Laryngology

Direct autofluorescence during CO_2 laser surgery of the larynx: can it really help the surgeon?

Autofluorescenza diretta in corso di laserchirurgia transorale con laser CO_2: un reale aiuto per il chirurgo?

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SUMMARY

Herein we assessed the impact of direct autofluorescence during intraoperative work-up on obtaining superficial free resection margins, identifying new areas of malignant transformation and altering disease-free survival and local control at 3 years in patients submitted to transoral laser surgery (TLS) for early glottic cancer. Prospective cohort evaluation was carried out on the diagnostic accuracy of the superficial extent and TNM staging in 73 patients with glottic carcinoma undergoing transoral CO_2 laser surgery. The use of direct autofluorescence was associated with superficial disease-free margins in 97.2% of cases, and with superficial close margins in 2.8%. The improvement in diagnostic accuracy was 16.4%; in 8.2% of cases, there was upstaging of the TNM classification (in one case, a second neoplastic area in a different laryngeal site was observed and considered to be a second endolaryngeal primary). The sensitivity of direct autofluorescence was 96.5% with a specificity of 98.5%. Overall, 3-year disease-specific survival and local control with laser alone were, respectively: T1a (97.5%, 100%), T1b (86.7%, 86.7%), T2 (88.9%, 88.9%). This study demonstrates that direct autofluorescence can help to identify positive superficial margins, and has a favourable impact on disease-specific survival and local control at 3 years.

KEY WORDS: Autofluorescence • Transoral laser surgery • Resection margins • Endoscopic surgery • Laryngeal cancer • Glottic tumour

RIASSUNTO

Obiettivo di questo studio è stato valutare l'impatto dell'autofluorescenza diretta durante il work-up intraoperatorio nell'ottenere margini di resezione superficiale indenni, nell'individuare nuove aree di trasformazione maligna e nel migliorare a 3 anni la sopravvivenza libera da malattia/controllo locale nei pazienti sottoposti a laserchirurgia transorale (TLS) per cancro della glottide in fase iniziale. (Disegno dello studio: studio di coorte prospettico). Una valutazione prospettica sull'accuratezza diagnostica dell'estensione superficiale e sulla stadiazione TNM è stata condotta in 73 pazienti con carcinoma della glottide sottoposti a laserchirurgia transorale. L'utilizzo dell'autofluorescenza diretta ha determinato margini superficiali liberi da malattia nel 97,2 % dei casi e margini superficiali esigui nel 2,8 %. Un miglioramento nell'accuratezza diagnostica si è verificato nel 16,4 % mentre nell'8,2 % dei casi si è assistito ad un up-staging della classificazione TNM (in un caso un'area neoplastica in una sede laringea distinta è stata considerata un secondo tumore endolaringeo primitivo). La sensibilità dell'autofluorescenza diretta è stata del 96,5%, la specificità del 98,5%. Nel complesso la sopravvivenza malattia-specifica ed il controllo locale con laser a 3 anni sono stati rispettivamente: T1a (97,5 % ,100 %), T1b (86,7%, 86,7%), T2 (88,9%, 88,9%). Concludendo questo studio dimostra che l'autofluorescenza diretta può aiutare il chirurgo nell'identificare i margini superficiali positivi e risulta associata ad un impatto favorevole sulla sopravvivenza malattia-specifica e sul controllo locale a 3 anni.

PAROLE CHIAVE: *Autoflorescenza • Chirurgia laser transorale • Margini di resezione • Chirurgia endoscopica • Carcinoma laringeo • Tumore glottico*

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Introduction

Correct loco-regional work-up of laryngeal squamous cell carcinoma (SCC), especially in early stage glottic carcinoma amenable to transoral carbon dioxide laser surgery (TLS), includes accurate endoscopic examination to evaluate the superficial extent of the tumour and precise imaging to assess the extent of depth to muscular, fibrous and cartilage structures and laterocervical lymph node status [1 2]. Regardless of treatment modality (TLS or radiation therapy), the treatment goals of early stage laryngeal glottic carcinoma are good oncological outcome and preservation of vocal function [3].

To optimise the oncological results of TLS, without compromising functional outcomes, many authors have stressed the utility of intraoperative endoscopic work-up with 0° and angled telescopes [1 4 5] to accurately identify the superficial extent of the tumour and provide disease-free mucosal margins of at least 1-3 mm [6].

Endoscopic tools for examination of the upper aero-digestive tract, especially autofluorescence and narrow band imaging (NBI), have undergone significant developments in recent years [7-9]; compared to endoscopic evaluation using white light alone, these endoscopic methods provide important biological information that is useful to improve intraoperative assessment of the superficial extent of the lesion and to identify new areas of malignant transformation that are distinct from the primary lesion.

Oncology in general, and in particular, head and neck oncology, is increasingly oriented towards therapeutic choices that are "custom tailored" to obtain both organ and functional preservation [10-12]; in this regard, a precise definition of the superficial extent of a tumour and correct evaluation of laryngeal motility in addition to high-quality imaging are strategic and fundamental aspects in evaluating all features of the lesion.

