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Dipartimento di Scienze Mediche e Chirurgiche, Materno-Infantili e dell'Adulto

Unità Operativa Complessa di Otorinolaringoiatria

PROSPECTIVE STUDY ON THE APPLICABILITY OF HISTOLOG® SCANNER IN ORAL CAVITY CANCER SURGERY: ANALYSIS OF TIME, COSTS AND MARGIN OUTCOMES

RELATORE:
PROF. FRANCESCO MATTIOLI

CORRELATORE:
DOTT. SAMUELE BALDELLI

TESI DI LAUREA DI:
EMMA BEDINI

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Alla piccola Emma, che in quinta elementare disse:
"Io da grande farò il liceo scientifico Tassoni,
e poi l'università di medicina a Modena"

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1 INTRODUCTION

1.1 Oral cavity cancer

Oral cavity cancer (OCC) refers to a heterogeneous group of malignant neoplasms arising within the oral cavity and belonging to the broader group of head and neck malignancies.

The oral cavity is bounded anteriorly by the lips, inferiorly by the mylohyoid muscle and mandibular alveolar ridge, superiorly by the hard palate and maxillary alveolar ridge, laterally by the buccal mucosa, and posteriorly by the circumvallate papillae and the anterior tonsillar pillars, which mark the transition to the oropharynx (1).

OCC may involve the buccal mucosa, floor of mouth, oral tongue, alveolar ridges, retromolar trigone, hard palate, or mucosal lips. Among these subsites, the oral tongue is the most frequently affected location (2).

Approximately 90% of oral cavity cancers originate from the squamous epithelium and are therefore commonly classified as oral cavity squamous cell carcinoma (OCSCC) (3). Other malignant tumors may originate from connective tissue, minor salivary glands, lymphoid tissue and melanocytes, or rarely they may represent metastasis from a distant primary tumor (4).

Given that OCSCC accounts for the vast majority of oral cavity malignancies, this thesis will focus exclusively on this histological subtype.

1.2 Epidemiology

Oral cavity cancer ranks as the sixteenth most common malignancy worldwide. In 2022, a total of 389,486 new cases of lip and oral cavity cancer were reported. A higher incidence is observed in men compared with women, and in both sexes, incidence increases markedly with advancing age. Globally, age-standardized incidence rate is 4.0 per 100,000 (5,6).

Oral cavity cancer is also the fifteenth leading cause of cancer-related mortality worldwide. In 2022, a total of 188,438 deaths were attributed to lip and oral cavity cancers. Mortality is higher in men compared with women and increases in parallel

with aging in both sexes. Globally, age-standardized mortality rate is estimated at 1.9 per 100,000 (5,6).

The disease shows marked geographical variation, reflecting differences in exposure to risk factors. The highest number of cases is reported in South-Central Asia, particularly in India, Sri Lanka, Bangladesh and Pakistan, and in Melanesia, with Papua New Guinea bearing a particularly high burden due to the widespread habit of chewing betel quid (3,7,8). Asia also shows the highest age-standardized mortality rate (2.4 per 100,000) and the highest number of deaths globally (5,6).

Within Europe, the highest incidence rates are observed in Central and Eastern Europe where tobacco use and alcohol consumption represent the main risk factors (9).

1.3 Risk factors

Tobacco smoking and alcohol consumption are the two major risk factors for OCSCC, accounting for up to 90% of cases. Their combined use exerts a strong synergistic effect, increasing the risk of cancer up to 35-fold. In contrast, in specific populations of South-Central Asia and Papua New Guinea, betel quid and tobacco chewing remain the predominant risk factors (3,8).

Smoking is associated with a 3-fold increased risk of oral cavity squamous cell carcinoma. Risk increases proportionally with both smoking intensity and duration and decreases substantially after cessation, with former smokers achieving a 50% risk reduction within seven years (10). Passive exposure to tobacco smoke is considered a significant risk factor as well (3).

Alcohol consumption is associated with an increased risk of oral cavity squamous cell carcinoma, even at low intake levels (<12.5 g or 1 drink per day). The risk is dose-dependent, with up to a 5-fold increase observed among heavy drinkers (>50 g or >4 drinks per day), and decreases progressively after cessation. Long-term abstinence (≥20 years) is associated with a 55% risk reduction (11–13).

Additional risk factors include hereditary and immunological conditions. Fanconi Anemia is associated with a markedly increased risk of head and neck cancers, with the oral cavity being the most affected site (14). Immunosuppression, related to both Human Immunodeficiency Virus (HIV) infection and post-transplant treatments, is also implicated. Similarly, poor oral hygiene and dietary deficiencies, particularly low

intake of fruits, carotenoids, and green vegetables, have been identified as risk factors for OCSCC (15).

Conversely, the role of Human Papillomavirus (HPV) remains controversial. The International Agency for Research on Cancer (IARC) classified Human Papillomavirus 16 as Group 1 carcinogen for cancer of oral cavity and pharynx, whereas evidence for Human Papillomavirus 18 remains limited (16). However, HPV is estimated to be involved in only a small portion of OCSCC cases, and its impact on survival appears limited (17).

1.4 Oral potentially malignant disorders

Although most OCSCCs develop de novo, a subset arises from pre-existing precursors collectively referred to as oral potentially malignant disorders (OPMDs) (18). A recent systematic review and meta-analysis reported that approximately 29% of OCSCCs arise from OPMDs, although lower proportions have been described in other studies (19,20).

OMPDs comprise a heterogeneous group of oral mucosal lesions and conditions associated with an increased risk of malignant transformation. These include lesions such as oral leukoplakia, oral erythroplakia, proliferative verrucous leukoplakia, lesions in reverse smokers and actinic cheilitis, as well as conditions like oral submucous fibrosis, oral lichen planus, lupus erythematosus, oral lichenoid reactions, and graft-versus-host disease. Certain hereditary conditions including dyskeratosis congenita and epidermolysis bullosa are likewise considered within the spectrum of OPMDs (21,22). Among these entities, oral leukoplakia is the most common OPMD in Europe, whereas oral submucous fibrosis predominates in South Asian populations (23).

OPMDs may remain stable, regress or progress to carcinomas over time, with an overall reported malignant transformation rate of approximately 8% (24).

The risk varies significantly among different entities, with higher rates observed in proliferative verrucous leukoplakia and oral erythroplakia, and is influenced by both lesion-related and patient-related factors. The presence and severity of epithelial dysplasia on histopathological examination are among the most important predictors of malignant transformation (24–26). Additional risk factors include female sex, older age, lesion size greater than 200 mm^2, and anatomic location, with lateral and ventral

tongue and floor of mouth being frequently affected. Moreover, malignant transformation appears to occur more frequently within the first five years after diagnosis (26).

1.5 Clinical presentation

The tongue is the most common site for the development of OCSCC, accounting for approximately 40% of all cases. The floor of the mouth is the second most frequently affected site, particularly in Western populations, whereas the buccal mucosa is more commonly involved in regions where betel quid chewing is a widespread habit (27,28). Clinical presentation varies significantly according to the stage of disease. Early lesions are often asymptomatic but may be clinically evident as alterations in the texture and appearance of the mucosal lining. Early OCSCCs may present as white patches, red patches, or mixed red-and-white lesions. As the disease progresses, superficial ulceration may develop on the mucosa surface, and the lesion may further enlarge through either an exophytic or endophytic growth pattern, appearing respectively as a papillary or fungating mass, or as a depressed ulcer with raised and rolled margins (27,29). Later-stage symptoms include pain, bleeding, halitosis, dysarthria, dysphagia, odynophagia, and loosening of teeth. Trismus may also occur, suggesting involvement of the medial pterygoid muscle and the masticator space (27,30). In locoregionally advanced stages cervical lymphadenopathy may be observed, whereas in distant metastatic disease hemoptysis, dyspnea, weight loss, and general functional decline may be present (30). The lungs represent the most common site of distant metastasis (27).

1.6 Diagnostic workup

Despite the oral cavity being readily accessible to clinical examination, OCSCC is frequently diagnosed at advanced stages (3).

Early detection is essential, as prognosis is closely related to the stage of disease at diagnosis: 5-year survival rate is approximately 80% for localized disease, decreasing to 40-50% in the presence of regional metastases and to less than 10% in patients with distant metastatic disease (28).

1.6.1 Medical history and clinical examination

The diagnostic workup begins with a thorough medical history and a comprehensive clinical examination.

Patients should be evaluated for risk factors, including alcohol or tobacco use, and undergo visual inspection and digital palpation to detect tissue abnormalities and assess the epicenter and the extent of the lesion (28,30). Flexible nasopharyngolaryngoscopy is an integral component of the comprehensive clinical examination as it enables visualization of anatomical sites not readily accessible by direct inspection and palpation, allowing assessment of tumor extent and identification of additional primary lesions (30,31).

Clinical evaluation should also include careful assessment of the neck, since regional nodal metastases, both clinical and occult, are present in up to 40% of oral cavity cancers at diagnosis. Cervical metastases most commonly involve ipsilateral submental and submandibular nodes (level I) and upper jugulodigastric nodes (level II). Middle jugulodigastric nodes (level III) may also be affected, whereas metastatic spread to levels IV and V is less frequently observed. In case of advanced locoregional disease or lesions located near the midline, the risk of contralateral nodal metastasis increases. Any palpable neck mass should be assessed in terms of size, location, number and fixation to skin or adjacent deep tissues (28,30,32).

