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Struttura complessa di Otorinolaringoiatria

Prospective real time margin assessment in laser cordectomy using Histolog® Scanner

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1 INTRODUCTION

1.1 ANATOMY OF THE LARYNX

The larynx is an unpaired, median musculoligamentous organ supported by a cartilaginous framework. Located in the anterior neck (extending from C3 to C6), it connects the pharynx superiorly to the trachea inferiorly.

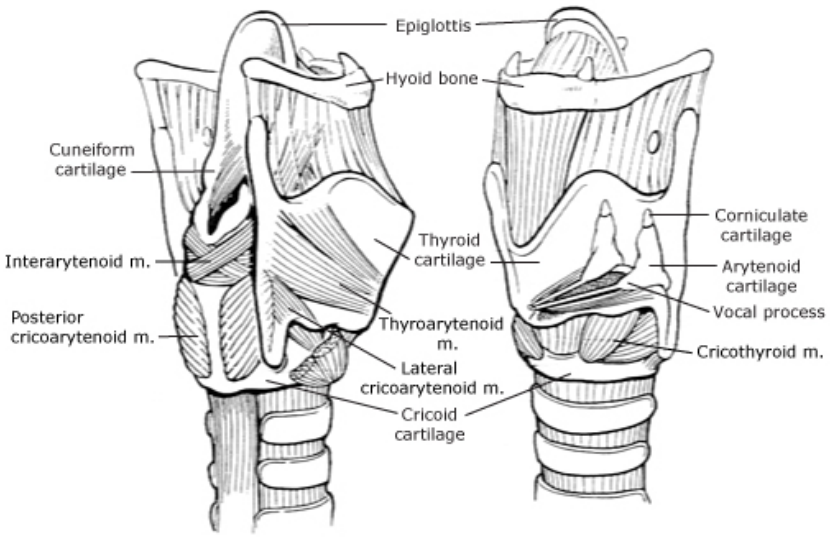

Figure 1: Anatomy of the larynx- Garrett CG, Ossoff RH. Hoarseness. Med Clin N Am 1999

The larynx is composed of:

- Three large, unpaired cartilages: cricoid, thyroid and epiglottis
- Three pairs of smaller cartilages: arytenoid, corniculate and cuneiform

This framework is stabilized by an elastic membrane and regulated by two muscle groups: extrinsic muscles (responsible for elevating and depressing the organ during swallowing) and intrinsic muscles (responsible for modulating vocal fold tension and airway patency). (1)

THE CAVITY OF THE LARYNX

The laryngeal cavity is lined by mucosa and divided into three distinct regions by the vestibular folds (false vocal cords) and vocal folds (true vocal cords):

- Supraglottis: the superior chamber extending from the laryngeal inlet down to the vestibular folds.
- Glottis: the middle region containing the true vocal folds and the rima glottidis (the variable triangular space between them). It includes the laryngeal ventricles.
- Subglottis: the inferior chamber extending from the vocal folds to the first tracheal ring, kept permanently patent by the cricoid cartilage. (1)

VASCULARIZATION AND INNERVATION

The vascular supply of the larynx is primarily derived from the superior and inferior laryngeal arteries, with venous drainage following a corresponding path toward the internal jugular and brachiocephalic veins. Lymphatic drainage is divided by the vocal folds; these act as a crucial watershed area with nearly no lymphatic network, resulting in a low rate of early nodal metastasis in glottic tumors. Innervation is entirely provided by the vagus nerve (CN X) through the superior laryngeal nerve, which supplies sensation above the vocal folds and motor control to the cricothyroid muscle, and the recurrent laryngeal nerve, which provides sensory supply below the glottis and controls all remaining intrinsic muscles crucial for vocal fold mobility. (1)

1.2 FUNCTION OF THE LARYNX

The larynx functions as a highly coordinated protective sphincter and a primary acoustic organ through four main mechanisms:

- Respiration: actively regulates airflow. During quiet breathing, the rima glottidis is triangular. During forced inspiration, the posterior cricoarytenoid muscles abduct the vocal folds, widening the airway into a rhomboid shape.
- Swallowing (airway protection): during deglutition, the larynx moves upward and forward while the glottis tightly closes. This forces the epiglottis to tilt downward, sealing the airway and directing the food bolus into the esophagus.
- Phonation: vocal folds adduct, narrowing the airway. Exhaled air forces its way through, creating mucosal vibrations. Pitch and tone are modulated by the vocalis and cricothyroid muscles, and the primary sound is amplified by the upper resonance cavities.

- Effort Closure: forceful, simultaneous closure of the true and false vocal cords traps air inside the lungs, stabilizing the thoracic cage during heavy lifting and increasing intra-abdominal pressure for physiological functions. (1)

1.3 NEOPLASTIC PATHOLOGY OF THE VOCAL FOLDS

A wide clinical spectrum of tumors can arise within the laryngeal framework; these are primarily categorized into benign and malignant neoplasms.

BENIGN VOCAL FOLD LESIONS

Benign lesions of the vocal folds include a variety of non-malignant alterations that primarily affect voice quality and require distinct therapeutic approaches. The most common entities encountered in clinical practice are:

- Vocal Fold Nodules
- Vocal Fold Polyps
- Laryngeal Papilloma
- Reinke's Edema
- Contact Ulcers
- Intracordal Cysts
- Post-Intubation Granuloma (2)

MALIGNANT LARYNGEAL NEOPLASMS

Malignant tumors of the larynx are classified into the following histological categories:

- Carcinomas
- Adenocarcinomas
- Lymphomas
- Sarcomas

Among these, carcinomas of epithelial origin represent the most prevalent group. Specifically, in the vast majority of cases (approximately 90-95%), laryngeal neoplastic disease manifests as squamous cell carcinoma (SCC). (3)

Due to the overwhelming prevalence of squamous cell carcinoma compared to these other exceptionally rare histological variants, the subsequent sections of the thesis regarding

epidemiology, risk factors, natural history, clinical presentation, and diagnosis will focus exclusively on laryngeal squamous cell carcinoma.

EPIDEMIOLOGY

Laryngeal cancer accounts for approximately 1% of all cancer cases and deaths worldwide. (4) Among head and neck cancers, laryngeal cancer is the second most common after oral cavity cancers, representing about 20–25% of head and neck malignancies. (5)

- Globally, the age-standardized incidence rate (ASIR) of laryngeal cancer is 1.9–2.3 per 100,000 population, with higher rates in males (3.5 per 100,000) than females (0.5 per 100,000).The highest incidence rates are observed in Central and Eastern Europe, the Caribbean, and parts of South America. (4)
- In Italy, laryngeal cancer is among the most common head and neck cancers, with a historically high incidence rate. It is characterized by a higher incidence and mortality in males compared to females. The most recent national data indicate that laryngeal cancer remains a significant contributor to the overall cancer burden, but its rates are lower than in past decades, reflecting reductions in tobacco and alcohol consumption. (6)

Laryngeal cancer is predominantly a disease of the elderly, with incidence and mortality rates increasing with age. The majority of cases occur in individuals aged 50 years and older, with the highest incidence typically observed in the 60–79 year age group. (7)

RISK FACTORS

The most important risk factors for laryngeal cancer are:

- Tobacco
- Alcohol

Tobacco remains the predominant risk factor and the risk increases with cumulative exposure. Alcohol acts synergistically with tobacco, further elevating risk, especially with heavy or chronic use. (8)

Additional risk factors include occupational exposures (such as to asbestos, diesel fumes, and sulfuric acid), human papillomavirus (HPV) infection, and gastroesophageal reflux disease (GERD). HPV, particularly high-risk types like HPV16, is increasingly recognized

in patients without traditional risk factors. GERD is associated with a modestly increased risk, independent of smoking and alcohol. (9)

NATURAL HISTORY

Laryngeal carcinoma may, in some cases, be preceded by precancerous lesions. These are defined as morphological alterations of the epithelium where, statistically, malignant transformation has been observed with greater frequency compared to surrounding tissues. Three types of precancerous lesions can be described in the larynx:

- Leukoplakia: a lesion with a moderate incidence of malignant transformation (5 %-10 %), presenting as a persistent white patch that cannot be removed by scraping.
- Erythroplakia: a term used to indicate a reddish area, not attributable to mechanical or inflammatory events, which persists even after the elimination of the most evident etiological factors.
- Keratosis: characterized by marked acanthosis and cornification of the superficial layers, which is responsible for the whitish appearance of the lesion. (10)

Any of these clinical manifestations can harbor varying degrees of architectural and cellular disorientation, biologically defined as laryngeal dysplasia. The current World Health Organization (WHO) 2017 classification system for laryngeal dysplasia uses a simplified two-tiered approach:

- Low-grade dysplasia
- High-grade dysplasia (11)

Invasive squamous cell carcinoma expands beyond the basement membrane, involving the stroma, muscle, and potentially the cartilage. Macroscopically, the tumor may present in three forms: exophytic, ulcerated or infiltrating.

Depending on the degree of differentiation, tumors can be classified as:

- Well-differentiated
- Moderately differentiated
- Poorly differentiated.

Glottic tumors are usually moderately or well-differentiated, exhibiting lower local aggressiveness and fewer metastases. Conversely, most supraglottic tumors are moderately

or poorly differentiated, resulting in a higher risk of metastasis and greater local aggressiveness.

SYMPTOMS

Hoarseness is the most common and earliest symptom of laryngeal cancer, particularly for glottic tumors, due to involvement of the vocal cords. Other symptoms may include voice changes, dysphonia, aphonia, pain, dysphagia, chronic cough, hemoptysis, stridor and respiratory distress. The symptom profile varies by tumor location:

- Supraglottic carcinomas: often present with silent clinical signs, such as pharyngeal tickling (the most frequent symptom), a foreign body sensation, or pharyngeal paresthesia. In advanced stages, they may cause odynodysphagia, referred otalgia and dysphonia. Among laryngeal cancers, this type has the highest incidence of lymph node metastasis.

- Glottic Tumors: the primary and earliest symptom is dysphonia. Due to early diagnosis and the relative absence of lymphatic drainage in this region, which limits lymph node metastasis, these tumors typically carry a better prognosis.

- Subglottic Tumors: the main symptoms are dyspnea and stridor, which usually appear at a late stage of the disease. Consequently, subglottic tumors are associated with a very poor prognosis. (5)

DIAGNOSIS

The diagnosis of laryngeal tumors is established through a combination of clinical evaluation, endoscopic assessment, imaging, and histopathologic confirmation.
Patients presenting with persistent dysphonia for more than four weeks should be referred to an otolaryngologist, as this symptom may represent an early manifestation of a glottic tumor. (12)

Endoscopic examination, utilizing either flexible or rigid laryngoscopy, is essential to visualize the lesion, assess vocal fold function (mobility). Furthermore, the use of Narrow Band Imaging (NBI) has significantly improved diagnostic accuracy by highlighting

neoangiogenesis and abnormal vascular patterns, which are indicative of malignancy even in early-stage or flat lesions. This technology aids in performing targeted biopsies, thereby increasing the reliability of histopathological confirmation. (13)

Further staging relies on advanced diagnostic imaging, such as Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). These modalities are crucial for evaluating the deep extension of the tumor into the paraglottic or pre-epiglottic spaces, as well as detecting regional lymph node involvement or distant metastases. (14)

Ultimately, a definitive diagnosis is provided by histopathological examination.

1.4 STAGING OF LARYNGEAL CANCER

Staging provides a concise, standardized representation of tumor extension and dissemination at the time of diagnosis. Accurate staging is essential, as it serves as a universal language for multidisciplinary communication, directly influencing both the therapeutic strategy and the prognostic outlook. The gold standard for classification is the TNM system.

The classification is further divided into two distinct phases:

- Clinical Staging (cTNM): determined before treatment through a comprehensive clinical work-up, including physical examination, ORL panendoscopy, imaging (CT, MRI, Ultrasound), and initial histological assessment via biopsy or Fine Needle Aspiration (FNA).
- Pathological Staging (pTNM): determined postoperatively. It is based on the definitive histopathological analysis of the resected specimen (T) and regional lymph nodes (N).

While the cTNM is the primary driver for selecting the initial therapy, the pTNM provides critical data regarding prognosis, surgical radicality (margins), and the potential necessity for adjuvant treatments. Following the determination of T, N, and M parameters, patients are categorized into stages (I–IV) to ensure prognostic homogeneity. Specifically for laryngeal cancer, the TNM classification is site-specific, with distinct criteria for the supraglottic,

glottic, and subglottic regions. Staging is based on the latest AJCC Cancer Staging Manual (8th Edition) (15), as outlined in the following tables.

Primary Tumor (pT) — Supraglottis	
Category	**Criteria**
pTX	Primary tumor cannot be assessed
pTis	Carcinoma in situ
pT1	Tumor limited to 1 subsite of supraglottis with normal vocal cord mobility
pT2	Tumor invades mucosa of > 1 adjacent subsite of supraglottis or glottis or region outside the supraglottis (e.g. mucosa of base of tongue, vallecula, medial wall of pyriform sinus) without fixation of the larynx
pT3	Tumor limited to larynx with vocal cord fixation or invades any of the following: postcricoid area, pre-epiglottic space, paraglottic space or inner cortex of thyroid cartilage
pT4a	Moderately advanced local disease: invades through the outer cortex thyroid cartilage or invades tissues beyond the larynx (e.g. trachea, soft tissues of neck including deep extrinsic muscle of tongue, strap muscles, thyroid or esophagus)
pT4b	Very advanced local disease: invades prevertebral space, encases carotid artery or invades mediastinal structure

Table 1: Primary tumor- Supraglottis.

