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REVIEW

Micrometastasis and Isolated Tumor Cells in Oral Squamous Cell Carcinoma: Refining Nodal Staging with Emerging Technologies

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Abstract

Purpose Cervical lymph node metastasis significantly influence prognosis in oral squamous cell carcinoma (OSCC), guiding staging, treatment decisions, and overall survival. Sentinel lymph node biopsy (SLNB) offers a minimally invasive approach for early detection of subclinical nodal metastasis, including micrometastases (0.2–2 mm) and isolated tumor cells (ITCs, < 0.2 mm). Despite its success in melanoma and breast cancer, the clinical relevance of micrometastases and ITCs in OSCC remains incompletely defined. This narrative review explores the biological significance, diagnostic challenges, and emerging strategies for detecting micrometastasis and ITCs in OSCC, aiming to inform their potential role in refining staging systems and treatment algorithms.

Methods We performed a comprehensive literature review of SLNB in OSCC, examining data on histopathological detection techniques, molecular markers, artificial intelligence (AI), and radiomics-based tools that enhance diagnostic sensitivity and specificity for occult metastases.

Results While current guidelines in some countries endorse SLNB for early-stage OSCC, integration of micrometastasis and ITC data into staging remains inconsistent. Studies suggest that ITCs represent early metastatic events with variable prognostic significance. Advanced techniques such as step-serial sectioning, immunohistochemistry, and molecular diagnostics—including ctDNA, gene-expression profiling, and AI-assisted pathology—have shown promise in improving detection accuracy. However, robust prospective data are lacking, and a consensus on the management of minimal nodal disease is yet to be reached.

Conclusion Accurate identification and interpretation of micrometastasis and ITCs in OSCC represent an evolving frontier in head and neck oncology. Future staging systems should incorporate these elements supported by standardized protocols and high-level evidence. The integration of AI, molecular diagnostics, and radiomics holds the potential to enhance risk stratification and personalize surgical decision-making, reducing overtreatment while ensuring oncologic safety.

Keywords Neoplasm · Micrometastasis · Isolated tumor cells · Oral squamous cell carcinoma · Mouth neoplasms · Sentinel lymph node biopsy · Occult metastasis · Lymphatic mapping · Prognostic markers · Molecular detection · Artificial intelligence · Radiomics · Lymph nodes

Introduction

Lymphatic dissemination is a principal determinant of prognosis in oral squamous cell carcinoma (OSCC), with cervical lymph node metastasis serving as a critical factor in staging, therapeutic decisions, and long-term survival [1]. Tumor cells from the primary lesion typically follow defined lymphatic drainage patterns, first colonizing anatomically predictable sentinel lymph nodes (SLNs) before progressing to other nodes within the cervical basin. While sentinel lymph node biopsy (SLNB) is a well-established staging tool in melanoma and breast cancer, its application in oral squamous cell carcinoma (OSCC) has only recently emerged and is not yet universally embedded in clinical

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practice. However, its role is gaining prominence, with several national guidelines now endorsing its use. Notably, in the UK, SLNB is recommended over elective neck dissection (END) for staging the clinically node-negative (T1–T2 N0) OSCC, as outlined in NICE guideline [2, 3]. Also, in the Netherlands and the United States, SLNB is recommended for staging these tumors according to national guidelines [4, 5].

The histopathologic assessment of SLNs offers a unique opportunity to identify subclinical nodal disease in patients with clinically node-negative (cN0) necks. Micrometastases—defined as tumor deposits measuring between 0.2 and 2.0 mm—and isolated tumor cells (ITCs)—individual cells or small clusters smaller than 0.2 mm—represent early metastatic events. This commonly used definition of ITCs is based on size rather than designation of the metastatic tumor deposit. ITC is considered a precursor of micrometastasis ‘‘waiting to grow’’. More specific histopathological characteristics have been described for ITC: no contact with vessel or lymph sinus walls, no extravasation(invasion or penetration of vessel/lymph sinus wall), no extravascular (extrasinusoidal) stromal reaction, and no extravascular tumor cell proliferation [6]. However, these definitions are more difficult to use in clinical practice. These definitions, based on size, initially introduced in the context of breast cancer staging, are not currently incorporated into the American Joint Committee on Cancer (AJCC) 8th edition staging criteria for head and neck cancers. This lack of granularity in staging may underestimate the prognostic and therapeutic implications of minimal nodal disease in OSCC [7].

Evidence from melanoma, including findings from the landmark Multicentre Selective Lymphadenectomy Trial I (MSLT-I), has demonstrated that early excision of SLNs containing micrometastatic disease is associated with improved regional control and survival [8–11]. In contrast, the observation group had only wide excision of the primary melanoma and were monitored; Completion lymph node dissection) CLND was performed later only if clinical nodal recurrence occurred. The biological rationale underlying this approach is the concept of the SLN as a metastatic “incubator”—an early site where malignant cells establish before widespread dissemination has occurred. In MSLT-I, delayed lymphadenectomy permitted progression of nodal disease, whereas early SLN removal mitigated further spread and improved outcomes. These observations raise important questions regarding the timing and extent of nodal intervention in OSCC, particularly in patients with micrometastatic disease [8].

Despite this, the prognostic relevance of micrometastases and ITCs in OSCC remains poorly defined. While some studies in melanoma and breast cancer suggest that exceedingly small deposits (ITCs) may not portend worse survival, and in fact are still classified as pN0(i+) or (mol+), although $pN1_{mi}$ for micrometastases, others argue that even minimal metastatic disease may signify a more aggressive tumor phenotype. In breast cancer, the AJCC 8 has introduced a molecular category with the introduction of pN0(mol+), denoting subclinical metastases identified only by molecular assays. Such molecular stratification, however, is currently absent from OSCC staging [9–12].

Given the limitations of traditional histopathology in detecting micrometastases, there is a growing emphasis on integrating newer diagnostic approaches in oral cancer. Emerging molecular markers such as ctDNA and microRNAs, advanced techniques like spatial transcriptomics and proteomics, and artificial intelligence-driven image analysis show promise in improving diagnostic precision and risk stratification [11]. This review highlights the current understanding of micrometastasis and ITC in oral cancer and explores emerging concepts in molecular diagnostics, artificial intelligence, and novel technologies that may transform future staging and treatment strategies.

