

Serial step sections at narrow intervals with immunohistochemistry are required for accurate histological assessment of sentinel lymph node biopsy in oral squamous cell carcinoma

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Abstract

Background: Sentinel lymph node (SLN) biopsy is an accurate staging modality in early oral squamous cell carcinoma (OSCC), but its accuracy relies on labor-intensive histopathology protocols. We sought to determine whether serial step sections with immunohistochemistry (SSSIHC) at narrow intervals of the entire SLN are required to accurately exclude metastasis.

Methods: Consecutive SLN biopsies over a 13-year period were retrospectively evaluated. If the index section was negative for carcinoma, the entire SLN was subjected to SSSIHC at 150 µm intervals. The first section level and total number of section levels to contain carcinoma were recorded.

Results: One hundred and eighteen SLN+ from 90 patients were included. SSSIHC upstaged the nodal status in 19.5% of patients. Metastasis was identified in 16.7% and 10.2% beyond section levels 4 and 6, respectively. Among SLNs requiring SSSIHC, 47.5% contained carcinoma in a single section level.

Conclusion: SSSIHC of the entire SLN at 150 µm intervals are required to identify occult metastasis in OSCC.

KEYWORDS

isolated tumor cells, micrometastasis, oral squamous cell carcinoma, sentinel node, serial step sections

1 | INTRODUCTION

Accurate staging of the neck is essential in the management of oral cavity squamous cell carcinoma (OSCC) because lymph node metastasis is described as the single

most important prognostic factor.^{1–3} Clinical and radiological methods alone are inadequate staging modalities in early OSCC as approximately 20%–40% of T1–2 tumors harbor occult nodal metastasis.^{4–6} The high rates of occult metastasis have driven the move toward elective

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neck dissection (END) for all T1-2 N0 OSCCs. However, routine END is likely to be unnecessary surgery in 70%–80% in this group of patients. Against this background, several recent meta-analyses and clinical trials have demonstrated sentinel lymph node biopsy (SLNB) to be a viable neck staging procedure that can reduce unnecessary END.^{7–11} The reduced morbidity of SLNB compared to END has led several authorities to recommend the former as standard of care in early stage OSCC.^{12–14}

The sensitivity and negative predictive value of SLNB is known to be dependent on laboratory technique.^{8,15} While serial step sections (SSS) with immunohistochemistry (IHC) improves diagnostic accuracy, it creates additional burden on laboratory resources and pathologist's time. Furthermore, there is considerable laboratory methodological variation in the literature (Table 1) with no consensus regarding the number of SSS required, nor the optimal interval thickness between step levels. Reducing the number, and/or increasing the interval thickness between step sections may be expedient in

relation to laboratory workload but runs the risk of missing metastatic deposits thereby reducing the sensitivity of the technique. Our standard practice has been to undertake SSS with IHC at 150 μ m intervals of the entire sentinel node (SN) according to the method used in a large multicenter European trial.⁶

The aim of this study was to determine whether this labor-intensive protocol is required to accurately exclude the presence of metastatic carcinoma cells within the SN. To achieve this aim, we reviewed consecutive cases of OSCC SLNBs to identify (1) the first histological section level containing carcinoma cells, and (2) the total number of section levels containing carcinoma cells.

2 | PATIENTS AND METHODS

All SNs in this single-institution study were processed according to a standard operating procedure. SNs of <3 mm thickness were submitted whole, nodes of 3–

TABLE 1 Methodological heterogeneity

Reference	Method				Cohort size (number of patients)	Positive rate (%)
	Grossing	Number of step sections	Interval thickness	Stain		
Melkane et al. ¹⁶	Not stated	Not stated	150 μ m	H&E, AE1/AE3	166	25
Riese et al. ¹⁷	Not stated	Not stated	150 μ m	H&E, AE1/AE3	36	33
Schilling et al. ⁶	Not stated	Through the block	150 μ m	H&E, AE1/AE3	415	27
Abdul-Razak et al. ¹⁸	Not stated	Not stated	200 μ m	H&E, AE1/AE3	30	50
Boeve et al. ¹⁹	Not stated	Not stated	500 μ m	AE1/AE3	91	27
Moya-Plana et al. ²⁰	Not stated	4	200 μ m	H&E, AE1/AE3	215	21
Den Toom et al. ²¹	Not stated	At least 6	150 μ m	H&E, AE1/AE3	488	22
Vigili et al. ²²	Longitudinal from hilum	At least 6	150 μ m	H&E, AE1/AE3	48	31
Vishnoi et al. ²³	Bisected through hilum	Not stated	Not stated	Pan-CK (clone not stated)	94	28
Molstrom et al. ²⁴	Not stated	Not stated	Not stated	H&E, AE1/AE3	220	25
Loree et al. ²⁵	Not stated	3	Not stated	No IHC performed	103	20
Ishiguro et al. ²⁶	Not stated	Not stated	2.0 mm	H&E	26	19
Marttila et al. ²⁷	Not stated	Not stated	Not stated	Not stated	70	11

Notes: Selected recent reports of sentinel lymph node biopsy in oral squamous cell carcinoma demonstrating inter-study methodological heterogeneity.