Primary Tumor (pT) — Glottis	
Category	**Criteria**
pTX	Primary tumor cannot be assessed
pTis	Carcinoma in situ
pT1	Tumor limited to the vocal cord(s) (may involve anterior or posterior commissure) with normal mobility
pT1a	*Limited to 1 vocal cord*
pT1b	*Involves both vocal cords*
pT2	Tumor extends to supraglottis or subglottis or with impaired vocal cord mobility
pT3	Tumor limited to the larynx with vocal cord fixation or invasion of paraglottic space or inner cortex of the thyroid cartilage
pT4a	Moderately advanced local disease: invades through the outer cortex of the thyroid cartilage or invades tissues beyond the larynx (e.g. trachea, cricoid cartilage, soft tissues of neck including deep extrinsic muscle of the tongue, strap muscles, thyroid or esophagus)
pT4b	Very advanced local disease: invades prevertebral space, encases carotid artery or invades mediastinal structures

Table 2: Primary tumor- Glottis.

Primary Tumor (pT) — Subglottis	
Category	**Criteria**
pTX	Primary tumor cannot be assessed
pTis	Carcinoma in situ
pT1	Tumor limited to subglottis
pT2	Tumor extends to vocal cord(s) with normal or impaired mobility
pT3	Tumor limited to larynx with vocal cord fixation or invasion of paraglottic space or inner cortex of the thyroid cartilage
pT4a	Moderately advanced local disease: tumor invades cricoid or thyroid cartilage or invades tissues beyond the larynx (e.g. trachea, soft tissues of neck including deep extrinsic muscles of the tongue, strap muscles, thyroid or esophagus)
pT4b	Very advanced local disease: tumor invades prevertebral space, encases carotid artery or invades mediastinal structures

Table 3: Primary tumor- Subglottis.

Pathological Regional Lymph Nodes (pN)	
Category	**Criteria**
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in a single ipsilateral lymph node ≤ 3 cm in greatest dimension and extranodal extension (ENE)−
pN2a	Single ipsilateral lymph node ≤ 3 cm and ENE+ or single ipsilateral lymph node > 3 cm but ≤ 6 cm in greatest dimension and ENE−
pN2b	Metastases in multiple ipsilateral lymph nodes, none > 6 cm in greatest dimension and ENE−
pN2c	Metastases in bilateral or contralateral lymph node(s), none > 6 cm in greatest dimension and ENE−
pN3a	Metastasis in a lymph node that is > 6 cm in greatest dimension and ENE−
pN3b	Metastasis in either: single ipsilateral lymph node, > 3 cm and ENE+; or multiple ipsilateral, contralateral or bilateral lymph nodes, any with ENE+; or single contralateral lymph node of any size and ENE+

Table 4: Pathological Regional Lymph Nodes.

Distant Metastasis (pM)	
Category	**Criteria**
pM0	No distant metastasis
pM1	Distant metastasis

Table 5: Distant Metastasis.

AJCC Prognostic Stage Grouping			
Stage	**T**	**N**	**M**
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1–T3	N1	M0
Stage IVA	T4a	N0–N1	M0
	T1–T4a	N2	M0
Stage IVB	Any T	N3	M0
	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Table 6: AJCC Prognostic Staging Grouping.

1.5 TREATMENT OF LARYNGEAL CANCER

The management of laryngeal tumors is highly complex, as therapeutic strategies differ significantly depending on the specific anatomical subsite involved (supraglottis, glottis, or subglottis). Because each subsite possesses distinct lymphatic drainage and clinical behaviors, treatment protocols must be strictly customized. In particular, this section will focus on the treatment strategies for glottic tumors, which represent the vast majority of laryngeal malignancies.

For glottic disease, it remains essential to differentiate between early stages (T1 and T2) and advanced stages (T3 and T4). In early-stage glottic tumors, both conservative surgery and radiotherapy can be employed, offering similar cure and survival rates. Conversely, advanced-stage glottic lesions require a multimodal approach, typically involving surgery followed by postoperative radiotherapy, or integrated chemoradiotherapy protocols.

1.5.1 SURGICAL MANAGEMENT

TRANSORAL LASER MICROSURGERY (TLM)

Endoscopic cordectomy was introduced by Lynch in 1920 (16), but it became the cornerstone of laryngeal cancer treatment only after Strong introduced the CO2 laser in 1975. (17)

A. The ELS classification of endoscopic cordectomy

To standardize the nomenclature based on depth and anatomical extent, the European Laryngological Society (ELS) proposed a classification in 2000, which originally included five types of cordectomies. (18)

This framework was subsequently refined 2007 into six distinct categories to better classify resections involving the anterior commsure and bilateral extensions: (19)

- Type I: Subepithelial Cordectomy

  This technique involves the resection of the vocal fold epithelium, passing above Reinke's space and sparing the vocal ligament and muscle. It can be performed for both diagnostic and therapeutic purposes. It is considered therapeutic if histopathology confirms hyperplasia, dysplasia, or carcinoma in situ, lesions which are confined to the epithelium. If there is evidence of deeper tumor infiltration, the procedure serves a purely diagnostic role, requiring additional therapeutic intervention.

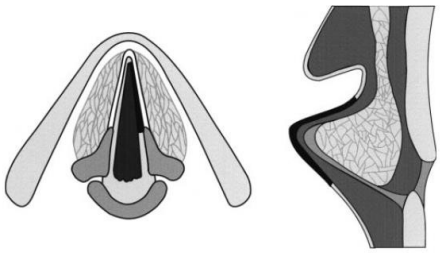

Figure 2: Type I- Andy Bertolin © 2026

- Type II: Subligamental Cordectomy

  This procedure involves the resection of the epithelium, Reinke's space, and the vocal ligament, while the vocal muscle is preserved. The dissection plane is identified between the muscle and the vocal ligament. Clinically, it is warranted for severe leukoplakia with signs of neoplastic transformation. From a therapeutic

standpoint, it is indicated for microinvasive carcinoma or carcinoma in situ with suspected microinvasion.

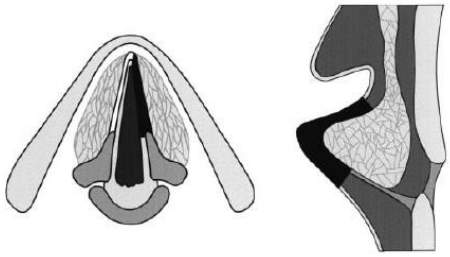

Figure 3: Type II- Andy Bertolin © 2026

- Type III: Transmuscular Cordectomy

  It is the most widely adopted procedure (approximately 80% of cases). It entails the incision into the thyroarytenoid muscle. The resection includes the epithelium, lamina propria, and part of the muscle, spanning from the vocal process to the anterior commissure. It is indicated for small, superficial vocal fold carcinomas, provided that vocal cord mobility is preserved.

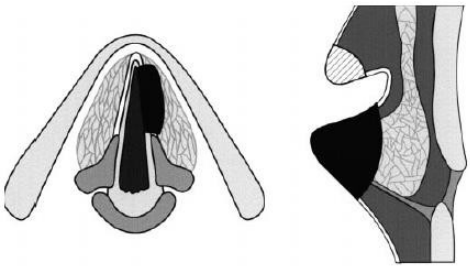

Figure 4: Type III- Andy Bertolin © 2026

- Type IV: Total Cordectomy

  This resection extends from the vocal process to the anterior commissure, requiring the detachment of the vocal ligament from the thyroid cartilage. It is specifically indicated for T1a tumors with deep infiltration into the vocal muscle.

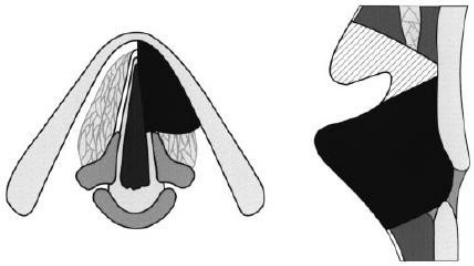

Figure 5: Type IV- Andy Bertolin © 2026

- Type V: Extended Cordectomy

  When the tumor extends beyond the vocal folds, other laryngeal structures are included in the resection. Type V is subdivided into four subgroups:

  - Type Va: the resection includes the anterior commissure and, depending on tumor extent, a segment or the entirety of the contralateral vocal fold. Resection of the contralateral ventricular fold may be necessary for adequate exposure. This is indicated for superficial tumors reaching the commissure without infiltrating it or spreading toward the epiglottic base or subglottis.

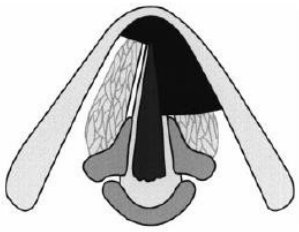

Figure 6: Type Va- Andy Bertolin © 2026

  - Type Vb: indicated for tumors infiltrating the vocal process posteriorly while sparing the arytenoid. The cartilage is partially or totally removed, while the posterior arytenoid mucosa is preserved.

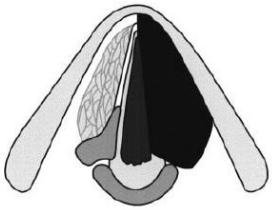

Figure 7: Type Vb- Andy Bertolin © 2026

  - Type Vc: extends the total cordectomy to include the ventricular fold. This is indicated for ventricular tumors or transglottic tumors spreading from the vocal fold to the ventricle.

Figure 8: Type VC- Andy Bertolin © 2026

- Type Vd: the resection extends approximately 1 cm below the glottis, exposing the cricoid cartilage.

Figure 9: Type Vd- Andy Bertolin © 2026

- Type VI: Cordectomy for the Anterior Commissure

  This anatomical site is particularly critical due to its lack of a protective perichondrium where the vocal ligaments insert into the thyroid cartilage ("Broyles' ligament"), providing a potential pathway for early tumor spread.

  Type VI involves the resection of the anterior commissure, which may extend to include the anterior third of both vocal folds. This procedure is indicated for tumors involving the anterior junction (often staged as T1b) that don't show signs of subglottic extension or macroscopic cartilage infiltration. In selected cases, this type of cordectomy is indicated for T2 stage carcinomas.

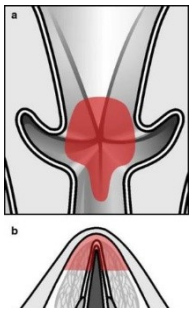

Figure 10: Type VI- Andy Bertolin © 2026

B. <u>Clinical Indications and Therapeutic Selection</u>

Overall, the indications for treatment via transoral laser microsurgery (TLM) within this glottic-centered context include early-stage tumors (Tis, T1, and T2) and highly selected cases of glottic and T3 lesions.

The choice between endoscopic surgery and radiotherapy (RT) must be evaluated on a case-by-case basis and remains a subject of ongoing debate in the literature. Both modalities aim to achieve comparable oncological control, but they differ significantly in terms of functional outcomes, cost-effectiveness, and potential future treatment options.

C. <u>Technical-Anatomical and Oncological Contraindications</u>

While endoscopic cordectomy is highly effective for early-stage glottic tumors, its selection must be guided by strict contraindications. These are categorized into technical-anatomical, oncological, and systemic criteria.

- Technical-Anatomical Contraindications: Laryngeal Exposure and the Laryngoscore.

The absolute technical prerequisite for a safe and oncologically sound TLM is the optimal, static visualization of the entire lesion and its healthy peripheral margins through a rigid laryngoscope. Laryngeal exposure is clinically graded using the modified Kleinsasser classification, which divides patients into four classes:

- Class I (Excellent exposure): Full and easy visualization of the entire glottic plane and both vocal folds.
- Class II (Good exposure): Adequate visualization of the glottis, though requiring minor external laryngeal counterpressure.
- Class III (Fair/Difficult exposure): Only partial visualization of the vocal folds; the anterior commissure cannot be fully brought into view without advanced maneuvers.
- Class IV (Impossible exposure): The glottic plane, and particularly the anterior commissure, cannot be visualized at all due to anatomical constraints.

Inability to achieve adequate visualization (Class III or IV) represents an absolute contraindication to endoscopic resection, mandating a conversion to open-neck partial laryngectomy or primary radiotherapy.

To objectively predict and quantify the risk of a Difficult Laryngeal Exposure (DLE) prior to surgery, Piazza et al. (2014) developed and validated the Laryngoscore. This standardized preoperative protocol assesses 11 clinical parameters: interincisors gap (IIG), thyro-mental distance (TMD), upper jaw dental status, trismus, mandibular prognathism, macroglossia, micrognathia, degree of neck flexion-extension, history of previous open-neck surgery/radiotherapy, modified Mallampati's score, and body mass index (BMI). A total Laryngoscore ≥ 6 serves as the clinical cutoff distinguishing favorable versus difficult exposures, while a score ≥ 9 indicates an extremely high probability (67%) of severe DLE or Class IV impossible exposure. (20)

- Oncological Contraindications: Subsite Involvement and Infiltration Patterns.