SLN, ITCs, and Micrometastasis

A SLN is a regional lymph node that receives direct afferent lymphatic drainage from a primary tumor site. More than one SLN may be present in a regional nodal basin, and some primary tumors may drain to more than one regional nodal basin [13, 14]. Sentinel nodes are identified through lymphatic mapping, which involves the uptake of a tracer injected near the primary tumor or within the affected organ. Commonly used agents for this purpose include radiotracers such as technetium-99 (^{99m}Tc) sulfur colloid or nano colloid, and vital dyes like isosulfan or patent blue. However, blue dye should only be used as an adjunct to the radiotracer, not as a standalone method, as relying solely on dye is considered unreliable for accurate SLN identification [15]. Tilmanocept is a new agent, a 99mTc-labeled non-particulate radiotracer that contains multiple mannose moieties with high affinity for the CD206 receptor found on macrophages and dendritic cells, enhancing targeting to these cells within the SLN. Tilmanocept may have improved clearance from the site of the primary tumor and enhanced retention within the SLN when compared to sulfur colloid, which results in a reduction of the shine-through effect and an improvement in SLN detection, especially in floor of mouth tumors. In small series, Tilmanocept has a much lower false negative rate (FNR) than reported in trials with other tracers. Using histopathological examination as the reference standard, the false negative rate was 2.56% (compared with 12–14% for traditional colloids) [16, 17]. However, in another small series, technetium-99 (^{99m}Tc) Tilmanocept was not

superior to technetium-99 (^{99m}Tc) nanocolloid [18]. Moreover, in a within-patient (head-to-head) comparison study of 20 OSCC patients, the diagnostic performance of these tracers was similar [19]: Results from a large phase II/III trial are awaited, in which Tilmanocept is being evaluated for its clinical utility. [20].

ITCs include single tumor cells and small clusters not larger than≤0.2 mm in greatest diameter, generally without stromal response in the lymph (Fig. 1). Specifically, multiple separate individual tumor cells or clusters may be seen in a single lymph node, but it is only the single largest contiguous cell cluster that is used; it is not the addition of all the clusters together nor the addition of the area distributed in multiple different levels of the same lymph node nor across different lymph nodes. Thus, it is only up to 200 cells in a single node profile that is counted. If there are more clusters, then micrometastasis should be used, recognizing that the upper limit of ITCs and lower limits of micrometastases inherently overlap. Such cells are usually found in the subcapsular nodal sinuses but may be seen within the nodal parenchyma. Because ITCs may represent in-transit tumor cells that are not proliferating within the node, lymph nodes with only ITCs are usually categorized as pN0(i+). Isolated tumor cells (ITCs) are rarely encountered in head and neck squamous cell carcinomas (HNSCC), in contrast to their relatively higher prevalence in breast cancer, melanoma, and endometrial cancers [13, 21, 22]. The clinical relevance and prognostic implications of ITCs in head and neck cancers remain poorly defined, with limited evidence guiding their interpretation and integration into clinical decision-making [23].

In the context of SLN evaluation, the presence of ITCs may result in an upstaging disease in certain solid tumors. For instance, in breast and endometrial cancers, current staging systems account for the presence of ITCs within SLN. However, their impact on treatment decisions—particularly treatment intensification—remains minimal [24–26]. In breast cancer, ITCs are frequently identified during SLN biopsy or axillary sampling, either in isolation or in conjunction with micrometastases or macro metastases. In such cases, it is the presence of micro- or macrometastasis that primarily drives staging and therapeutic decisions, while the contribution of ITCs alone is generally acknowledged histologically but does not influence staging or prognostication independently [27, 28].

In head and neck cancers, the identification of ITCs is uncommon and is not routinely reported in clinical practice or incorporated into current staging frameworks. When ITCs are observed, typically in the setting of intensive pathological evaluation or sentinel node studies, their clinical significance remains uncertain due to a lack of robust outcome data. In a review of 27 studies, comprising 511 patients with OSCC and positive SLNs and subsequent neck dissection, the pooled prevalence of non-SLN metastasis in patients with positive SLNs was 31%. Non-SLN metastases were detected (available from 9 studies) in 13, 20, and 40% of patients with ITC, micro-, and macrometastasis in the SLN, respectively. However, from most of these small studies with a limited number of patients, it was not clear which definition of ITC was used [26]. Consequently, there is no consensus on whether their presence should alter staging or influence adjuvant therapy decisions in HNSCC (Table 1). Several studies show a different overall survival for OSCC patients with SLNs containing no tumor deposit, ITC, micrometastasis and macrometastasis, although not always statistical significant [26]. Further prospective investigations are warranted to determine whether ITCs have any prognostic value in head and neck cancer and to assess their potential role in refining staging systems or guiding therapeutic strategies.

Micrometastases are defined as tumor deposits measuring more than 0.2 mm but not exceeding 2.0 mm in largest dimension (Fig. 2). In breast and melanoma staging, cases with micrometastases—without deposits exceeding 2 mm,

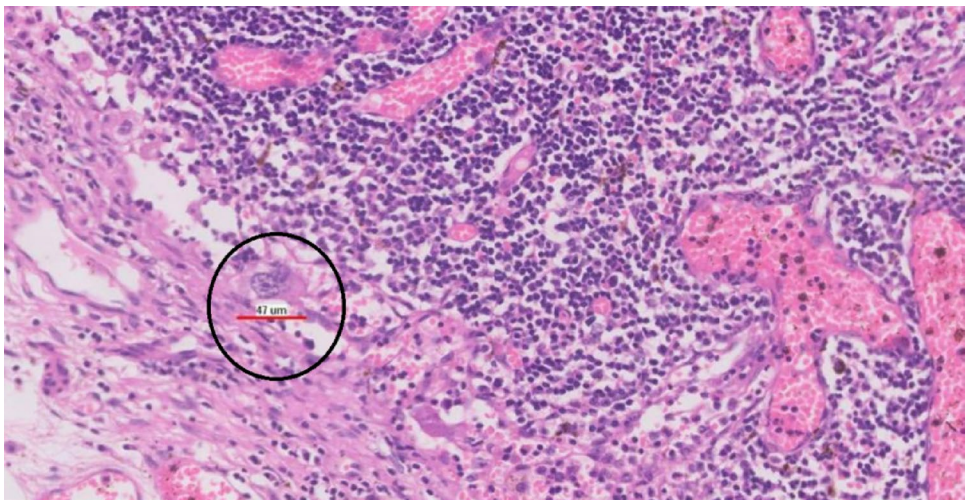

Fig. 1 H&E stained biopsy slide of lymph node depicting isolated tumor cells measuring 47 microns across (redline and circle)