A careful clinical examination is essential, as it enables the identification of up to 99% of oral cavity carcinomas (29).

1.6.2 Biopsy and diagnostic adjuncts

Physical examination findings suggestive of malignancy may include lesions with ulceration or necrosis, friable tissue prone to bleeding, submucosal induration, and alterations in mucosal texture, color or integrity, as well as fixation of the lesion to the surrounding tissues (28,33). When suspicious clinical features are identified and persist for more than two weeks, the lesion should be biopsied, according to the World Health Organization and the National Institute of Dental and Craniofacial Research. More recently, the American Dental Association has suggested shortening this timeframe to 10-14 days (34,35).

Biopsy followed by histopathological examination represents the gold standard for the definitive diagnosis of OCSCC. Oral biopsies may be either incisional or excisional.
Incisional biopsy is considered the preferred initial approach for suspicious oral lesions, as it preserves lesion architecture and margins. Incisional biopsy can be performed using either a punch or a scalpel. Punch biopsy is often favored because of its simplicity, reproducibility, and anatomic preservation. However, scalpel incisional biopsy remains the reference standard when assessment of depth of invasion (DOI), evaluation of larger tissue area, or more comprehensive architectural analysis is required, or when a previous punch biopsy is inconclusive.
By contrast, excisional biopsy is generally reserved for cases in which incisional biopsy is non-diagnostic or discordant with clinical suspicion, as it may compromise subsequent treatment planning through loss of clinically visible margins, tissue distortion, and scarring (36).

Over the years, additional adjunctive techniques have been developed to support lesion assessment, including brush biopsy, toluidine blue staining, and autofluorescence imaging. However, these methods are considered complementary tools and do not replace histopathological examination, which remains the gold standard for the definitive diagnosis of OCSCC (29,36).

1.6.3 Radiological imaging

Once the diagnosis has been established histologically, radiological imaging is undertaken in order to determine disease stage and guide therapeutic planning (29,39). The primary objectives of imaging are assessment of the primary tumor site, evaluation of cervical lymph node involvement, and detection of distant metastases.
According to the National Comprehensive Cancer Network (NCCN) Guidelines, the imaging workup for oral cavity cancer may include contrast-enhanced computed tomography and magnetic resonance imaging with and without contrast for primary tumor evaluation, chest computed tomography with or without contrast when clinically indicated, and fluorodeoxyglucose positron emission tomography/computed tomography in advanced stages (2).

In order to evaluate the primary tumor site and cervical lymph node involvement, both computed tomography (CT) and magnetic resonance imaging (MRI) can be performed (2). The choice between contrast-enhanced CT and MRI depends on patient-related and tumor-related factors, such as the presence of dental artefacts, the patient's ability to remain still during the examination, and the size and anatomical location of the primary tumor. However, in the diagnostic workup of OCSCC, both CT and MRI are typically required as they provide complementary information (37). Specifically, contrast-enhanced CT offers excellent assessment of cortical bone involvement, whereas MRI provides superior soft tissue resolution and improves the detection of perineural spread and bone marrow involvement (38).

Compared with MRI, CT is more susceptible to dental metallic artefacts, whereas MRI has lower spatial resolution and is more prone to motion artefacts due to longer acquisition times (38). Both modalities can be used preoperatively to estimate depth of invasion (DOI), defined as the distance from the basement membrane of the adjacent normal mucosa to the deepest point of tumor infiltration, although MRI shows a slightly higher correlation with histopathological measurements (39). Intraoral ultrasonography (IOUS) demonstrates an even higher correlation with pathological DOI and may serve as an alternative to CT and MRI for preoperative DOI assessment, particularly for oral tongue cancers (38).

Fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) may provide additional information by facilitating localization of the primary tumor, identifying regional lymph node involvement, and detecting distant metastases and synchronous malignancies (38).

FDG-PET/CT is recommended in patients with locoregionally advanced disease in combination with contrast-enhanced CT and MRI, and for the evaluation of cervical nodal metastases when no primary tumor is identified on clinical examination or conventional imaging (2,38).

When FDG-PET/CT is not performed, chest CT is recommended to assess pulmonary metastases and mediastinal lymph node involvement (2).

Additional imaging modalities include neck ultrasound, useful for the evaluation and biopsy guidance of cervical lymph nodes, and panoramic dental radiography for pre-treatment dental assessment (2,38).

1.7 Staging

1.7.1 TNM classification and stage grouping

The universally adopted staging system for oral cavity squamous cell carcinoma is the tumor-node-metastasis (TNM) classification developed by the American Joint Committee on Cancer (AJCC), currently in its 8th edition. The TNM system evaluates three components: primary tumor (T), regional lymph nodes (N), and distant metastases (M). The T category is defined by tumor size and depth of invasion (DOI), the N category is based on the number, size, laterality and presence of extranodal extension (ENE) of lymph nodes, whereas M category reflects the presence or absence of distant metastatic disease. These three components are combined to assign an overall prognostic stage group ranging from Stage I to Stage IVC (2).

The AJCC 8th edition distinguishes between clinical staging (cTNM) and pathological staging (pTNM) (2). Clinical staging is determined prior to treatment based on physical examination and radiological imaging and guides treatment selection, whereas pathological staging is established postoperatively through histopathological examination of the resected specimen and is used to guide adjuvant therapy and estimate prognosis (3). Discrepancies between clinical and pathological staging are common in OCSCC, with pathological upstaging frequently observed after surgical resection (40).

The AJCC 8th edition TNM classification for oral cavity carcinoma is summarized in Table 1, Table 2, and Table 3; corresponding prognostic stage groups are summarized in Table 4.

Table 1. Primary Tumor (T).

Category	Definition
Tx	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Tumor ≤2 cm with DOI ≤5 mm
T2	Tumor ≤2 cm with DOI >5 mm and ≤10 mm, or tumor >2 cm and ≤4 cm with DOI ≤10 mm
T3	Tumor >2 cm and ≤4 cm with DOI >10 mm, or tumor >4 cm with DOI ≤10 mm
T4	Moderately advanced or very advanced local disease
T4a	Moderately advanced local disease Tumor >4 cm with DOI >10 mm, or invasion of adjacent structures (e.g., cortical bone of mandible/maxilla, maxillary sinus, skin of face)
T4b	Very advanced local disease Invasion of masticator space, pterygoid plates, skull base, and/or encasement of internal carotid artery

DOI = Depth of invasion
Adapted from the AJCC Cancer Staging Manual, 8th Edition (41).

Table 2. Regional Lymph Nodes (N).

Category	Clinical N (cN)	Pathological N (pN)
NX	Regional lymph nodes cannot be assessed	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node ≤3 cm, ENE (–)	Metastasis in a single ipsilateral lymph node ≤3 cm, ENE (–)
N2	Metastases in a single ipsilateral node >3 cm and ≤6 cm, ENE (–); *or* metastases in multiple ipsilateral lymph nodes, none >6 cm, ENE (–); *or* in bilateral or contralateral lymph nodes, none >6 cm, ENE (–)	Metastasis in a single ipsilateral lymph node ≤3 cm and ENE (+); *or* single ipsilateral >3 cm and ≤6 cm, ENE (–); *or* metastases in multiple ipsilateral lymph nodes, none >6 cm, ENE (–); *or* in bilateral or contralateral lymph nodes, none >6 cm, ENE (–)
N2a	Metastasis in a single ipsilateral lymph node >3 cm and ≤6 cm, ENE (–)	Metastasis in single ipsilateral lymph node ≤3 cm and ENE (+), or a single ipsilateral >3 cm and ≤6 cm, ENE (–)
N2b	Metastases in multiple ipsilateral lymph nodes, none >6 cm, ENE (–)	Metastases in multiple ipsilateral lymph nodes, none >6 cm, ENE (–)
N2c	Metastases in bilateral or contralateral lymph nodes, none >6 cm, ENE (–)	Metastases in bilateral or contralateral lymph nodes, none >6 cm, ENE (–)

N3	Metastasis in a lymph node >6 cm, ENE (–); *or* metastasis in any node(s) and clinically overt ENE (+)	Metastasis in a lymph node >6 cm, ENE (–); *or* metastasis in a single ipsilateral node >3 cm and ENE (+); *or* multiple ipsilateral, contralateral or bilateral nodes any with ENE (+); *or* single contralateral node of any size and ENE (+)
N3a	Metastasis in a lymph node >6 cm, ENE (–)	Lymph node >6 cm, ENE (–)
N3b	Metastasis in any node and clinically overt ENE (+)	Metastasis in a single ipsilateral node >3 cm and ENE (+), or multiple ipsilateral, contralateral or bilateral nodes any with ENE (+), or single contralateral node of any size and ENE (+)

ENE = Extranodal extension
Adapted from the AJCC Cancer Staging Manual, 8th Edition (41).

Table 3. Distant Metastasis (M).

Category	Definition
M0	No distant metastasis
M1	Distant metastasis

Adapted from the AJCC Cancer Staging Manual, 8th Edition (41).

Table 4. Prognostic Stage Groups.

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

Adapted from the AJCC Cancer Staging Manual, 8th Edition (41).

1.7.2 DOI and ENE in the TNM classification

The AJCC 8th edition introduced two major modifications: the incorporation of depth of invasion (DOI) and the inclusion of extranodal extension (ENE) into the T and N categories, respectively (42).