The landmark criteria established by Peretti et al. and the European Laryngological Society (ELS) guidelines define the precise oncological boundaries where endoscopic resection is contraindicated due to an unacceptably high risk of positive margins or local recurrence:

- Vertical cranio-caudal extension at the anterior commissure: while horizontal trans-commissural spread across the anterior commissure can be managed via an ELS Type VI cordectomy, vertical extension along this subsite constitutes a major contraindication. Because the mucosa lies directly over the thyroid cartilage via the Broyles' ligament, lacking a protective perichondrium, vertical infiltration leads to early micro-invasion of the cartilage or downward subglottic propagation toward the cricothyroid membrane, making complete endoscopic margins unattainable.
- Deep arytenoid infiltration and cricoarytenoid joint impairment: infiltration extending deeply into the arytenoid body, the posterior paraglottic space, or causing mechanical fixation of the cricoarytenoid joint represents an absolute contraindication to standard endoscopic cordectomy. Preserving at least one fully functional cricoarytenoid unit (comprising the cricoid, arytenoid, cricoarytenoid joint, and the recurrent laryngeal nerve) is mandatory to guarantee postoperative sphincteric function. Deep posterior extension jeopardizes this unit; attempting an endoscopic resection in this setting causes irreversible postoperative aspiration and chronic swallowing dysfunction. (21)
- Massive paraglottic space (PGS) infiltration and vocal fold fixation: superficial or minimal involvement of the anterior PGS can be managed via an extended Type V cordectomy. However, massive infiltration of the paraglottic space, clinically presenting as complete vocal fold fixation (glottic T3 disease), is a strong relative or absolute contraindication for pure endoscopic approaches in most standard protocols, requiring open partial laryngectomies to ensure radicality.
- Macroscopic cartilage invasion and extralaryngeal spread: any radiological (CT/MRI) or clinical evidence of macroscopic erosion through the thyroid or cricoid cartilage, as well as tumor extension outside the laryngeal framework into the soft tissues of the neck, constitutes an absolute contraindication for TLM, requiring total or open partial laryngectomy.

- Systemic and General Contraindications

- Severe pulmonary comorbidities: patients with severe, end-stage chronic obstructive pulmonary disease (COPD) or poor pulmonary reserve are poor candidates for extensive endoscopic resections (especially those approaching the posterior glottis), as they cannot tolerate even minor episodes of postoperative micro-aspiration.
- Inability to tolerate general anesthesia or prolonged neck extension: Conditions such as unstable cardiovascular disease or severe cervical spine instability (e.g., severe rheumatoid arthritis) preclude the surgical positioning required for suspension laryngoscopy. (22)

D. Advantages and Disadvantages of TLM

Transoral Laser Microsurgery (TLM) represents a cornerstone of "organ-preserving" treatment for laryngeal cancer. Its primary advantages include a minimally invasive approach that ensures rapid recovery of swallowing and eliminates the need for a tracheostoma. Strategically, TLM is highly cost-effective and, unlike radiotherapy, is repeatable in case of recurrence, as it doesn't limit future therapeutic options. (23)

Despite its clinical efficacy, TLM presents some disadvantages. Primarily, the vocal outcomes are significantly impacted by the depth of the excision: deeper resections (Types III–VI) inevitably result in incomplete glottic closure, leading to a breathy voice and chronic dysphonia. Furthermore, the technique is highly operator-dependent and its success is strictly tied to adequate laryngeal exposure. In patients with difficult anatomy (e.g. limited neck extension), achieving safe oncological margins can be challenging, potentially requiring a shift toward alternative treatments. (24)

This anatomical challenge is further compounded by the technique's minimally invasive profile. While the use of biological endoscopic imaging and high-magnification microscopy allows for ultra-narrow surgical margins, this precision often represents a "double-edged sword". Indeed, the literature reports a high incidence of close or positive margins (reaching up to 50%), a finding that underscores one of the most significant limitations of TLM in ensuring oncological radicality. (25,26)

OPEN PARTIAL HORIZONTAL LARYNGECTOMY (OPHL)

When glottic tumors extend beyond the boundaries of safe endoscopic resection (such as in advanced T2 or selected T3 lesions), Open Partial Horizontal Laryngectomies (OPHL) represent the primary open-neck alternative to total laryngectomy. The fundamental objective of OPHL is organ and function preservation. By avoiding a permanent tracheostoma, these techniques aim to safeguard the physiological functions of the larynx, including phonation, respiration, and sphincteric deglutition.

To standardize these conservative procedures, a comprehensive international classification was established in 2014 (27), categorizing OPHLs into three main types based on the inferior anatomical limit of the resection:

- OPHL Type I (Supraglottic laryngectomy): The resection is limited to the supraglottic compartment, keeping the glottic plane completely intact.

- OPHL Type II (Supracricoid laryngectomy): The resection extends down to the cricoid cartilage. It is the most relevant open conservative option for advanced glottic tumors, as it entails the removal of both vocal folds while preserving at least one functional arytenoid unit to guarantee swallowing and airway protection.

- OPHL Type III (Supratracheal laryngectomy): The resection is extended further downward to the first tracheal ring, indicated when the glottic tumor exhibits significant subglottic propagation.

Each of these horizontal procedures can be further customized with specific extensions to adjacent laryngeal or pharyngeal structures, balancing oncological radicality with functional preservation.

TOTAL LARYNGECTOMY (TL)

Introduced historically by Watson (1866) and later detailed by Billroth, total laryngectomy remains a fundamental procedure in laryngeal oncology. Despite the advancement of organ-preservation protocols, TL is still indicated in approximately 25% of cases, primarily as a salvage surgery for advanced-stage tumors or when conservative treatments fail.

The primary indications include:

- Extensive tumors unsuitable for conservative surgery.
- Recurrence following radiotherapy or reconstructive partial surgery.
- Non-laryngeal malignancies (e.g. thyroid or base of tongue) invading the larynx. (28)

The procedure involves the complete removal of the laryngeal framework (epiglottis, thyroid, cricoid, and arytenoid cartilages), sometimes extending to the hyoid bone, infrahyoid muscles and the thyroid gland. A definitive permanent tracheostomy is created, achieving a complete separation between the respiratory and digestive tracts. This anatomical partition eliminates the risk of aspiration, as air passes directly into the trachea while food is diverted into the esophagus.

1.5.2 MEDICAL MANAGEMENT

RADIOTHERAPY AND CHEMORADIOTHERAPY

Radiotherapy (RT) is a cornerstone of laryngeal cancer management, utilizing ionizing radiation, primarily X-rays, to eliminate malignant cells. In clinical practice, the role of RT is dictated by tumor stage and laryngeal function:

- Early-Stage Disease (T1-T2): radiotherapy serves as a definitive, organ-preserving therapy. While standard fractionation involves daily doses of 1.8–2.0 Gy (totaling 66–74 Gy), hypofractionated regimens are increasingly adopted for glottic cancers to improve efficiency and treatment duration.
- Locally Advanced Disease (T3-T4): for patients with a functional larynx and good performance status, concurrent chemoradiotherapy (CCRT) with platinum-based agents is the gold standard for organ preservation. Conversely, total laryngectomy followed by adjuvant radiotherapy or chemoradiotherapy is reserved for bulky T4 lesions, significant laryngeal dysfunction, or high-risk pathological features such as positive margins and extranodal extension.

CHEMOTHERAPY

The clinical applications of systemic chemotherapy in the the management of laryngeal cancer are primarily twofold:

- Neoadjuvant chemotherapy: this approach is employed for advanced tumors that have not yet developed distant metastases. The primary objective is to achieve a

reduction in tumor volume, thereby increasing the likelihood of a radical surgical resection and, in some cases, facilitating organ preservation.

- Palliative chemotherapy: it is indicated for extremely advanced stages or for recurrences following radiotherapy where curative treatment is no longer feasible. In these instances, chemotherapy aims to alleviate symptoms, improve quality of life, or slow down tumor progression. (29)

1.6 SURGICAL MARGINS

One of the most important prognostic factors in the treatment of the laryngeal squamous cell carcinoma is the surgical margin status. Therefore, ensuring R0 resection margins remains a primary surgical goal, effectively reducing the necessity for adjuvant treatments and their related sequelae.

A major challenge of CO2 TOLMS is balancing radical tumor excision with the preservation of healthy tissue for optimal vocal function. However, this ultraconservative approach may lead to a higher incidence of positive margins, subsequently increasing the risk of local recurrence. The surgical margins ideally should be clean of neoplastic cells, with a rim of healthy tissue surrounding the excised tumor. Nonetheless, in the context of a malignancy characterized by excellent clinical outcomes, paramount importance is given to the quality-of-life and functionality. Consequently, in the case of glottic cancer, the preservation of viable vocal fold tissue and the avoidance of excessive resection are foremost considerations when determining the optimal primary therapy. (30–32)

1.6.1 ASSESSMENT OF GLOTTIC TUMOR

Pathological assessment of resection margins is the gold standard for achieving the completeness of tumor excision. However, intraoperative and definitive margin evaluation in glottic TLM is intrinsically challenging and complicated by two distinct orders of limitations:

- Specimen and histological artifacts: factors such as the lack of a standardized margin definition, laser-induced thermal damage, and tissue shrinkage introduce major structural alterations, complicating the formal histopathological reading and

contributing to a high rate of false-positive margins. Consequently, these artifacts can lead to unnecessary additional treatments in more than 50% of instances.

- Intrinsic limitations of traditional intraoperative techniques: frozen section analysis exhibit severe operational, diagnostic, and functional drawbacks when applied to the millimetric scale of the glottic larynx.

DEFINITION OF SURGICAL MARGINS

In general terms, surgical margins are classified as positive when neoplastic cells extend to the specimen's edge, and close when the distance between the tumor and the margin is insufficient to ensure oncological safety.
Currently, a universal consensus on the exact millimetric definition of negative, close, and positive surgical margins is still lacking in the literature, a factor that complicates the direct comparison of oncological data between different studies. (33)

This lack of standardization is directly attributed to the unique anatomical and embryological characteristics of the glottic region. Early glottic carcinomas exhibit a distinct exophytic nature, frequently leaving the deeper vocalis muscle uninvolved, and develop within a compartment virtually devoid of lymphatic drainage. (30)
Consequently, a far more conservative, tissue-sparing approach is clinically justified in this setting. Unlike other head and neck subsites (such as the oral cavity or oropharynx), which traditionally require wide safety margins of at least 5 mm to achieve local control (34), the peculiar anatomy of the glottis allows for significantly narrower safety clearances.

To address these site-specific features, the classification proposed by Fiz et al. has become the standard reference for the majority of authors (25,35–38):

- Negative when the distance from the tumor exceeds 1 mm
- Close when the clearance is less than 1 mm
- Positive when carcinoma in situ or invasive disease is identified at the surgical margin.

Furthermore, while some authors group all positive margins into a single category, others stratify them based on their location and number. Specifically, margins can be classified as superficial or deep depending on their anatomical site, and as single or multiple according

to their number. Both deep positive margins and positive multiple superficial margins are associated with an increased risk of local recurrence. (31)
It's crucial to emphasize that the experience of the surgeon with the laser is very important for a radical tumor excision.

THERMAL ARTIFACTS INDUCED BY LASER

Second, the CO2 laser induces significant alterations and thermal damage to the surgical specimen, thereby complicating the histopathological assessment of margins, which remains the gold standard for confirming radical tumor excision. The incidence of positive margins associated with Transoral Laser Microsurgery (TLM) is estimated to be as high as 50%.

This phenomenon is primarily attributable to the technical limitations of laser dissection within the narrow glottic space, further exacerbated by both anatomical constraints and functional considerations. (39,40)
The CO2 laser induces an area of carbonization of 0.3 mm around the incision, which should be considered in the evaluation of margin status. (41) The thermal damage often obscures the histological differentiation between positive and unclear resection margins. Consequently, some researchers (42,43) treat this category as a single clinical entity, recommending the same treatment approach, which is second look or close follow-up according to the surgeon's evaluation. Preuss et al. (44) performed routinely at least two second looks for all patients regardless of the status of the surgical margin to circumvent the diagnostic limitations imposed by tissue charring. Furthermore, literature indicates that CO2 laser can also cause submucosal thermal damage. (45)

Beyond inherent anatomical constraints, the magnitude of thermal damage to the surgical specimen is dictated by intraoperative laser configurations, such as beam spot size and power density.
Moreover, the surgeon's experience in TLM plays a pivotal role in fine-tuning these variables to minimize specimen degradation and ensure more accurate margin assessment. (23)

Regarding the optimal laser power settings, the existing literature presents diverging perspectives. One school of thought suggests that higher power settings minimize peripheral tissue damage due to the increased speed of the surgical stroke, which reduces the duration of thermal interaction with the cells. In support of this, Buchanan et al. (45) demonstrated that higher power outputs, coupled with a narrow spot size (0.25 mm), enhance surgical

precision and limit carbonization. This approach was further corroborated by Charbonnier et al.(46), who employed power settings between 5W and 10W. In their cohort of 110 patients, despite 30 cases of positive resection margins, only 10 of the 23 recorded recurrences occurred in patients with positive margins, aligning with Buchanan's observations on the reliability of high-power dissection.

