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Frozen section analysis in detection of occult metastasis in sentinel nodes from oral squamous cell carcinoma: A Systematic review and meta-analysis

Authors and affiliations:

- Clare Schilling (University College London Hospital, London, United Kingdom)
- Axel Sahovaler (University College London Hospital, London, United Kingdom)
- Mark McGurk (University College London Hospital, London, United Kingdom)
- Selvam Thavaraj (King's College London, London, United Kingdom)
- Munir Abukhder (University College London Hospital, London, United Kingdom)
- Panagiotis Vrakas (University College London Hospital, London, United Kingdom)

Corresponding author: Clare Schilling; clare.schilling@nhs.net

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Abstract

Background: Sentinel node biopsy (SNB) is an accurate surgical staging test for occult metastasis in oral squamous cell cancer (OSCC), however there is a wait of 4-10 days to obtain results of serial step sectioning (SSS) analysis requiring staged completion neck dissection in the case of a positive result. On-table frozen section (FS) sentinel node analysis offers a potential one-stage analysis and treatment, although the accuracy of this technique has not been fully explored.

Aim: To evaluate the accuracy of FS analysis in the identification of occult metastasis in sentinel nodes from patients with cT1-T2 N0 OSCC.

Methods: A systematic review of cT1-T2 N0 OSCC patients undergoing SNB with FS analysis. Protocol registered with PROSPERO and reported in accordance with the Preferred Reporting for Items for Systematic Reviews and Meta-Analyses (PRISMA). Comprehensive electronic search strategies between January 2000 to January 2023 were developed by a librarian. Studies were screened by two independent reviewers.

Results: Seventeen articles met the eligibility criteria identifying 878 patients that underwent intraoperative FS analysis of sentinel nodes, in most cases confirmatory SSS was also performed on the remaining nodal tissue. Overall, occult metastasis was found in 30% of patients (263/878). Of the 263 patients with cervical nodal involvement, frozen section analysis was able to identify 65.7% (n=173). Following the completion of SSS, an additional 90 positive results were identified, leading to 47 patients undergoing staged completion neck dissection. Pooled sensitivity of FS was 0.711; CI [0.6, 0.802], diagnostic odds ratio was 110, and false negative rate was 34.2%.

Conclusion: Intraoperative FS analysis showed a reasonable sensitivity, diagnosing 65.7% of occult metastasis. However, there was still a significant proportion of positive results detected on subsequent SSS, suggesting FS is not sensitive enough as a sole technique. In many studies it was unclear how much of the sentinel node was subsequently available for histopathological analysis raising the possibility that very small metastatic deposits could be missed. On-table diagnosis is advantageous for the SNB pathway and non-destructive analysis such as whole node imaging may improve on the result shown by frozen section.

Introduction

Oral squamous cell carcinoma (OSCC) is an aggressive cancer with a high recurrence and mortality rate [1]. It is the most common oral malignancy, and it continues to increase in incidence worldwide [2]. According to the data collected by the Global Cancer Observatory, there were 377,713 cases of OSCC worldwide in 2020 [3]. Approximately 20-40% of T1-T2 oral cancer patients will have occult metastasis to cervical lymph nodes [4–7]. Nodal involvement has a significant impact on survival, therefore accurately identifying involved nodes is important to stratify clinical management [8,9].

The sentinel lymph node (SLN) is defined as the first node to receive drainage from the primary tumour site. Therefore, this node would be the first recipient of metastatic tumour cells and as such, can be considered a representation of the histopathological status of the neck [10]. This is of particular importance for the clinically N0-neck, as this minimally invasive technique allows for the accurate detection of occult metastasis, and therefore reliably selecting patients for completion neck dissection. Consequently, patients for which there is no SLN involvement are spared a neck dissection, and therefore avoid the cost and morbidity associated with this procedure [11].