Table 1 Comparison of ITC incidence and management across key SLNB studies/trials

Study	Year	Patient group	ITC Incidence	Management	Prognostic findings	Conclusion on ITC
Civantos et al. (ACOSOG Z0360) [34]	2010	T1–T2N0 oral SCC ($n=140$)	Not reported	Treated all nodes equally, no ITC distinction	Not evaluated	Prognostic/therapeutic role unclear
Brogile et al. [86]	2012	T1/T2N0 oral/oropharyngeal cancer	10/111 (24%)	ND done for ITC+	Better survival	Predictive of additional nodal disease
Flach et al. (Dutch trial) [87]	2014	T1–T2N0 oral cavity	3/62(5%)	ND done	Lower survival in ITC+	Predictive of additional nodal disease
Den Toom et al. [88]	2014	T1–T2N0 OSCC	15%	ND done	Better prognosis than micro/macro mets	Predictive of additional nodal disease
Pederson et al. [89]	2015	T1–T2N0 OSCC	2/88	ND done	ITC+micromets worse than negative	Predictive of additional nodal disease
Schilling et al. (SENT Trial) [90]	2015	Early oral SCC (~415)	~9%	ND done	ITC+had better OS	Predictive of additional nodal disease
Boeve et al. [91]	2018	T1–T2N0 OSCC	7/25 (28%)	ND done	No recurrence in ITC-only	Predictive of additional nodal disease
Garrel et al. (Senti-MERORL) [36]	2020	T1–T2N0 oral/oropharyngeal SCC ($n=307$)	11/140 (7.9%)	No ND for ITC-only	No neck recurrence	Predictive of additional nodal disease
Den Toom et al. [46]	2020	T1–T2N0 OSCC	15/107 (14%)	ND done	Better survival than macro	Predictive of additional nodal disease
Yokoyama et al. [92]	2020	T2–T3N0 oral SCC ($n=18$)	12.7% (not ITC-specific)	No ND for ITC-only	No adverse prognosis	No adverse prognostic impact
Hasegawa et al. [38]	2021	T1–T2N0 oral SCC ($n=275$)	9/134 (6.8%)	ND done	No adverse survival impact	Predictive of additional nodal disease

ND, Neck dissection; ITC, Isolated tumor cell

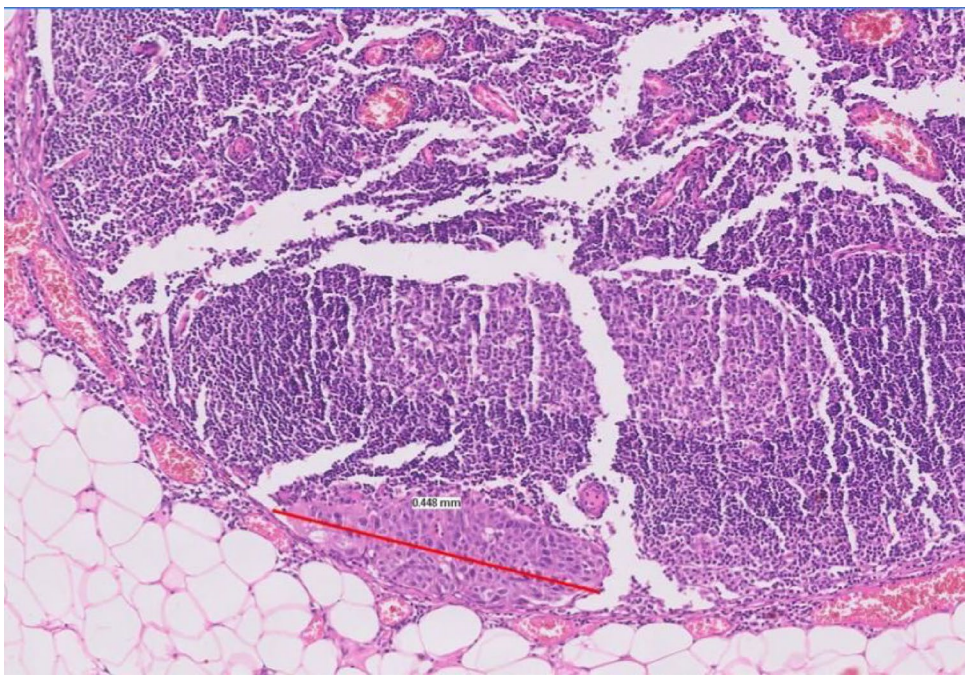

Fig. 2 H&E-stained biopsy slide of lymph node depicting a micrometastasis measuring 0.488 mm across (red line)

are designated as pN1mi or pN1mi(sn), depending on the SLNB status. Importantly, only the largest contiguous tumor deposit is considered in classifying the lymph node, regardless of the number of deposits or their overall distribution within the node. The prognostic implications of macro metastatic involvement in SLNs are well-documented, correlating strongly with poorer disease-free survival [10, 12]. However, the impact of micrometastases in SLNs remains controversial, particularly in HNSCC [27, 28].

In breast oncology, the role of micrometastases has been extensively studied. Level 1 evidence from the ACOSOG Z0011 trial demonstrated that micrometastases (≤2 mm)

were present in 44.8% of patients with sentinel lymph node (SLN) involvement, specifically within the SLNs themselves—not in nodes retrieved during subsequent axillary lymph node dissection (ALND). Long-term follow-up showed that SLN biopsy (SLNB) alone provided non-inferior 10-year overall survival compared to ALND (86.3% vs 83.6%), supporting treatment de-escalation in selected patients and the omission of more invasive procedures or radiotherapy. [29, 30].

Unfortunately, such robust data do not exist for HNSCC. While studies have reported micrometastasis incidence rates between 2 and 14%, their prognostic weight and influence on clinical decisions remain uncertain. It is still unclear whether detection of a single tumor cell within an SLN warrants a completion neck dissection.