Herein, a prospective study was carried out to determine the accuracy of diagnosis of superficial extent and TNM staging in 73 patients with early neoplastic lesions of the glottis undergoing TLS, with the aim of determining whether the intraoperative use of direct autofluorescence helps the surgeon to obtain disease-free resection margins and identify separate areas of malignant transformation. An additional goal was to assess the impact of direct autofluorescence on disease-free survival and local control with laser alone at 3 years in patients submitted to TLS for early glottic cancer.

Materials and methods

In the period between January 2005 and December 2009, a prospective study was carried out at the ENT Department of the University of Turin on 73 patients, all of whom were current smokers (65 males and 8 females; mean age 63 years) affected by a suspected, previously untreated, early squamous cell carcinoma (SCC) with glottic localisation. Overall, in the same period, autofluorescence endoscopy was applied in a cohort of 286 patients with suspected SCC of the larynx and hypopharynx. However, any case of cancer at a more advanced stage or with localisation other than glottic was excluded from the study to obtain a group characterised by homogeneous lesions, for which endoscopic treatment using TLS was planned.

Pre-operative work-up included physical examination, routine blood test, chest X-ray and careful evaluation of the superficial and depth extent of the tumour, which was preoperatively examined using flexible videolaryngoscopy (Olympus Medical Systems Corporation, Tokyo, Japan) and/or rigid videolaryngoscopy coupled to a high definition television (HDTV) and videolaryngostroboscopy (Karl Storz, Tuttlingen, Germany). Magnetic resonance imaging (MRI) of the neck was performed in cases of bulky T1a and in all cases of T1b and T2 to exclude infiltration of the laryngeal framework and assess the extension of the lesion to the paraglottic and pre-epiglottic spaces. Disease was classified according to the 2002 (6th Edition) Union Internationale Contre le Cancer (UICC)/American Joint Committee on Cancer (AJCC) TNM system [13].

Demographic characteristics and clinical staging of the study cohort are shown in Table I. All patients were subjected to cordectomy (preferably en bloc technique, in a single procedure) using a CO_2 laser (Sharplan 1055S CO_2 laser; Sharplan, Tel Aviv, Israel) under microlaryngoscopy with superpulse delivery in continuous mode (1 to 5 W), coupled with an AcuBlade micromanipulator (270 mm spot size). The type of resection was graded according to the European Laryngological Society (ELS) Classification which includes six types of cordectomy: subepithelial (type I), subligamental (type II), transmuscular (type III), total (type IV), extended (type V) and anterior commissure (type VI) [14 15].

Table I. Demographic characteristics of the study cohort.

No. of patients	73
Gender	
Male	65
Female	8
Age	
Range	48-79 yrs
Mean	63 yrs
TNM staging (level C2)	
Glottic	
T1a	47
T1b	13
T2	13
Impairment of motility	4
Subglottic/supraglottic extension	9

The intraoperative work-up protocol involved examination of the larynx of each patient with direct endoscopy under white light and in direct autofluorescence, in a stepwise fashion. All endoscopies were recorded for documentation and for immediate re-evaluation before proceeding with TLS.

First, the evaluation of the superficial extent of the lesion was carried out using rigid 0° and 70° angled telescopes with white light (WL) coupled to HDTV (Fig. 1A, step 1); on the basis of this assessment, after careful enlargement of the ventricular bands with dilating forceps, the area of excision was marked with several laser spots, maintaining an apparent margin of healthy tissue of approximately 2 mm compared to the visible limits of the suspected neoplastic lesion (Fig. 1B, step 2). Next, an additional endoscopic evaluation with direct autofluorescence was carried out coupled with a 3CCD camera (D-light Autofluorescence System, Karl Storz, Tuttlingen, Germany) using rigid 0° and angled 70° telescopes. In agreement with literature data, any area showing a bright green fluorescence was considered normal, while any well-defined blue/dark violet area compared to the surrounding mucosa was considered positive.

Following re-evaluation with direct autofluorescence, comparing area(s) found by autofluorescence to be positive with the surgical margins outlined by laser spots, all cases were assigned to one of three categories: (a) area of surgical excision analogous to the positive area determined by autofluorescence, (b) area of surgical excision insufficient compared to that found by autofluorescence and (c) area positive by autofluorescence in another site compared to the area of surgical excision.

In all cases where the areas positive by autofluorescence were different from those planned on the basis of evaluation under white light (Fig. 1C, step 3), surgical intervention consisted of widening the mucosal resection of the respective area to obtain apparently free superficial margins of at least 2 mm, which were considered to be definitive superficial margins (DSMs); in the case of area(s) positive by direct autofluorescence that were immediately adjacent to the lesion evident by white light enlargement, the resection was performed in monobloc; in some locations, due to the technical difficulty, it was preferred to obtain a biopsy or carry out a separate resection of the area considered to be positive. In the case of suspected superficial extension to the bottom/roof of the ventricle or to the false cord, at the time of vestibulectomy, particular care was taken to precisely coincide the inferior extreme of the vestibulectomy with the lateral extreme of the area previously marked for excision (Fig. 2A,B).

