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Diagnostic accuracy of intraoperative margin assessment techniques in surgery for head and neck squamous cell carcinoma: A meta-analysis

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ABSTRACT

Background: Positive margins following head and neck squamous cell carcinoma (HNSCC) surgery lead to significant morbidity and mortality. Existing Intraoperative Margin Assessment (IMA) techniques are not widely used due to limitations in sampling technique, time constraints and resource requirements. We performed a meta-analysis of the diagnostic performance of existing IMA techniques in HNSCC, providing a benchmark against which emerging techniques may be judged.

Methods: The study was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines. Studies were included if they reported diagnostic metrics of techniques used during HNSCC surgery, compared with permanent histopathology. Screening, manuscript review and data extraction was performed by multiple independent observers. Pooled sensitivity and specificity were estimated using the bivariate random effects model.

Results: From an initial 2344 references, 35 studies were included for meta-analysis. Sensitivity (Sens), specificity (Spec), diagnostic odds ratio (DOR) and area under the receiver operating characteristic curve (AUROC) were calculated for each group (n, Sens, Spec, DOR, AUROC): frozen section = 13, 0.798, 0.991, 309.8, 0.976; tumour-targeted fluorescence (TTF) = 5, 0.957, 0.827, 66.4, 0.944; optical techniques = 10, 0.919, 0.855, 58.9, 0.925; touch imprint cytology = 3, 0.925, 0.988, 51.1, 0.919; topical staining = 4, 0.918, 0.759, 16.4, 0.833.

Conclusions: Frozen section and TTF had the best diagnostic performance. Frozen section is limited by sampling error. TTF shows promise but involves administration of a systemic agent. Neither is currently in widespread clinical use. Emerging techniques must demonstrate competitive diagnostic accuracy whilst allowing rapid, reliable, cost-effective results.

Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is the 7th most common form of cancer worldwide. It is associated with alcohol, tobacco, and high-risk forms of the Human Papillomavirus (HPV). HNSCC has a high mortality rate and significant morbidity, including pain, disfigurement, speech and swallowing dysfunction, and psychosocial distress [1].

Current standard treatment of HNSCC is either primary chemo-radiotherapy (CRT), or surgical resection. Both approaches have comparable survival, so post-treatment morbidity is a key influence on treatment decision-making. Small primary tumours, operable oral cancer, and locally recurrent HNSCC are usually offered surgery [2,3].

The success of surgical resection relies on complete removal of the tumour. The current gold standard for assessing completeness of resection is histopathological examination of the formalin-fixed, paraffin-embedded resection specimen [4]. Malignant cells present on the cut surface imply that tumour was transected during resection, and therefore that viable tumour cells are left *in situ*. This is referred to as a *positive margin*.

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Resection with positive margins is a poor prognostic indicator [5]. Local control decreases from 87 to 91% at 5 years with > 5 mm margins to 44 – 65% with involved margins [6–9]. Higher local recurrence rates correlate with closer pathological margins [10,11], and overall mortality is around three-fold higher with positive margins [12]. The increased risk of recurrence in this patient group means that eligible patients are offered adjuvant CRT, adding the morbidity of these treatments to that of surgery. Adjuvant head and neck radiotherapy frequently leads to long term xerostomia and poor speech and swallow outcomes [13,14], which are high priority outcomes for patients [15]. Despite this additional treatment, patients still have a higher risk of recurrence and death from disease.

The reasons for positive margins are complex and multifactorial [5,16], however it remains the only prognostic factor in HNSCC under the direct control of the operating team. Intraoperatively, surgeons rely on macroscopic visual and tactile properties of the tumour to determine the extent of the lesion and plan the resection. This approach has significant limitations. Surgical experience and acumen appear to have a sensitivity of 88.9% and specificity of 81.1% for the *mucosal* margin [17]. Surgical performance at the deep margin is even poorer, with positive margins occurring at this margin alone in 17% of cases of oral cancer [18].

A major goal of surgical oncology research is development of an intraoperative margin assessment (IMA) technique that can address these shortcomings and reduce positive margin rates. An ideal IMA technique would alert the surgeon to positive margins, facilitating complete resection at the first attempt. Results should be provided rapidly, preferably in real time, to avoid increased operative duration and complexity. A wide variety of IMA techniques have been proposed, however, no single technique meets these criteria completely and so none have achieved widespread acceptance by head and neck surgeons.