SLNB and the Role of ITCs and Micrometastases in Head and Neck Cancer:

Level 1 evidence from multicenter randomized trials demonstrates that SLNB is noninferior to elective neck dissection for early-stage oral cancer, providing comparable oncologic outcomes and similar postoperative functional results. These studies underscore the safety of SLNB when performed by surgeons with specialized expertise in nodal mapping and within institutions equipped with validated pathological protocols. Furthermore, SLNB represents the optimal diagnostic strategy for targeted identification and histopathological sampling of sentinel nodes harboring ITCs or micrometastases, thereby reducing unnecessary morbidity while maintaining staging accuracy [31, 32] (Table 1).

In a seminal 2004 multicenter clinical trial, Ross and colleagues pioneered the evaluation of SLNB in early-stage oral/oropharyngeal squamous cell carcinoma, establishing foundational evidence for its technical feasibility and diagnostic accuracy [33]. The study demonstrated a 93% sensitivity rate for SLNB in identifying metastatic nodal disease when followed by END, marking a pivotal advancement in staging precision for clinically node-negative patients. While this landmark trial validated SLNB as a minimally invasive alternative to routine END, it did not assess the presence or clinical implications of ITCs or differentiate between macro metastases and micrometastases, likely reflecting that the concepts of ITCs and micrometastases were yet evolving [33].

Civantos et al. evaluated SLNB in 140 patients with pT1–T2, clinically N0 OSCC across 25 centers. SLNB was followed by completion neck dissection, with a reported negative predictive value (NPV) of 94%, improving to 96% using step sectioning and immunohistochemistry, especially in tongue and T1 tumors. However, the study did not stratify metastases into ITCs, micrometastases, or macro metastases, limiting its prognostic insights. This omission likely reflects the lack of robust evidence on the prognostic significance of ITCs at the time, as their clinical relevance in head and neck cancers was not well-established [34].

In the 3-year analysis of the prospective multicenter Sentinel European Node Trial (SENT), the clinical relevance of ITCs in SLNs was evaluated in the context of early oral cancer staging and prognosis. Among patients with nodal involvement, ITCs represented 14.7%, whereas micrometastases and macro metastases comprised 35.7% and 49.6%, respectively. Survival analyses demonstrated that patients with ITCs had significantly better outcomes compared to those with larger-volume nodal metastases: overall survival (OS) rates were 100% for ITC, 85% for Micrometastasis, and 65% for macrometastasis. These results underscore the prognostic importance of ITCs as a biologically distinct and less aggressive form of nodal dissemination [35].

Garrel and colleagues conducted a multicenter, randomized equivalence trial evaluating SLNB versus END in 307 patients with operable, clinically node-negative (cT1–T2N0) oral and oropharyngeal squamous cell carcinoma [36]. The trial demonstrated oncologic equivalence between the two approaches, with comparable 2-year neck node recurrence-free survival (90·7% in the SLNB group vs 89·6% in the END group), as well as similar overall and disease-specific survival at 5 years. Notably, ITCs were detected in 11 patients within the SLNB arm, none of whom underwent neck dissection and no patient developed regional recurrence during follow-up. These findings suggest that the presence of ITCs, in isolation, may not confer significant prognostic risk and do not appear to necessitate further neck dissection, supporting a more conservative approach in this subgroup [36, 37].

Hasegawa et al. conducted a multicenter, randomized, noninferiority trial in Japan comparing SLNB–guided management to END in 271 patients with pT1–T2, cN0 OSCC [38]. The 3-year overall survival and disease-free survival in the SLNB group were noninferior to those in the END group, and SLNB demonstrated superior postoperative neck function. Among the 54 metastasis-positive cases identified postoperatively in the SLNB group, 9 cases (6.8%) were classified as ITCs. In contrast to some previous trials, ITCs in this study were considered metastasis-positive and managed with completion neck dissection, either during initial surgery or as a staged procedure within six weeks. However, the study did not specify whether additional disease was found in the non-sentinel nodes of these ITC-positive cases [38].

Clinical staging and treatment decisions in OSCC have traditionally relied on the binary presence or absence of lymph node metastasis. However, SLNB enables a more nuanced assessment by evaluating not only the presence but also the size of the metastatic deposits within the sentinel node, thereby allowing for refined pathological

classification [39–41]. Early identification of SLN metastasis offers the potential to prevent subsequent non-SLN involvement, which is strongly associated with poorer prognosis. For SLNB to fulfil this role effectively, it must be sufficiently sensitive to detect micrometastases, guiding the need for further management of the neck [42–45].

In cases where cancer cell deposits in sentinel nodes are limited to micrometastases or ITC, these are small but not dormant enough to preclude further regional spread [39, 44]. There is a need to identify those patients who can avoid completion neck dissection. The presence of multiple positive SLNs, the absence of negative SLNs, and a positive SLN ratio(Number of positive sentinel lymph nodes / Total number of sentinel lymph nodes removed) of more than 50% may be predictive for non-SLN lymph node metastasis which may necessitate completion neck dissection [46]. More research is needed to determine if these parameters can be used in clinical practice to select patients for completion neck dissection or not. Best clinical practice/ expert recommendation given in Fig. 3

Role of Pathological Techniques

Conventional pathologic evaluation, which primarily relies on microscopic examination of hematoxylin and eosin (H&E)-stained tissue sections, has limited sensitivity for detecting small metastatic deposits within lymph nodes [47]. Consequently, patients harboring occult metastases may be misclassified as node-negative, potentially resulting in undertreatment and suboptimal clinical outcomes [27]. This limitation highlights the urgent need for more sensitive diagnostic modalities capable of accurately identifying occult nodal metastases, particularly in patients with clinically node-negative necks. The identification of reliable biomarkers for metastasis is therefore critical to optimizing patient management and improving therapeutic outcomes in this population [48].