DOI is defined as the distance from the basement membrane of the closest adjacent normal mucosa to the deepest point of tumor invasion, measured along a line perpendicular to the tumor base. It differs from tumor thickness, which is measured from the tumor surface to the deepest point of invasion (43). Conversely, ENE is defined as the extension of metastatic tumor beyond the lymph node capsule into the surrounding connective tissue (44).

Both DOI and ENE have been demonstrated to be independent prognostic factors for oral cavity squamous cell carcinoma, leading to improved prognostic stratification in the AJCC 8th edition compared with the previous version (45).

1.8 Prognostic factors

The prognosis of OCSCC is influenced by a wide range of factors, which can be broadly categorized into patient-related and tumor-related factors. Among these, tumor-related factors exert the greatest independent prognostic impact (46).

Disease stage at diagnosis remains one of the strongest determinants of outcome, with overall survival progressively decreasing with advancing stage, as summarized in Table 5 (28).

Beyond disease stage, several additional tumor characteristics have been independently associated with overall survival. These include T and N stage, the presence of distant metastases, and histopathological features such as margin status, depth of invasion (DOI), perineural invasion (PNI), lymphovascular invasion (LVI), histological grade, and extranodal extension (ENE). By contrast, patient-related factors exert a more limited influence, with tobacco use being the only variable independently associated with worse overall survival, although its effect size remains modest. The evidence supporting an independent prognostic role for advanced age remains limited (46).

Table 5. Five-year Overall Survival Rates for OCC by AJCC Stage.

Stage	5-year Overall Survival
I	80%
II	70%
III	60-65%
IVA-IVB	35-45%
IVC	<10%

Adapted from Howard and al. (28).

1.9 Treatment strategies

1.9.1 General principles

Accurate staging is fundamental for selecting the most appropriate treatment strategy (2).

The primary goal of therapeutic management is to achieve the highest possible cure rate while preserving organ function and minimizing treatment-related morbidities. The oral cavity plays a central role in essential functions such as speech, swallowing, mastication, and facial aesthetics, and all these functions should be considered during therapeutic decision-making. Therefore, a multidisciplinary evaluation, involving head and neck surgeons, radiation and medical oncologists, dental and nutritional specialists, and speech and language therapists, among others, is required before the initiation of any therapy (2,37).

The three main treatment modalities for oral cavity squamous cell carcinoma are surgery, radiotherapy (RT), and chemotherapy (CT), used alone or in combination. Treatment selection depends on several factors, including tumor location, size, and stage, as well as patient comorbidities, nutritional status, treatment tolerance, and personal preferences (3).

Among the available treatment modalities, surgery is the preferred approach for resectable OCSCC, as the oral cavity is readily accessible through a transoral approach, enabling favorable oncologic outcomes with limited morbidity while providing detailed histopathological information that may guide subsequent treatment planning (47,48).

1.9.2 Stage-based treatment approach

In early-stage disease (stage I–II) and in locally advanced resectable disease (stage III-IVA) surgery represents the standard of care (2).
Definitive radiotherapy may be considered as an alternative in early-stage patients who are not surgical candidates or who decline surgery (2), although RT to the oral cavity is associated with significant toxicities, including xerostomia, dysphagia, and osteoradionecrosis, which further support surgery as the preferred treatment modality when feasible (31).
In addition, in locally advanced resectable disease, perioperative immunotherapy with pembrolizumab has recently been introduced as an adjunct to surgery in selected PD-L1-positive patients (2).

In unresectable disease, which includes tumors invading critical structures (T4b), patients unfit for surgery, unresectable primary tumor, or unresectable nodal disease, treatment is generally based on non-surgical approaches. Concurrent cisplatin-based chemoradiotherapy represents the standard of care in patients with adequate performance status, whereas in other cases palliative radiotherapy, single-agent systemic therapy, or best supportive care may be considered (2).

In the metastatic setting (stage IVC), treatment is primarily palliative and may include platinum-based chemotherapy and/or immune checkpoint inhibitors (2).

1.9.3 Surgical management

The goal of surgery is complete tumor removal with histologically confirmed tumor-free margins, while preserving optimal functional and aesthetic outcomes (2,37).
The surgical procedure consists of an ablative phase, in which the tumor is excised, and a reconstructive phase, aimed at restoring oral cavity form and function (37).
Surgical resection should be planned on the basis of the extent of the tumor, as determined by clinical examination and radiographic imaging (2). Smaller tumors can be accessed through a transoral approach, whereas larger or posterior lesions may require a lip-split mandibulotomy or pull-through technique (49). When feasible, en

bloc resection is recommended to improve pathological assessment of tumor margins (2,50).

After surgical resection, reconstruction is performed with options ranging from primary closure or local flaps for smaller defects to microvascular free tissue transfer, which remains the gold standard for extensive defects (51).

Surgical treatment is frequently associated with concomitant neck dissection, which consists of the surgical removal of lymph node-bearing tissue from defined cervical levels. Neck dissection is generally classified as selective, when fewer than five nodal levels are removed, or comprehensive, when all cervical levels I-V are included (50,52).

In clinically node-negative (cN0) disease, selective neck dissection including levels I-III is recommended when DOI is greater than 3 mm. Conversely, in clinically node-positive (cN+) disease, selective or comprehensive neck dissection may be performed in N1–N2 disease, whereas comprehensive neck dissection is recommended in N3 disease (2).

Neck dissection is generally performed ipsilateral to the primary tumor, while bilateral dissection is indicated for midline or crossing lesions and subsites with bilateral lymphatic drainage (2). In cases of direct tumor extension into the neck, en bloc resection with the primary tumor is recommended (2).

Recently, sentinel lymph node biopsy (SLNB) has emerged as an alternative to elective neck dissection in early-stage disease, demonstrating comparable oncologic outcomes in terms of regional recurrence, disease-free survival, and overall survival, while significantly reducing morbidity and improving aesthetic outcomes (2,53). In cases of a positive SLNB, completion neck dissection is recommended (2).

1.9.4 Adjuvant treatment

Surgery may be followed by adjuvant treatment, including radiotherapy and/or chemotherapy, depending on postoperative histopathological findings. Adjuvant treatment is indicated in the presence of adverse pathologic features, including extranodal extension, positive or close surgical margins, pT3 or pT4 primary tumor, pN2 or pN3 nodal disease, nodal involvement of levels IV or V, and perineural or lymphovascular invasion (2).

In the presence of extranodal extension, concurrent chemoradiotherapy with cisplatin is recommended, whereas in cases of positive margins, re-resection should be attempted if feasible, followed by radiotherapy if negative margins are achieved. In all other cases, adjuvant radiotherapy alone or concurrent chemoradiotherapy may be considered. Adjuvant radiotherapy alone is also considered in patients with a single positive lymph node in the absence of other adverse features (2).

Within this context, the identification of adverse pathologic features is a cornerstone in the management of oral cavity squamous cell carcinoma, and among these characteristics, the status of surgical margins deserves particular attention.

1.10 Surgical margins in oral cavity squamous cell carcinoma

1.10.1 Definition and classification

A surgical margin is defined as the measurable distance between the closest edge of the invasive tumor and the resection surface of the excised specimen.
According to the Royal College of Pathologists, a margin is considered clear when the distance between tumor and resection edge is greater than 5 mm, close when it ranges from 1 to 5 mm, and positive when invasive carcinoma is present within 1 mm of the resection edge (54).
This three-tier classification system is the most widely accepted classification for oral cavity squamous cell carcinomas, and the 5 mm threshold is traditionally considered the reference standard for defining margin clearance. However, the optimal cutoff for an adequate surgical margin remains controversial. Several studies have proposed alternative thresholds derived from outcome-based analysis, but the heterogeneity among these studies, including differences in tumor location, tumor stage, and margin compartment, has limited the establishment of an alternative universally accepted cutoff, and a margin greater than 5 mm remains the most broadly accepted criterion for defining a clear surgical margin in oral cavity squamous cell carcinoma (54,55).

1.10.2 Role of dysplasia at the surgical margin

Dysplasia is defined as architectural and cytological alterations in the epithelium and is traditionally classified as mild, moderate, or severe according to the extent of epithelial involvement (56).

Both severe and moderate dysplasia have been included in the definition of a positive margin by The College of American Pathologists, as they have been associated with an increased risk of locoregional recurrence (57).

1.10.3 Determinants of surgical margin status and prognostic significance

Surgical margin status may be influenced by both tumor-related and anatomical factors.

Higher T category and histological tumor grade have been associated with an increased risk of inadequate margins, mainly due to larger tumor size and more infiltrative growth patterns; similar associations have been reported for lymphovascular invasion and lymph node positivity (58).

Tumor location represents another key determinant, with subsites such as the floor of the mouth, buccal mucosa, and retromolar trigone carrying a significantly higher risk of inadequate resection, due to the complex anatomical of the oral cavity and proximity to critical anatomical structures (59).

Inadequate surgical margins are recognized as an adverse pathological feature associated with poorer oncological outcomes, including increased risk of local recurrence and reduced survival (55). A stepwise decline in prognosis has been observed as margin status worsen from clear to close and ultimately to positive, with positive margins being strongly associated with both higher recurrence rates and worse overall and disease-specific survival (60,61). These findings underscore the importance of achieving tumor-free surgical margins in OCSCC surgery (55).

1.10.4 Deep and mucosal surgical margins

Surgical margins can be further distinguished into deep margins or mucosal margins according to their anatomical location. Deep margin refers to the distance between the deepest point of tumor invasion and the surgical cut surface, whereas mucosal margin

represents the distance from the tumor edge to the closest mucosal resection surface (62).