Frozen section is a well-recognised IMA technique, mostly used in selected cases where margins are of particular concern. After resection of the main specimen, the surgeon takes samples from the tumour bed, targeting areas that they feel are at risk. This is known as a *defect-driven* approach. Alternatively, the entire resection specimen can be sent to the pathologist, who can then remove the faces of the specimen and examine them for tumour: the *specimen-driven* approach [19].

Several IMA techniques employ stains or dyes that exploit structural or metabolic differences between normal and pathological mucosa to provide visual contrast to guide resection [20,21]. One prominent example is Toluidine Blue, which binds to DNA and thus predominantly stains rapidly dividing cells [22].

Touch imprint cytology (TIC) is a simple technique in which the cut face of the specimen is pressed to a glass slide. Cells from the cut surface adhere, then are fixed, stained, and microscopically examined. This offers an indication of whether malignant cells are present at the specimen margin [23].

Tumour-targeted fluorescence (TTF) techniques couple an injectable fluorescent dye with a tumour-specific targeting mechanism, such as monoclonal antibodies [24,25] or encapsulated within a pH-sensitive delivery mechanism that releases the dye in the acidic tumour microenvironment [26]. Tumour tissue then displays a fluorescent signal that is readily distinguished from the background by dedicated detectors. This signal allows evaluation of the tumour bed for residual disease after resection, or rapidly assessment of the specimen margins [27].

Numerous related techniques exploit the differing optical properties of cancer to allow intraoperative margin assessment. These optical techniques employ light of a variety of wavelengths to detect the differential emission of electromagnetic radiation from tumour and normal tissue. The detection element is variable, including a broad range of techniques such as visual inspection of tissue fluorescence [28], Raman spectroscopy [29] and *in situ* microscopy [30].

In addition to these more established techniques, exciting new techniques are emerging. Intraoperative mass spectrometry exploiting the different metabolic features of malignant tissue (the 'iKnife'), has been demonstrated using various acquisition techniques in solid tumours including breast, colorectal and brain tumours [31].

It is important to establish the current benchmark for diagnostic performance against which innovative approaches may be evaluated. This study aims to define the diagnostic metrics of tools and techniques used during surgical resection of HNSCC that can identify and correct close or positive margins in real time.

Materials and methods

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [32]. It was registered on Prospero before starting the searches (registration number CRD42021287618).

Literature search

To identify relevant studies, bibliographic searches were performed in Medline, Embase, Cochrane Library and Scopus databases. Relevant studies were identified using electronic bibliographic searches in Medline, Cochrane Library, Scopus, and EMBASE. MeSH terms and all-field search terms were searched for "Head and Neck Squamous Cell Carcinoma" [head and neck, pharyn*, hypopharyn* oropharyn*, laryn*, oral, tongue][squamous cell carcinoma, carcinoma, cancer, tumour, tumor] AND "margin" OR "margin assessment" or "margins of excision" [MeSH]. The search included all study designs. Potentially relevant studies identified from bibliographies of manuscripts included in the full text screen were added to the database of studies to screen. Individual searches were also performed in Google Scholar as suggested by full text screening. No limitations were placed on the search date range. The last search was performed in March 2022.

Two investigators (JAH and OB) screened all titles and abstracts independently. Any title or abstract identified as potentially relevant by either investigator was included for full-text review.

Inclusion criteria

Studies that included diagnostic margin assessment data provided by one or more intraoperative techniques used during curative surgery for HNSCC in adults (18 years or over) were eligible for inclusion.

Studies were only included for meta-analysis if they provided raw diagnostic data (true positive, false positive, true negative, false negative) for the index test compared with permanent section histopathology, or sufficient derived diagnostic metric data (total sample numbers, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)) to reconstruct the raw diagnostic data.

Exclusion criteria

Abstracts, conference proceedings, opinion articles, case reports and reviews were excluded, as were articles that were not available in English. Studies not performed in humans or not in an operative environment were excluded. Studies were excluded if they did not report raw diagnostic data, or sufficient derived diagnostic metric data. Disagreement regarding article inclusion was resolved by consensus, and inter-rater reliability for the full text inclusion decision was estimated using Cohen's kappa (κ).[33].

Study quality

Each study was assessed for methodological quality using two separate validated scoring systems.

Studies were independently rated for both scoring systems by two investigators (JAH and AHT).