The NICE guidelines showed that SNB must have a sensitivity of > 84% to be more cost-effective than END. While techniques like step serial sectioning (SSS) and immunohistochemistry (IHC) with cytokeratin markers (ie: such as, AE1/AE3CK8/18, CK5/6, HMWCK) can indeed enhance SLNB sensitivity, they also place a significant strain on histopathology departments. This resource demand may partly explain the limited adoption and hesitancy surrounding the routine implementation of this approach. To address these challenges, some researchers have proposed streamlining the lab protocol by reducing the number of section levels examined. For instance, Bell et al. found that a single H&E and IHC section of the sentinel node provided an acceptable negative predictive value in a cohort of 35 patients [49]. Similarly, Jefferson et al. re-examined sentinel nodes from 10 patients previously deemed tumor-free using a single H&E plus IHC section and found no micrometastases upon further analysis with SSS and IHC. However, both studies are constrained by small sample sizes and few or no positive sentinel nodes [50]. In contrast, King et al. found that 83% of metastatic tumors were detected within the first four section levels, but omitting deeper levels could overlook 16.7% of tumor deposits, most of which were ITCs [51]. It is important to recognize that retrospective comparisons of laboratory protocols have inherent limitations, and the clinical significance of these methodological differences remains unclear due to a lack of comparative outcome data. Still, until more robust data emerges, performing SSS with IHC to include the whole face of sentinel node seems prudent [52].

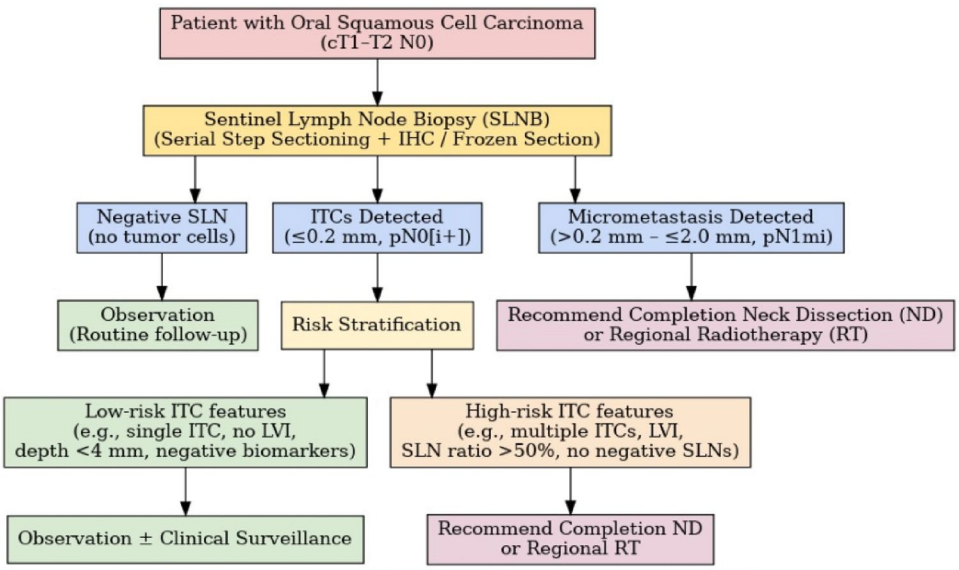

Fig. 3 Best clinical practice / expert recommendations

Additionally, a recent systematic review regarding frozen section of SLN in patients with OSCC showed that the evaluated studies, although employing different techniques, collectively achieved a pooled sensitivity of 0.71, which now serves as a benchmark for comparing alternative one-step approaches. However, given the substantial false-negative rate—impacting nearly one-third of patients—intraoperative frozen section (FS) analysis must be consistently paired with serial sectioning to ensure diagnostic accuracy [53]. Following frozen section analysis, which allows for direct completion neck dissection if SLN is positive, SSS with IHC on the rest of the SLN can be performed, and if positive, a completing neck dissection is done in a second operation. Using this strategy, majority of the patients with a positive SLN can be treated with the neck dissection directly, and the other small group of patients at a second operation.

Novel Diagnostic Tools to Predict ITC and Micrometastasis

Personalized management of the cN0 neck in patients with OSCC would benefit greatly from staging techniques that enhance the accuracy of nodal disease assessment, particularly those that are minimally or not at all dependent on the size of the metastatic deposits [44, 54, 55]. While tumor depth of invasion remains the only clinicopathologic feature consistently associated with nodal metastases, the lack of consensus on a definitive cutoff for END underscores the need for more reliable biomarkers [55–59]. Recent advances in gene-expression profiling using microarrays and next-generation sequencing (NGS: e.g. RNAseq) have ushered in a new era of tumor classification and prognostication [60, 61]. In a multicenter validation study conducted across all head and neck oncology centers in the Netherlands, a gene-expression profile predictive of lymph node metastasis was successfully transferred to a diagnostic platform and validated in an independent series of 222 oral and oropharyngeal SCC samples, yielding a negative predictive value of 89% in early-stage (cT1–T2N0) OSCCs. These findings suggest that gene-expression profiling could significantly reduce unnecessary END, enabling more personalized treatment strategies [62]. Furthermore, genome-wide assessment of genetic changes using, for example, comparative genomic hybridization (arrayCGH/SNP arrays) or NGS platforms have demonstrated additional value in N-staging, supporting the integration of molecular diagnostics into clinical decision-making for the detection of ITC and micrometastasis [63, 64].

Existing literature comparing molecular diagnostics and SLNB for staging the cN0 neck in early-stage OSCC offers important clinical insights. Multicenter studies, including those by Van Hooff et al. on gene-expression profiling and Alkureishi et al. on SLNB, demonstrate that while SLNB provides a high negative predictive value, it remains an invasive procedure involving radioactive tracers and is associated with potential morbidity and logistical challenges, such as the need for a second surgical intervention if metastases are identified [62, 65]. In contrast, gene-expression profiling has been validated as an accurate method to stratify patients by their risk of nodal metastasis [65]. Current evidence supports a combined diagnostic strategy: patients staged as cN0 by conventional imaging undergo initial gene-expression profiling(gep); those classified as low risk (cN0[gep]) may be managed conservatively with surveillance, whereas high-risk patients (cN0[+gep]) are directed to SLNB for further evaluation [65]. This paradigm has the potential to markedly reduce unnecessary elective neck dissections and the attendant risks of overtreatment. Nevertheless, both approaches carry a small but significant false-negative rate, underscoring the need for meticulous follow-up to mitigate the risk of undertreatment. Taken together, the integration of molecular diagnostics with selective SLNB represents a promising, personalized approach to optimize neck management in early oral SCC [66].