This distinction is clinically relevant, and the National Comprehensive Cancer Network (NCCN) Guidelines recommend to assess histologically both deep and mucosal margins (2). Indeed, recent evidence suggests that prognostic significance may differ between these two compartments, with inadequate deep margin being significantly associated with worse local recurrence-free survival, overall survival, and disease-specific survival (62).

This is particularly relevant since the deep margin is more challenging to assess intraoperatively (57).

1.11 Intraoperative margin assessment

1.11.1 Rationale for intraoperative margin assessment

Given the strong association between margin status and oncological outcomes, achieving clear surgical margins represents a primary goal in OCSCC surgery (55). Notably, surgical margin status is the only parameter among all oncological prognostic factors that can be directly influenced by the surgical team (54).

During resection, surgeons primarily rely on visual inspection and palpation to distinguish tumor from surrounding healthy tissue and to guide the extent of resection (63). Surgeons are advised to achieve a gross margin of at least 1 cm of clinically normal tissue around the tumor at the time of resection to maximize the likelihood of obtaining adequate histopathological margins. This recommendation accounts for postoperative tissue shrinkage, which may range from 20% to 40% as a result of muscle contraction. This phenomenon is particularly pronounced in specific subsites such as ventral tongue, floor of the mouth, soft palate, and buccal mucosa, where a wider gross margin of 1.5 cm is generally recommended in order to obtain adequate margins (64,65).

However, visual inspection and palpation alone are often insufficient in achieving adequate resections, particularly given the complex anatomy of the oral cavity, and adequate tumor resection is achieved only in a minority of cases (54,63).

This underlines the need for adjunctive intraoperative techniques that may enable immediate evaluation of margin status and, when necessary, revision of inadequate resections (63).

1.11.2 Margin sampling methods

A key methodological consideration in intraoperative margin assessment is how tissue samples are obtained.

Two methods have been described for intraoperative margin sampling: sampling from the tumor bed and sampling directly from the resection specimen. These strategies are commonly referred to as defect-driven and specimen-driven approaches, respectively (66).

Traditionally, tumor bed sampling has been preferred by surgeons over the specimen-driven approach, as it is less time-consuming and technically easier (65). However, a gradual shift in clinical practice has been observed over time, as shown by the American Head and Neck Society surveys reporting an increase in surgeons favoring specimen-driven margins from 14% in 2005 to 55% in 2020 (66).

This shift is supported by an increasing body of evidence demonstrating the advantages of the specimen-driven approach. By preserving the intact three-dimensional architecture of the resected specimen, this method enables accurate tumor orientation, precise measurement of tumor-to-margin distance, and independent assessment of all margin planes, including the deep margin, which may be difficult to evaluate from the surgical defect at certain subsites. Additionally, specimen-driven sampling has been associated with lower rates of final positive margins and improved local control. Conversely, tumor bed-based sampling has shown no prognostic value and low sensitivity in detecting positive margins on the resection specimen. Moreover, the fragmentation inherent to defect-driven sampling hampers the reconstruction of spatial relationships among tissue fragments, potentially leading to an underestimation of margin positivity (64,66).

Accordingly, the specimen-driven approach is considered the standard of care in clinical practice (64), consistent with the latest American Joint Committee on Cancer recommendations (67).

1.11.3 Frozen section analysis

Frozen section analysis (FSA) is currently the most widely used method for intraoperative margin assessment, enabling rapid evaluation of surgical margin status and, when necessary, immediate re-resection (66).

1.11.3.1 Technique and workflow

Frozen section analysis is performed in the pathology department, where the en bloc resection specimen is transported directly from the operating room.

First, the pathologist orients the specimen anatomically, a step often facilitated by sutures or clips placed by the surgeon to mark key anatomical landmarks. Similarly, when supplemental margins are obtained from the surgical defect, these must be properly oriented to allow correct spatial correlation with the main specimen.

After orientation, the specimen undergoes gross examination, and the different resection planes are inked in distinct colors, with particular attention to the mucosal and deep soft tissue margins.

The margins are then evaluated by serial sectioning at 5–10 mm intervals along the resection planes, with areas of concern selected for histological assessment. Perpendicular sections are preferred when measurement of tumor distance from the margin is required, whereas parallel sections are used when margins appear clearly free of tumor.

Selected tissue samples are embedded in optimal cutting temperature compound, rapidly frozen and sectioned at approximately 5 μm in a cryostat, stained with hematoxylin and eosin, and examined microscopically.

The results are then promptly communicated to the surgical team, typically by telephone.

An average turnaround time of approximately 20 minutes is reported for simple specimens, although complex en bloc resections may require longer processing times.

Following the completion of procedure, the specimen undergoes definitive histopathological evaluation (66).

1.11.3.2 Diagnostic performance and limitations

Compared to final pathology, frozen section analysis has demonstrated high diagnostic reliability, with reported accuracy rates of 96.7%, sensitivity of 83.1%, and specificity of 97.9% (68).

However, frozen section analysis carries inherent limitations.

First, the technique is time-consuming, with an average turnaround time of approximately 20 minutes for simple specimens, increasing to 45 minutes or longer in complex cases. This time includes specimen orientation, freezing, sectioning, staining, and interpretation, with additional time required for specimen transport and potential delays related to pathology service availability (66).

Second, the procedure is affected by technical constraints: the freezing process may alter cytological details and tissue architecture, thereby predisposing to interpretative errors, whereas the sectioning step results in tissue consumption. In addition, frozen section analysis has an intrinsic sampling vulnerability, as only a small fraction of the resection margin can be examined. Consequently, large areas of the specimen remain unsampled, particularly in extensive resections, increasing the risk of undetected positive margins (66,68).

Beyond these limitations, frozen section analysis is also a resource-intensive procedure, requiring an experienced pathologist, a dedicated pathology laboratory, and efficient specimen transport between the operating room and pathology department, and is associated with considerable costs (69). *DiNardo et al.* estimated an average cost of frozen section analysis of up to $3,123 per patient, including both pathological evaluation and operating room expenses (70).

Despite its limitations, frozen section analysis remains the gold standard for intraoperative margin assessment (66).

However, new approaches have been developed to overcome its drawbacks and improve margin evaluation during surgery (71).

1.11.4 Emerging techniques

Several alternative or complementary techniques to frozen section analysis have been investigated for intraoperative margin assessment in oral cavity squamous cell carcinomas, with the aim of improving margin evaluation. These approaches can be broadly categorized into wide-field and narrow-field techniques (71).

1.11.4.1 Wide-field techniques

Wide-field techniques allow real-time visualization of large tissue areas and have been applied intraoperatively both to guide surgical resection and to assess margin adequacy. These techniques include narrow-band imaging, autofluorescence imaging, fluorescence-guided imaging with tumor-targeted probes, and vital dyes such as toluidine blue (71).

In particular, toluidine blue (TB) is a metachromatic dye with high affinity for DNA and RNA, capable of staining cells with increased nucleic acid content, including malignant cells. TB has been applied directly to resection specimens to detect positive mucosal margins and to guide frozen section sampling, with reported sensitivity of 100% and specificity approaching 94%. However, its use is inherently limited to the superficial mucosal assessment and does not allow reliable evaluation of deep surgical margins (72).

1.11.4.2 Narrow-field techniques

Narrow-field techniques provide higher spatial resolution and the potential to evaluate tissue at a microscopic level. These techniques include optical coherence tomography (OCT), spectroscopy and confocal microscopy (71).

OCT is an imaging technique that exploits differences in light refraction and scattering within tissues to generate cross-sectional images, enabling the identification of architectural alterations and increased epithelial thickness in malignant tissues (71,73). Recently, OCT has been used to examine surgical margins immediately after tumor resection in OCSCC, with reported sensitivity and specificity of 81.5% and 87%, respectively. However, it has shown a limited tissue penetration depth of

approximately 2 mm, which restricts its ability to evaluate deeper tissue layers beyond this threshold (73).

Among spectroscopic techniques, Raman spectroscopy has been similarly used to assess fresh resection margins. This technique quantifies variations in molecular bond vibrations to characterize tissue composition, demonstrating high diagnostic accuracy in discriminating tumor from healthy tissue, with reported sensitivity of 99% and specificity of 92% (71,74). To overcome its limited sampling depth of 40–50 μm, a fiber-optic needle probe can be integrated into the system, allowing estimation of the distance between the resection surface and the tumor border and thus enabling the assessment of deep margins. Nevertheless, the small sampling area of each measurement still requires multiple sequential acquisitions to evaluate the entire resection surface, which limits its use in routine clinical practice (71,75).

Despite their promising performance, none of these techniques has yet achieved sufficient validation for routine clinical implementation to complement or replace frozen section analysis in intraoperative margin assessment (71).

Among narrow-field approaches, confocal microscopy represents another potentially valuable tool for intraoperative margin assessment. Confocal microscopy captures light emissions through a small pinhole aperture and assembles multiple tissue planes through a process called optical sectioning, producing images that can be analyzed in a manner similar to conventional histopathology. To enhance image quality, this technique uses illumination sources such as lasers, and may be further combined with contrast agents such as acetic acid, fluorescein, or acriflavine hydrochloride (71).

Recently, fluorescence confocal microscopy (FCM) has been applied ex vivo to fresh tissue specimens for rapid margin assessment. Preliminary clinical studies have demonstrated encouraging diagnostic accuracy in the assessment of surgical margins across various tumor types, with sensitivity and specificity exceeding 80% in most reports (76). One of the technologies based on this approach is the Histolog® Scanner, a fluorescence confocal microscopy system developed for rapid intraoperative tissue assessment (69).