6 mm thickness were longitudinally hemisected through the hilum, and those >6 mm thickness were longitudinally sliced every 3 mm. Blocks were trimmed minimally following which four serial sections (designated Level 1) were obtained, the second of which was stained for hematoxylin and eosin (H&E). If metastatic carcinoma was identified on this index H&E, no further laboratory procedure was needed to report the SN as positive. If no carcinoma was identified on the index H&E, four further serial sections were obtained at every 150 μm intervals (designated Level 2, etc.) throughout the SN until all tissue within the block was depleted. The third serial section at each level (including Level 1) was then submitted for pan-keratin (AE1/AE3, Dako Omnis, Agilent, Stockport, United Kingdom) IHC on an automated platform (Benchmark Ultra, Ventana Medical Systems, Cambridgeshire, United Kingdom). All cases were reported by specialist head and neck histopathologists. If present, metastatic carcinoma was categorized as isolated tumor cells (ITCs, tumor deposits of <200 cells and/or <200 μm), micrometastasis (Mi, deposits 200 μm -2 mm) or macrometastasis (Ma, deposits larger than 2 mm).

Consecutive patients undergoing SLNB for T1-2 N0 OSCC between May 2007 and October 2020 were retrospectively identified using a pathology database. All slides for cases previously reported as positive for carcinoma were reviewed and the first level to contain carcinoma was identified. For cases progressing to step

sections with IHC, the number of levels containing carcinoma cells were also recorded. This study was registered as part of a service evaluation audit (Guy's & St Thomas' NHS Foundation reference 10965) and exempt from formal research ethics committee approval.

3 | RESULTS

Two hundred and seventy-two patients underwent SNLB during the study period giving a total of 901 nodes (mean 3.3, range 1-10 SNs per patient). Per SN, the mean, median, and range of slides were 41.6, 44.0, and 4-128, respectively. The mean number of slides per patient was 128.3. Carcinoma cells were present in 118 (13.1%) SNs from 90 (33.1%) patients. Of the SNs positive for carcinoma, there were 56 (47.3%), 29 (24.5%), and 33 (27.7.0%) Ma, Mi, and ITC, respectively (value in parenthesis is percentage of positive SNs). Sixteen SNs from 14 patients demonstrated extranodal extension. SSS with IHC resulted in upstaging of nodal status in 61 (6.8%) lymph nodes in 53 patients (19.5% of all patients). Of the LNs revealed to contain carcinoma cells on SSS with IHC, 9 (14.8%), 21 (34.4%), and 31 (50.8%) were Ma, Mi, and ITC, respectively. Details of completion neck dissection were available from 36 patients, 4 (11.1%) of whom had additional metastasis in non-sentinel nodes.

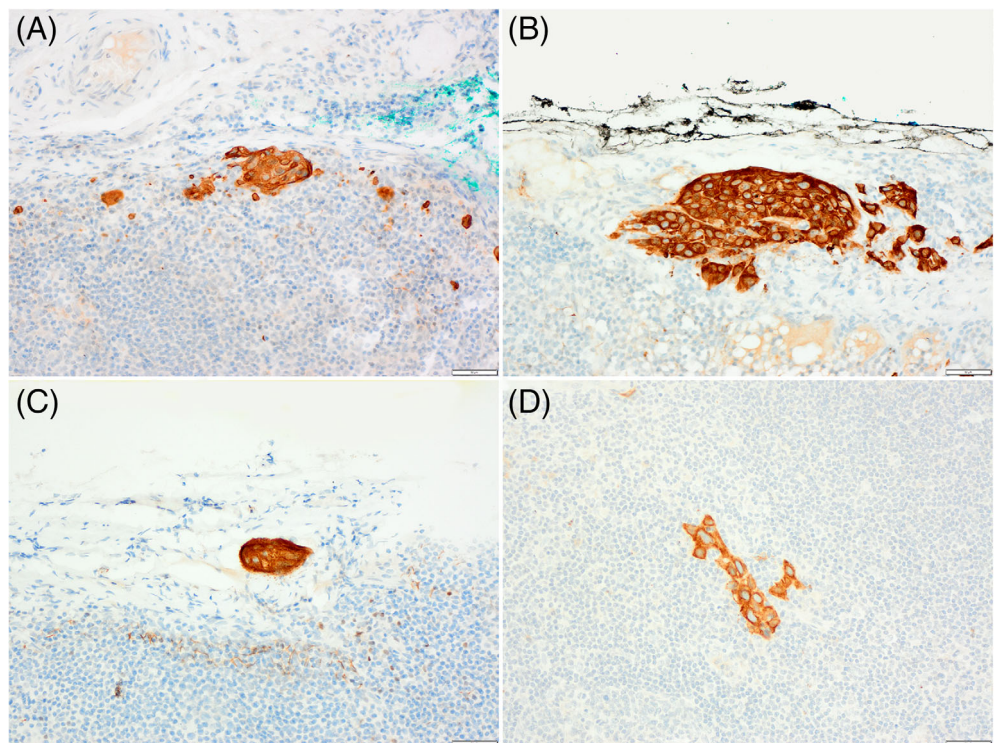


FIGURE 1 First positive level at deeper section levels. Photomicrographic examples where carcinoma cells were identified in levels greater than Level 4 (A, Level 8; B, Level 5, C, Level 7, D, Level 7) [Color figure can be viewed at wileyonlinelibrary.com]

3.1 | First positive level

Carcinoma cells were detected at Level 1 in 70 LNs (59.3% of positive SNs). Of these, carcinoma cells were visible on H&E in 57 (48.3%) SNs, whereas in 13 (11.0%) tumor cells were only detectable by IHC. In 61 SNs, carcinoma was only detected following SSS with IHC (51.7% of positive SNs). For SNs progressing to SSS, the first level to contain carcinoma was Level 2 in 12 (10.2%) nodes, Level 3 in 12 (10.2%) nodes and Level 4 in 4 (3.4%) nodes. Tumor cells were identified beyond Level 4 in 20 (16.7%) and beyond Level 6 in 12 (10.2%) positive SNs (Figure 1). Values in parentheses are as percentages of all positive SNs. These data, together with the relative proportions of Ma, Mi, and ITC are summarized in Figure 2.