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist was used to provide a transparent rating of bias and

applicability.[34] All seven QUADAS-2 questions were used. For each question, the study was scored as having a low, unclear, or high risk of bias or concern regarding applicability. Studies were regarded as being at high risk of bias, or high concern for applicability, if the answer to any of the questions indicated high or unclear bias.

When evaluating the risk of bias, we considered how margins were obtained and processed, how results were interpreted, and the difference between the number of patients enrolled in the study and the number reported in outcome data.

The Strength of Recommendation Taxonomy (SORT) scoring system was used to assess the diagnostic quality of the studies.[35] Each study was rated from 1 to 3. High quality prospective trials or cohort studies were assigned a score of 1, lower quality cohort studies or diagnostic case-control studies were assigned a score of 2. Disease-centred research, opinion pieces and similar were scored 3.

Data collection

All data were extracted using Covidence[36] and stored in a dedicated, secure online database. Where raw diagnostic data were explicitly included in the study manuscript, these were entered directly into the database. Where raw diagnostic data were not explicitly stated, but derived diagnostic metrics and total sample numbers were provided, the raw data were reconstructed using simple arithmetic. If this data reconstruction was not possible, we contacted the corresponding author to ask them to provide the raw data.

Meta-analysis

Results were synthesised using the meta and mada packages in R.[37–39] Pooled sensitivity and specificity data for all studies together and for each subgroup were computed using the bivariate model. The thresholds relating sensitivity and specificity were examined using a summary receiver operating characteristic (sROC) model; sROC curves were plotted to illustrate the diagnostic performance of each subgroup. A 95% confidence area was also plotted. Heterogeneity was evaluated using the Higgins I^2 test.[40].

Publication bias was quantified using the *trim and fill* method,[41] evaluating the distribution of reported accuracy (Diagnostic Odds Ratio (DOR) in this analysis) compared with the precision (standard error in this analysis) of each study. The trim and fill model evaluates the symmetry of this plot, and estimates the position of studies that would be 'missing' from this plot in a truly representative meta-analysis. The number of 'missing' studies, K_0, acts as a quantification of publication bias. $K_0 = 0$ indicates no publication bias.

Results

Study selection

Electronic literature searches and careful bibliographic examination identified a total of 2344 citations for initial screening. Following removal of duplicate citations, 2082 unique citations remained. 1911 of these were excluded based on the title and abstract. Of the remaining 169 studies that underwent full text screening, 134 were excluded. The most common reasons for exclusion were because raw diagnostic data were not presented or calculable from data presented in the manuscript (57), or because the reference identified in the search was an abstract only, not a full peer-reviewed paper (45). 35 papers fulfilled the eligibility criteria and were included for review and meta-analysis. 25 papers provided all raw diagnostic data; 10 provided sufficient derived diagnostic metrics to reconstruct all diagnostic data. One paper compared two separate methods for the use of frozen section,[42] and so 36 sets of diagnostic data were included in the final meta-analysis. The interrater reliability κ for full text inclusion before resolution of disagreements was 0.67, indicating substantial agreement.

Six corresponding authors were contacted to request raw diagnostic data; disappointingly, we received no responses. The PRISMA diagram is shown in Figure 1.

The frozen section category included 13 studies and 14 datasets for meta-analysis. Optical techniques included 10 studies. This was the most heterogeneous group, with eight different techniques presented. Tissue targeted fluorescence included five papers. Topical staining techniques were reported in four studies and touch imprint cytology included three.

Study characteristics

Of the included studies, 27 used prospective methodology, 9 were retrospective. All the retrospective papers reported frozen section results. The publication dates spanned almost 50 years, from 1973 to 2021, though 26 of the 35 studies were published in or after 2016. Quality assessment outcomes using the QUADAS-2 and SORT scoring systems are shown in Table 1 (Detailed answers to QUADAS-2 questions are shown in Table S1).

Meta-analysis

The forest plots showing the individual and pooled sensitivity and specificity results from the bivariate analysis are shown in Figures 2 and 3. The Diagnostic Odds Ratio (DOR) forest plot is shown in Figure S1.