1.11.5 Histolog® Scanner

Histolog® Scanner (SamanTree Medical SA, Lausanne, Switzerland) is a fluorescence confocal microscopy system that enables rapid, high-resolution imaging of fresh tissue specimens. It has been primarily used for intraoperative assessment of surgical margins.

Histolog® provides en-face visualization of the freshly excised tissue surface, allowing evaluation of the entire surgical margin with subcellular histological detail directly in the operating room.

Tissue preparation is straightforward and does not require fixation, embedding, or slide mounting. Specimens are immersed for 10 seconds in an acridine orange-based photoreactive solution (Histolog® Dip, SamanTree Medical SA), rinsed with 0.9% saline solution, gently dried, and then placed on the scanner for image acquisition.

In less than a minute, Histolog® provides high-resolution images with a spatial resolution of approximately 2 μm over a large field of view (4.8 × 3.6 cm). No calibration or manual parameter adjustment is required.

Images are displayed on a touchscreen monitor with artificial purple coloration, allowing the surgeon to zoom and navigate across the image (69,77).

Evidence on clinical performance of the Histolog® Scanner is growing but still limited.

Histolog® has been primarily evaluated for intraoperative margin assessment across different surgical specialties, including urologic, breast, and dermatologic surgery, showing high specificity and diagnostic accuracy compared to the reference standard. Sensitivity appears more variable, ranging from 30% to 100% depending on the clinical setting and specimen type.

Overall, these preliminary findings appear promising and suggest a potential role for Histolog® as an adjunct to conventional methods for intraoperative margin assessment (69).

In head and neck surgery, evidence on the use of Histolog® remains limited. Histolog® Scanner has been evaluated for ex vivo lymph node assessment in patients with head and neck cancer undergoing sentinel lymph node biopsy, selective neck dissection, or complete neck dissection. In this setting, Histolog® achieved an overall

diagnostic accuracy of 95.5%, with a specificity of 98.8% and a sensitivity of 76.7% compared with conventional frozen section analysis (78).

Despite these encouraging findings, the role of Histolog® in the assessment of surgical margins in head and neck cancer has not yet been adequately investigated. This setting may be particularly relevant, as intraoperative decision-making in head and neck oncology is complicated by the complex anatomy of the oral cavity and the proximity of critical functional structures (69).

These considerations highlight the need for further studies evaluating the feasibility of Histolog® Scanner in the assessment of surgical margins in head and neck oncology.

2 AIM OF THE STUDY

The aim of this preliminary study was to evaluate the feasibility and diagnostic performance of the Histolog® Scanner for intraoperative assessment of resection margins in oral cavity squamous cell carcinoma, using conventional frozen section analysis as the reference standard. Workflow times were also compared between Histolog®-based assessment and conventional frozen section evaluation.

3 MATERIALS AND METHODS

3.1 Study design

This was a prospective single-center pilot diagnostic accuracy and workflow study conducted at the University Hospital of Modena between October 2025 and April 2026. The study aimed to evaluate the diagnostic performance and workflow time of the Histolog® Scanner for intraoperative assessment of tongue resection margins in patients undergoing surgery for oral cavity squamous cell carcinoma, using conventional intraoperative frozen section analysis as the reference standard.

3.2 Study population

All consecutive adult patients (>18 years old) undergoing surgery for oral cavity squamous cell carcinomas involving the tongue with intraoperative margin assessment at our institution during the study period were assessed for inclusion.

Patients were excluded when either Histolog® Scanner acquisition or conventional frozen section evaluation was not feasible or incomplete.

3.3 Surgical specimens and margin assessment

In all cases, margins were obtained intraoperatively by the operating surgeon from surgical specimens. The number of margins collected per case varied according to tumor size, tumor location, and extent of surgical resection.

To ensure proper orientation, the tumor-facing side of each margin was marked with blue ink (Bio Marking Dye, Bio Optica®). Margins were subsequently processed for both Histolog® Scanner imaging and conventional frozen section analysis.

3.4 Index test: Histolog® Scanner

Histolog® scans were performed in the operating room prior to conventional frozen section analysis. Image acquisition was conducted by an otolaryngology surgeon using the scanner available in the surgical suite following a dedicated training in the use of the device.

Immediately after excision, fresh margins were immersed for 10 seconds in an acridine orange-based photoreactive solution (Histolog® Dip, SamanTree Medical SA), rinsed with 0.9% saline solution, gently dried with absorbent paper, and placed on the scanner surface for image acquisition. Scanning was performed on the non–tumor-facing surface of each margin, identified as the side without blue ink marking. A digital image of the specimen surface was generated and displayed on a touchscreen interface using histology-like purple pseudo-staining. The process was repeated for each margin.

The time required to acquire images of all margins in each case was recorded and defined as Histolog® acquisition time.

The Histolog® Scanner and its acquisition workflow are illustrated in Figure 1.

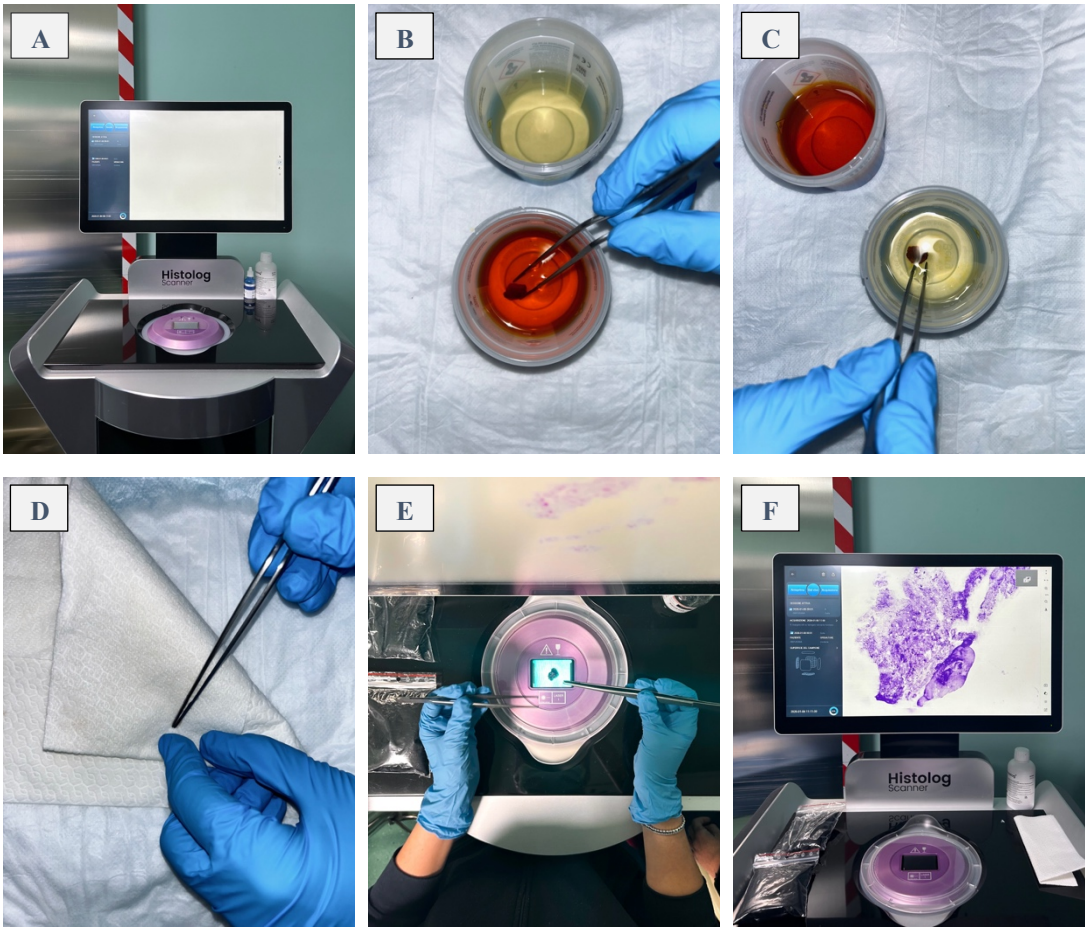

Figure 1. Histolog® Scanner and acquisition workflow. *(A) Histolog® Scanner device; (B) Margin immersion in acridine orange-based solution; (C) Rinsing with saline solution; (D) Drying with absorbent paper; (E) Specimen placement on the scanner surface; (F) Representative digital image displayed with histology-like purple pseudo-staining.*

3.5 Reference standard: frozen section analysis

After Histolog® image acquisition, margins were submitted to the pathology department for conventional intraoperative frozen section analysis.

Margins were grossly examined, including measurement in three dimensions and assessment of ink orientation, then embedded in optimal cutting temperature compound, frozen at -40°C for 60 seconds, and sectioned at approximately 5 µm in a cryostat. Sections were stained with hematoxylin and eosin and evaluated microscopically by the on-call pathologist.

Each margin was classified as positive in the presence of carcinoma in situ, high-grade dysplasia, or squamous cell carcinoma at or within 5 mm of the resection surface. For the purposes of this study, both involved and close margins were grouped into a single

positive category. Margins without carcinoma involvement were classified as negative. Low-grade dysplasia was considered negative as well.

The result was promptly communicated to the operating room by telephone. The interval between specimen submission and communication of frozen section result was recorded and defined as the frozen section turnaround time.