Several considerations arise in the pursuit of effective metastasis markers. Biomarkers derived from the primary tumor (such as Podoplanin, D2-40, VEGF-C, and LYVE-1) should ideally be assessed from initial biopsy specimens rather than postoperative tissue, as detection of suspected nodal metastasis after surgery may necessitate a second operation. Additionally, expression-based markers like matrix metalloproteinases (e.g., MMP-9) and epithelial-mesenchymal transition (EMT)–related genes (e.g., Snail, Twist) have been implicated in promoting metastatic spread in head and neck cancers. Importantly, markers of occult metastasis often exist along a continuum rather than as binary variables, making the establishment of appropriate threshold values essential for accurately stratifying patients into metastasis-positive and negative groups to guide clinical decision-making [67–71].

Although the concept of identifying a single, powerful marker to predict occult nodal involvement is appealing, it may fail to capture the complexity of metastatic processes influenced by multiple factors. Combining several biomarkers could enhance predictive accuracy (Table 2). Integrating diverse molecular markers may ultimately enable the development of a novel scoring system with improved predictive value for occult lymph node metastasis, thereby refining risk stratification and informing tailored therapeutic strategies.

Table 2 Established and investigational biomarkers of occult lymph node metastasis in HNSCC patients

Marker name	Detection Method/Specimen type	Diagnostic efficiency	Sensitivity	Specificity	Significance
miR-205 [67]	qRT-PCR/Lymph Node biopsy	AUC 1.0, accuracy 100%	100%	100%	High precision for macro-, micro-, and isolated tumor cells
miR-203 [67]	qRT-PCR/Lymph Node Biopsy	AUC 1.0, accuracy 100%	100%	100%	Excellent for micrometastatic detection
miR-200a [67]	qRT-PCR/Lymph Node Biopsy	Accuracy 84.2%, PPV 100%, NPV 68.4%	84.2%	100%	Moderate for micrometastasis
miR-200c [67]	qRT-PCR/Lymph Node Biopsy	Accuracy 92.1%, PPV 100%, NPV 81.2%	94.7%	100%	Good for macro-metastasis
CK14 + DSG3 [69, 93]	qPCR + IHC/Lymph Node Biopsy	Sensitivity 88%, Specificity 85%	88%	85%	Combo improves intraoperative detection
CK19 (OSNA) [94]	RT-PCR, OSNA/Lymph Node Biopsy	Accuracy 95%, PPV 75%, NPV 98.5%	90%	95.6%	Strong intraoperative marker
HPV-16 DNA [95, 96]	RT-PCR/Blood & Lymph Node Biopsy	High viral load correlates with metastasis	Variable	Uncertain	Potential false positive
MYO5A, RNF145, FBXO32, CTONG2002744 [71]	Affymetrix + qPCR/Lymph Node Biopsy	AUC 0.85	Not stated	Not stated	Outperforms tumor size as a predictor
SCCA [97]	Real-time qPCR/Lymph Node Biopsy	Overexpressed in metastasis	Not specified	Better than CK13	Promising for lymph node involvement
Activin A [98, 99]	IHC, ELISA/Tumour & Lymph Node Biopsy	$p = 0.006$ for occult metastasis	Not quantified	Not quantified	Independent prognostic factor
Cyclin D1 [100–102]	FISH/ Immunofluorescence Lymph Node Biopsy	$p < 0.007$, RR = 8.68	High (implied)	High (implied)	Reliable for early-stage occult metastasis
E-cadherin [103–106]	IHC/Tumour & Lymph Node Biopsy	$p = 0.007$ for nodal spread	Not specified	Not specified	Correlates with aggressive spread
β-catenin [103]	IHC/Lymph Node Biopsy	$p = 0.02$ for nodal involvement	Not specified	Not specified	Aggressiveness marker
Podoplanin [104]	IHC/Lymph Node Biopsy	Sensitivity 36%, Specificity 83%	36%	83%	Moderate predictor of sentinel node involvement
Cornulin [107]	2D-DIGE, Mass Spectrometry/ Tumour-adjacent Mucosa	Overexpressed in adjacent normal tissue	Not specified	Not specified	Indicates high-risk epithelial environment
CD133, NANOG, NOTCH1 [108]	IHC/Tumour & Lymph Node Biopsy	Significant association ($p < 0.05$)	Not specified	Not specified	Cancer stem cell markers for early OSCC metastasis
PROX1 [109]	IHC/Lymph Node Biopsy	Sensitivity 60%, Specificity 98%, Accuracy 88%	60%	98%	Predictor via lymphatic vessel density
LINE-1 methylation [110]	COBRA, Methylation Analysis/ Lymph Node Biopsy	AUC 0.806 (uCmC), 0.716 (mCuC)	Not explicitly stated	Not explicitly stated	Epigenetic indicator of metastatic activity
Panitumumab-IRDye800 [111]	Fluorescence Imaging/Lymph Node Biopsy	Sensitivity 100%, Specificity 85.8%, NPV 100%	100%	85.8%	High accuracy intraoperative tool
Circulating Tumor Cells (CTCs) [112, 113]	Blood assay systems/Blood	Associated with poor prognosis in several studies	Variable	High	Early marker of metastasis and progression
HPV-DNA (blood) [114]	PCR from serum/plasma/Blood	Elevated in recurrent/ metastatic HPV + cases	Moderate to High	Moderate to High	Non-invasive tracking of disease in HPV + HNSCC
CD31 [109]	Flow cytometry / ELISA/Blood	Linked to tumor angiogenesis and nodal spread	Not quantified	Not quantified	Endothelial marker of tumor vascularization
NLR (Neutrophil/ Lymphocyte Ratio) [115]	Standard blood count/Blood	Prognostic value for survival and nodal disease	Moderate	Moderate	Systemic inflammatory response marker
PLR (Platelet/ Lymphocyte Ratio) [116]	Standard blood count/Blood	Correlated with advanced/ metastatic disease	Moderate	Moderate	Another systemic inflammation-based predictor
Bone Marrow Tumor Cells [117]	Bone marrow aspiration + PCR/ Bone Marrow Aspirate	High accuracy in detecting systemic metastasis	High (when present)	High	Early dissemination indicator, surpasses nodal biopsy

Role of Artificial Intelligence (AI)

In the near future AI will likely revolutionize the detection of early lymph node metastasis across various cancers, including oral cancer, by enhancing diagnostic accuracy and efficiency. The CONFIDENT-B trial, demonstrated that AI-assisted pathology significantly improves the detection of SLN metastases in breast cancer, particularly, while reducing the reliance on time-intensive and costly immunohistochemistry (IHC). The AI-based tool (Visiopharm©) demonstrated high diagnostic accuracy, achieving a sensitivity of 95.8% for micrometastases and 100% for macrometastases, though sensitivity for isolated tumor cells (ITCs) was lower at 44.4%. Notably, its implementation resulted in a 32% reduction in immunohistochemistry usage, translating to an estimated cost savings of approximately €3,000 for every 100 cases assessed in the intervention arm. Pathologists reported increased diagnostic confidence, faster assessments, and improved workflow satisfaction. These findings highlight the urgent need to integrate AI into routine pathology to enhance diagnostic accuracy and efficiency [72].