Conversely, other investigators advocate for lower power settings to better preserve the histological integrity of the specimen. Ansarin et al.(35), for instance, preferred a lower power range (0.8W to 4.7W) with an even smaller spot size (0.15 mm). Similarly, Lee et al. (43) performed TLM using settings between 1W and 2W in a study of 118 patients. Their findings, showing only 14 recurrences despite 43 cases of positive margins, suggest that low-power configurations do not necessarily compromise the histopathological assessment, potentially allowing for a sufficiently accurate evaluation of the resection edges.

Recent technological advancements have further mitigated these issues. The introduction of UltraPulse CO2 lasers has significantly reduced the extent of thermal artifacts; these devices produce a minimal layer of cellular necrosis, with a mean coagulation zone of only 20 ± 11 μm along the incision line. (47)

In addition to thermal artifacts, the physical loss of tissue due to laser-induced vaporization must be considered. According to the literature (41,42,48), this phenomenon reduces the actual surgical margin by approximately 0.05–0.5 mm. Consequently, the margin measured by the pathologist is inherently smaller than the one achieved by the surgeon intraoperatively, potentially leading to an underestimation of the oncological safety distance.

SHRINKAGE

Current literature suggests that specimen shrinkage occurs both before and during fixation. It is secondary to a combination of the intrinsic elastic properties of the tissue and the biochemical effects of formalin processing.

It is well established that human tissues, particularly the mucosa, possess an inherent tendency to undergo post-surgical shrinkage. This phenomenon is primarily driven by the presence of contractile proteins within the connective tissue and their subsequent release from surrounding anatomical structures. While this process is well-documented in various anatomical sites, such as the skin, objective reports in the head and neck region have focused mainly on the lips and oral cavity. For instance, it has been demonstrated (49) that surgical

margins of the lip contracted by 41% to 47.5% following resection and fixation. Notably, the most significant shrinkage (17.2–21.5%) was observed immediately post-excision, whereas formalin fixation accounted for a smaller reduction of 13.6–14.3% after 24 hours. Similarly, clinical studies (50) reported a mean reduction in oral cavity margins of 3.71 mm (23.5%) in tongue cancer patients. Further research (51) corroborated these findings, describing a 14.9% mean shrinkage rate from pre- to post-excision, compared to only 3.7% during the fixation phase.

In the context of laryngeal surgery, only Mariani et al. (52) introduced a precise measurement methodology, evaluating specimens both in situ and post-resection. Their study revealed a mean mucosal shrinkage of 3.8 ± 0.3 mm, corresponding to a 29% average loss in anteroposterior length. This significant contraction is fundamentally explained by the intrinsic glottic anatomy: the vocal folds are horizontally tensioned between the thyroid cartilage and the vocal process of the arytenoids. Biomechanical studies have confirmed that the human vocal folds exhibit anisotropic elastic properties due to collagen and elastin fibers aligned along the anteroposterior axis. Consequently, the most pronounced shrinkage occurs along this longitudinal axis following incision.

Furthermore, a critical observation is that healthy tissue margins exhibit significantly greater retraction compared to neoplastic tissue, as tumor infiltration tends to replace the contractile elements of the stroma. This is consistent with experimental data (53), which reported a 19% shrinkage in normal skin versus only 11% in malignant lesions. Aligning with these results, Mariani et al. (52) observed that while shrinkage affects both healthy and tumor tissues, the retraction of anterior and posterior margins was substantially higher than that of the tumor itself (49% vs. 20% and 45% vs. 20%, respectively).

Therefore, post-cordectomy shrinkage can significantly alter the macroscopic margins achieved intraoperatively, resulting in narrower measurements upon histological examination. Surgeons must also account for increased retraction in cordectomies involving the intermediate and deep layers of the lamina propria, likely due to the elastic properties of the vocal ligament. This is in line with clinical reports (52), which have demonstrated that type II and III cordectomies exhibit statistically significant higher shrinkage compared to type I resections.

While the aforementioned studies focus on the intrinsic biomechanical contraction of the tissue immediately following incision, it is crucial to distinguish this from the secondary

shrinkage induced by histological processing. Beyond the immediate elastic recoil of the fibers, the chemical action of formalin fixation further exacerbates specimen deformation and may compromise the margin assessment. Upile et al. (54) observed that tissue fixation prompt a shrinking of >30% and can therefore impact the assessment of margins on final pathology.

Consistent with these findings, several studies have reported that in cases of 'positive' or 'close' margins, up to 80-90% of patients exhibit no residual disease during revision surgery (55–57). This evidence strongly suggests that positive margin reports frequently stem from tissue processing artifacts rather than actual tumor persistence. In such clinical scenarios, the detection of neoplastic cells specifically during secondary revision surgery appears to be a far more accurate predictor of poor oncologic outcomes than the initial, potentially confounded, margin status alone.

INTRAOPERATIVE MARGIN ASSESSMENT

In oncology, intraoperative frozen section analysis traditionally represents a valuable technique to achieve an immediate evaluation of the surgical bed. By providing real-time pathological feedback, this modality aims to allow the surgeon to instantly enlarge the resection if necessary, thereby ensuring a higher rate of clear margins and significantly improving the overall oncological outcome.

However, in the setting of glottic squamous cell carcinoma treated via TLM, frozen section analysis has several practical and clinical limitations. From an operational standpoint, frozen sections require significant hospital resources, including a fully equipped pathology laboratory, dedicated technical staff, and specialized pathologists, along with the logistical burden of specimen transportation between the operating room and the lab, which are not always in close proximity. This entire workflow contributes to high costs and significant intraoperative time consumption.

Moreover, from a diagnostic perspective, the reliability of a margin verdict is often challenging due to the limited tissue volume available for assessment and the millimetric size of the surgical margins. Furthermore, the diagnostic accuracy can be severely compromised by additional artifacts introduced during tissue handling and cryostat preparation. In addition, prior treatments like radiation or previous surgeries can alter the tissue, making the pathological diagnosis much more difficult due to inflammation and

scarring. As a result, the surgeon may not always receive reliable, real-time feedback regarding the precise status of superficial and deep margins. Finally, taking multiple, serial tissue samples contradicts the conservative goal of TLM, which aims to preserve vocal cord structure and voice quality. (58)

1.6.2 PROGNOSTIC IMPACT

The prognostic role of surgical margins in patients undergoing Transoral Laser Microsurgery (TLM) for laryngeal squamous cell carcinoma has been a subject of extensive investigation. The association of positive margins and survival has been reported with different results.

A landmark systematic review and meta-analysis by De Virgilio et al. (30) synthesizes evidence from 26 retrospective studies, encompassing a total of 5,463 patients. The study aims to offer a high-level evaluation of oncological outcomes, specifically Local Control (LC), Disease-Free Survival (DFS), and Overall Survival (OS), by comparing patients with positive versus negative resection margins. Data from multiple centers were pooled using a random-effects model. The meta-analysis revealed a pooled log-HR of -0.76 for LC, 0.16 for OS, and 0.93 for DFS. Statistically, a log-HR greater than 0 indicates a higher risk of recurrence or mortality for patients with positive margins. The results demonstrated that positive margins were significantly associated with worse DFS ($p < 0.05$). However, no statistically significant relationship was observed for Local Control or Overall Survival. The interpretation of these results is affected by the differences between the included studies. While the data for Local Control were too inconsistent to be clinically decisive, the results for Disease-Free Survival (DFS) showed high consistency. This confirms that positive resection margins are a reliable predictor of recurrence. The variability in Overall Survival (OS) data, however, suggests that long-term mortality is influenced by a wider range of factors beyond margin status alone.

Regarding Local Control (LC) specifically, the literature offers a complex landscape. The influence of margin status following TLM for glottic carcinoma remains a subject of significant controversy, as evidenced by the divergent findings reported in the literature. A group of pivotal studies (26,35,59), suggests that patients with close or positive margins face an increased risk of adverse oncological outcomes. Conversely, this correlation has been challenged by other authors (41,48,57), whose research showed contradictory results, failing to establish a statistically significant link between margin status and local failure.

A recent study specifically addressing oncological outcomes based on margin status was conducted by Mariani et al. (31) This research evaluates the impact of resection margins on local control, survival rates, and clinical treatment recommendations. The findings underscore high survival across the cohort, with a Disease-Specific Survival (DSS) of 99.6% for all patients and 100% for those with negative margins. Regarding Recurrence-Free Survival (RFS), the overall rate was 92.9%. However, a significant discrepancy was observed based on the margin type, with RFS decreasing from 93.2% for negative margins to 80.9% for deep margins. Similarly, while the Local Control with laser alone (LCL) reached 94.6% for the entire group, it was significantly lower, at 57.5%, in cases involving positive deep margins. The Overall Laryngeal Preservation (OLP) rate was 98.2%, falling to 86.9% for deep margins.

Concerning Overall Survival (OS), the study by Jumaily et al. (60) represents one of the largest analyses comparing survival outcomes based on margin status in patients treated with TLM for T1-T2 glottic squamous cell carcinoma. This study specifically examined the OS rates of patients with positive versus negative tumor margins. The authors demonstrated that, even in the presence of positive margins, patients did not exhibit significantly inferior survival rates compared to those with negative margins. These findings suggest that, in the context of early-stage disease, margin status may not be a definitive predictor of overall mortality.

This clinical paradigm has been definitively reinforced by a recent 2026 large-scale multicenter study on 1,216 patients by Chu et al. (61), which shifts the prognostic evaluation from a binary positive/negative perspective toward a detailed evaluation of "margin burden". Their multivariable analysis confirmed that while a single superficial positive margin behaves comparably to free margins in terms of disease-free survival (DFS), deep margin involvement acts as the true driver of disease-specific mortality, carrying a 3.96-fold increased risk of recurrence and a 6.82-fold increased risk of disease-specific death.

Furthermore, several investigators (25,62,63) have observed that the clinical significance of a positive margin is highly dependent on its topographical location and multiplicity. Specifically, evidence suggests that a single superficial positive margin does not inherently compromise local control (LC) and may not necessitate an immediate 'second-look' procedure. Conversely, the presence of multiple superficial or deep positive margins has

been consistently correlated with less favorable oncological outcomes, thereby calling for further surgical intervention or adjuvant therapy.

1.6.3 MANAGEMENT

The difficulty in the histopatological assessment of the resection margins, combines with the lack of consensus regarding the optimal margin to tumor distance, leads to significant variations in management policies for positive, close and negative margins. Options include:

- Planned early revision TLM
- Adjuvant RT
- Strict endoscopic surveillance (close follow-up)
- Open surgery

MANAGEMENT OF POSITIVE SURGICAL MARGINS

In cases of positive resection margin, a second-look surgery is the most followed approach. (23,30)

- Other authors (42,43,48) propose a second-look TLM or close follow-up according to the surgeon's judgment during surgery or in cases of suspicion of recurrence during the follow-up.
- On the other hand, some researchers (25,38,64) adopt a distinct strategy based on the number and depth of the positive margins. They select the close follow-up only if a superficial margin is positive, and the second-look procedure open surgery or RT protocols if more than one superficial margin or the deep margins are involved. By using this strategy, Fiz et al achieved a 5-year DFS in 77.2%, DSS in 98.3%, and LP in 96.2% of their sample, while Lucioni reported a 3-year DFS in 84,7%, a 3- year DSS in 97,8% and a 5-year OS in 91,4% of their cohort.
- Conversely, Ansarin et al. (35) conduct a second look with TLM only if a resection margin is positive, and RT in case of more than 1 positive margin, achieving a 5-year OS in 90.01%, LP in 97.1%, and a 8-year DFS in 88.2% of their sample.
- Other authors (46,65), prefer a close follow-up approach in cases of positive margins, regardless of the depth of the infiltration and the number of positive margins. Thus, in 2015, Hoffmann et al. achieved a 5-year OS in 79.2%, DSS in 91.5%, DFS in 61.7%, LP in 93.4%, and LC in 74.4% of their sample. In their case series,

Charbonnier et al. had similar findings, with a 5-year OS in 88%, DFS in 73%, and LC in 79% of their sample.

By comparing the results of these different performances regarding the 5-year DFS, DSS and LP, the strategy of the second-look surgery has better results than the close follow-up in cases of positive resection margins, providing a lower risk of recurrence and of invasive surgery, which can compromise organ preservation.

- Preuss et al. (44) recommend undertaking second- and third-look TLM in all patients independent of the status of the resection margins, with a 5-year DFS in 96.3%, and a 5-year OS in 100% of their sample. Indeed, in their study, they detected an increased incidence of tumor persistence with two successive second look TLMs, which allowed them to reach complete resection of the tumor and early detection of recurrence, as demonstrated by a 5-year DFS in 96.3% of their sample, compared with a 5-year DFS in 73% and 61,7% in the studies by Charbonnier and Hoffmann respectively.

The optimal pathway for positive margins has been further clarified by the multicenter data of Chu et al. (61), demonstrating that second-look surgery achieves significantly superior DFS compared to radiotherapy or surveillance alone. Crucially, the study identifies histopathological findings at the time of revision as the ultimate survival discriminator: patients achieving successful re-radicalization (negative final histopathology at SLS) experience identical survival curves to those with primary free margins, whereas the presence of true residual carcinoma identifies a high-risk subgroup with aggressive tumor biology and poor oncologic outcomes.