3.2 | Number of positive levels

Of the positive SNs requiring SSS, carcinoma cells were present in only a single level in 29 nodes (47.5% of positive SNs, Figures 3 and 4), of which 8 (13.1%) and 21 (34.4%) of all SN progressing to SSS were Mi and ITC, respectively. Where tumor cells were present in multiple levels, there was discontinuous tumor (multiple deposits) in 7 (5.9%) nodes, of which 2 (28.6%) were Ma and 5 (71.4%) ITC (Figure 5).

4 | DISCUSSION

Our study identified occult lymph node metastasis in 33.1% of patients with cT1-2 N0 OSCC, a rate

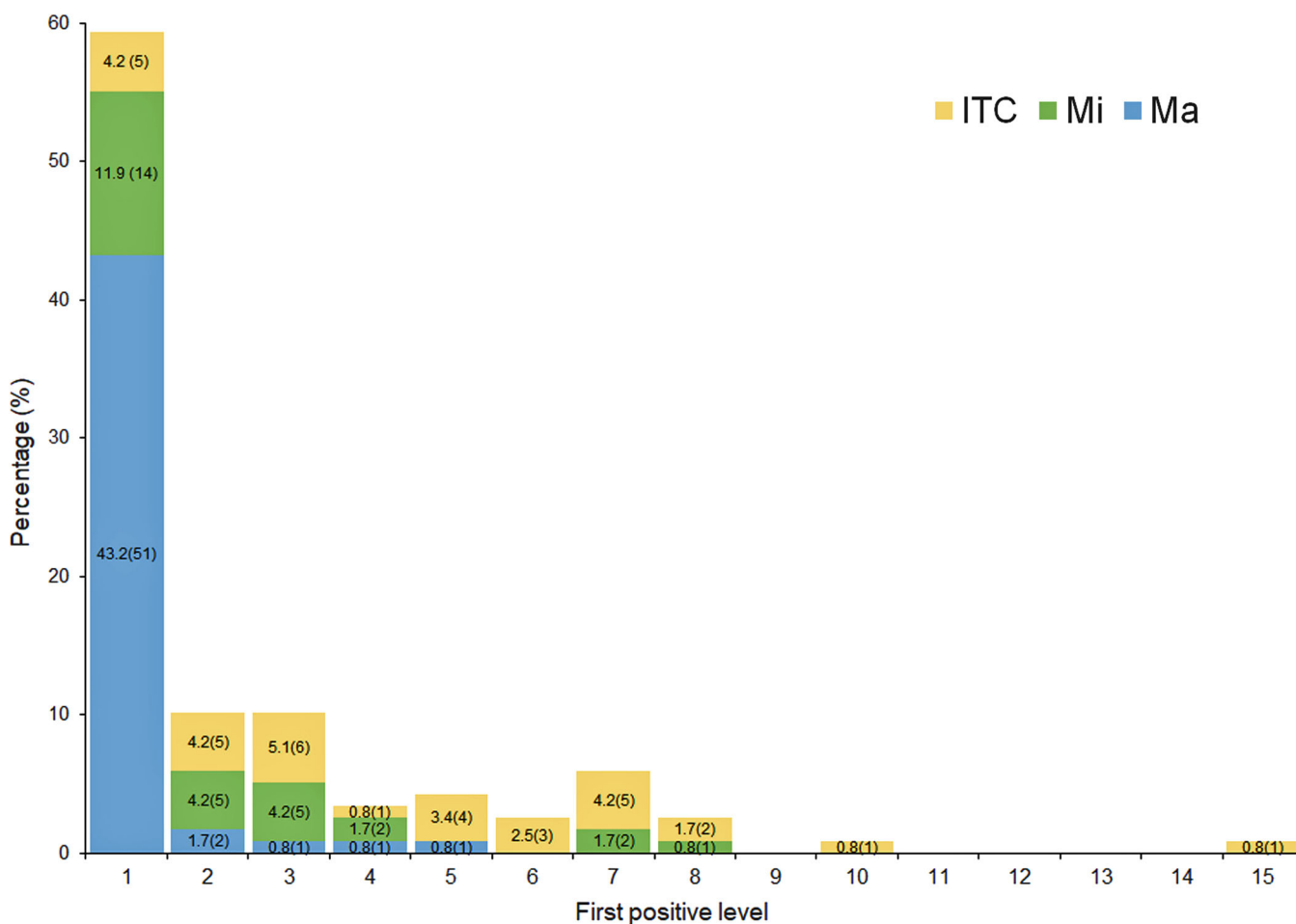
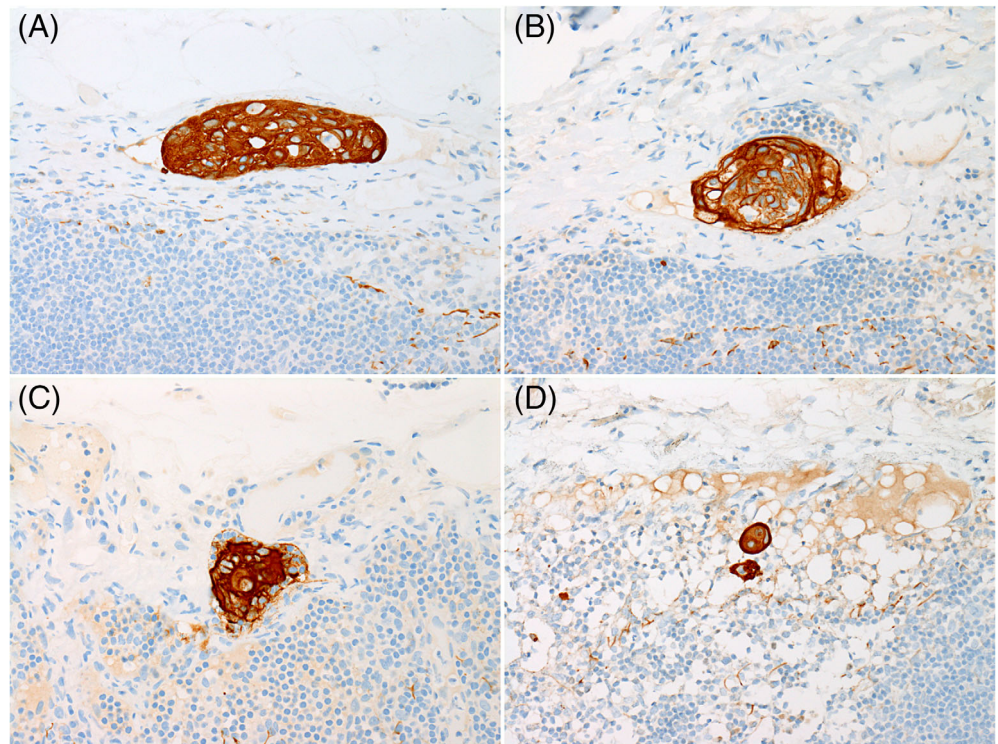