The deep margins of surgical specimens were marked with black ink, and both the specimen and the eventual enlargement of the resection were sent for histological examination to the same pathologist (GA) on a paper diagram of the larynx with precise indications of the procedure to identify the actual DSMs.

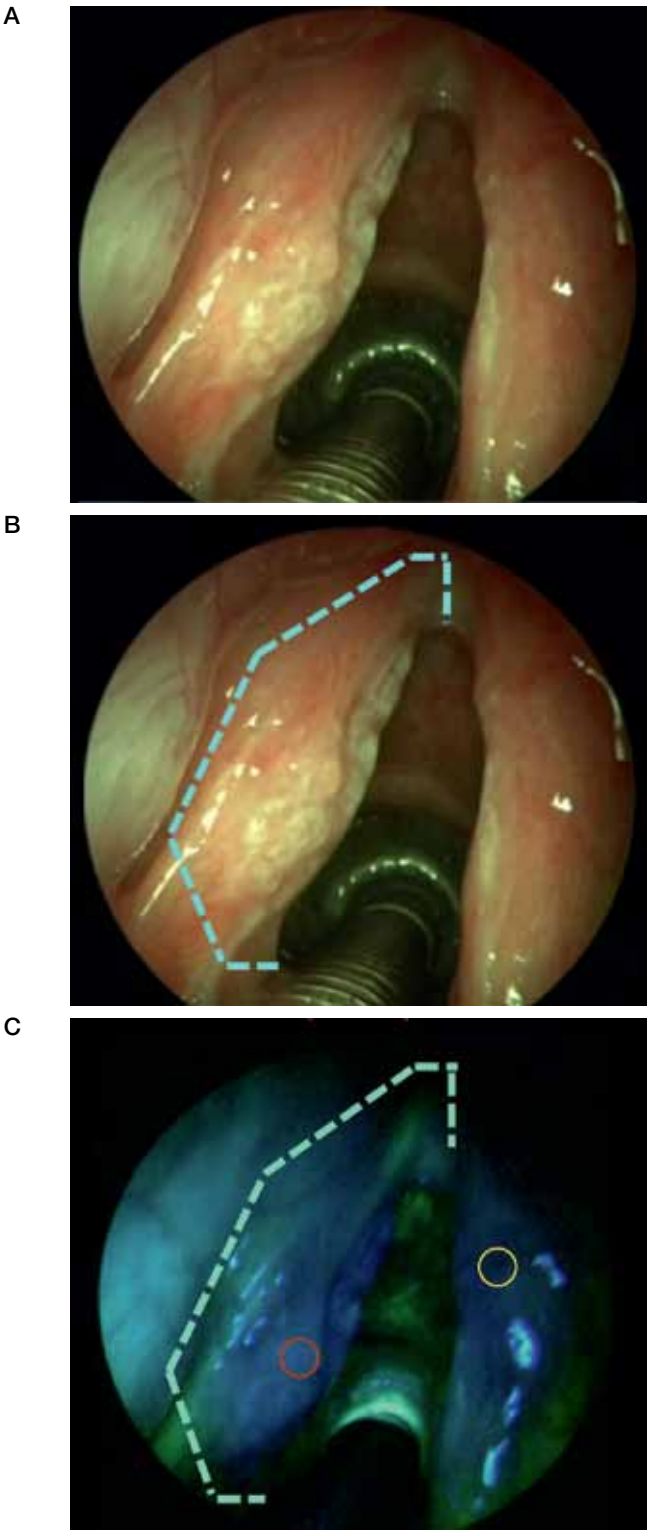

Fig. 1. Stepwise protocol used for intraoperative work-up. **A**: during direct microlaryngoscopy, initial assessment in white light of a suspected left vocal cord SCC staged cT1a; **B**: the area of excision is marked with several laser spots, maintaining an apparent margin of healthy tissue of approximately 2 mm compared to the visible limits of the suspected neoplastic lesion; **C**: assessment of field using direct autofluorescence showing an area of surgical excision insufficient compared to that found by autofluorescence in the dark [the histological examination on the surgical specimen and biopsy on the contralateral vocal cord found an invasive SCC in both the site of the clinically visible tumour (red circle) and in the contralateral vocal cord (yellow circle) → upstaging from glottic T1a to T1b].

