we performed a CO2 laser transection of the tumor (Fig. 1b) to better understand its extension in depth. Depending on the lesion site and exposition, microscope, surgical loupes, or high-definition endoscopes were used as magnification instruments during the resection. The first samples corresponding to the main half-bodies of the tumor were oriented with sutures and directly sent to the pathologist for definitive histological examination. As a third step, we performed margin mapping. Both superficial and deep margins were taken from the surgical bed: 1) superficial margins consisted of 3 to 4 mm thick stripes of tissue around the tumor, the external surface of which corresponded to the limits of the NBI tattoo (Fig. 1C); and 2) deep margins consisted of one or two bowls of tissue underlying the site of the resected tumor (Fig. 1D). Staining of the most lateral surface of each surgical sample was performed with medical tissue marking dye and presented by the second surgeon to the pathologist for frozen section examination. Each tissue sample was then cut at the cryostat by the pathologist into two or three slices depending on its dimensions. If all the slices did not show any trace of dysplasia or cancer on the inked surface on frozen section analysis, the procedure was stopped. On the flip side, in case of positivity, a

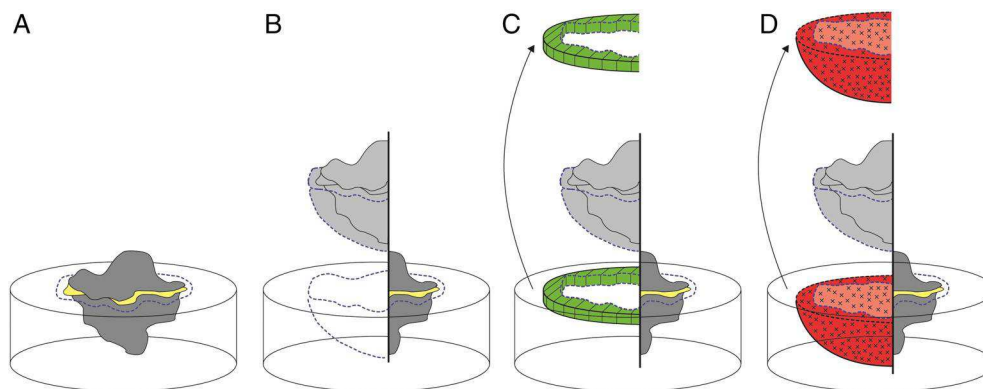


Fig. 1. Combined use of NBI and piecemeal technique for cancer resection. (A) The gray mass represents the main tumor; the yellow area is the dysplastic area beyond the macroscopic superficial tumor extension identified with NBI. (B) The vertical line indicates the first cut to transect the tumor and understand its deep extension; superficial and deep resection margins are represented with a dashed line. (C) Superficial margin mapping: frozen sections from the superficial margins consist of 3 to 4 mm thick stripes of tissue around the tumor (green lined area). (D) Deep margin mapping: frozen section from the deep margins consists of one or two bowls of tissue underlying the site of the resected tumor (red area with crosses). NBI = narrow band imaging.

laser enlargement was performed if possible and the staining procedure repeated. Each tissue sample evaluated on frozen section during surgery was then entirely revised after formalin fixation and hematoxylin and eosin staining. Only margins that scored negative at final histology and were at least 3 mm from the tumor mass were classified as negative. The number of frozen sections (superficial and deep) for each tumor was variable among patients depending on tumor site and dimension. We defined each patient as *superficial negative* or *deep negative* if none of the superficial or deep margins defined as negative on frozen section showed any traces of dysplasia or cancer after revision at definitive histological examination. Conversely, we defined patients as *superficial positive* or *deep positive* if one or more margin (superficial or deep) defined as negative on frozen section was revised as positive at definitive histological examination.

The rate of patients defined as superficial positive or deep positive in the present cohort (group 1) was compared with that observed in a historical cohort (group 2). Group 2 included 60 patients, enrolled following the same criteria listed above and treated by the same surgeon (G.T.) between January 2013 and April 2015 for oral and oropharyngeal cancer with conventional en bloc resection (Fig. 2) using electrothermal bipolar vessel sealing system (n = 42) or harmonic scalpel (n = 18) with intraoperative NBI-HDTV using a transoral approach. In this group, superficial and deep margins were entirely analyzed en face by the pathologist on the main specimen after formalin fixation, whereas intraoperative frozen sections consisted of little fragments of tissue sampled from tumor bed. As stated above, we classified margins as clear when > 3 mm.