FIGURE 2 First positive level. Bar chart summarizing the first positive section level to contain metastatic carcinoma and the relative proportions of macrometastasis (Ma), micrometastasis (Mi), and isolated tumor cells (ITC). Data labels indicate percentage of all positive SNs, absolute numbers in parenthesis [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Isolated tumor cells within a single section level. Photomicrographic examples of isolated tumor cells (A–D) present in only a single section level within the entire SN [Color figure can be viewed at wileyonlinelibrary.com]



commensurate with that reported in the literature.^{4–6,15} The high rate of occult metastasis mandates active management of the neck in this group of patients. However, subjecting all patients with clinically undetectable neck disease to END is likely to result in unnecessary surgery in an estimated 60%–80% of individuals. Against this background, SLNB is now considered by many to be the standard of care since it accurately stages the neck thereby avoiding the need for neck dissection in patients who do not require it.^{13,14}

The benefits of SLNB over END are only apparent if the sensitivity of the former exceeds 83.7%.¹³ However, while SSS and IHC increases the sensitivity rates of SLNB, this results in considerable burden on histopathology services and may explain the lack of widespread availability and reluctance to implement this technique.²⁸ In this context, some groups have suggested that simplifying the laboratory procedure with fewer section levels may be clinically expedient.^{29,30} For example, Bell et al. reported that a single H&E and IHC section of the SN was sufficient to provide an acceptable negative predictive value for the nodal basin status in a cohort of 35 patients.²⁹ Similarly, Jefferson et al. re-evaluated SNs from 10 patients previously assessed as tumor free on a single H&E with IHC and showed no micrometastases when these nodes were subsequently subjected to SSS with IHC.³⁰ Both these studies are limited by low patient numbers, low numbers or lack of positive SN in the test cohort and absence of negative controls. In contrast, our

data show that while the majority (83%, Figure 2) of metastatic carcinomas are identified within the first four section levels, failure to sample beyond this may result in 16.7% of tumor deposits being missed, the majority of which were ITCs. We acknowledge that comparison of laboratory methods between studies are limited by their retrospective nature. Furthermore, the clinical significance of differing laboratory protocols remains largely unknown since there is a lack of comparative patient outcome data. Nevertheless, until such data becomes available, and in keeping with previous consensus guidelines, we recommend that exhaustive SSS with IHC should be undertaken on the entire SN.^{31,32}

The presence of ITCs in SLNB for OSCC remains controversial primarily since the clinical significance of this category is poorly understood. Two European prospective studies demonstrated that patients with ITCs had adverse outcomes compared to SN negative individuals despite going on to receive completion neck dissections.^{6,33} Similarly, Trivedi et al. reported a neck recurrence rate of 30% in patients with ITCs.³⁴ By contrast, a recent prospective clinical trial showed that patients with ITCs who do not proceed to neck dissection had similar outcomes to those with negative SNs.¹⁰ These apparent contradictory findings may be explained, at least in part, by the low ITC cohort sizes ($n = 10–12$) in these studies. The clinical significance of ITCs is further complicated by the somewhat arbitrary maximum size definition of $<200 \mu\text{m}$ which is largely a result of extrapolation from other cancer sites.