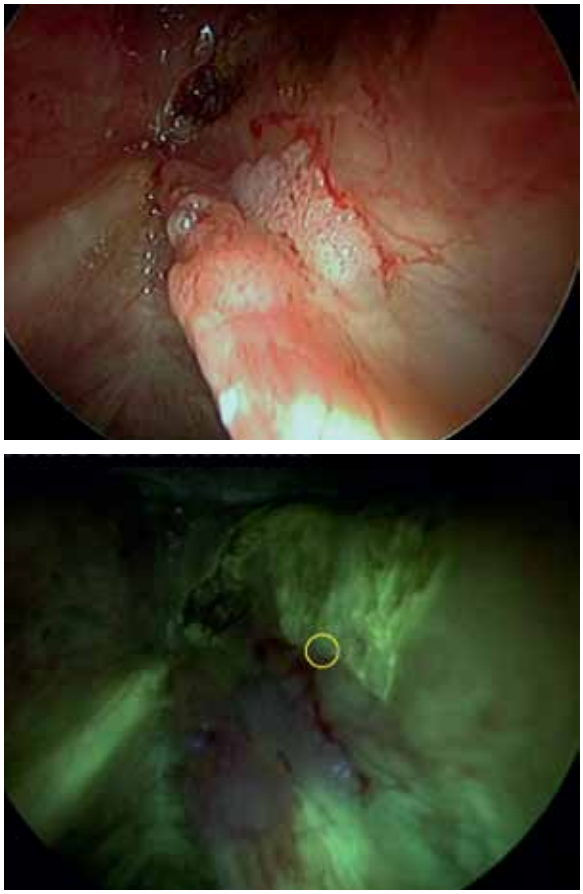

Fig. 2. A: during direct microlaryngoscopy, initial assessment in white light of a suspected right vocal cord SCC staged cT2; **B**: assessment of field using direct autofluorescence showing the safe margin between the inferior extremity of vestibulectomy (yellow circle) and the lateral extremity of the area previously marked for excision (histological examination on the surgical specimen found a free margin = 2 mm).

Histopathological evaluation focused on the following aspects: presence of dysplasia, carcinoma in situ or invasive carcinoma at the level of DSMs and deep margins (positive margins); presence of dysplasia, carcinoma in situ or invasive carcinoma at a distance less than 1 mm from the DSMs and deep margins (close margins); presence of dysplasia, carcinoma in situ or invasive carcinoma in specimens containing lesions evident both to white light and direct autofluorescence (tumour) or containing area(s) positive to direct autofluorescence, but negative by white light endoscopy (white light endoscopy negative areas; WLENA).

No elective neck dissection was performed in this patient population. In this study, we focused our attention on early diagnosis of persistence/recurrence of disease and identification of secondary tumours. Endoscopic retreatment was performed 30-60 days after the first procedure in all cases with positive deep margins. In cases with close surgical margins (< 1 mm), during the first post-operative year, endoscopic follow-up with monthly endoscopic controls was chosen. All patients underwent follow-up that included flexible videolaryngoscopy and/or rigid videolaryngoscopy coupled with HDTV every 3 months and neck MRI in cases of suspected submucosal recurrence. In the case of indirect endoscopy with suspected recurrence/secondary tumour, patients were subjected to microlaryngoscopic re-evaluation under general anaesthesia followed by excisional biopsy of the suspect area.

For the calculation of sensitivity and specificity of autofluorescence, the following endoscopic and pathological criteria were adopted: among the 73 patients treated, all cases who had negative superficial margins of more than 1 mm at histological examination and for the 12-month period after TLS with endoscopic examination judged as "not suspicious" for recurrence (and therefore not receiving a histological evaluation) were considered true negatives; in contrast, false negatives were considered to be any positive or close (< 1 mm) superficial margin and every superficial recurrence occurring within the first 12 months after TLS, considering this as a failure criterion of autofluorescence to detect areas of epithelial transformation "upstream" of the line of resection; true positives were considered to be any area(s) positive to direct autofluorescence in which histological examination was able to find areas of dysplasia, in situ carcinoma or invasive carcinoma; false positives were considered to be those area(s) positive to direct autofluorescence in which histological examination did not reveal epithelial transformation (dysplasia, in situ carcinoma or invasive carcinoma) and for 12 months after TLS, endoscopic examinations were judged as "not suspicious" for recurrence (and therefore not receiving histological evaluation).

Statistical methods

The SPSS statistical package was used for statistical analysis. Three-year survival was estimated. The entry point was the date of surgery. The endpoint for disease-specific survival was the date of first recurrence. The endpoint for local control with laser alone was the date of local recurrence requiring open-neck surgery or nonsurgical salvage treatment.

Results

The study group included 73 patients with glottic carcinoma with TNM staging (level of evidence C2) as follows: T1a glottic, 47 patients (64.3%); T1b glottic, 13 patients (17.8%); T2 glottic, 13 patients (17.8%) (four for hypomobility of the vocal cord, nine for superficial extent of the ventricular or subglottic mucosa). Cordectomies were classified according to the classification system of the European Laryngological Society (ELS): Type I, 4 (5.5%); Type II, 10 (13.7%); Type III, 26 (35.6%); Type IV, 8 (11.0%); Type V, 25 (34.2%).