With regard to the second aim of our study (reliability of frozen section analysis), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated in group 1. We defined true positive (TP), true negative (TN), false positive (FP), and false negative (FN) margins as follows: TP, a positive frozen section confirmed as positive at definitive histological examination; TN, a negative frozen section confirmed as negative at definitive histological examination; FP, a positive frozen section resulted as negative at definitive histological examination; and FN, a negative frozen section resulted as positive at definitive histological examination.

Statistical Analysis

Patients demographics and tumor characteristics, as the comparison of the rate of positive margins between the two groups was assessed using Fisher test with a threshold of statistical significance set at a *P* value < 0.05. Statistical analysis was

performed with GraphPad InStat 3 Ink (GraphPad Software, La Jolla, CA).

RESULTS

Patient demographics, pathological T category (pT), and tumor site in the two groups are summarized in Table I. The two groups were comparable in terms of tumor site and stage, considering T1 to T2 (early stage tumors) and T3 to T4 (advanced stage tumor) (*P* = NS).

At definitive histologic examination, in group 1 superficial and deep margins were positive in two (14.3%) and four (9.52%) patients, respectively, whereas in group 2 they were four (6.7%) and 16 (26.7%), respectively (Table II). None of the patients in the two groups had both superficial and deep positive margins.

The difference between positive deep margins in the two groups was statistically significant (*P* = 0.042).

The difference between positive superficial margins in the two groups was not statistically significant (*P* = NS), consistent with the fact that these margins were similarly analyzed by NBI in both groups.

A total of 283 frozen section analysis of margins was performed in group 1. Comparison between frozen section and definitive histological examination found 88 TP, 180 TN, nine FP, and six FN. Sensitivity, specificity, PPV, and NPV were 93.6%, 96.8%, 90.7%, and 96.8%, respectively.

DISCUSSION

Over the last decades, the surgical treatment for oral and oropharyngeal squamous cell carcinoma has clearly evolved. New surgical trends, supported by the so-called *transoral surgery* (TOS), such as TLM, TORS, and more recently TOUSS, avoid the need of mandibular swing or pull-through approaches, which negatively affect quality of life²⁷ and allow shorter hospitalization and lower complication rates.²⁸ Besides these unequivocal advantages of TOS, the potential risk of an incomplete resection of the tumor could be argued. Thus, the reliability of frozen sections appears crucial to grant oncological radicality.

Starting with the standpoint of Steiner more than 40 years ago,²⁹ the noteworthy advances in laser

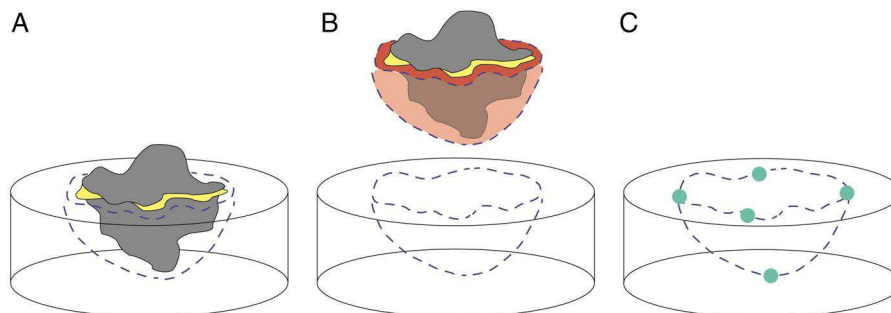


Fig. 2. Combined use of NBI and en bloc technique for cancer resection. (A) The gray mass represents the main tumor; the yellow area is the dysplastic area beyond the macroscopic superficial tumor extension identified with NBI. Superficial and deep resection margins are represented with a dashed line. (B) The yellow area is comprised in the superficial resection margins; the light orange area is the tissue comprised in the resection of the deep margin of the tumor. (C) Frozen sections from both superficial and deep resection margins are taken as point samples from the tumor bed. NBI = narrow band imaging.