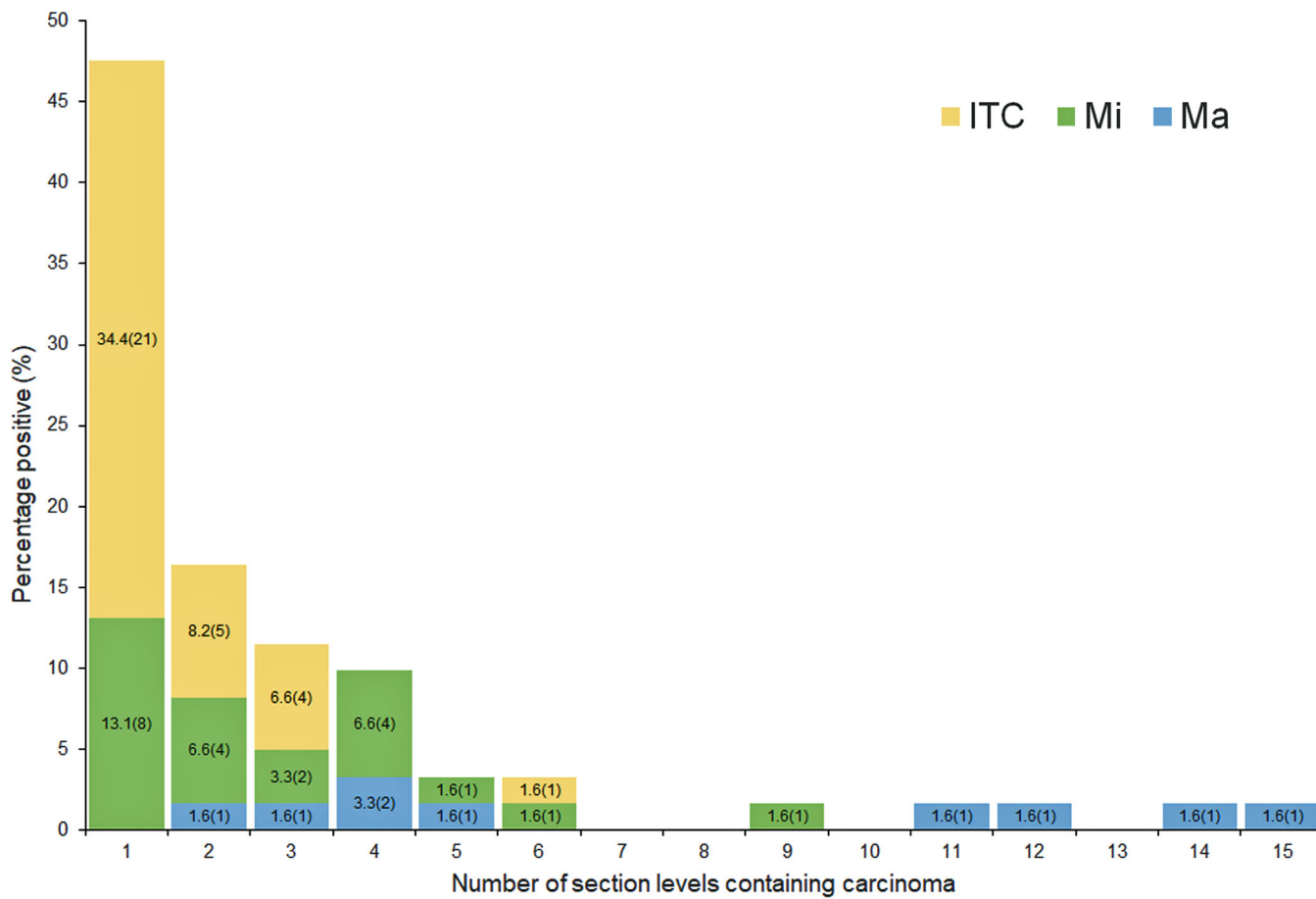


FIGURE 4 Total number of section levels containing carcinoma. Bar chart summarizing the total number of section levels containing metastatic carcinoma cells as macrometastasis (Ma), micrometastasis (Mi), or isolated tumor cells (ITC) within a single sentinel node. Data labels indicate percentage of all positive sentinel nodes requiring serial step sections with immunohistochemistry, absolute numbers in parenthesis [Color figure can be viewed at wileyonlinelibrary.com]