The frozen section workflow is illustrated in Figure 2.

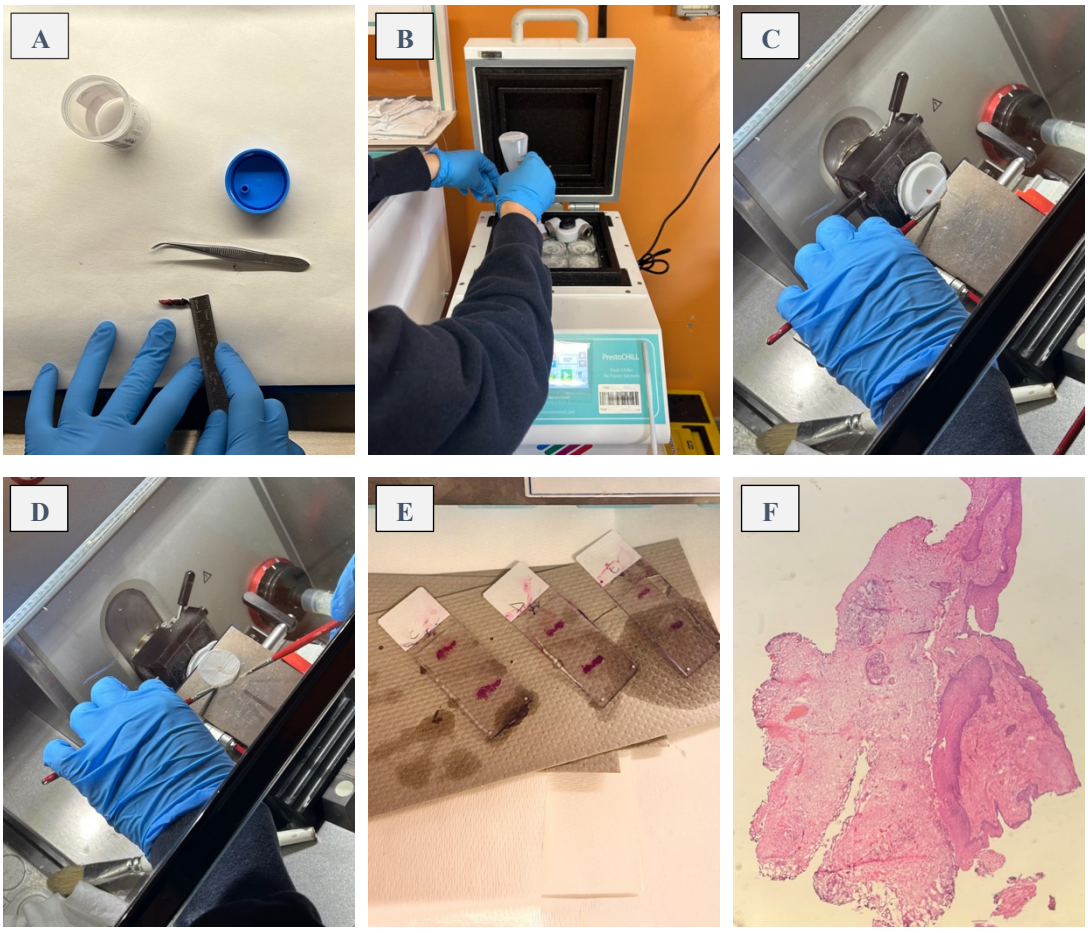

Figure 2. Frozen section workflow. *(A) Gross examination of the surgical margin; (B) Embedding in optimal cutting temperature compound; (C, D) Sequential cryostat sectioning; (E) Hematoxylin and eosin-stained frozen sections on glass slides; (F) Microscopic evaluation.*

3.6 Image interpretation

Histolog® images were assessed retrospectively on a computer monitor by a single pathologist blinded to the frozen section results. The reviewing pathologist had undergone dedicated training in reading and interpreting the images generated by the device and was therefore familiar with Histolog® imaging and its operating principles.

Margin status was classified as positive or negative using the same criteria described in Section 3.5. An additional category, indeterminate, was assigned in cases of insufficient image quality for reliable interpretation.

The time required for image interpretation was recorded for each case and defined as Histolog® interpretation time.

3.7 Outcomes

The study aimed to assess the diagnostic performance of the Histolog® Scanner compared with conventional frozen section analysis through the evaluation of concordance, sensitivity, specificity, positive predictive value, and negative predictive value. Workflow times were also compared between the two techniques.

3.8 Statistical analysis

The primary unit of analysis was the individual margin.

Histolog® Scanner and frozen section results were classified as positive or negative for margin involvement and compared at the margin level using a 2×2 contingency table, with frozen section analysis as the reference standard. Margins were categorized as true positive, true negative, false positive, or false negative according to the reference standard. Overall concordance was calculated as the proportion of margins for which Histolog® and frozen section analysis provided the same result. Indeterminate Histolog® margins were to be excluded from the primary diagnostic accuracy analysis and reported separately.

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated when estimable. Cohen's kappa was planned to assess agreement beyond chance.

Workflow times were analyzed per case. Mean frozen section turnaround time, mean Histolog® acquisition time, mean Histolog® interpretation time, and mean total Histolog® workflow time were calculated. Total Histolog® workflow time was defined as the sum of image-acquisition time and cumulative pathologist interpretation time for all scanned margins in a given case. Mean per-margin Histolog® workflow time was also assessed.

Paired differences between frozen section and Histolog® workflow times were tested using the Wilcoxon signed-rank test. The correlation between the number of intraoperative margins and workflow time was evaluated using Spearman's correlation coefficient. A p value < 0.05 was considered statistically significant.

This was a pilot study, and no formal sample size calculation was performed; the sample reflects the initial consecutive series available during the study period.

4 RESULTS

The flow of patients and margins through the study is illustrated in Figure 3.

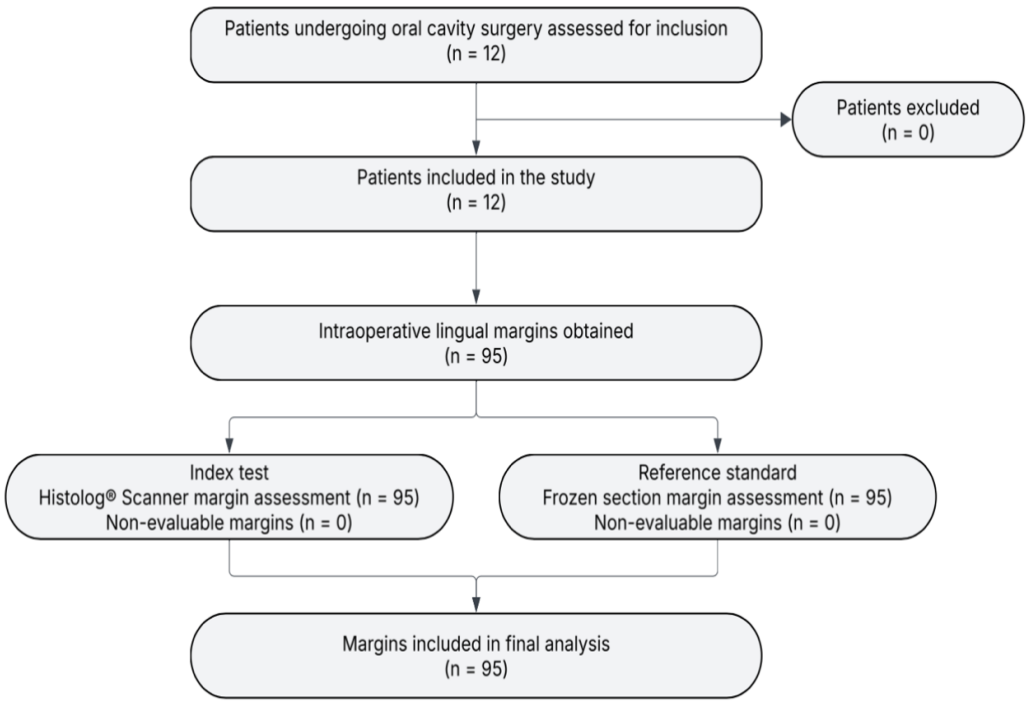

Figure 3. STARD flow diagram of patient inclusion and margin-level analysis. *Twelve patients undergoing oral cavity surgery were included. A total of 95 surgical margins were obtained and evaluated by both Histolog® Scanner and reference-standard frozen section analysis. All 95 margins were included in final analysis.*

4.1 Study population and margin characteristics

Twelve consecutive patients undergoing surgery for oral cavity squamous cell carcinoma involving the tongue were enrolled in the study. Both primary and recurrent tumors were included. The cohort comprised nine men and three women, with a mean age of 65.3 years at the time of surgery.

Surgical procedures included transoral hemiglossectomy (n = 4), pull-through compartmental hemiglossectomy (n = 7), and salvage surgery following a previous compartmental hemiglossectomy (n = 1).

Baseline demographic and surgical characteristics of the study population are summarized in Table 6.

A total of 95 intraoperative tongue margins were obtained, corresponding to a mean of 7.92 margins per patient. All margins were successfully assessed using both the Histolog® Scanner and conventional frozen section analysis, with no margin classified as non-evaluable.

Margin characteristics are summarized in Table 7.

Table 6. Baseline demographic and surgical characteristics of the study population.