MANAGAMENT OF CLOSE OR NON-VALUABLE SURGICAL MARGINS

Regarding close or non-valuable margins, close follow-up represents the most widely accepted approach. (25,37,43,57,65)

However, Hendriksma et al. (42) and Hartl et al. (48) define the most appropriate management between close follow-up and second-look surgery based on individual clinical grounds. According to these authors, a surgeon's intraoperative evaluation is just as important as the histological assessment. Moreover, Hartl et al. maintain that in cases of disagreement between the surgeon and the pathologist (regarding positive or non-valuable margins), they usually performed a second look TLM. In the literature, on the contrary, other

authors (36,37,44) recommend the second-look approach systematically if the margins are close or non-valuable. Some studies omitted this kind of surgical-margin status, considering only positive and negative ones. (46,65,66)

MANAGEMENT OF NEGATIVE SURGICAL MARGINS

In the literature, there is unanimity regarding the optimal approach in cases of negative resection margins. Thus, all authors suggest the follow-up. Only Preuss et al. (44) executed a minimum of two second-look microlaryngoscopies in presence of negative surgical margins.

However, numerous reports (25,48,66,67) have documented the possibility of local recurrence even in cases of negative resection margins. Given this perspective, follow-up, ideally utilizing narrow-band imaging (NBI), is important to identify precocious relapse. In fact, NBI is an endoscopic system that facilitate an enhanced analysis of the microvascular pattern in cases of preneoplastic and neoplastic lesions of the mucosa that are not detectable via with white-light endoscopy. (68) So, NBI enables the detection of the early recurrence of the tumor and its surface spread but cannot evaluate the invasive depth.

The analysis shows that there is not a shared strategy. Upon evaluating the contemporary literature, the European Laryngological Society (ELS) published in 2014 a series of evidence-driven statements; specially, it defined that second-look TLM is mandatory in cases of positive margins, and recommended if the margins are close or non-valuable. (63)

1.7 HISTOLOG® SCANNER

Overall, current evidence highlights that the primary limitation of TLM does not stem from its oncological efficacy, but rather from the technical challenges associated with margin assessment. Based on this background, a reliable method for rapid intraoperative evaluation of both superficial (mucosal) and deep margins would therefore be of substantial clinical value.

Conventional hematoxylin-eosin (H&E) histopathological examination remains the gold standard for definitive margin assessment. However, this process requires tissue fixation, embedding, sectioning, and staining, and therefore does not provide immediate feedback during surgery. Furthermore, standard histology only analyzes selected sections of the

specimen rather than the entire epithelial surface, and tissue processing can introduce artifacts that complicate the interpretation. For these reasons, there is a growing interest in new tools that can evaluate fresh tissue in real time, supporting conventional histology.

Histolog® Scanner, as shown in Figure 12, is an innovative digital microscopy device designed for ultra-fast confocal imaging of freshly excised specimens directly inside the operating room. Operation is straightforward and can be performed by standard medical staff after brief training using a touchscreen interface. The fresh tissue is simply treated with a fluorescent dye solution and placed on the imaging window. The device uses a 488 nm laser to excite tissue fluorescence, displaying high-resolution images in real time with an artificial purple coloring, without requiring any complex calibration or post-processing. It offers a fast mode providing a specimen overview in 5 seconds and a high-resolution mode that visualizes tissue morphology down to the cell nuclei level in just 50 seconds. Moreover, this technology completely bypasses the need for tissue fixation, embedding or slide mounting. Its potential advantages include rapid image acquisition, reduced processing-related artifacts, the ability to visualize a broader epithelial surface, and the evaluation of multiple margins within a single scan. (69)

Figure 12: The Histolog® Scanner device for real-time fresh tissue imaging. Source: Courtesy of SamanTree Medical SA (© 2026).

Currently, its clinical application has been explored across various surgical specialties, including urologic, breast, dermatologic, and head and neck surgery. In these oncologic settings, the device is implemented through three main modalities: as a tool for direct intraoperative interpretation by the surgeon, as a support system for the pathologist, or as an adjunct to traditional methods like frozen section analysis. Although clinical data for head and neck surgery are still limited, this anatomical region represents a highly promising field of application for Histolog® technology. Indeed, intraoperative decisions in head and neck oncology are uniquely demanding due to the intricate local anatomy, the need to preserve narrow functional margins, and the heavy logistics of standard frozen section analysis. Consequently, the successful outcomes achieved in other surgical fields provide a solid rationale for expanding research into head and neck procedures, where this device could significantly optimize the surgical workflow.

The aim of the present study was to perform a preliminary validation of the Histolog® Scanner for intraoperative surgical margin assessment in patients undergoing transoral laser cordectomy, using conventional hematoxylin-eosin (H&E) histopathology as the reference standard. Specifically, we compared the two methods (Histolog® Scanner vs H&E) in terms of margin classification concordance and diagnostic performance.

2 MATERIALS AND METHODS

This is a prospective pilot diagnostic accuracy study conducted at the University Hospital of Modena between October 2025 and March 2026.

The objective of the study is to preliminarily validate the diagnostic performance of Histolog® Scanner for intraoperative assessment of surgical margins of the specimen in patients undergoing transoral laser cordectomy, comparing the results with conventional hematoxylin-eosin histopathology as the reference standard. STARD 2 015 reporting guidelines were used for the construction and elaboration of this study. (70)

All adult patients (aged > 18 years) undergoing transoral laser cordectomy (from cordectomy type I to type VI) for glottic lesions at the treating center during the study period were evaluated for eligibility.

Patients were excluded when either Histolog® Scanner acquisition or final histopathological evaluation of the specimen was not feasible or remained incomplete.

SURGICAL SPECIMENS AND MARGIN ASSESSMENT

For each cordectomy specimen, surgical margins were oriented and assessed according to a predefined institutional protocol. Five margins were systematically examined for each specimen: anterior, posterior, superior, inferior, and deep. Each margin constituted a unit of analysis in the comparative assessment with conventional histology.

INDEX TEST: HISTOLOG® SCANNER ASSESSMENT

Immediately following resection, the fresh surgical specimens were oriented and pinned on a cork board to maintain correct anatomical alignment. Subsequently, they were analyzed intraoperatively by the surgeon and the pathologist using the Histolog® Scanner system. Histolog® Scanner permits real-time image acquisition on fresh tissue without the need for formalin fixation, paraffin embedding, or sectioning. Specimens are immersed in an acridine orange-based photoreactive solution (Histolog® Dip, SamanTree Medical SA) for 10 seconds, then rinsed with 0.9% saline solution, carefully dried with absorbent paper and placed on the scanner to capture the images. A digital image of the specimen surface is displayed with artificial purple staining on a touchscreen, which allows the surgeon to zoom in and move around the image.

This process, as illustrated in Figure 13, is repeated for the following sides: superior side, inferior side, whole epithelial side and deep side. At the end of the acquisitions the quality and reliability of the images were evaluated by the surgeon and the pathologist. This

technique enables visualization of the epithelial surface over a broad field and allows simultaneous assessment of multiple margins within the same scan.

Each margin was categorized by the pathologist as negative or positive (defined either Rclose or R1) according to standard histological criteria. Specifically, positive margin was considered in case of detection of carcinoma in situ/ high grade dysplasia or squamous cell carcinoma at the margin or close to it (less than 1 mm from the border). On the other hand, the margin was classified as negative when there was no carcinoma involvement at the margin, low grade dysplasia was considered negative as well. Eventually the margin was considered indeterminate in case of poor image quality, uninterpretable scan, uncertain deep assessment.

The assessment of Histolog® images was performed without knowledge of the final conventional histopathological result.

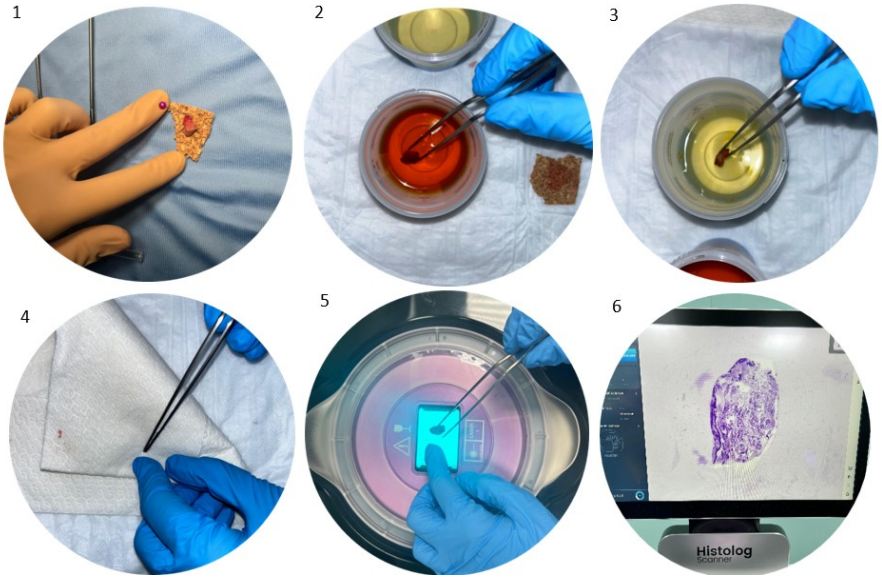

Figure 13: Intraoperative preparation protocol for Histolog® Scanner assessment.
(1) Fresh cordectomy specimen oriented and pinned on a cork board to maintain correct anatomical alignment immediately after resection. (2) Immersion of the specimen in the acridine orange-based photoreactive solution (Histolog® Dip, SamanTree Medical SA) for 10 seconds. (3) Rising of the specimen with 0.9% saline solution to remove excess dye. (4) Careful drying of the specimen with absorbent paper prior to scanning. (5) Placement of the prepared specimen on the Histolog® Scanner for real-time image acquisition. (6) Representative Histolog® Scanner output displayed on the touchscreen with artificial purple

staining, enabling intraoperative assessment of the specimen surface at variable magnifications.

REFERENCE STANDARD: CONVENTIONAL HISTOPATHOLOGY

Once the surgical specimens were scanned intraoperatively using the Histolog® scanner, they subsequently underwent the standard histopathological processing protocol. Specimens were immediately immersed in 4% buffered formalin for fixation (lasting 12-48h). Following fixation, macroscopic grossing was performed by inking the surgical margins with different colors and sectioning the specimen to isolate each individual margin for separate cassette embedding, ensuring complete evaluation of all margins, as shown in Figure 14. Finally, these sections underwent automated dehydration and clearing prior paraffin embedding, microtome sectioning, and hematoxylin–eosin staining.

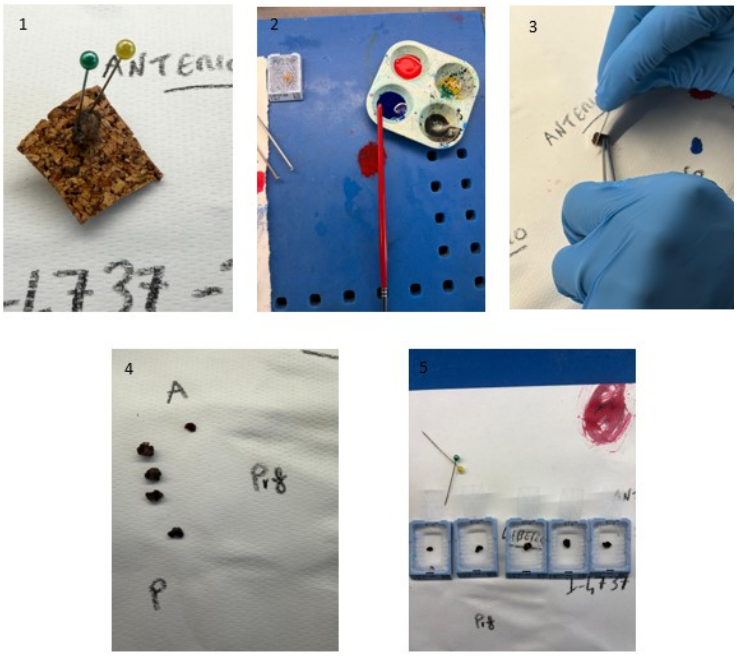

Figure 14: Macroscopic grossing of the cordectomy specimen for definitive histopathological analysis. *(1) Fresh specimen after formalin fixation, oriented on a cork board with colored pins identifying the surgical margins. (2) Ink-based margin painting: each surgical margin is marked with a different color to allow precise orientation throughout the histopathological process. Following margin inking and specimen measurement, the orienting pins are removed and the specimen is sectioned. (4) Individual margins samples laid out and oriented prior to cassette embedding: anterior (A) and posterior (P) margins are places at the extremities and the central sections, containing the free and deep (Prf) margins, are arranged in between. (5) Placement of the margin sections into histological*

cassettes, each containing the tissue fragment sandwiched between foam pads to maintain orientation during sectioning.

Subsequently, a second group of pathologists evaluated the surgical margins on hematoxylin–eosin–stained slides, providing a definitive histopathological diagnosis as well as margin status classification with the same protocol used for the index test (e.g. negative or positive). These findings were then compared, for each individual margin, with the results obtained from the analysis of images acquired using the Histolog® scanner and categorized as either "concordant" or "discordant".

Final histopathological assessment was performed independently and blinded to the Histolog classification.