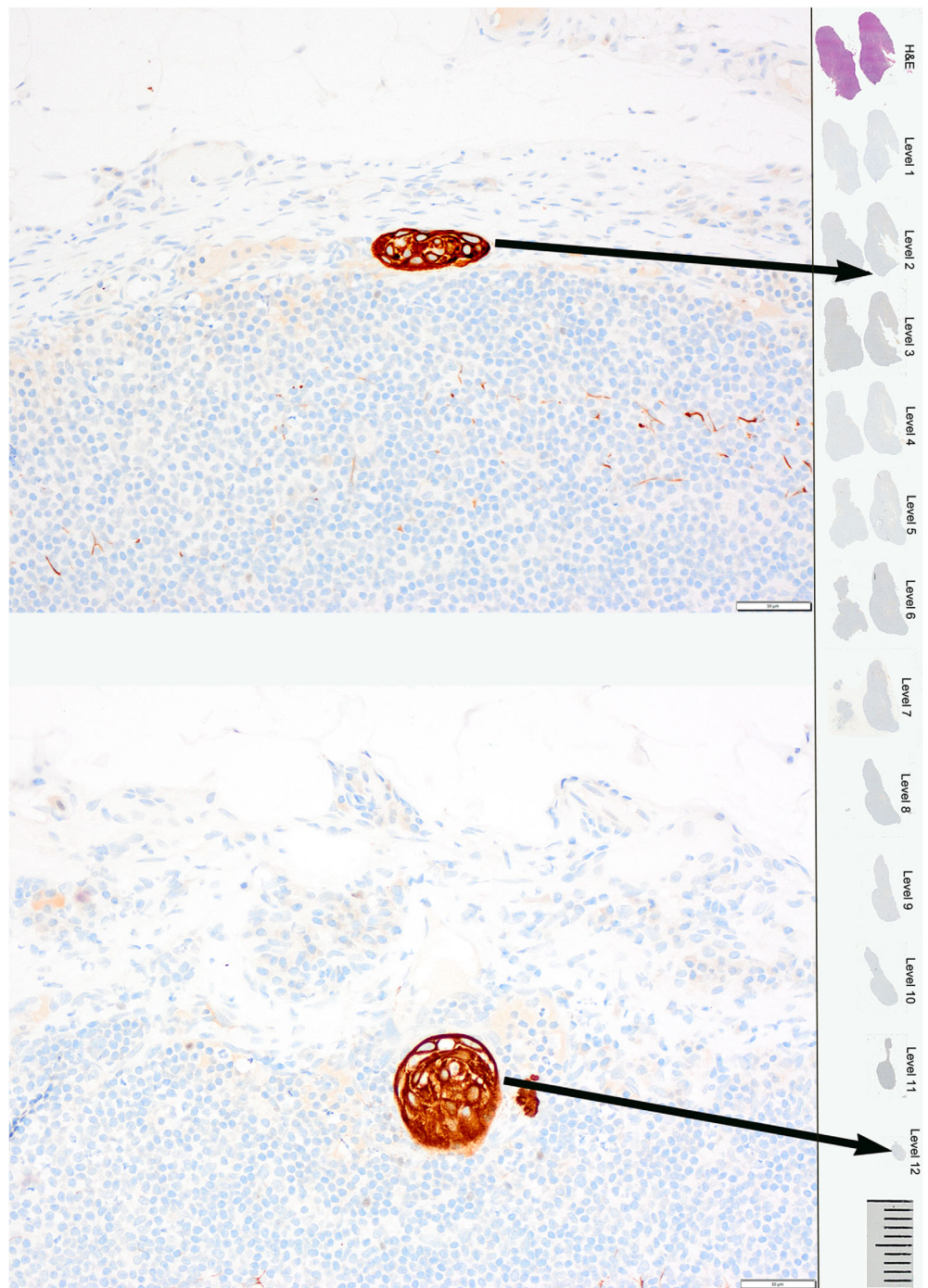
Moreover, some authorities also exclude contact with vessel or lymph sinus wall, extravasation, extravascular stromal reaction, and extravascular tumor cell proliferation as part of the definition of ITCs.³⁵ However, these additional criteria apply to other cancer sites and have not currently been validated in OSCC.

Since there is currently no low-end size cut-off definition, this category includes a broad histomorphological range from a single cell to clusters of up to 200. The fate of the single metastatic oral squamous carcinoma cell remains unknown and its histological detection is likely to be poorly reproducible, which may further explain the apparently contradictory clinical significance of ITCs. In our study, the majority (50.8%) of SNs diagnosed as positive by SSS with IHC were classified as ITC. Our findings indicate that SSS with IHC of the entire SN remains necessary because the current standard of care stipulates completion neck dissection following identification of ITCs.^{15,36} Further work is required to elucidate the clinical significance of ITCs against patient outcomes, in

particular whether the presence of a single cell mandates completion neck dissection. Until this is known, prospective acquisition of detailed morphological features of ITCs, including size, estimated number of cells, stromal appearance, and possible tumor and/or microenvironment molecular profiling may help to define a low-end cut-off value, above which completion neck dissection is indicated.

This study also questioned whether a reduction in workload achieved by increasing the interval thickness between section levels would compromise diagnostic accuracy. The rationale for intervals of 150 μm stems from the definition of ITCs (i.e., deposits of carcinoma cells up to 200 μm) and possibly assumes that disregarding any cells that would be otherwise present in the discarded material between section levels would not impact on patient outcomes.³² Our data show that 47.5% of positive SNs requiring SSS contained carcinoma cells in a single section level. Interestingly, although the majority of carcinoma cells present in a single

FIGURE 5 Noncontiguous metastatic carcinoma. Example of a sentinel node containing isolated tumor cells in nonadjacent section levels. Isolated tumor cells present in section level 2 (upper panel) and section level 12 (lower panel). Right panel shows whole mount composite of all section levels [Color figure can be viewed at wileyonlinelibrary.com]



section level were categorized as ITCs, 27.6% (8 of 29) were Mi, the latter unequivocally requiring completion neck dissection.