TABLE I.
Patients Demographics, Tumor Site, Subsite and Stage in the Two Groups

	Group 1 (n = 42) (piecemeal resection)	Group 1 (n = 60) (en bloc resection)	P Value
Age, median (range) y	70 (48–90)	71 (47–88)	P = NS
Sex, No. (%)			
Male	26 (61.9)	44 (73.3)	P = NS
Female	16 (38.1)	16 (26.7)	
Tumor site			P = NS
Oral cavity	22	40	
Floor of the mouth	8	16	
Tongue	8	14	
Buccal mucosa	4	4	
Hard palate	2	6	
Oropharynx	20	20	
Tonsil	10	6	
Base of tongue	6	3	
Soft palate	4	11	
Tumor stage (pT)			P = NS
T1–T2 (early)	26 (61.9)	28 (46.7)	
T3–T4 (advanced)	16 (38.1)	32 (53.3)	

pT = pathological T category; T = tumor.

technology have led to an expansion of TLM indications over the last decades.^{30,31} Indeed, whereas in the past, TLM was limited to few stages and sites tumors, the introduction of flexible fibers has now extended its indications.^{32,33} From a practical point of view, the tool does not need the laser beam to be aligned with the microscope (as for micromanipulator CO2 lasers), which makes its use easier for the surgeon, who can work at a distance of up to 5 mm in a noncontact mode with different angles of cut.³⁴ CO2 laser surgery overall preserves a higher amount of normal tissue, avoiding the need of reconstruction and its potential complications, functional deficits and prolonged hospitalization.^{11,28,33,35} Transoral laser microsurgery represents a Copernican revolution because it overcomes the oncologic dogma of the en bloc resection, switching to the modern concept of piecemeal resection.²¹ In this way, the surgeon has the opportunity to deliberately remove manageable sections of tumor, moving the laser inside an exposing tool such as a laryngoscope or a mouth retractor.¹⁷ This should allow a better intraoperative evaluation of the depth of invasion.^{12,17–21} Indeed, deep margins represent a major problem for the surgeon³⁶ compared to mucosal margins, which are more visible, palpable, and assessable by optical imaging such as

NBI.^{7,10,37} Woolgar and Triantafyllou,³⁸ in a very detailed appraisal on 301 specimens of oral and oropharyngeal cancers, found 70 cases with positive margins (23.25%), 50 of which (71%) had only deep infiltrated margins. Some reports tried to face this crucial problem using vital staining or digital microscopy to detect deep residual cancer tissue.^{39,40} Steiner et al.,⁴¹ in a series of 48 patients with base-of-tongue carcinoma, found nine patients (19%) with positive margins following the initial resection. Haughey et al.,¹¹ in a multicenter study on 204 advanced oropharyngeal carcinoma, found 7% of positive margins. Others reported a rate between 3% and 10.5%.^{12,20,38} However, none of these studies analyzed separately deep and superficial margins to objectively determine the potential advantage of TLM on deep margins control. Our study first aims at differentiating between superficial and deep margins status in a series of oral and oropharyngeal cancer treated with CO2 laser and piecemeal resection.

At definitive histological evaluation, six patients (14.2%) in group 1 presented positive margins: two patients (14.3%) were defined as superficial positive, whereas four (9.52%) were deep positive. In contrast, in group 2 we found 16 patients (26.7%) with involved deep margins, which was statistically different from group

TABLE II.
Superficial and Deep Margin Status in the Two Groups

	Group 1 (P = 42) (piecemeal resection)	Group 2 (n = 60) (en bloc resection)	P Value
Superficial negative	40	56	P = NS
Superficial positive	2	4	
Deep negative	38	44	P = 0.042
Deep positive	4	16	

1 ($P = 0.042$). The lack of a significant difference in superficial margins was expected because NBI-guided resection was performed in both groups. The limited number of superficial positive margins confirms the results found in our previous works.^{7,9,10}

Our results are in agreement with those of the only other study that tried to objectively assess the advantage of piecemeal resection in achieving deep margins control.⁴² Differently from Choi et al.,⁴² who reported a series of T1 to T2 tongue carcinomas, in our study we considered both oral and oropharyngeal cancers and included patients with both early and advanced tumors. Thus, our results further underline the efficacy of piecemeal resection in assessing tumor extension in depth, irrespective of site and stage.