Frozen section

Of the included studies, 13 reported data for frozen section, including one[42] that reported two cohorts, one with defect-driven frozen section analysis, and one with specimen-driven analysis. Pooled sensitivity for all studies was 0.795 (95% Confidence Intervals (CI) 0.668–0.882); pooled specificity was 0.991 (0.979–0.996). The DOR was 309.8 (153.5–625.6). Heterogeneity for univariate analysis (DOR) was substantial, with Higgins $I^2 = 68\%$, ($p < 0.01$). The bivariate sROC curve is shown in Figure 4, and the pooled weighted area under the sROC curve (AUC) was 0.976.

Tumour-targeted fluorescence

Five studies presented data on TTF techniques. Pooled sensitivity (95% CI) was 0.957 (0.839–0.990); pooled specificity was 0.827 (0.747–0.886). The DOR was 66.4 (37.2–118.7) These studies had a very low level of heterogeneity: DOR Higgins $I^2 = 0\%$, ($p = 0.58$). The bivariate sROC curve is shown in Figure 5, and the pooled weighted AUC was 0.944.

Optical techniques

Data for 10 studies using optical IMA techniques were included. Pooled sensitivity was 0.919 (95% CI 0.861–0.954); pooled specificity was 0.855 (0.707–0.935). The DOR was 58.9 (21.3–163.1). There was substantial heterogeneity: DOR Higgins $I^2 = 75\%$, ($p < 0.01$). The bivariate sROC curve is shown in Figure S2, and the pooled weighted AUC was 0.925.

Touch imprint cytology

Three studies presented data on the intraoperative use of touch imprint cytology. Pooled sensitivity was 0.925 (95% CI 0.351–0.996); pooled specificity 0.988 (0.212–1.00). The DOR was 51.1 (12.7–205.4). There was a low level of between study heterogeneity: DOR Higgins $I^2 = 22\%$, ($p = 0.28$). The bivariate sROC curve is shown in Figure S3. The pooled, weighted AUC was 0.919.

Topical staining

Four studies reported diagnostic accuracy data for direct intraoperative tissue staining. Pooled sensitivity was 0.918 (95% CI 0.770–0.974); pooled specificity was 0.759 (0.414–0.933). The DOR was 16.4 (4.3–62.1). Heterogeneity was moderate: DOR Higgins $I^2 = 43\%$, ($p = 0.15$).