Technical note: margins evaluated microscopically on hematoxylin–eosin–stained slides and those assessed on Histolog images do not strictly represent the same margin in terms of the "analyzed image," due to the sampling protocol used for vocal cord specimens in histopathological processing. Specifically, once appropriately fixed and processed, the specimens are sectioned along coronal planes, yielding serial slices of the vocal cord that cannot be reproduced from the fresh specimen imaged with the Histolog® scanner. Conversely, the comprehensive assessment of both the entire deep surface and the epithelial surface achievable on the fresh specimen using the scanner cannot be replicated on histological slides.

Figure 14 compares conventional hematoxylin–eosin-stained sections with Histolog® scanner acquisitions of vocal folds with negative and positive margins. Panels A–C show a vocal fold with a negative margin, with hematoxylin–eosin staining in panel A and Histolog® images in panels B and C. Panels D–F show a vocal fold with a positive margin, with hematoxylin–eosin staining in panel D and Histolog® images in panels E and F.

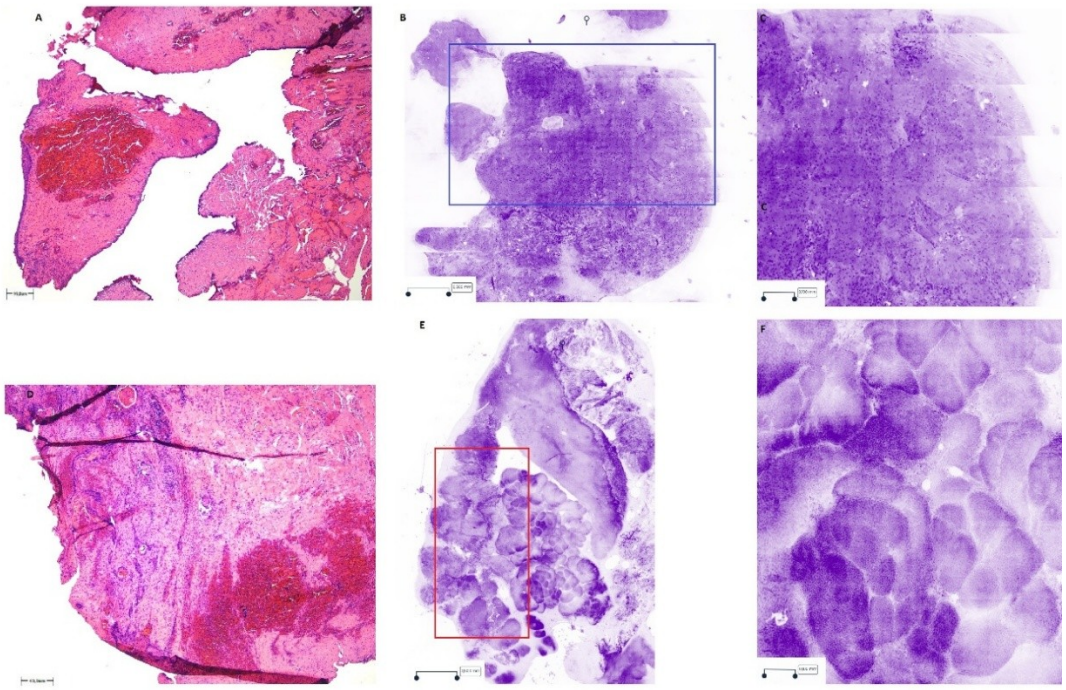

Figure 14: Example of vocal fold with negative and positive margins*. (A) Hematoxylin-Eosin (10x) and Histolog® scans at various magnifications (B:100x digital viewer, C:200x digital viewer) of a vocal fold with a negative margin; the blue box highlights a well-differentiated and maturing squamous epithelium. (D) Hematoxylin-Eosin (10x) and Histolog® scans at various magnifications (E: 100x digital viewer, F:200x digital viewer) of a vocal fold with a positive margin; the red box highlights a hyperchromic and poorly maturing squamous epithelium.*

Figure 15 provides additional representative examples of Histolog® Scanner image interpretation. Panel A shows a negative margin with acanthosis on conventional H&E histopathology, while panel B illustrates the corresponding appearance of a negative margin with acanthosis as visualized by th Histolog® Scanner, demonstrating the visual correspondence between the two methods in the identification of benign epithelial changes. Panel C shows a Histolog® Scanner image of the deep margin.

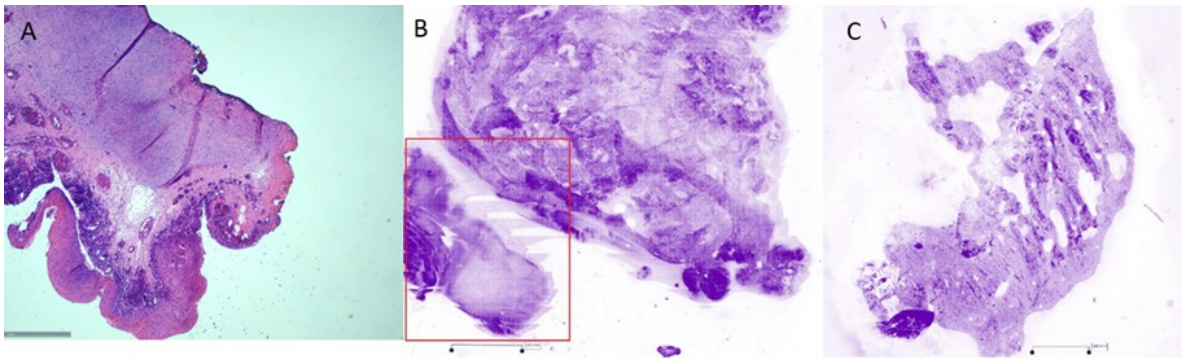

Figure 15: Representative images of margin assessment using conventional H&E histopathology and Histolog® Scanner. *(A) Conventional H&E histopathology (4x) showing a negative margin with acanthosis. (B) Histolog® Scanner image (100x digital viewer) of a negative margin with acanthosis, demonstrating the visual correspondence with conventional histopathology. (C) Histolog® Scanner image (100x digital viewer) of the deep margin.*

DATA COLLECTION

For each patient, demographic and clinical data were collected, including age, sex, indication for surgery, lesion site, and the specific type of cordectomy performed. For each specimen, margin-specific information were recorded for the anterior, posterior, superior, inferior, and deep margins. For every individual margin, the Histolog® result, final histopathological result, and evaluability status were documented.

Technical feasibility of Histolog® and its potential usefulness for intraoperative surgeon-pathologist interaction were descriptively assessed.

OUTCOMES

The primary outcome of the study was the overall concordance between Histolog® and conventional histopathology in the assessment of surgical margins.

Secondary outcomes included sensitivity of Histolog® images for positive margins, specificity of Histolog® images for negative margins, positive predictive value, negative predictive value, overall diagnostic accuracy, agreement beyond chance as measured by Cohen's kappa coefficient and proportion of non-evaluable margins.

Furthermore, a secondary per-patient analysis was scheduled to assess the overall case classification, which was defined as the presence of at least one positive margin.

STATISTICAL ANALYSIS

A primary analysis was performed on a per-margin basis. Histolog® results were compared with those of conventional histopathology using 2×2 contingency tables.
Overall concordance was calculated as the proportion of identically classified margins among all evaluable margins. Diagnostic performance was quantified through the evaluation of sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy, each with 95% confidence intervals, calculated using the exact binomial (Clopper-Pearson) method.
Agreement beyond chance between Histolog® and conventional histopathology was assessed using Cohen's kappa coefficient. Missing data, if encountered, were reported and excluded from pairwise comparisons.

Because multiple margins were collected from the same specimen and were therefore not fully independent, these results were interpreted with caution and considered preliminary. A secondary per-patient descriptive analysis was also performed.

Given the pilot nature of this study, no formal sample size calculation was undertaken; the sample reflects the initial consecutive series available during the study period.

3 RESULTS

During the study period, 24 consecutive patients undergoing transoral laser cordectomy were screened for eligibility, and 19 were ultimately included. Of 24 consecutive patients assessed, the first 5 were excluded from the diagnostic accuracy analysis because they were treated during the initial run-in phase, before full implementation of the predefined 5-margin acquisition protocol. These cases were considered for feasibility/workflow refinement but were not included in the final protocol-compliant accuracy analysis.

Consequently, data analysis was performed on all 19 included patients. The cohort comprised 18 men and 1 woman, with a mean age of 70 years. A comprehensive breakdown of participant inclusion and margin-level analysis are summarized in Figure 15.

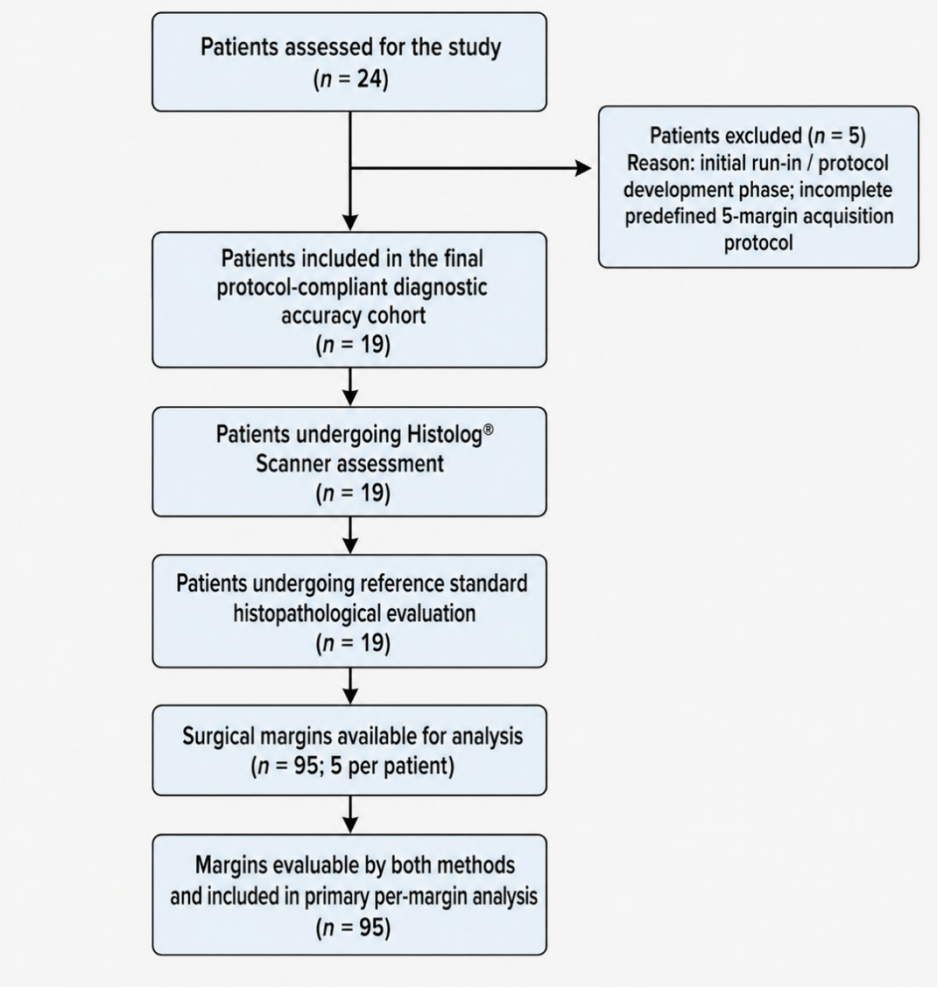

Figure 15: STARD flow diagram of patient inclusion and margin-level analysis*. Twenty-four consecutive patients were assessed. The first 5 patients were excluded from the final diagnostic accuracy cohort because they were treated during the initial run-in phase, before completion of the predefined 5-margin acquisition protocol. The final protocol-compliant*

cohort included 19 patients, yielding 95 predefined margins, all evaluable by both Histolog® Scanner and conventional H&E histopathology.

A total of 19 cordectomy specimens were collected, providing 95 theoretical surgical margins for analysis (5 margins per specimens: anterior, posterior, superior, and deep). All 95 margins were successfully evaluated using both the Histolog® Scanner and conventional histopathology, thereby permitting their inclusion in the primary per-margin analysis. Notably, no margins were classified as non-evaluable by either diagnostic technique.

Based on the surgical procedures performed, the distribution of cordectomy types was as follows: type I (n=4), type II (n=4), type III (n=8), type Va (n=2), type VI (n=1). The baseline demographic and procedural characteristics of the study cohort are detailed in Table 7.

Variable	Value
Patients, n	19
Age, mean ± SD, years	70 ± 2
Sex, male, n (%)	18 (94.7)
Sex, female, n (%)	1 (5.3)
Cordectomy type I, n	4
Cordectomy type II, n	4
Cordectomy type III, n	8
Cordectomy type Va, n	2
Cordectomy type VI, n	1
Total theoretical margins, n	95
Evaluable margins, n (%)	95 (100)
Non-evaluable margins, n (%)	0 (0)

Table 7: Baseline demographic and surgical characteristics of the study population. *Data are presented as number (%) unless indicated otherwise. A total of 19 patients undergoing transoral laser cordectomy were enrolled; all associated surgical margins were completely evaluable using both Histolog® Scanner and standard histopathology.*

Overall concordance between Histolog® Scanner assessment and the reference standard histopathological evaluation was achieved in 88 out of 95 margins (92.6%), whereas discordant classification occurred in 7 margins (7.4%). Among these 7 discordant margins, 3 were identified within a single patient, while the remaining 4 discrepancies were

distributed across 4 different patients. Regarding the nature of these discordant cases, the Histolog® Scanner yielded 5 false-positive and 2 false-negative assessments.