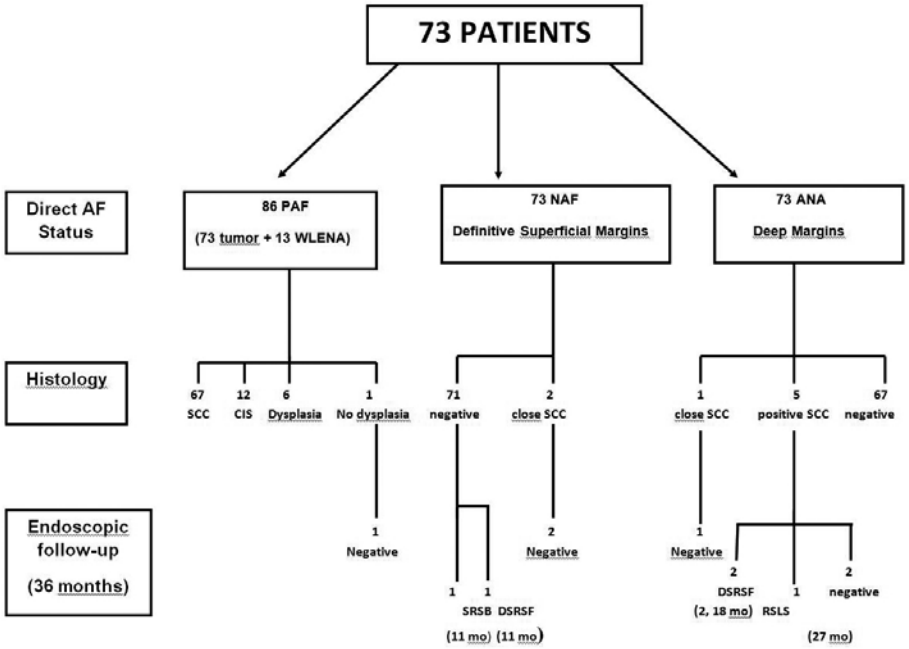

Fig. 3. Study design showing results of histological evaluation and endoscopic follow-up in 73 patients. Each evaluation is described with respect to direct autofluorescence status [positive to autofluorescence (PAF), negative to autofluorescence (NAF), autofluorescence not applicable (ANA)], location of examined areas [tumour, white light endoscopy negative area (WLENA), definitive superficial margins, deep margins], status to endoscopic follow-up Negative (N), superficial recurrence on surgical boundaries (SRSB) deep submucosal recurrence in surgical field (DSRSF), recurrence in separate laryngeal site (RSLS)].

The use of direct autofluorescence was associated with better definition of superficial margins of excision in seven patients (9.6%), all who received wider superficial resections, without changing the T category (2 T1a, 3 T1b, 2 T2), while in six patients (8.2%) direct autofluorescence led to up-staging of the TNM category. The study design as well as data on specimens obtained and categorised by direct autofluorescence status, histology and endoscopic follow-up are shown in Figure 3.

In total, there were 12 WLENA considered to be positive by direct autofluorescence (PAF) that were also shown to be positive by histological analysis [severe dysplasia, carcinoma in situ (CIS), SCC], while there was one PAF that was negative by histological evaluation (hyperplasia). All DSMs were negative by direct autofluorescence (NAF); the histological analysis of the surgical specimens (for the 13 patients in whom the results of direct autofluorescence led to use of wider surgical margins, the latter was considered the last area of superficial resection) showed that 71 (97.3%) had negative superficial margins, while superficial margins were found in two cases (2.7%). Histological analysis of the deep margins of the surgical specimens (on which direct autofluorescence did not provide additional information) was positive in five cases (6.8%) and adjacent in one case (1.3%) (Table II).

Comparison between clinical TNM (cTNM) and pathological TNM (pTNM) showed that two cases were up-staged from T1a to T1b for involvement of the anterior commissure mucosa or contralateral vocal cord (Fig. 4A,B), two cases passed from T1a to T2 for involvement of the mucosa of the bottom/roof of the ventricle, one case passed from T1a to T2 for a second primary CIS on the contralateral false cord, and one case from T1b to T2 due to involvement of the supraglottic mucosa overlying the anterior commissure. Five patients (6.8%) received endoscopic retreatment for positive deep margins. Two of these patients (40%) underwent supracricoid open partial laryngectomy: one for positive margins after endoscopic re-excision and one for early recurrence after re-excision. In two cases (40%), there was no evidence for the presence of disease, while in one case (20%) recurrent disease was completely excised with clean margins. In these cases, only strict endoscopic and radiological follow-up was carried out.

The minimal follow-up was 36 months (mean 50, range

Table II. Status of resection margins.

Margin status	N (%)
Negative	65
Positive	5
superficial	0
deep	5
Close (< 1 mm)	3
superficial	2
deep	1