The identified SLNs can be potentially subjected to different pathologic investigations with varying clinical utility. Gold standard processing of excised sentinel nodes includes serial step sectioning (SSS) and immunostaining (IHC) using anti-pancytokeratin primary antibody (AE1/3). Recent research suggests maintaining step section interval thickness at 150 micrometres is advisable to prevent overlooking isolated tumour cells within the specimen [12]. However, this approach imposes a high demand on laboratory resources, the time of the pathologist and introduces a turnaround time of 4-5 working days [13]. Utilizing frozen section (FS) analysis may enable surgeons to identify occult metastasis intraoperatively, eliminating the need for a subsequent surgery. However, it is imperative to recognize the inherent limitations associated with FS analysis, such as an increase in operative time, need for flexibility in theatre scheduling, and in labour costs. Moreover, a lack of consensus exists regarding the optimal approach for examining the SLN using this technique. While some studies advocate for multislice analysis, others suggest simpler methods, such as bisecting the sample [14–16]. Despite the potential for more in-depth analysis with multislice preparation, it remains inferior to established techniques like SSS with IHC. In most cases patients staged intraoperatively by FS will also have confirmatory histopathology performed on the whole or remaining sentinel node, allowing a comparison of the detection rates of both techniques.

In this study, we aimed to perform a systematic review of the literature and meta-analysis to evaluate the accuracy of frozen section analysis in the identification of occult metastasis within SLNs in patients with OSCC.

Methods

Methods of the analysis and inclusion criteria were defined in advance and registered with PROSPERO (CRD42022380476). The review was reported following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Eligibility Criteria

Inclusion criteria were primary research papers including cohort studies, case series and randomised control trials, investigating the accuracy of frozen section analysis in the identification of occult metastasis within SNB in patients with cT1-T2 N0 OSCC; and articles published or translated into English. Exclusion criteria were articles which did not investigate cT1-T2 N0 OSCC; articles which did not include parameters of sensitivity and specificity; abstracts; and non-English articles. Whenever feasible, non-OSCC lesions were excluded from the final analysis. However, in studies where non-OSCC lesions were of limited occurrence, and their exclusion was not feasible, they were incorporated into the final analysis. Detailed description of inclusion and exclusion criteria can be found on table 1.

Information Sources and Search Strategy

A total of 5 bibliographic databases were searched (Embase, MEDLINE, CINAHL, Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Trials). Comprehensive electronic search strategies using a combination of relevant keywords and index headings were developed for each of the databases searched. The search strategy was modified so that the index headings relevant to each specific database were selected. The search strategy was peer-reviewed by an experienced librarian. Bibliographies of selected articles were also reviewed for additional relevant studies. Full search strategies and results are contained in Supplement 1.

Study Selection and Data extraction

Duplicate papers were identified and removed in Endnote 20 before being uploaded to online analysis software Rayyan for screening [17]. Two independent reviewers screened titles and abstracts according to the inclusion and exclusion criteria (Table 1). The remaining articles were downloaded in full-text format and re-screened. Discussion with a senior author to achieve consensus resolved any conflicts between the two reviewers.

The following information was extracted from full-text articles onto a customised Microsoft Excel spreadsheet: 1) Study characteristics, including title, author, year of publication, sample size, country, and study design; 2) Patient demographics; 3) Stage and site of tumour; 4) Modality of sentinel lymph node detection; 5) Technique of frozen section analysis; 6) Histopathological technique used to

evaluate the accuracy of frozen section analyses; 7) true positive, true negative, false positive and false negative rates; 8) follow-up duration; and 9) Incidence of isolated neck recurrence.

Risk of bias Assessment

Two reviewers independently evaluated the risk of bias in each study using the Diagnostic Precision Study Quality Assessment Tool (QUADAS-2) using the Review Manager Software [18]. Any conflicts were discussed with a senior author to achieve consensus.

Statistical analyses and definitions

Sensitivity and specificity were measured with a 95% confidence interval based on the true positive (TP), true negative (TN), false positive (FP) and false negative (FN) rates extracted from the studies suitable for inclusion. Sensitivity, also known as the true positive rate, is the probability of a positive test result being truly positive. Sensitivity was calculated as: true positive/true positive + false negative. Specificity, also known as the true negative rate, is the probability for a negative test result being truly negative. This was calculated as: true negative/true negative + false positive.

In order to establish the accuracy of the combined technique of FS and SSS a false negative SNB result was defined as neck recurrence after a negative SNB result (both FS and definitive histopathology) and the false negative rate followed (FN/TP+FN). Table 1 summarises the definitions utilised within this study.

A meta-analysis was then performed for the accuracy of frozen section analysis on a patient basis using R, and Meta-DiSc 2.0 web-based application [19,20]. These applications pooled the results of the included studies under the bivariate random effects model.