Variable	Value
Patients, n	12
Age (year), mean	65.3
Sex (male), n (%)	9 (75)
Sex (female), n (%)	3 (25)
Primary disease, n (%)	11 (91.7)
Recurrent disease, n (%)	1 (8.3)
Transoral hemiglossectomy, n (%)	4 (33.3)
Pull-through compartmental hemiglossectomy, n (%)	7 (58.3)
Salvage surgery, n (%)	1 (8.3)

Table 7. Margin characteristic.

Variable	Value
Total tongue margins assessed, n	95
Margins per patient, mean	7.92
Evaluable margins, n (%)	95 (100)
Non-evaluable margins, n (%)	0 (0)

4.2 Diagnostic performance

All 95 margins were classified as positive or negative at both the Histolog® and frozen section assessment. No margin was classified as indeterminate.

At the reference standard, all 95 margins were negative. Histolog® correctly classified 94 margins as negative and incorrectly classified one margin as positive, resulting in a single false-positive finding. No false-negative Histolog® results were observed.

The cross-classification of Histolog® Scanner and reference standard results at margin level is reported in Table 8.

Table 8. Margin-level 2x2 contingency table comparing Histolog® Scanner with the reference standard.
The table shows 0 true-positive, 94 true-negative, 1 false-positive, and 0 false-negative margins.

	Reference standard positive	Reference standard negative	Total
Histolog® positive	0	1	1
Histolog® negative	0	94	94
Total	0	95	95

Overall concordance between Histolog® Scanner-based assessment and the reference standard was 98.95% (94/95).

Specificity for reference-standard negative margins was 98.95% (94/95). Negative predictive value was 100% (94/94).

Sensitivity and positive predictive value could not be estimated, as no positive margins were identified by the reference standard.

Cohen's kappa was not reported as the absence of positive reference-standard margins precluded a reliable estimate of agreement beyond chance.

Diagnostic performance measurements are summarized in Table 9.

Table 9. Diagnostic performance of the Histolog® Scanner compared with the reference standard.

Parameter	Result
Overall concordance	98.95% (94/95)
Specificity	98.95% (94/95)
Negative predictive value	100% (94/94)
Sensitivity	Not estimable *
Positive predictive value	Not estimable *

**Not estimable because no positive margins were identified by the reference standard.*

4.3 Workflow analysis

Mean frozen section turnaround time was 63.92 minutes per case (range 45–125 minutes). Mean total Histolog® workflow time was 27.81 minutes per case (range 14.54–40.75 minutes), comprising a mean image-acquisition time of 22.50 minutes and a mean pathology interpretation time of 5.31 minutes per case.

Mean per-margin Histolog® workflow time was 4.06 minutes.

The mean time difference between frozen section and total Histolog® workflow was 36.11 minutes, with shorter times observed for Histolog®.

In a sensitivity analysis excluding the longest frozen section turnaround time (125 minutes), the mean difference remained 31.55 minutes.

The paired comparison was statistically significant (Wilcoxon signed-rank test, $p = 0.000488$).

A statistically significant positive correlation was found between the number of margins and Histolog® workflow time (Spearman's rho = 0.600, $p = 0.039$) and an

increase of 1.70 minutes per additional margin was estimated. No similar proportional relationship was observed for frozen section turnaround time (Spearman's rho = 0.332, p = 0.292).

Workflow times and statistical comparison are summarized in Table 10.

Table 10. Workflow times and statistical comparison between Histolog® Scanner and frozen section analysis.

Parameter	Result
Frozen section turnaround time, mean (range), min	63.92 minutes (range 45–125)
Histolog® image acquisition time, mean, min	22.50 minutes
Histolog® image interpretation time, mean, min	5.31 minutes
Total Histolog® workflow time, mean (range), min	27.81 minutes (range 14.54–40.75)
Histolog® workflow time per margin, mean, min	4.06 minutes
Statistical comparison	
Mean time difference (frozen section – Histolog®), min	36.11 minutes
Mean time difference (frozen section – Histolog®) after the exclusion of longest frozen section case	31.55 minutes
Paired workflow comparison	Wilcoxon signed-rank test, p = 0.000488
Number of margins vs Histolog® workflow time	Spearman's rho = 0.600, p = 0.039
Number of margins vs frozen section workflow time	Spearman's rho = 0.332, p = 0.292

5 DISCUSSION

Achieving tumor-free surgical margins remains one of the most important determinants of oncologic outcome in oral cavity squamous cell carcinoma, as positive margins are associated with higher recurrence rates and poorer survival. Reliable intraoperative margin assessment is therefore essential to confirm resection adequacy and guide immediate re-excision when needed (55,63).

Although frozen section analysis remains the standard approach for intraoperative margin evaluation, its implementation is affected by turnaround time, workflow complexity, sampling limitations, and dependence on pathology service availability. Within this context, technologies capable of providing rapid assessment of fresh surgical margins directly in the operating room are particularly appealing (66,69).

Histolog® Scanner is a confocal microscopy system used for intraoperative margin assessment. While previous studies in breast and urologic surgery have shown promising feasibility and diagnostic performance, evidence in head and neck surgery remains limited (69). To the best of our knowledge, no previous study has evaluated the Histolog® Scanner for intraoperative margin assessment in oral cavity squamous cell carcinoma, despite the particular challenges of intraoperative margin evaluation in this setting and the potential values of this technology (69).

We therefore investigated the use of the Histolog® Scanner in oral cavity squamous cell carcinoma surgery, evaluating its diagnostic performance and workflow efficiency for intraoperative assessment of tongue resection margins.

5.1 Diagnostic performance

An overall concordance of 98.95% was observed between Histolog® Scanner and conventional frozen section analysis. The single discordant result was a false-positive finding attributed to glandular structures mistaken for tumor cells on the Histolog® image. Notably, no false-negative Histolog® result was observed in this preliminary cohort. All margins were successfully imaged and interpreted, with no indeterminate cases, supporting the technical feasibility of the Histolog® Scanner in the intraoperative setting.

Specificity was 98.95% and negative predictive value was 100%. These preliminary results suggest that a negative Histolog® assessment may be reliable in confirming margin adequacy, which is clinically relevant as it may support intraoperative decision-making and help avoid unnecessary additional resection.

The observed concordance, specificity, and negative predictive value are consistent with values reported in studies conducted in breast and urologic surgery (Table 11) suggesting that confocal imaging with the Histolog® Scanner may be a feasible tool also for intraoperative assessment of oral cavity negative margins. In prostate surgery, de Angelis et al. reported an overall diagnostic accuracy of 91.1%, a specificity of 91.7%, and a negative predictive value of 97.5% (79), and comparable diagnostic performance rates of 95.2% accuracy, 92.3% specificity, and 100% negative predictive value were reported by Colard-Thomas et al. when evaluating Histolog® Scanner for intraoperative margin assessment in breast-conserving surgery (80). In both studies, images were interpreted by experienced pathologists, consistent with the approach adopted in the present study. However, comparable diagnostic performance has also been achieved when image interpretation was performed by trained surgeons: Lux et al. reported 97.8% accuracy, 99.5% specificity, and 98.1% negative predictive value in breast-conserving surgery (77), suggesting that Histolog® diagnostic performance may be maintained across different users and supporting the feasibility of broader clinical implementation.

Overall, our findings are in line with values reported in the literature. However, their interpretation warrants particular caution.

As no positive margins were obtained at the reference standard, the present study could not evaluate the ability of the Histolog® Scanner to detect margin involvement, and reliable estimation of sensitivity and positive predictive value was precluded. Additionally, given the exclusive presence of negative cases at the reference standard, the observed concordance and negative predictive value should be interpreted prudently.

Compared with the cited studies, which included both positive and negative margins allowing full assessment of diagnostic performance metrics, the present study could only evaluate Histolog® performance in negative margin assessment (Table 11). Further prospective studies including positive cases are therefore required to fully characterize the diagnostic performance of Histolog® in oral cavity cancer surgery.

Table 11. Comparison of Histolog® Scanner diagnostic performance across studies.

Study	Setting	Interpreter	Diagnostic accuracy	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Present study	Oral cavity surgery	Pathologist	98.95%	NE	98.95%	NE	100%
de Angelis et al. (79)	Prostate surgery	Pathologist	91.1%	88.2%	91.7%	68.2%	97.5%
Colard-Thomas et al. (80)	Breast surgery	Pathologist	95.2%	100%	92.3%	88.9%	100%
Lux et al. (77)	Breast surgery	Surgeon	97.8%	80.9%	99.5%	94.4%	98.1%

NE = Not Evaluable

5.2 Workflow efficiency

Compared with conventional frozen section analysis, Histolog® Scanner may offer practical advantages in terms of specimen management and intraoperative workflow efficiency.

First, Histolog® may address several recognized technical limitations of frozen section analysis, including freezing artifacts, tissue consumption during specimen preparation, and sampling errors inherent to the evaluation of selected tissue sections (66). Histolog® image acquisition does not require tissue freezing or sectioning, thereby preserving specimen integrity and avoiding freeze-thaw-related artifacts and tissue loss; this is clinically relevant, as freezing artifacts and loss of tissue may affect the quality and reliability of the subsequent final histopathologic assessment (81). Histolog® also enables visualization of a large surface area of the specimen, allowing a broader assessment than conventional frozen section analysis and potentially reducing sampling limitations and the risk of missing focal abnormalities (68).