Cross-tabulation of Histolog® Scanner results against the reference standard is reported in Table 8. Using a dichotomized classification for margin status (defined as positive for Rclose/R1 and negative for R0), the Histolog® Scanner demonstrated 12 true-positive, 76 true-negative, 5 false-positive, and 2 false-negative results.

Histolog® Scanner	Reference standard positive	Reference standard negative	Total
Positive	12	5	17
Negative	2	76	78
Total	14	81	95

Table 8: Cross-tabulation of Histolog® Scanner findings against the reference standard at the margin level. *Margin status was dichotomized into positive (Rclose or R1) or negative categories, utilizing conventional histopathology as the reference standard. Within this framework, Histolog® Scanner identified 12 true-positive, 76 true-negative, 5 false-positive, and 2 false-negative margins*.

On per-margin analysis, Histolog® Scanner showed a sensitivity of 85.7% (95% CI, 57.2–98.2), a specificity of 93.8% (95% CI, 86.2–98.0), a positive predictive value of 70.6% (95% CI, 44.0–89.7), a negative predictive value of 97.4% (95% CI, 91.0–99.7), culminating in an overall diagnostic accuracy of 92.6% (95% CI, 85.4–97.0), as graphically illustrated in Figure 16.

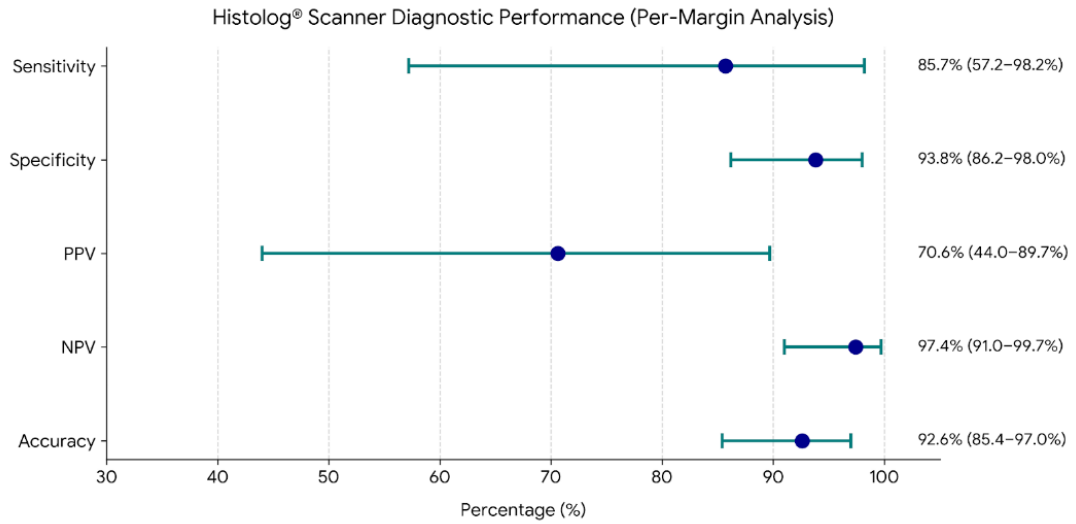

Figure 16: Forest plot of Histolog® Scanner diagnostic metrics (Per-Margin Analysis)*. Overview of the device's performance based on the margin-specific evaluation, demonstrating a sensitivity of 85.7% (95% CI: 57.2–98.2%), a specificity of 93.8% (95% CI: 86.2–98.0%), a PPV of 70.6% (95% CI: 44.0–89.7%), an NPV of 97.4% (95% CI: 91.0–99.7%), and an overall accuracy of 92.6% (95% CI: 85.4–97.0%). Dots represent point estimates; horizontal lines indicate 95% confidence intervals.*

The agreement beyond chance between Histolog® Scanner and conventional histopathology was substantial, with a Cohen's kappa coefficient of 0.73.

Notably, one of the false-negative cases involved the deep margin and highlighted an inherent technical limitation of Histolog® Scanner in the assessment of Rclose status at the deep plane. Specifically, while the system enables a comprehensive evaluation of the specimen surface along axial or sagittal planes relative to the margin, it lacks the capacity for a coronal-plane assessment comparable to that obtained with conventional histological sectioning. Consequently, this constraint limits the interpretation of close deep margins in selected cases.

At the patient level, discordant discrepancies were observed in 5 of 19 individuals, including one patient in whom 3 margins were discordant. Conversely, complete concordance across all assessed margins was achieved in the remaining 14 patients.

Histolog® Scanner	Reference standard positive patient	Reference standard negative patient	Total
Positive	8	1	9
Negative	1	9	10
Total	9	10	19

Table 9: Cross-tabulation of Histolog® Scanner findings against the reference standard at the patient level. *A patient was classified as positive when at least one surgical margin was positive (Rclose or R1) and as negative when no positive margins were identified. Conventional histopathology served as the reference standard. Histolog® Scanner identified 8 true-positive, 9 true-negative, 1 false-positive, and 1 false-negative patients.*

Patient-level cross-tabulation of Histolog® Scanner results against the reference standard is presented in Table 9. In the secondary analysis, 9 of 19 patients had at least one positive margin according to the reference standard, whereas 10 had no positive margins. Histolog® Scanner accurately detected 8 of 9 patients with at least one positive margin and 9 of 10 patients with no positive margins, showing a sensitivity of 88.9% (95% CI, 51.8%–99.7%), a specificity of 90.0% (95% CI, 55.5%–99.7%), a positive predictive value of 88.9% (95% CI, 51.8%–99.7%), a negative predictive value of 90.0% (95% CI, 55.5%–99.7%), and an overall accuracy of 89.5% (95% CI, 66.9%–98.7%), as graphically illustrated in Figure 17.

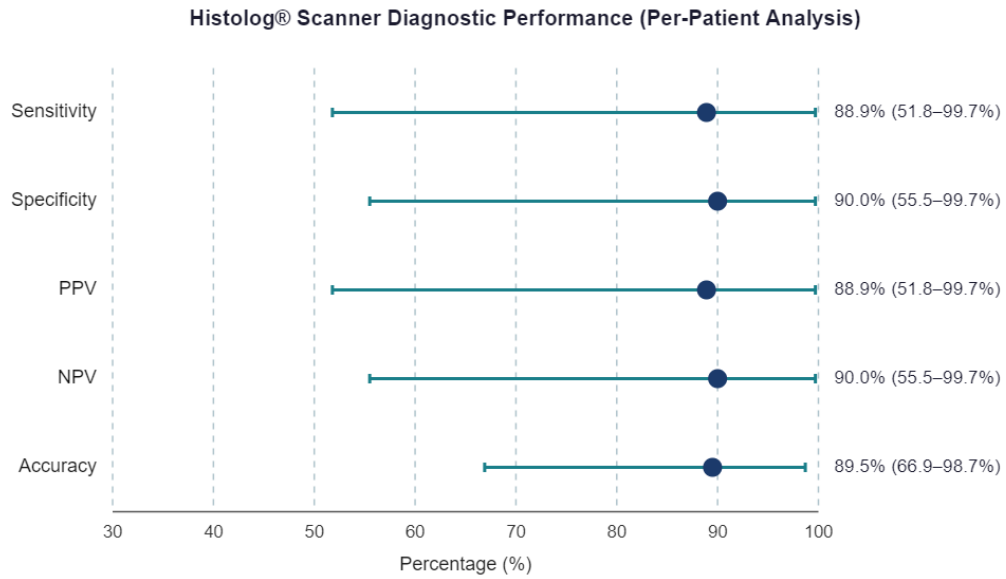

Figure 17: Forest plot of Histolog® Scanner diagnostic metrics (Per-Patient Analysis)*. Overview of the device's performance based on the patient-level evaluation, demonstrating a sensitivity of 88.9% (95% CI: 51.8–99.7%), a specificity of 90.0% (95% CI: 55.5–99.7%), a PPV of 88.9% (95% CI: 51.8–99.7%), an NPV of 90.0% (95% CI: 55.5–99.7%), and an overall accuracy of 89.5% (95% CI: 66.9–98.7%). Dots represent point estimates; horizontal lines indicate 95% confidence intervals.*

Regarding procedural feasibility, Histolog® acquisition was successfully completed in all cases. The mean acquisition time per specimen was 13±2 minutes.

Neither missing acquisitions nor non-assessable scans were recorded during the study. Additionally, no adverse events associated with either the Histolog® Scanner or standard histopathological processing were observed.

Representative histological images illustrating concordant margin assessments obtained with the Histolog® Scanner and conventional histopathology are shown in Figure 18. Panels A–D depict a concordant true-positive case: panel A shows a Histolog® Scanner image in which both the positive margin and the invasive tumor are simultaneously visible within a single wide-field acquisition, panels B and C show H&E-stained sections illustrating the invasive tumor at different magnifications (4x and 10x respectively), and panel D shows cytokeratin immunohistochemistry confirming tumor involvement. Panels E–F illustrate a concordant true-negative case at the deep margin: panel E shows the Histolog® Scanner image of the negative deep margin, and panel F shows the corresponding H&E-stained section confirming the negative deep margin status.

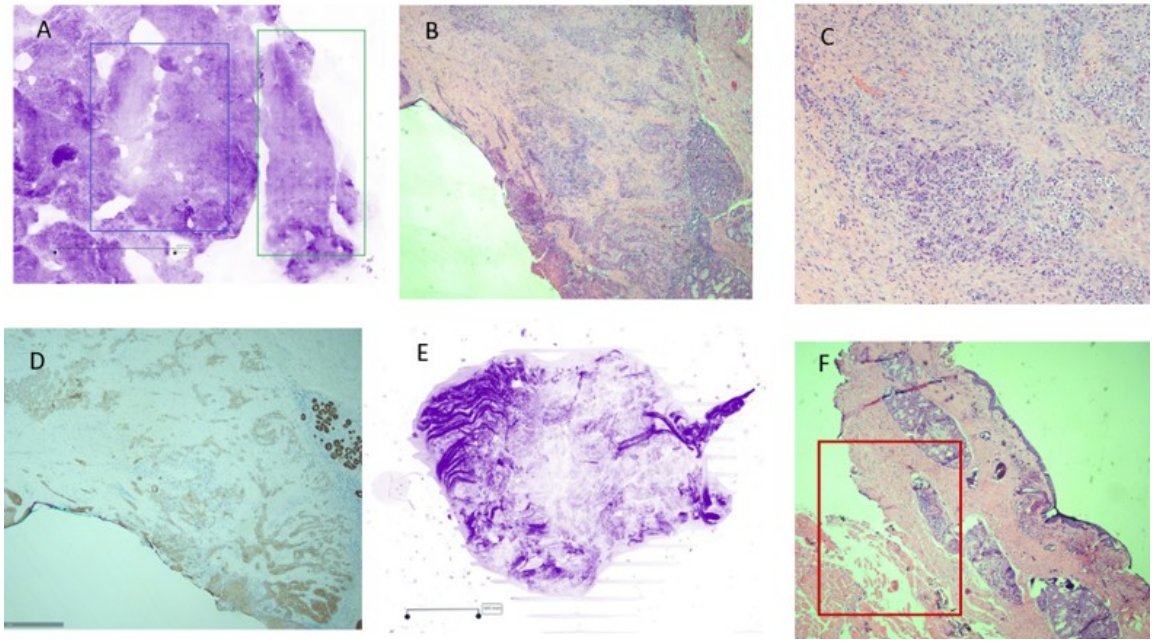

Figure 18. Representative concordant cases of margin assessment using Histolog® Scanner and conventional histopathology. *(A) Histolog® Scanner image (100x digital viewer) in which both the positive margin (green box) and the invasive tumor (blue box) are simultaneously visible within a single wide-field acquisition. (B) H&E-stained section illustrating the invasive tumor (4x). (C) H&E-stained section illustrating the invasive tumor at higher magnification (10x). (D) Cytokeratin immunohistochemistry (4x) confirming tumor involvement. (E) Histolog® Scanner image (100x digital viewer) of a negative deep margin. (F) Corresponding H&E-stained section (4x) confirming the negative deep margin (red box).*

4 DISCUSSION

MARGIN ASSESSMENT AND CURRENT LIMITATIONS IN TLM

The primary strength of transoral laser microsurgery (TLM) for glottic lesions lies in its ability to combine oncologic radicality with functional preservation, tailoring the excision to the tumor extent while minimizing the removal of the healthy laryngeal tissue. (71)

In this setting, pathological assessment of resection margins remains the gold standard for achieving complete tumor excision, representing one of the most critical prognostic factors in transoral laser microsurgery (TLM) for glottic lesions. However, secure margin evaluation is historically limited by a combination of specific factors, including the lack of a standardized definition of close margins, severe laser-induced thermal artifacts at the cutting edge (which potentially obscure epithelial and stromal details), and significant tissue shrinkage occurring both post-excision and during formalin fixation. (52,72)

Unlike open oncological resections, cordectomy specimens are inherently small, thin and occasionally fragmented, complicating macroscopic orientation and histopathological interpretation. Furthermore, intraoperative frozen section analysis is routinely difficult to perform due to the limited tissue volume and additional structural damage introduced during cryostat preparation. (73)

Collectively, these diagnostic challenges contribute to a high rate of false-positive margins in traditional pathology, which can lead to unnecessary additional treatments or second-look surgeries in more than 50% of instances. (30)

Within this complex clinical framework, a fresh-tissue imaging modality capable of rapidly evaluating a broad epithelial surface directly within the operating room (potentially minimizing processing artifacts) is exceptionally appealing.