Results

A total 1690 published articles were identified following a comprehensive literature search. These articles were filtered for relevance and duplication, resulting in twenty-nine articles. Subsequent full-text assessment reduced the number of articles suitable for inclusion to seventeen [4,14–16,21–33]. Articles were analysed for sensitivity and specificity of frozen section analyses based on patient outcomes. Table 2 summarises the characteristics of the patients within the included studies. Table 3 summarises the studies included in this review. The PRISMA flow diagram is presented in figure 1**.**

Frozen section technique and occult metastatic rate

Following SLN excision within the included studies, frozen section analysis was performed through multislice analysis of the specimen in five studies (n=263 patients). The remaining studies described a simpler method of preparing the SLN for frozen section analysis, such as bisecting the sample. Following intra-operative analysis, the remaining transected SLN was re-evaluated in permanent specimens via paraffin preparation and hematoxylin-eosin staining (15 studies), immunohistochemical staining (10 studies), and serial step sectioning (5 studies).

The prevalence of occult metastasis within SLN between the studies varied from 10% -55%, (median 25.3%, mean 29.18%). Of the 263 patients with cervical nodal involvement, frozen section analysis was able to identify 65.7% (n=173). Frozen section sensitivity was variable amongst the included studies, ranging from 44% - 100%. Pooled sensitivity was 0.711; CI [0.6, 0.802]. Overall diagnostic odds ratio was 110. Cochrane's Q value was 15.62 (df=16, p-value=0.48) and tau^2 = 0.3598. Figure 2 demonstrates the variation in sensitivity and specificity of frozen section analysis in the included studies. Patients that did not have SLNs identified within the studies were excluded from the statistical analysis. Table 3 summaries FS technique, FS accuracy, and definitive histopathological technique chosen in each included study.

Based on the intraoperative FS results 173patients underwent completion neck dissection during the same surgery. When formal histopathology was completed, a further 90 positive results were detected and 47 additional patients underwent staged completion neck dissection.

Recurrences & follow-up

Follow-up information was reported in nine studies [4,15,21,23,24,28,31–33]. Disease recurrence was reported as regional or locoregional in 31 cases, as local in 11 cases, and as distant in one case. Only two studies reported no recurrences on follow-up of their patient cohort. Table 4 summarises the follow-up and recurrence information.

Not all patients underwent completion neck dissection following a positive formal histology result. In the study by Terada et al. 2011, the two false negative patients (based on frozen section analysis) refused second surgery [24]. On follow-up, one patient died of disease while the other remained disease free. In the study by Trivedi et al, all patients who were identified to have occult metastasis (micrometastasis and isolated tumor cells) by SSS and IHC were observed, and did not undergo completion neck dissection [31]. However, 30% of the patients with isolated tumour cells developed neck recurrence within the follow-up period. In the study by Terada et al. 2006, neck dissection was recommended in the two false negative patients, however follow-up data was not available within the study [22]. The 8 false negative patients in the study by Stoeckli et al. underwent neck dissection as a staged procedure [4]. Similarly, in the study by Melkane et al, 18 patients underwent a completion neck dissection when positive nodes were diagnosed on histology [33]

Quality of the Studies

With regards to risk of bias, most studies were low risk of bias concerning patient selection, and flow and timing. More than half of the studies were unclear risk with regards to the index test as they did not specify the threshold for diagnosis, i.e. isolated tumour cells, or micro/metastasis. The majority of studies were also classified as unclear with regards to reference standard as the papers did not state whether the interpretation of the reference standard results were completed without the knowledge of the results of the index test. Regarding applicability concerns, all studies had low risk of bias for patient selection, index test, and reference standard. Figure 3 summarises detailed judgement of each of the assessment items of the QUADAS-2.

Discussion

In this systematic review and meta-analysis, the pooled sensitivity of FS analysis in the detection of occult metastasis within SNB in predominantly cT1-T2 N0 OSCC patients was 0.711; CI [0.6, 0.802]. FS analysis was able to diagnose 65.7% of all the occult metastasis within this patient cohort. This review provides evidence that on-table diagnosis is advantageous for the SNB pathway. However, there is a high false negative rate of 34.2% with frozen sectioning meaning this technique is not currently accurate enough without confirmatory serial sectioning.