A recent study from the Netherlands group highlighted the potential of AI-assisted pathology in enhancing lymph node (LN) assessment across breast cancer, melanoma, and head and neck cancer (HNC), particularly for detecting micro-metastases and ITCs [73]. In HNC, DeepPath-LYDIA© (DP) demonstrated excellent performance with 100% sensitivity for both macro- and micro-metastases, as well as ITCs, even when used outside its intended regulatory use, outperforming Visiopharm©, another AI model which missed some micro-metastases and most ITCs. The consistent performance of these tools across tumor types suggests the feasibility of implementing a single AI solution, which could streamline diagnostics, reduce pathologist workload, and significantly improve accuracy in resource-constrained settings. However, further validation in larger, prospective cohorts is essential to confirm these benefits and ensure safe integration into standard clinical practice [73].

Kim et al. introduced an efficient, interpretable AI framework leveraging transcriptomic data and protein–protein interaction networks to predict nodal metastasis [74]. By employing neural network–based subnetwork representation learning and an attention-weighted classifier, the model identifies biologically meaningful gene clusters (e.g., EMT, E2F, TNF-NFκB) that signal metastatic potential. Validated across racially and geographically distinct cohorts, this approach achieved significantly higher predictive accuracy (AUC 0.9174) than conventional machine learning models. Importantly, it offers explainable outputs in the form of patient-specific network biomarker maps, aligning with the principles of precision oncology. This tool represents a promising advance toward molecularly guided, non-invasive risk stratification in early OSCC and a potential reduction in unnecessary surgical morbidity [74].

The Role of Radiomics

Traditional imaging methods such as CT and MRI rely heavily on morphological criteria, such as lymph node size thresholds, which have demonstrated poor specificity and sensitivity, often resulting in overtreatment or missed micrometastases. Furthermore, clinical palpation and even fine-needle aspiration biopsies are insufficient in detecting subclinical nodal disease. In this context, radiomics—quantitative analysis of medical imaging—has emerged as a promising non-invasive tool capable of capturing intra tumoral and peritumoral heterogeneity that correlates with underlying tumor biolog y [75–77].

Tianjun Lan et al. developed predictive models for occult lymph node metastasis in early-stage SCC using handcrafted radiomic features and deep learning features (DLFs) from preoperative MRI, with the ResNet-50 model showing superior performance across all cohorts (AUC: 0.796–0.928) [78]. This combined deep learning–radiomics approach offers a transformative advance in the preoperative risk stratification of cervical lymph node status. Notably, 13 out of 18 top-performing features in the ResNet-50 model were deep learning-derived, underscoring the ability of AI algorithms to extract high-dimensional, abstract imaging biomarkers not accessible through human interpretation from CT, MRI, and PET/CT. The model not only predicted occult lymph node metastasis with high accuracy but also showed strong correlation with overall survival, reinforcing its potential role as a dual diagnostic and prognostic tool. While prior radiomics research has been limited by single-center designs, small sample sizes, or lack of external validation, this study represents a significant step forward in clinical applicability [78].

Giannitto et al. conducted a comprehensive systematic review evaluating the role of radiomics-based machine learning (ML) in diagnosing lymph node metastases in patients with HNC. The review reported pooled AUCs of approximately 91% for CT-based models, 84% for MRI, and up to 92% for PET/CT, with deep learning models achieving comparable performance. However, challenges remain regarding standardizing radiomic workflows, automation of tumor segmentation, and integration into clinical decision-making emphasize the need for prospective validation, standardized imaging protocols, and integration into clinical pathways to ensure reproducibility and clinical applicability. Bridging the “clinical transition gap” will require prospective validation, regulatory clarity, and alignment with machine learning best practices. Nonetheless, this