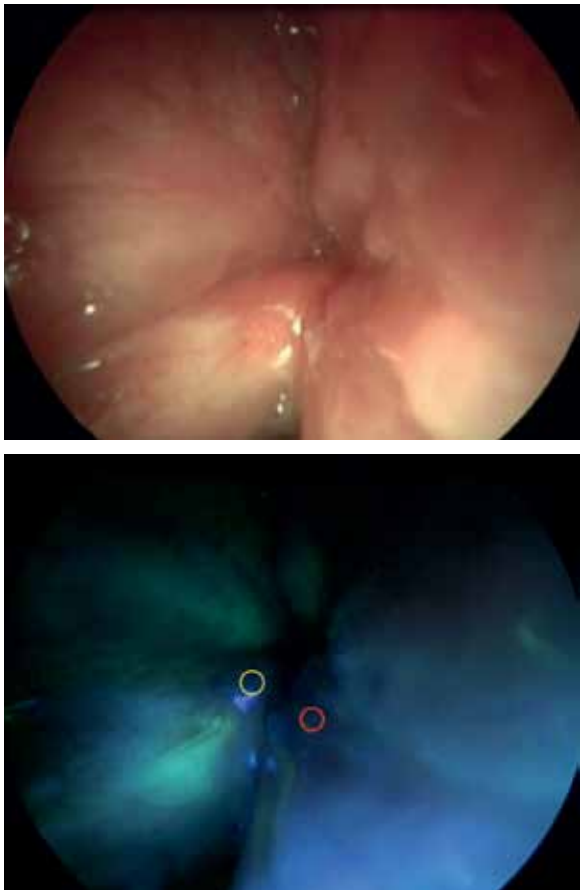

Fig. 4. A: during direct microlaryngoscopy, initial assessment in white light of a red hyperplastic lesion at the anterior part of the vocal cord; **B**: assessment of field using direct autofluorescence showing an area positive to direct autofluorescence encompassing the anterior commissure (histological examination on the surgical specimen found a CIS both at the site of a clinically more visible red lesion (red circle) and in the contralateral vocal cord (yellow circle).

36-70 months) during which five recurrences were observed and classified according to the mode of presentation: three deep submucosal recurrences in the surgical field, respectively, at 2, 11 and 18 months after surgery (two in positive deep margins, one in negative superficial and deep margins), one superficial recurrence on surgical boundaries (in a patient with NAF superficial margins) within 11 months and therefore considered to be a false negative to autofluorescence and one recurrence (in a patient with positive deep margins) at a separate supraglottic laryngeal site at 29 months after TLS. No loco-regional or regional recurrences were observed during follow-up.

Four patients affected by deep submucosal recurrence or recurrence at a separate laryngeal site underwent open partial laryngectomies, while the only superficial recurrence was treated by TLS. The 3-year disease-specific survival and local control rates with laser alone were, respectively, 97.5% and 100% for T1a, 86.7% and 86.7% for T1b, and 88.9% and 88.9% for T2 (Table III).

Discussion

Work-up in a patient undergoing CO_2 laser surgery for laryngeal SCC is no longer a matter of debate. Preoperative evaluation is based on flexible fibrolaryngoscopy and/or rigid videolaryngoscopy with a 70°/90° telescope, while CT or MRI and ultrasound examination of the neck (fine needle aspiration cytology in cases with suspect lymph node metastases) are carried out in the majority of cases and avoided only for selected cT1a lesions with a superficial pattern of growth [1]. Moreover, intraoperative rigid endoscopy with 0° and angled telescopes, particularly if coupled with HDTV, complete the work-up by providing accurate information on the superficial extent of the lesion, especially in cases of extension to the bottom and roof of the ventricle, anterior commissure involvement or subglottic extension [12]. It is clear that any additional endoscopic examination will provide further information, and that each investigation is useful in reducing the proportion of patients with laryngeal lesions that are under- or over-staged during the pre-treatment phase.

In the present study, we assessed the benefits of direct autofluorescence endoscopy in pre-operative work-up of glottic SCC to allow more precise planning of surgical excision, obtain superficial disease-free margins and identify additional areas of malignant transformation. Autofluorescence endoscopy enhances intraoperative work-up, providing information about the biological characteristics of the lesion, and especially about surgical margins. In fact, after direct endoscopy in white light during microlaryngoscopy, performing the same exam in direct autofluorescence allows identification of superficial pathological areas on the basis of chromatic differences compared to healthy tissue [16]. Accordingly, direct autofluorescence can only reveal pathologies that involve the mucosal surface of the larynx, but not those involving submucosal or deep layers [17].

In head and neck oncology there are several studies that help to explain the observed differences in autofluorescence between normal and neoplastic tissues [17], which have demonstrated that the aspect and degree of fluorescence of each tissue depend on three factors: amount of fluorophore, morphological aspects and wavelength of excitation [16]. The interaction of light with tissue has generally been found to highlight changes in the structure and metabolic activity of the areas optically sampled. Specifically, the loss of autofluorescence is believed to reflect a complex mixture of alterations to intrinsic fluorophore distribution in tissue (such as the breakdown of the collagen matrix and a decrease in flavin adenine dinucleotide concentration due to tissue remodelling), increased metabolism associated with neoplastic development in both the epithelium and lamina propria (e.g. thickening of the epithelium, hyperchromatism and increased cellular/nuclear pleomorphism or increased microvascularity)

Table III. Disease specific survival and local control with laser alone (n = 73) according to pT and status of definitive margins.

Variables	Patients (N)	3-year disease specific survival	3-year local control with laser alone
pT categories			
pT1a	40	97.5%	100%
pT1b	15	86.7%	86.7%
pT2	18	88.9%	88.9%
		p = ns	p = ns
Margins	65	96.9%	96.9%
Negative	3	100%	100%
Close	–	–	–
Positive superficial	5	40%	60%
Positive deep			

leading to increased absorption and/or scattering of light, which in turn reduces and modifies the detectable autofluorescence [18-20]. In healthy tissues, riboflavins are in an oxidised state and show strong fluorescence emission at wavelengths around 520 nm, leading to bright green fluorescence; in contrast, in dysplasia, in situ carcinoma, and particularly in malignant lesions, since reduced riboflavins are present at a lower concentration there is a marked reduction or an absence of green fluorescence, and the lesion appears as blue/dark violet [20].