The retrospective nature of our study raises several limitations: (1) any conclusions relating to interval thickness are based on multiples of 150 μm , and (2) ITCs may be present in the tissue discarded between section levels. However, the presence of Mi in single section levels, together with the undetermined size cut-off for clinically significant ITCs, indicate that the interval thickness should remain no greater than 150 μm . The labor-intensive nature

of this protocol and its resultant burden on histopathology workload highlight the necessity for careful resource planning prior to implementing this service.

Multifocal deposits of carcinoma within a single SN have been previously described.³⁷ However, our data indicate that their occurrence in nonadjacent section levels is rare (5.9% of positive nodes). Classification of multifocal metastasis as Ma, Mi or ITC remains problematic as it is uncertain whether pathologists should categorize the SN according to the largest deposit or if a sum estimation of all foci should be made. Categorization based on total

number of deposits are likely to upstage a subset of positive SNs (e.g., ITCs to Mi). This unresolved issue currently does not impact on subsequent patient management since any category of positivity necessitates completion neck dissection. However, as the low-end cut-off for ITCs becomes more clearly defined, the cumulative total size of multifocal deposits may be required to inform patient management. As such, accurate categorization according to total size estimates in multifocal deposits within a single SN is not possible without SSS and IHC.


5 | CONCLUSION

SSS with IHC of the entire SN 150 μ m intervals are required to identify occult metastasis in OSCC. Further work is needed to determine the criteria for clinically significant ITCs.

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REFERENCES

- Shah JP. Patterns of cervical lymph node metastasis from squamous carcinomas of the upper aerodigestive tract. *Am J Surg*. 1990;160(4):405-409.
- Gourin CG, Conger BT, Porubsky ES, Sheils WC, Bilodeau PA, Coleman TA. The effect of occult nodal metastases on survival and regional control in patients with head and neck squamous cell carcinoma. *Laryngoscope*. 2008;118(7):1191-1194.
- Leemans CR, Tiwari R, van der Waal I, Karim AB, Nauta JJ, Snow GB. The efficacy of comprehensive neck dissection with or without postoperative radiotherapy in nodal metastases of squamous cell carcinoma of the upper respiratory and digestive tracts. *Laryngoscope*. 1990;100(11):1194-1198.
- Pedersen NJ, Jensen DH, Hedback N, et al. Staging of early lymph node metastases with the sentinel lymph node technique and predictive factors in T1/T2 oral cavity cancer: a retrospective single-center study. *Head Neck*. 2016;38(suppl 1):E1033-E1040.
- Civantos FJ, Zitsch RP, Schuller DE, et al. Sentinel lymph node biopsy accurately stages the regional lymph nodes for T1-T2 oral squamous cell carcinomas: results of a prospective multi-institutional trial. *J Clin Oncol*. 2010;28(8):1395-1400.
- Schilling C, Stoeckli SJ, Haerle SK, et al. Sentinel European Node Trial (SENT): 3-year results of sentinel node biopsy in oral cancer. *Eur J Cancer*. 2015;51(18):2777-2784.
- Govers TM, Hannink G, Merx MA, Takes RP, Rovers MM. Sentinel node biopsy for squamous cell carcinoma of the oral cavity and oropharynx: a diagnostic meta-analysis. *Oral Oncol*. 2013;49(8):726-732.
- Liu M, Wang SJ, Yang X, Peng H. Diagnostic efficacy of sentinel lymph node biopsy in early oral squamous cell carcinoma: a meta-analysis of 66 studies. *PLoS One*. 2017;12(1):e0170322.
- Kim DH, Kim Y, Kim SW, Hwang SH. Usefulness of sentinel lymph node biopsy for oral cancer: a systematic review and meta-analysis. *Laryngoscope*. 2020;131(2):E459-E465. <https://doi.org/10.1002/lary.28728>.
- Garrel R, Poissonnet G, Moya Plana A, et al. Equivalence randomized trial to compare treatment on the basis of sentinel node biopsy versus neck node dissection in operable T1-T2N0 oral and oropharyngeal cancer. *J Clin Oncol*. 2020;38(34):4010-4018. <https://doi.org/10.1200/JCO.20.01661>.
- Hasegawa Y, Tsukuhara K, Yoshimoto S. Neck dissections based on sentinel lymph node navigation versus elective neck dissections in early oral cancers: a randomized, multicenter, non-inferiority trial. *J Clin Oncol*. 2019;37:6007.
- Network NCC. *NCCN Clinical Practice Guidelines: Head and Neck Cancers*; 2020.
- National Institute for Health and Care Excellence. *Cancer of the Upper Aerodigestive Tract: Assessment and Management in People Aged 16 and Over*; 2016.
- Lai SY, Ferris RL. Evolving evidence in support of sentinel lymph node biopsy for early-stage oral cavity cancer. *J Clin Oncol*. 2020;38(34):3983-3986. <https://doi.org/10.1200/JCO.20.02716>.
- Bilde A, von Buchwald C, Therkildsen MH, et al. Need for intensive histopathologic analysis to determine lymph node metastases when using sentinel node biopsy in oral cancer. *Laryngoscope*. 2008;118(3):408-414.
- Melkane AE, Mamelle G, Wycisk G, et al. Sentinel node biopsy in early oral squamous cell carcinomas: a 10-year experience. *Laryngoscope*. 2012;122(8):1782-1788.
- Riese CGU, Karstadt JA, Schramm A, et al. Validity of sentinel node biopsy in early oral and oropharyngeal carcinoma. *J Craniomaxillofac Surg*. 2018;46(10):1748-1752.
- Abdul-Razak M, Chung H, Wong E, et al. Sentinel lymph node biopsy for early oral cancers: Westmead Hospital experience. *ANZ J Surg*. 2017;87(1-2):65-69.
- Boeve K, Schepman KP, Schuurin E, et al. High sensitivity and negative predictive value of sentinel lymph node biopsy in a retrospective early stage oral cavity cancer cohort in the northern Netherlands. *Clin Otolaryngol*. 2018. <https://doi.org/10.1111/coa.13107>.
- Moya-Plana A, Auperin A, Guerlain J, et al. Sentinel node biopsy in early oral squamous cell carcinomas: long-term follow-up and nodal failure analysis. *Oral Oncol*. 2018;82:187-194.
- den Toom IJ, Boeve K, Lobeek D, et al. Elective neck dissection or sentinel lymph node biopsy in early stage oral cavity cancer patients: the Dutch experience. *Cancers (Basel)*. 2020;12(7):1783. <https://doi.org/10.3390/cancers12071783>.
- Vigili MG, Rahimi S, Marani C, Natale ME, Tartaglione G. Radioguided sentinel node biopsy to avoid unnecessary neck dissection in T1-T2N0 oral cavity squamous cell carcinoma: personal experience with same day protocol. *Eur Arch Otorhinolaryngol*. 2020;277(12):3479-3487. <https://doi.org/10.1007/s00405-020-06107-3>.