Moreover, another significant difference is the tools used to divide and resect the tumor: Choi et al.⁴² used a monopolar cautery to transect the tumor and harmonic scalpel to perform resection. Conversely in our experience in group 1, the CO2 laser was used both to transect the tumor and resect it. In our opinion, the use of the laser in piecemeal resection is of primary importance for two reasons: first the interaction of the laser beam with the tissue is clearly different, especially under magnification when it cuts the tumor and carbonization happens, while healthy tissues are easily dissected (Fig. 3); moreover, it has been demonstrated that monopolar cautery and harmonic scalpel produce a higher thermal damage compared to CO2 laser.⁴³ If we consider that piecemeal resection is based on frozen section analysis and there is a tissue damage related to the freezing process itself,^{44,45} it is essential to choose surgical instruments that produce minimal thermal damage that could compromise the histopathological assessment of margins.⁴⁶

Our second aim was to investigate the reliability of frozen section analysis. Previous studies reported a high accuracy with the use of frozen section but underlined the risk of sampling errors.^{47,48} During TLM, larger samples

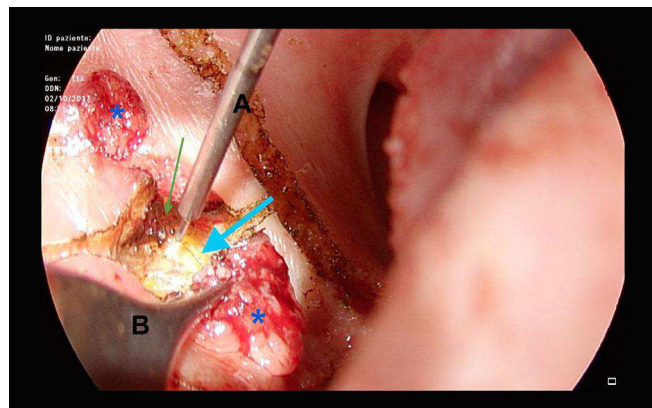


Fig. 3. Intraoperative photograph of a pT2 squamous cell carcinoma of the right cheek mucosa. The blue stars indicate the superior and inferior parts of the tumor; the letter A indicates the wave guide CO2 laser; the letter B indicates the surgical forceps to maintain the specimen in traction; the green thin arrow indicates the carbonization produced by the laser interacting with the tumor; and the cyan large arrow indicates the healthy tissue that is not carbonized by the laser. The deep interface between tumoral and healthy tissue is clearly visible.

representative of the whole margin—and not point samples as in traditional en bloc surgery—are sent for frozen section analysis. In our opinion, this type of sampling could reduce the risk of sampling errors.

Our results in group 1 also confirm the high sensitivity (94.6%) and specificity (94.7%) of frozen section analysis in the examination of larger samples corresponding the whole margin, which again is consistent with recent available literature.^{46,47}

In both groups, tissue to be analyzed on frozen section was collected from the tumor bed because we prefer the so-called *defect-driven approach*. Indeed, even if recent studies^{49,50} seem to recommend the *specimen-driven approach*, the problem is still far from being solved, as highlighted by the American Head and Neck Surgery Society review.⁵¹ In our experience, if meticulously performed, the defect-driven approach is the most precise way to help the surgeon during tumor resection because it allows an easier relocation of the sampling site if a surgical enlargement is required. In fact, because of the specimen handling and shrinkage, the specimen and the surgical bed do not perfectly match anymore and the exact relocation of the margin to be enlarged is more difficult if frozen sections have been collected from the specimen. As the surgical specimen is removed, the shrinkage phenomenon begins. This implies that an initially adequate margin can become close or even positive, and that the frozen section collected from the surgical specimen could result falsely positive.

The number of patients enrolled and the limited period of follow-up are limitations of this study. The analysis of a larger sample and the impact of deep margins control on local recurrence and survival will be the aim of our future studies.

CONCLUSION

In our opinion, all transoral approaches such as TLM, TORS, or TOUSS represent a step forward in the future of the oncologic surgery. Specifically, the opportunity to transect the tumor, which has traditionally been considered a heresy in oncologic surgery; and the different point of view on margin surveillance, which provides a better intraoperative evaluation of the deep resection margins, represent the main advantages of these approaches.

Does piecemeal resection necessarily require laser CO2? In our opinion, the instrument may be of secondary importance compared to the resection method. Whereas laser CO2 seems to be the instrument of choice for the larynx and the hypopharynx, the real advantage of using CO2 laser rather than other radiofrequencies or ultrasonic tools for oral and oropharyngeal cancer still has to be demonstrated. Future studies focusing on the thermal damage and postoperative pain using different cutting tools are necessary to understand if one must be preferred to others.

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