Considering its ability to distinguish normal mucosa from dysplastic/neoplastic tissue, some features can have a negative impact on the sensitivity and specificity of autofluorescence and must be kept in mind to avoid misinterpretation of the endoscopic picture. Leukoplakia/hyperkeratosis without dysplasia shows a field of intense bright white autofluorescence, while the presence of underlying dysplasia/cancer can lead to a slight change in the underlying colour to dark red/light brown. In these cases, it is important to focus examination on the mucosal margins of the lesion, avoiding the vegetating and keratotic portion, especially in narrow areas (bottom of the ventricle, anterior commissure) without causing bleeding (which would hinder proper examination; Fig. 5A,B). Lesions characterised by abnormal hyperplasia and speckled leukoplakia, such as observed in cases of severe gastrointestinal reflux with extra-oesophageal lesions are associated with a reduction in normal fluorescence, with various degrees of colour from bright green/bright white to blue/violet. Hypervascularised lesions, chronic laryngitis and lesions with bacterial infection are associated with a reduction in autofluorescence compared to normal tissues, and scarring from prior surgical treatment, biopsy or radiotherapy is associated with a reduction in autofluorescence similar to that seen in pre-neoplastic and neoplastic lesions.

Considering rigid endoscopy in white light and HDTV, in 13 cases in the present study, the positive areas seen by direct autofluorescence were wider; at histological analysis on surgical specimens and areas of widened resection/separate biopsy, 12 true positives and one false positive were found (scar on the contralateral vocal fold due to a previous procedure for leukoplakia 8 years before), while

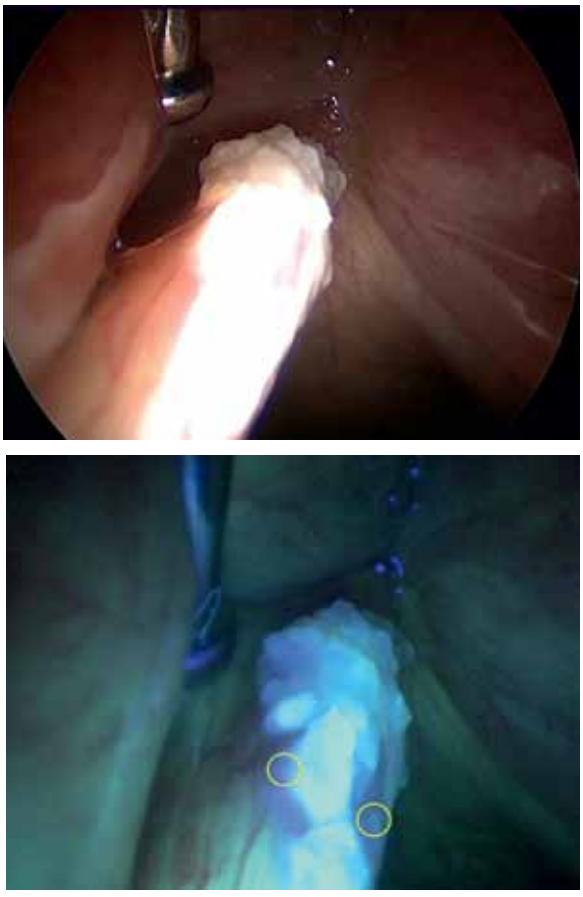

Fig. 5. A: during direct microlaryngoscopy, initial assessment of the left vocal cord in white light; **B**: assessment of field using direct autofluorescence showing a bright white hyperkeratotic lesion with slight positivity to direct autofluorescence on the mucosal margins of the lesion (yellow circles) (histological examination on the surgical specimen found a SCC in the areas marked with the yellow circles).

only one superficial recurrence occurred within the first 12 months after surgery. In all other cases, follow-up endoscopic examinations were judged to be "not suspicious" for superficial recurrence. Therefore, in the entire cohort of patients, direct autofluorescence showed a sensitivity of 96.5% and a specificity of 98.5%.

The extensive and combined utilisation of direct autofluorescence before TLS resulted in several advantages compared to the traditional diagnostic methodology in white light because it allowed better definition of the superficial extent of the lesion, which is useful in widening a superficial laser resection and can help the surgeon to obtain disease-free superficial margins. This has a positive impact on endoscopic treatment modulation and, subsequently, on both disease-free survival and local control with laser [21].