23. Vishnoi JR, Kumar V, Gupta S, et al. Outcome of sentinel lymph node biopsy in early-stage squamous cell carcinoma of the oral cavity with methylene blue dye alone: a prospective validation study. *Br J Oral Maxillofac Surg*. 2019;57(8):755-759.
24. Molstrom J, Gronne M, Green A, Bakholdt V, Sorensen JA. Topographical distribution of sentinel nodes and metastases from T1-T2 oral squamous cell carcinomas. *Eur J Cancer*. 2019; 107:86-92.
25. Loree JT, Popat SR, Burke MS, Frustino J, Grewal JS, Loree TR. Sentinel lymph node biopsy for management of the N0 neck in oral cavity squamous cell carcinoma. *J Surg Oncol*. 2019;120(2):101-108.
26. Ishiguro K, Iwai T, Izumi T, et al. Sentinel lymph node biopsy with preoperative CT lymphography and intraoperative indocyanine green fluorescence imaging for N0 early tongue cancer: a long-term follow-up study. *J Craniomaxillofac Surg*. 2020;48(3):217-222.
27. Marttila E, Keski-Santti H, Hagstrom J, Snall J, Wilkman T. Sentinel lymph node biopsies in early stage oral and oropharyngeal carcinoma: a retrospective single-centre experience. *Br J Oral Maxillofac Surg*. 2020;58:1078-1083.
28. Bowe CM, Shastri M, Gulati A, et al. Challenges and outcomes in establishing a sentinel lymph node biopsy service for oral squamous cell carcinoma in a regional district specialist hospital. *Br J Oral Maxillofac Surg*. 2020;59:217-221.
29. Bell RB, Markiewicz MR, Dierks EJ, Gregoire CE, Rader A. Thin serial step sectioning of sentinel lymph node biopsy specimen may not be necessary to accurately stage the neck in oral squamous cell carcinoma. *J Oral Maxillofac Surg*. 2013;71(7): 1268-1277.
30. Jefferson GD, Sollaccio D, Gomez-Fernandez CR, Civantos F Jr. Evaluation of immunohistochemical fine sectioning for sentinel lymph node biopsy in oral squamous cell carcinoma. *Otolaryngol Head Neck Surg*. 2011;144(2):216-219.
31. Ross GL, Shoaib T, Soutar DS, et al. The first international conference on sentinel node biopsy in mucosal head and neck cancer and adoption of a multicenter trial protocol. *Ann Surg Oncol*. 2002;9(4):406-410.
32. Stoeckli SJ, Pfaltz M, Ross GL, et al. The second international conference on sentinel node biopsy in mucosal head and neck cancer. *Ann Surg Oncol*. 2005;12(11):919-924.
33. Broglie MA, Haile SR, Stoeckli SJ. Long-term experience in sentinel node biopsy for early oral and oropharyngeal squamous cell carcinoma. *Ann Surg Oncol*. 2011;18(10):2732-2738.
34. Trivedi NP, Ravindran HK, Sundram S, et al. Pathologic evaluation of sentinel lymph nodes in oral squamous cell carcinoma. *Head Neck*. 2010;32(11):1437-1443.
35. Hermanek P, Hutter RV, Sobin LH, Wittekind C. International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. *Cancer*. 1999;86(12):2668-2673.
36. Den Toom IJ, Bloemena E, van Weert S, Karagozoglu KH, Hoekstra OS, de Bree R. Additional non-sentinel lymph node metastases in early oral cancer patients with positive sentinel lymph nodes. *Eur Arch Otorhinolaryngol*. 2017;274(2):961-968.
37. Sloan P. Head and neck sentinel lymph node biopsy: current state of the art. *Head Neck Pathol*. 2009;3(3):231-237.

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