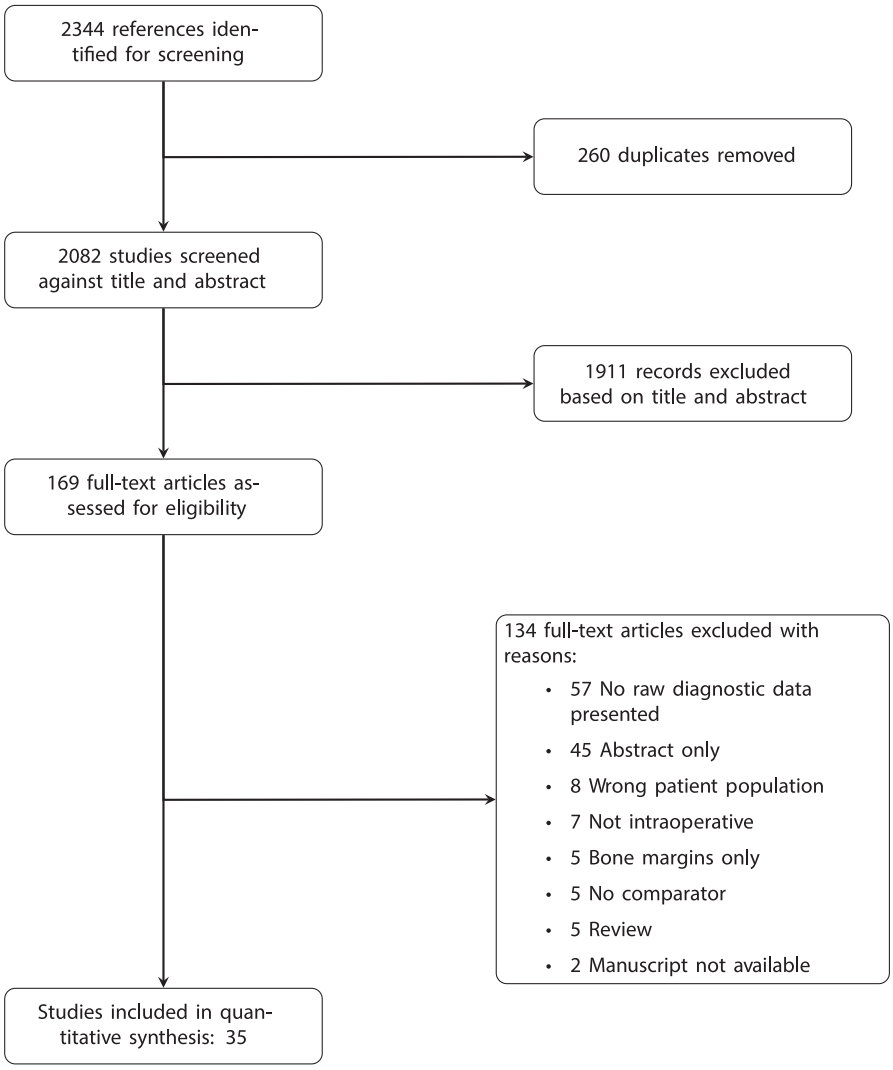

Figure 1. PRISMA diagram, showing how the final studies for inclusion within the meta-analysis were selected.

The bivariate sROC curve is shown in Figure S4, with a pooled weighted AUC of 0.883.

Publication bias

The funnel plot (Figure 6) emphasises the considerable heterogeneity identified in the forest plots. However, trim and fill analysis returned $K_0 = 3$, indicating low publication bias. The funnel plot shows the reconstructed 'missing' studies as having low treatment effect with low precision. This fits the expectation of publication bias, where smaller studies that do not show a significant, novel, or contradictory effect are less likely to be submitted or accepted for publication.

Discussion

This study presents a systematic review and meta-analysis of the diagnostic accuracy of intraoperative margin assessment techniques in HNSCC, providing a benchmark for comparison with emerging techniques. The rising incidence of HNSCC presents a need for development of reliable IMA techniques to minimise the risk of recurrence, and the morbidity associated with adjuvant treatment. The same need is being addressed in other solid tumours,[43,44] where early evidence suggests IMA techniques are driving improved clinical outcomes.[45].

Sensitivity and specificity are standardised metrics for understanding the performance of a diagnostic test, and as such for a fair comparison of diagnostic tests. Sensitivity measures the rate at which the presence of disease is correctly diagnosed; conversely, specificity measures the rate at which the absence of the disease is correctly diagnosed.

Sensitivity and specificity do not consider the prevalence of disease, unlike the related diagnostic metrics false positive rate and false negative rate.[46] In the context of IMA techniques, the patient is known to have cancer, the question is whether the tissue sampled contains cancer or not. The rate at which these contain disease is highly variable, and depends on many complex factors, such as the skill and experience of the operating team,[47–49] the ability of the test to provide rapid results, and whether the disease has an aggressive, infiltrative pattern.[7] Evaluating sensitivity and specificity allows more standardised comparison.[46].

When evaluating the diagnostic performance of IMA techniques, the purpose of the techniques should be borne in mind. The aim of IMA is to allow surgeons to remove disease entirely, to reduce risks of recurrence and the need for morbid adjuvant treatment.

Inappropriate removal of healthy tissue is to be avoided, but can be considered a secondary objective for IMA techniques. All else being equal, sensitivity is paramount.

Our data suggests that the two best IMA techniques are frozen section, with pooled sensitivity 0.795 and specificity 0.991, and tumour-targeted fluorescence techniques, with pooled sensitivity 0.957 and specificity 0.827, with an AUC of 0.976 and 0.944 respectively. Despite these promising results, neither technique is widely used in current

Table 1
Studies included in the meta-analysis, the groups to which they were assigned, the raw or reconstructed diagnostic data, and a summary of the quality assessment. (5-ALA; 5-aminolevulinic Acid; CRS: Coherent Raman Spectroscopy; Def-dr: Defect-driven; ESS: Elastic Scattering Spectroscopy; FN: False Negative; FP: False Positive; FS; Frozen section; gGlu-HMRG: γ-glutamyl hydroxymethyl rhodamine green; HRME: High-resolution microendoscopy; ICG: Indocyanine Green; NBI; Narrow band imaging; NOS: Not Otherwise Specified; OCT: Optical Coherence Tomography; QUADAS: Quality Assessment of Diagnostic Accuracy Studies; SORT: Strength of Recommendation Taxonomy; Spec-dr: Specimen-driven; TIC: Touch imprint cytology; TN: True Negative; TP: True Positive).