Considering correct modulation of resection during TLS, it must first be kept in mind that there is an increased risk of dysplastic and malignant changes in the mucosa surrounding the tumour after many years of smoking, alcohol abuse [6] and/or gastrointestinal reflux. The surgeon, therefore, must consider the possibility of premalignant and microinvasive disease that is not macroscopically evident. This can frequently be seen in pre-operative evaluation in white light (telescopic/microscopic), and even in expert hands, there is a tendency to under-stage the actual extent of disease, with the consequence that superficial resection margins are often positive. This is evident from several studies. In order to routinely obtain a high degree of disease-free resection margins, some authors recommend wide resection of vocal fold mucosa, and widen the resection near the deep margins of the lesion [22]; this particular type of functional oncological surgery tends to exaggerate the substantial compromise between the necessity of performing a wide resection with clean margins and that of preserving healthy tissue so that good vocal function can be maintained.

Peretti and colleagues reported positive superficial margins after TLS in 38.4% of cases, even though this did not appear to have a negative impact on disease-specific survival or loco-regional control at 5 years [1]. This demonstrates the importance of choosing the widest margins possible in order to resect a tumour with clean margins, thus avoiding any form of overtreatment that would negatively impact good functional preservation.

Achieving a high level of quality in TLS can be aided by correct pathological study of resection margins, which is greatly improved through the use of laser technology (low power, AcuBlade, SuperPulse) as it is associated with less charring along the line of excision [23-26]. The same considerations can be made if the surgeon has access to accurate "in vivo" information on apparently healthy tissue surrounding the tumour, and the ability to correctly determine the entity of resection will undoubtedly lead to a lower proportion of positive superficial margins. This will clearly have an impact on both disease-specific survival and local control.

Indirect evidence for an advantage of endoscopic tools in allowing precise calibration of the entity of superficial resection during TLS is provided by Lucioni and colleagues [3] who studied the impact of surgical margins obtained by CO_2 laser photocoagulation (LPC) on local disease control in patients submitted to endoscopic surgery for early glottic cancer. The authors reported a significant difference in recurrence rate among patients treated with surgical margin LPC compared to those treated with laser cordectomy without LPC ($P = 0.022$). In particular, a lower recurrence rate in LPC patients was seen in the case of close (≤ 1 mm), non-definable and positive margins with infiltration of the superficial border. No significant difference was observed in the case of negative edges (> 1 mm) or involvement of either deep margins or both superficial and deep edges. Carried out by expert laser surgeons, that study demonstrates that, after apparently radical laser surgery, systematic widening of visible disease-free margins by about 2 mm (the authors used photovaporisation of margins with a defocalised spot that was 1.6 mm in diameter) is associated with benefits in local control of disease and a reduction in recurrence.

In our experience, the use of direct autofluorescence was associated with greater accuracy in defining superficial resection margins in 12 of 73 cases (16.4%), which would have otherwise been positive or close. Following direct autofluorescence, superficial resection was widened by 3-5 mm to incorporate the positive area, which can however be difficult in narrow spaces such as the anterior commissure and bottom/roof of the ventricle.

In a study on 96 patients undergoing pre-operative work-up with narrow band imaging (NBI), Piazza et al. reported improved delineation of the superficial extent of disease category in 35 cases (36.4%), which is a consequence of better definition of tumour category (upstaging of 26 neoplasms) and wider superficial resection [8].

The similarity of results even between two studies using different techniques allows discussion about the usefulness of endoscopic tools that provide better definition and information about the biology of the lesions. Similar to HDTV-NBI, direct autofluorescence unquestionably provides a wealth of endoscopic data that leads to better clinical definition in terms of assessment of pre-neoplastic or neoplastic tissues and their superficial extent, especially along the boundaries of a macroscopic lesion, the bottom and roof of the ventricle and anterior commissure. Thus, these tools are valuable in helping the surgeon correctly plan resection during TLS, especially when an excision biopsy is planned.

Compared to AF, NBI endoscopy has the advantage of being able to obtain the same information using both a videoendoscope coupled with a HDTV camera and rigid

telescopes. In our opinion, compared to NBI, there is a shorter learning curve with direct AF since judgment is given on the basis of colour changes; at present, direct AF is not coupled with an HDTV camera and this can be a problem, especially for preliminary evaluation in white light. Finally, the overall ergonomics of the procedure is more complex than with NBI endoscopy, which also seems to show superiority in the ability to properly distinguish post-actinic changes from persistent/recurrent disease [27].

Conclusions

In the present study, the use of direct autofluorescence was associated with the absence of positive superficial margins and the presence of close superficial margins in two cases that clearly had a positive impact on both disease-free survival and local control.

It is obvious that the experience of the surgeon has a compensatory mechanism if direct autofluorescence is not used: the choice of a widened superficial resection or photovaporisation of superficial resection margins can lead to similar results in terms of radicality and prognosis. In our opinion, endoscopic tools such as direct autofluorescence and NBI add value to intraoperative work-up, especially for surgeons with less experience.

While the learning curve for autofluorescence is rapid and the number of false positives in previously untreated patients appears to be low in our experience, one must be very careful in assessing any condition that may obstruct visualisation of mucosal margins, such as bulky keratotic lesions, and to consider autofluorescence less useful in conditions in which the number of false positives tend to be much higher, such as in patients previously treated by TLS or radiotherapy since, in these cases, specificity may be greatly reduced [28].

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