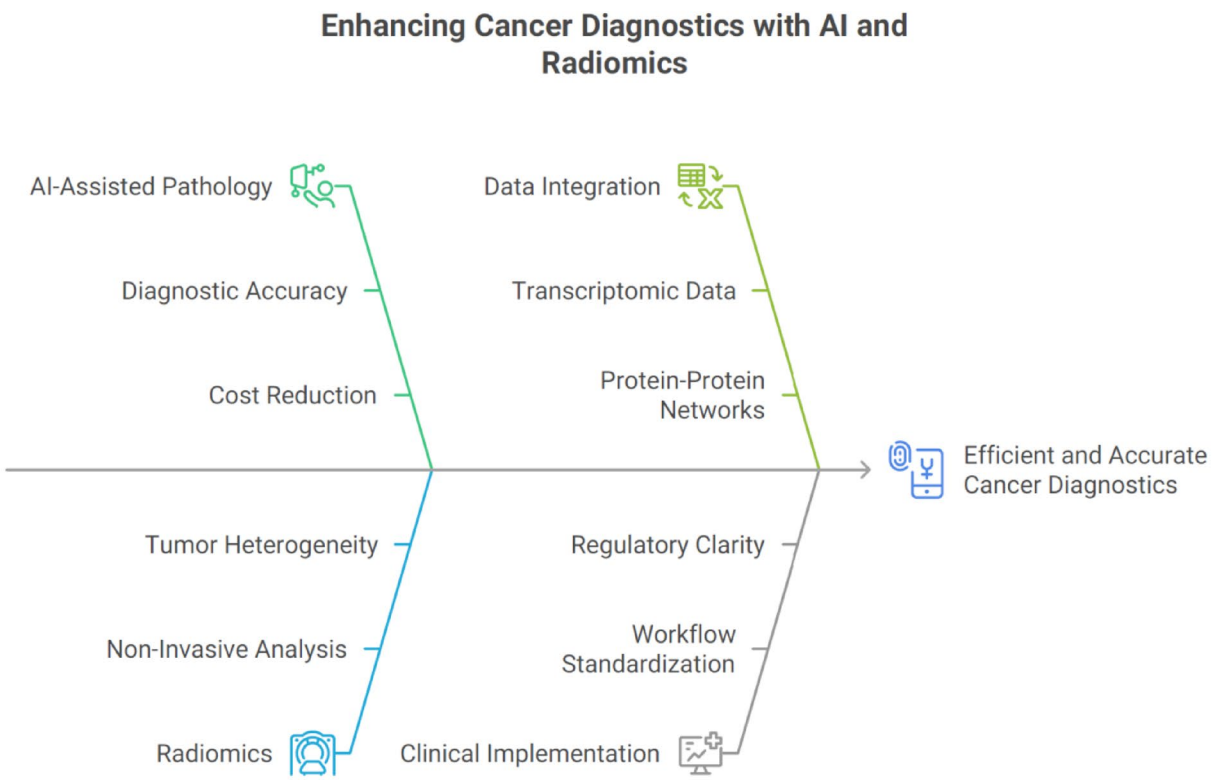

Fig. 4 Enhancing cancer diagnostics with AI and radiomics

radiomics-based strategy presents a compelling, data-driven alternative to conventional imaging, with the potential to reduce unnecessary surgery, guide personalized treatment planning, and ultimately improve oncologic outcomes in HNC care [78, 79] (Fig. 4).

Challenges to Clinical Translation

AI currently functions largely as a "black box," making complete clinical transition challenging and dependent on future developments [80]. For successful adoption, AI-generated results must be both interpretable and trustworthy, requiring targeted training for clinicians, radiologists, and pathologists. This must be followed by rigorous validation and regulatory approvals. Integrating such systems into hospital workflows remains complex, but once achieved, it could enable seamless clinical adoption. Long-term studies in large patient cohorts are essential to determine cost-effectiveness, time savings, and overall economic feasibility. Importantly, these technologies must be scalable and accessible to low- and middle-income countries to ensure equitable benefit [80].

Future Challenges

Identifying ITCs and micrometastases in lymph nodes presents a complex and evolving challenge with potential prognostic implications. Traditional histopathology, while standard, often fails to detect these occult metastases due to their minuscule size and limited morphological disruption of lymph node architecture. However, emerging evidence underscores that lymph node metastases are not a single, uniform entity; rather, they display molecular heterogeneity that reflects distinct biological behaviors and prognostic risks. The molecular classification of lymph node metastases, as demonstrated in HNSCC, reveals three distinct subtypes—immune (Group 1), invasive (Group 2), and metabolic/proliferative (Group 3)—with the invasive subtype showing markedly poorer locoregional control and survival outcomes [81]. Notably, these prognostically relevant molecular subtypes are often not reflected in the matched

primary tumors, highlighting the inadequacy of primary tumor profiling alone in predicting disease development. This suggests that molecular profiling of lymph nodes, even in the absence of overt metastases, could provide more accurate risk stratification. Complementing this, the concept of the pre-metastatic niche (PMN) provides a mechanistic framework wherein primary tumors release soluble factors, extracellular vesicles, and immune-modulating signals that precondition distant organs, including lymph nodes, to be more receptive to future metastases. These insights challenge the traditional sequential view of metastasis and suggest that some lymphatic environments may already be biologically "primed" before tumor cells arrive. Therefore, the utility of advanced molecular diagnostics, such as transcriptomics, miRNA profiling, NGS, circulating tumor DNA (ctDNA), and even exosome-based assays, must be explored for routine clinical use to identify these subclinical changes. To be truly transformative, such technologies must become affordable, standardized, and integrated into existing diagnostic routines. Moreover, future research should aim to develop dynamic biomarkers that not only detect occult metastases but also monitor evolving metastatic risk over time. The goal is to evolve from static anatomic staging to biologically-informed, personalized risk models that guide treatment intensity, surveillance strategies, and perhaps even targeted interventions to disrupt PMN formation before metastatic colonization occurs. This shift holds the potential to greatly enhance prognostication, reduce overtreatment in low-risk patients, and improve outcomes for those with aggressive yet still curable disease [82–85].

As previously mentioned, the disadvantages of SLNB are its invasiveness and a second operation to perform a neck dissection if SLNB is positive. The number of SLNB and second-stage subsequent completed neck dissection could be reduced by performing a neck dissection in the same operation as primary tumor resection in patients in whom occult lymph node metastases can be predicted with high positive predictive value using fluor-18 (^{18}F) FDG PET/CT. By varying cut-off levels of fluor-18 (^{18}F) FDG uptake, scoring criteria can be developed and optimized to predict the presence of lymph node metastases with high positive predictive value rather than high sensitivity, which is the usual focus in existing studies. When aiming for a high positive predictive value, sentinel lymph node biopsy (SLNB) can be performed after a negative fluorine-18 (^{18}F) FDG PET/CT scan [83].

Conclusions

A more practical and scientifically sound way forward in improving cancer staging is not to design new clinical trials with the primary aim of evaluating SLNB based solely on the detection of micrometastases. Instead, the focus should be on consistently reporting the incidence of ITCs and micrometastases in large, well-defined patient populations using standardized methods. This approach would generate high-level (Level I) evidence that can be used to refine cancer staging systems. Integrating such data into staging would strengthen two key principles: hazard consistency, meaning each stage reflects a clear and increasing risk of worse outcomes, and hazard discrimination, meaning the staging system can effectively separate patients into groups with distinctly different prognoses. These refinements would ensure that staging is not only biologically informed but also clinically meaningful. This aligns with the mission of the Precision Medicine Core (PMC), which emphasizes the need for evidence-based, validated prognostic tools that are relevant to today's clinical settings and patient care [84, 85]. By moving in this direction, staging systems can evolve from purely anatomical models to ones that reflect both the molecular pathogenesis of the disease and real-world patient outcomes, paving the way for more precise, personalized treatment decisions and future updates grounded in robust, population-level data.

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