Second, Histolog® Scanner enables fresh tissue imaging directly in the operating room, eliminating the need for specimen transport to the pathology department. This is clinically relevant, as it reduces dependence on pathology laboratory availability, cryostat access, and technical staff, which are factors that may limit the feasibility of conventional frozen section analysis (69). Additionally, it may help preserve specimen orientation, which is essential for accurate margin reporting and, in case of a positive margin, for guiding re-excision at the correct anatomical site (57).

These technical and procedural advantages are accompanied by a marked reduction in overall workflow time. In the present study, we observed a mean total Histolog® workflow time substantially shorter than the frozen section turnaround time (27.81 vs 63.92 minutes per case) with a mean saving of 36.11 minutes in favor of Histolog®. In a sensitivity analysis excluding the case with the longest frozen section turnaround time, the mean difference remained 31.55 minutes, confirming the robustness of the observed time advantage.

This time difference has relevant practical implications, as a shorter intraoperative margin assessment may translate into decreased anesthesia exposure, lower operating room occupancy and reduced periods of surgical inactivity while waiting for pathology

results. This may be relevant especially in oral cavity surgery, as oral cavity surgical procedures are often lengthy and usually require reconstructive steps to restore oral cavity form and function. Within this context, faster margin assessment may allow reconstruction to proceed earlier, ultimately improving intraoperative workflow efficiency.

The observed reduction in intraoperative margin assessment time using Histolog® is consistent with previous reports in different surgical settings, which have similarly demonstrated reduced workflow times with this technology (69), suggesting that this workflow advantage may be maintained across different clinical applications.

However, direct comparison of the observed overall workflow times with those reported in previous studies is challenging, as different specimen types were evaluated, different numbers of margins were assessed, and different reference standards were used.

Accordingly, a comparison at level of individual margin may be more informative. A mean per-margin workflow time of 4.06 minutes was observed in the present study, further supporting the feasibility of rapid intraoperative margin assessment and the potential for Histolog® to provide near-real-time feedback to the surgical team. In breast-conserving surgery, Lux et al. reported a comparable mean per-margin workflow time of approximately 3 minutes (77).

Additionally, a positive correlation between the number of submitted margins and Histolog® workflow time was observed, reflecting the additional image acquisition and interpretation time required for each extra margin. By contrast, frozen section turnaround time did not show a comparable proportional relationship. This is likely explained by the fact that frozen section is less directly dependent on the number of margins and more influenced by multiple organizational and technical factors, including specimen transport, laboratory workload, cryostat access, embedding and sectioning workflow, pathologist availability, and communication back to the operating room (69).

The observed proportional relationship between the number of submitted margins and Histolog® workflow time may be clinically relevant, as it suggests that the total assessment time could be predicted and estimated in advance, based on the number of margins to be evaluated. This may allow the surgical team to plan intraoperative workflow accordingly and ultimately facilitate a more efficient surgical management.

5.3 Cost analysis

From an economic perspective, the initial investment for the Histolog® Scanner is considerable, with a reported cost exceeding £200,000 (approximately €235,000). However, once acquired, ongoing consumable costs remain low (82). This is particularly relevant when compared with the substantial per-procedure costs associated with conventional frozen section analysis.

The cost of a single frozen section can reach up to €155, depending on complexity (83). This cost includes consumables and, most significantly, the labor of the pathologist and laboratory technician. Notably, each margin corresponds to a single frozen section analysis and, in oral cavity surgery, several margins per procedure are typically collected, thereby leading to increased costs. However, bundled pricing models covering multiple margins per case are also available.

Beyond direct laboratory costs, frozen section analysis is associated with prolonged operative times due to the intraoperative waiting period for pathology results, with significant economic implications for overall procedural costs. In the Italian healthcare system, operating room costs are estimated at approximately €20 per minute, encompassing personnel, anesthesia, pharmaceuticals, and equipment. The mean time saving of 36 minutes observed in the present study would therefore correspond to a mean reduction of approximately €720 per case in operating room-related expenditure alone.

When considering the combination of reduced laboratory costs and lower operating room expenditure due to shorter intraoperative times, the Histolog® Scanner may offer a substantial overall cost reduction compared with conventional frozen section analysis, particularly in high-volume centers.

However, it should be noted that Histolog® does not eliminate the need for pathologist involvement. Nevertheless, time required for image evaluation is substantially shorter than that needed for conventional frozen section workflow, as it does not require specimen preparation, freezing, sectioning, and staining. In the present study, mean Histolog® interpretation time was 5.31 minutes per case, compared with a mean frozen section turnaround time of 63.92 minutes. This reduced time commitment may translate into lower pathologist-related costs per case and may allow more efficient allocation of pathology resources, particularly in settings where pathologist availability is limited.

However, no formal cost-effectiveness analysis was performed in the present study, and these considerations remain preliminary. Dedicated cost-effectiveness studies, including cost-per-case comparisons and break-even analysis relative to case volume, are needed in order to determine the true economic impact of Histolog® Scanner in oral cavity surgery.

5.4 Limitations

This study has several limitations that should be acknowledged. First, this was a preliminary single-center pilot study with a small sample size of 12 patients and 95 margins, which limits the precision of diagnostic performance estimates and generalizability of the findings. Second, all margins were negative at the reference standard, precluding reliable estimation of sensitivity and positive predictive value. As a result, the diagnostic performance of Histolog® Scanner in detecting margin involvement could not be assessed completely, and the observed concordance, specificity, and negative predictive value should be interpreted with caution. Third, Histolog® images were reviewed by a single pathologist in a retrospective setting, which does not fully replicate conditions of intraoperative use, where time pressure and clinical context may influence interpretation. Finally, although both the surgeon and the pathologist underwent dedicated training on the Histolog® Scanner, clinical experience was progressively gained during the course of the study. Consequently, early cases may have been influenced by initial phase of the learning curve, although this was not formally assessed.

5.5 Future perspectives

The findings of this preliminary study provide a rationale for further investigation of the Histolog® Scanner in oral cavity oncologic surgery.

First, future prospective studies with larger sample sizes including both positive and negative margins are warranted to allow reliable estimation of sensitivity and positive predictive value, and comprehensively characterize the diagnostic performance of the Histolog® Scanner in oral cavity surgery. Multicenter designs would also strengthen the generalizability of the results across different clinical settings and patient populations.

Second, future studies should evaluate Histolog® image interpretation in real time during surgery, as this would more accurately reflect the conditions of clinical use. Several intraoperative implementation models can be envisioned, including digital review by the pathologist directly in the operating room, remote pathology consultation via network-connected devices, and shared surgeon-pathologist workflows in which the surgeon performs a preliminary assessment and the pathologist provides confirmation when needed. In this regard, the comparable diagnostic performance reported for trained surgeons and pathologists in previous studies (77,79,80) suggests that Histolog® could potentially serve as an intraoperative triage tool: the surgeon could perform an initial rapid assessment of margin status at the time of excision, while reserving conventional frozen section analysis for positive or suspicious margins. This approach could reduce the number of specimens sent for frozen section, shorten overall turnaround time, and optimize pathology resources.

Third, formal assessment of the learning curve in oral cavity surgery is warranted. In the present study, both the surgeon and the pathologist progressively gained experience with the technology during the course of the study, but no formal evaluation of learning-curve effects was performed. Previous studies in breast surgery have demonstrated that experience significantly improve both image acquisition and interpretation, resulting in improved diagnostic performance and reduced workflow times (77,84). Whether similar improvements can be achieved in oral cavity surgery remains to be determined in future dedicated studies.

Fourth, the integration of artificial intelligence (AI) represents a promising avenue for further development. Automated image interpretation algorithms and AI-assisted decision-support systems could support and standardize image analysis, potentially further reducing interpretation time and improving the diagnostic performance.

Future studies should also include formal health evaluations to determine the cost-effectiveness of Histolog® implementation in routine oral cavity surgical practice.

Finally, the potential applications of the Histolog® Scanner in oral cavity surgery may extend beyond the scope of the present study. On one hand, the use of Histolog® can be extended to other oral cavity subsites in addition to the tongue to assess whether the observed diagnostic performance and workflow advantages are maintained across different anatomical locations. On the other hand, this technology may also prove useful for the intraoperative evaluation of additional prognostic parameters.

Interestingly, in one patient of our series, the final surgical specimen was scanned with the pathologist present in the operating room, allowing intraoperative estimation of tumor depth of invasion, which was subsequently confirmed on definitive histopathology. Although anecdotal, this observation suggests that the potential application of fresh-tissue confocal imaging may extend beyond margin assessment to include the evaluation of additional clinically relevant tumor characteristics.

6 CONCLUSION

To our knowledge, this is the first study to evaluate the Histolog® Scanner for intraoperative margin assessment in oral cavity squamous cell carcinoma. This preliminary study suggests that fresh-tissue confocal imaging with the Histolog® Scanner is a feasible tool for intraoperative assessment of tongue resection margins. Histolog® demonstrated high concordance with conventional frozen section analysis, with excellent specificity and negative predictive value, supporting its reliability in confirming negative margin status. In addition, the technology offered practical advantages in terms of specimen preservation and reduced dependence on pathology laboratory infrastructure, and a marked reduction in workflow time, with potential benefits for operating room efficiency and healthcare costs. However, the small sample size and the absence of positive margins at the reference standard precluded estimation of sensitivity and positive predictive value, limiting the generalizability of the observed diagnostic performance results. Further prospective studies with larger cohorts including both positive and negative margins are needed to fully characterize the diagnostic performance of the Histolog® Scanner and define its role in the intraoperative management of oral cavity squamous cell carcinoma.

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