The present prospective pilot diagnostic accuracy study provides preliminary evidence supporting the feasibility and diagnostic potential of the Histolog® Scanner as an innovative solution for real-time intraoperative margin assessment.

ANALYSIS OF DIAGNOSTIC PERFORMANCE AND LEARNING CURVE

In the present study, a high overall concordance with an agreement rate of 92.6% was observed between the Histolog® Scanner and conventional definitive histopathology. This inter-method agreement was classified as substantial and well beyond chance, as demonstrated by a Cohen’s kappa coefficient of 0.73.

Crucially, the learning curve associated with the implementation of this novel technology proved to be remarkably rapid for both the surgical and pathological teams. Indeed, this swift proficiency was facilitated by the intraoperative cooperation between the surgical and pathological teams, with the pathologist present directly in the operating room to assist the surgeon during the initial evaluation of the Histolog® scans. Consequently, the initial run-in phase required only 5 patients to fully standardize and complete the image acquisition and analysis protocol. In all subsequent 19 cases, a 100% rate of correct image acquisition and adequate interpretation was achieved. This swift transition from initial technical calibration to procedural stability strongly underscores the user-friendly nature of the device, suggesting it can be easily integrated into standard surgical workflows without requiring extensive training.

From a diagnostic performance standpoint, the Histolog® Scanner demonstrated a high specificity (93.8%) and an exceptional negative predictive value (97.4%). These metrics suggest that a negative assessment obtained via fresh-tissue surface imaging is highly reliable for ruling out margin involvement (R0). This high specificity translates into a dramatic reduction in false-positive rates compared to historical literature data. As previously noted (30), conventional pathology following TLM is frequently confounded by laser-induced thermal carbonization and specimen shrinkage, yielding false-positive margin reports in up to 50% of cases and driving unnecessary, costly second-look surgeries. By contrast, the Histolog® Scanner successfully minimizes these processing artifacts by scanning fresh tissue, effectively preventing unneeded extensions of the cordectomy, maximizing the preservation of healthy vocal fold tissue, and optimizing functional phonatory outcomes.

Furthermore, the outstanding negative predictive value of 97.4% addresses another critical oncological dilemma: the occurrence of local recurrence in patients with formally negative margins on definitive histology. (25,48,66,67) Our findings indicate that when the Histolog® Scanner provides a negative verdict, the certainty of true oncological clearance is extremely high. This intraoperative reassurance offers the surgical team a robust shield against early relapse, drastically minimizing the risk of leaving undetected residual disease.

Conversely, sensitivity was encouraging but lower than specificity (85.7%), with a limited number of false-negative cases observed.

METHODOLOGICAL CONSIDERATIONS

Taken together, these preliminary findings suggest that the Histolog® Scanner currently serves as a robust "rule-out" adjuvant instrument rather than a definitive replacement for standard pathology. In other words, while the technique appears highly promising for real-time intraoperative guidance, it is not yet sufficient to supplant the definitive H&E examination. Conventional histology remains essential for final diagnosis, margin confirmation, tumor grading, assessment of invasion, and evaluation of features that cannot be fully appreciated by surface-based imaging.

A low number of diagnostic discordances was detected between the two methods. Notably, the single false-negative result involved the deep margin, reflecting an intrinsic technological limitation of the instrument itself rather than a mere subjective interpretative error. Specifically, the Histolog® Scanner operates on a surface-based assessment of the specimen and its outer margins, which does not allow for the evaluation of the vertical depth of tumor invasion. Conversely, definitive histopathological analysis is routinely performed on coronal sections after formalin fixation and paraffin embedding. This structural difference in spatial perspective is particularly critical at the deep surgical margin, where the assessment of an Rclose status may require a cross-sectional view that cannot be fully reproduced by fresh-tissue surface scanning. This structural mismatch between the index test and the reference standard should be considered when interpreting diagnostic discrepancies. Rather than representing a mere limitation of the study alone, this discrepancy reflects a fundamental difference between digital fresh-tissue imaging and conventional histological sectioning. Therefore, Histolog® Scanner appears highly appropriate for the rapid assessment of broad epithelial or superficial mucosal surfaces, while its role in the evaluation of deep margins may require further optimization. As illustrated in Figure 19, the Histolog® Scanner enables simultaneous visualization of both the invasive tumor and the positive margin within a single wide-field acquisition, whereas conventional histopathology requires two separate sections to capture the same information.

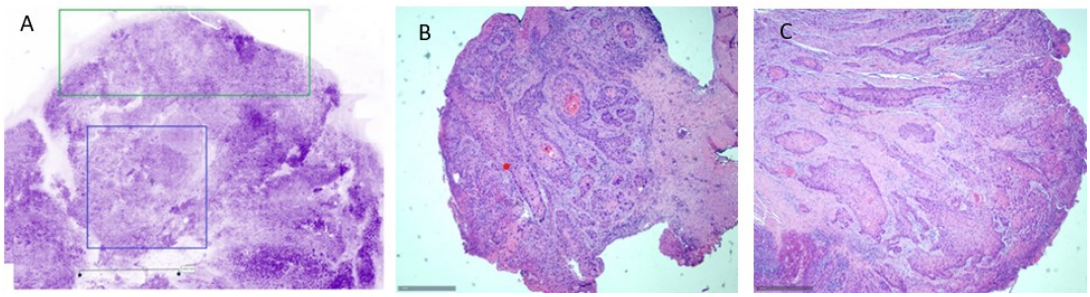

Figure 19: Comparative illustration of margin assessment in a true-positive concordant case. *(A) Histolog® Scanner image (100x digital viewer) demonstrating the ability to simultaneously visualize both the invasive tumor (blue box) and the positive margin (green box) within a single wide-field acquisition. (B) Corresponding H&E-stained section (4x) illustrating the positive margin. (C) Corresponding H&E-stained section (4x) illustrating the invasive tumor.*

Future protocols should investigate standardized specimen orientation, systematic scanning of multiple surfaces, ink-guided correlation with final histology, or combined use with targeted frozen sections in selected suspicious areas.

INTRAOPERATIVE COMMUNICATION

Beyond its diagnostic metrics, a major practical advantage of the Histolog® Scanner lies in its potential to optimize intraoperative communication between the surgical and pathological teams through real-time image sharing and the collaborative review of suspicious areas on the fresh specimen. This interactive capability represents a transformative operational shift in the framework of TLM, where the microscopic orientation of small specimens is notoriously complex and decisions regarding immediate margin enlargement must be made in real time.

Although this specific aspect was assessed qualitatively rather than formally quantified within our pilot protocol, our clinical experience strongly suggests that the Histolog® Scanner promotes a more dynamic, anatomically guided dialogue. Furthermore, this digital format enables remote interpretation by pathologists, bridging the physical distance between the operating theater and the laboratory, thereby reducing specimen transport and turn-around time. Additionally, the use of multiple digital evaluators can significantly strengthen diagnostic reproducibility, while opening promising future opportunities for AI-based image analysis to enhance both resection precision and surgical team confidence.

PER-MARGIN VERSUS PER-PATIENT ANALYSIS

Regarding the per-patient analysis conducted in this study, the findings necessitate a cautious interpretation due to their constrained clinical relevance in the specific framework of cordectomy margin assessment. This approach classifies a patient as positive when at least one positive margin is detected, both for the reference standard and for the Histolog® Scanner. However, such an analysis does not account for the fact that each patient may have multiple distinct margins assessed separately. As a result, both the Histolog® Scanner and conventional hematoxylin–eosin histology could record at least one positive margin for the same individual, while referring to different margins. In this scenario, the two methods would be classified as concordant in a per-patient analysis, despite being discordant at the clinically relevant margin-specific level.

This limitation is particularly critical in transoral laser cordectomy, a procedure where simply identifying a positive margin is insufficient for directing post-operative management or stratifying oncological risk. On the contrary, the precise identification of which margin is involved, as well as how many margins are positive, is essential. Different margins may carry distinct clinical implications, particularly when distinguishing between superficial and deep involvement, or between isolated and multiple positive margins. Consequently, margin-specific analysis yields more meaningful information than per-patient classification, as it aligns more closely with the intraoperative decision-making required during TLM and offers a truer reflection of the device's actual diagnostic accuracy.

Ultimately, this innovative approach is designed to integrate into, rather than substitute, current histopathological practices. Conventional H&E histology remains the reference standard, yet it is time-consuming and provides information only after the completion of tissue processing. On the other hand, frozen section analysis can provide intraoperative feedback, but it may be limited by sampling constraints, artifacts, and the small size of laryngeal specimens. In contrast, Histolog® Scanner offers a different type of information: rapid visualization of fresh tissue over a relatively broad surface, without fixation, embedding, or sectioning. This technology could function effectively as a preliminary screening method to target suspicious areas requiring further sampling or to support intraoperative decisions when the scanned surface appears clearly negative.

STUDY LIMITATIONS

Nevertheless, these encouraging preliminary results must be interpreted within the context of several inherent limitations. First, this research was designed as a pilot study with a relatively modest sample size; consequently, the resulting diagnostic performance metrics remain subject to a degree of statistical uncertainty. Second, the study was conducted exclusively at a single tertiary referral center, an operational constraint that may restrict the generalizability of our findings to other clinical settings. Third, because multiple margin specimens were frequently harvested and analyzed from the same patient, the diagnostic data points were not fully independent, introducing a potential clustering effect. Finally, there is a technical difference between the two methods. Fresh-tissue surface imaging and conventional cross-sectional histology look at the tissue from different spatial perspectives. This structural difference must be taken into account when analysing conflicting results.

COMPARISON WITH THE AVAILABLE LITERATURE

Despite the intrinsic challenges of laryngeal cancer, the current study addresses a clinically relevant and relatively underexplored problem in oncological surgery. Although the Histolog® Scanner has been investigated in several oncologic fields, its application in head and neck cancer remains extremely limited.

To date, the available literature includes only one study focused on head and neck oncology. Specifically, Abbaci et al. (74) evaluated the diagnostic accuracy of this technology for real-time tissue assessment, reporting an outstanding specificity of 98.8% and a Cohen's kappa coefficient of 0.80. Crucially, no previous studies have specifically assessed the Histolog® Scanner for margin evaluation in head and neck cancer, and absolutely none have focused on TLM cordectomy for glottic lesions. Therefore, the present research represents the first preliminary evaluation of this technology for intraoperative margin assessment in transoral laser surgery of the larynx. Taken together, both studies confirm that the Histolog® Scanner excels at ruling out malignancy with extreme precision, minimizing false-positive interpretations across different tissue types within the head and neck region.

To further contextualize these findings, the results of this study are highly consistent with the mean concordance values reported in the broader literature for other anatomical settings, which are generally above 70%. (69) In these larger oncological fields, this technology has already demonstrated high accuracy across various major anatomical districts. In breast cancer surgery, where the scanner has been most extensively investigated, a prospective

study, the POLARHIS study (75), demonstrated its clinical feasibility for entire lumpectomy surface evaluation, showing that its integration could potentially lead to a 30% to 75% reduction in re-operation rates compared to standard intraoperative protocols. Similarly, in robotic urological oncology, Mayor et al. (76) evaluated the scanner for whole-gland prostate surface assessment during radical prostatectomy. They reported a high specificity of 94% and a sensitivity of 79% for detecting clinically significant positive surgical margins, confirming fluorescence confocal microscopy as a reliable asset for real-time margin clearance. Finally, the effectiveness of this technology has been validated in dermatological surgery for micrographic resections. Specifically, a large-scale analysis by Peters et al. (77) on fresh basal cell carcinoma specimens demonstrated a specificity of 96% and a sensitivity of 73%, both of which reached 100% when evaluating punch biopsies.

Within this context, the striking alignment between the high diagnostic metrics observed in the present cohort and the emerging data from other fields reinforces the reproducibility and technological robustness of the device. Taken together, these robust data from larger oncological fields underscore the technical reliability and versatility of the Histolog® Scanner, reinforcing the clinical basis for its implementation and further validation in the highly challenging framework of glottic and laryngeal surgery.

5 CONCLUSIONS

In conclusion, from a clinical standpoint, the role of Histolog® scanner should currently be regarded as an adjunctive rather than substitutive tool. While this technique provides immediate and reliable intraoperative feedback, particularly for ruling out mucosal margin involvement, definitive histopathological examination remains indispensable, especially in cases with suspicious deep margins or borderline findings. Therefore, future studies should aim to confirm these diagnostic performance metrics in larger and preferably multicenter cohorts. Moreover, further research is needed to clarify the most appropriate clinical use of Histolog® scanner, evaluating whether it should be used as a triage tool, a complementary margin-mapping method, or a selective aid in technically challenging cases.

Finally, efforts should focus on standardizing image interpretation criteria, assessing interobserver agreement, and defining the performance of the technique across different cordectomy types and margin sites. Integrating these findings with structured surgical orientation protocols and refined pathological correlation will further enhance the value of this technology in routine clinical practice, ultimately aiming to optimize both oncological safety and post-operative functional voice outcomes for patients undergoing laryngeal surgery.

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