It is well reported that the incidence of occult metastasis in cT1-T2 OSCC is between 20-40%. This is of particular importance to diagnose as survival rates in this patient cohort can decrease by as much as 50% in the presence of N1 disease and may be ameliorated with additional treatment [6,7]. Due to the inability of clinical imaging at detecting occult metastasis in cervical lymph nodes, globally elective neck dissection (END) is the standard of care for the majority of patients with early OSCC. An alternative and increasingly utilised approach in the management of this patient cohort is by performing a SLN biopsy. Two recent randomised controlled trials comparing SNB to END showed equivalence of disease outcome in the two strategies, whilst allowing a minimum of 64% of patients to avoid neck dissection [15,34]. In both studies on-table analysis was employed but Garrel et al. was not included in this metanalysis as they used both imprint and frozen section analysis. Nevertheless, their results mirrored those shown here with sensitivity of 63.6% and false negative rate of 36.3% for on-table diagnosis.

A number of meta-analyses have now demonstrated safety of SNB compared to elective neck dissection in terms of tumour control, advocating that the technique can become routinely used in the management of these patients to reduce unnecessary neck dissection.[35,36]. However, a drawback to implementing SLN biopsy is the potential need for a subsequent surgery if positive cervical nodes are identified and such logistical issues may have reduced uptake of the technique.

Intra-operative FS analysis may offer a solution to this problem as it is capable of providing histological results at the time of primary surgery, allowing for completion surgery to continue if indicated. However, there is wide variability in the sensitivity within the included studies (0.44 - 1.00). A potential explanation for this variation may be due to the frozen section technique implemented by the studies. Fine-section FS analysis is a technique performed where fine sections ranging from 1-2mm are taken of the SLN and then analysed for metastasis. As highlighted in the study by King et al., carcinoma cells are not always present within the first section level, and in some cases, multiple section levels are required to be able to identify metastatic cells. Therefore, complete examination of the node will

improve the detection of metastasis which can be overlooked if only one or two cuts of the SLN are taken.

Within our analysis five studies reported the use of fine-section FS analysis, sensitivity ranged between 0.6 to 0.93. Hasegawa et al. were unable to identify 10 of the 20 micrometastasis cases with this technique, and a further 7 of 9 isolated tumour cells were also missed [15]. Possible explanations for the variation in sensitivity include technical factors, pathological factors, the development of frozen artifacts, or human error.

Sensitivity of FS analysis may be improved by targeting sites which are most likely to harbour metastasis, such as sites of increased radioactive intensity or fluorescence signal, although this association has not been investigated in clinical studies. If found to increase the sensitivity of FS analysis, an argument can be made that pathologist and laboratory efforts will not hugely increase, as the increased work for FS analysis will be offset by the number of positive FS SNB cases which will obviate the need for SSS and IHC.

Intraoperative FS analysis is most accurate in detecting occult metastasis >2mm, however, this was not the case for micrometastasis and isolated tumour cells [15,23,31]. The majority of these metastases were detected through IHC, and step-serial sectioning (SSS)) [37]. Furthermore, FS preparation is likely to result in tissue loss which consequently could lead to missed micrometastasis and ITC's. By contrast, formalin-fixed paraffin-embedded (FFPE) histology wastes almost no tissue during initial preparation. On the other hand, the influence of ITC in OSCC is still a matter for debate and in some centres is considered a negative result although consensus recommendations are to complete neck dissection when viable tumour cells are detected within sentinel lymph nodes [38].

A potential weakness of this report is the lack of granular data regarding the neck control rate (NCR) or false negative rate (FNR) with FS +/-SSS. If metastases are missed in discarded tissue during FS analysis, we would expect higher FNR and lower NCR than studies in which analysis was done by H&E and SSS alone. In the reviewed papers follow up data was only available in just over half and reported at a range of time points, although OS and DFS were within the expected range.

Finally, a single stage of diagnosis and treatment of the cN0 neck is likely to be appealing to both patients and clinicians whilst allowing for some flexibility in scheduling based in the on-table results. However, the accuracy of FS means that about one third of positive cases will still need to return for second surgery. Efforts should be made to standardise FS technique and if possible retain all tissue for subsequent H&E examination.

Conclusion

In conclusion, FS detected 65.7% of occult metastasis in the pooled cohort allowing potential for one stage treatment in the majority of patients with occult metastasis. However, there is significant heterogeneity of FS technique and in particular the handling of the remaining node tissue. This combined with difficulty in elucidating the false negative/neck relapse rate when using FS making true evaluation of the sensitivity of this technique difficult. Furthermore, there were different strategies in managing the positive neck with further confounded assessment. Nevertheless, this data is encouraging that one stage sentinel node assessment is possible and should be further investigated with a strict protocol which could incorporate non-destructive techniques such as whole node imaging or digital pathological examination.

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