Study	Technique	No. patients	Subsites	TN	TP	FN	FP	Study design	QUADAS-2 Risk	SORT score
Frozen section										
Saltzstein 1973 [93]	FS: NOS	not reported	Multiple sub-sites	73	22	0	1	Retrospective cohort study	High	2
Ord 1997[86]	FS: NOS	49	Oral Cancer	291	13	2	1	Retrospective cohort study	Low	2
DiNardo 2000 [94]	FS: Def-dr	80	Multiple sub-sites	389	24	3	4	Retrospective cohort study	Low	2
Chaturvedi 2014 [17]	FS: Spec-dr	141	Oral Cancer	529	27	9	0	Prospective cohort study	Low	2
Amit 2016[42]	FS: Def-dr	71	Oral Cancer	164	21	2	12	Randomised controlled trial	Low	1
Amit 2016[42]	FS: Spec-dr	71	Oral Cancer	30	7	25	0	Randomised controlled trial	Low	1
Du 2016[51]	FS: NOS	253	Multiple sub-sites	930	113	42	16	Retrospective cohort study	Low	2
Abbas 2017[52]	FS: Def-dr	77	Oral Cancer	62	8	3	4	Retrospective cohort study	Low	2
Layfield 2018 [50]	FS: Spec-dr	288	Multiple sub-sites	1452	289	35	20	Retrospective cohort study	Low	2
Tirelli 2019[95]	FS: Def-dr	182	Oral Cancer	726	31	14	14	Retrospective cohort study	High	2
Tirelli 2019[96]	FS: Def-dr	42	Multiple sub-sites	180	88	6	9	Prospective cohort study	High	2
Datta 2019[97]	FS: Spec-dr	1311	Oral Cancer	971	196	70	0	Retrospective cohort study	Low	2
Pandey 2019[98]	FS: Spec-dr	104	Oral Cancer	440	22	6	2	Retrospective cohort study	Low	2
Herruer 2020 [99]	FS: Spec-dr	61	Multiple sub-sites	45	1	4	0	Prospective cohort study	Low	2
Optical techniques										
Vila 2012[72]	HRME	38	Multiple sub-sites	60	183	3	6	Prospective cohort study	High	2
Tirelli 2015[69]	NBI	16	Multiple sub-sites	14	16	2	0	Prospective cohort study	High	2
Hamdoon 2016 [71]	OCT	28	Oral Cancer	80	18	4	10	Prospective cohort study	High	2
Miles 2015[30]	HRME	33	Multiple sub-sites	60	32	1	3	Non-randomised experimental study	High	2
Heuke 2016[76]	Multimodal nonlinear microscopy	10	Multiple sub-sites	11	16	1	2	Prospective cohort study	High	3
Ohnishi 2016 [28]	Tissue autofluorescence	20	Oral Cancer	11	13	1	35	Prospective cohort study	High	2
Hoesli 2017[74]	CRS microscopy	50	Multiple sub-sites	40	37	5	2	Prospective cohort study	High	3
Grillone 2017 [73]	ESS	34	Oral Cancer	70	64	12	28	Prospective cohort study	High	2
Tirelli 2018[70]	NBI	61	Multiple sub-sites	16	34	2	9	Prospective cohort study	Low	2
Lisul 2019[75]	Optomagnetic imaging spectroscopy	21	Oral Cancer	20	18	5	3	Prospective cohort study	High	2
Topical Staining Techniques										
Leunig 2001[68]	5-ALA stain	24	Oral Cancer	50	18	5	25	Prospective cohort study	High	2
Junaid 2012[22]	Toluidine blue stain	56	Oral Cancer	269	3	0	8	Prospective cohort study	High	2
Slooter 2018[67]	gGlu-HMRG stain	7	Oral Cancer	12	13	0	7	Prospective cohort study	High	2
Putri 2021[65]	Acetic acid & Iodine stain	26	Oral cancer	3	43	3	5	Prospective cohort study	High	2
Touch Imprint Cytology										
Yadav 2013[62]	TIC	30	Oral Cancer	125	164	16	43	Prospective cohort study	High	2
Naveed 2017[23]	TIC	52	Multiple sub-sites	2	50	0	0	Prospective cohort study	High	2
Zafar 2020[63]	TIC	32	Oral Cancer	75	24	31	0	Prospective cohort study	Low	2
Tumour-Targeted Fluorescence										
Warram 2016 [55]	Fluorescent-labelled cetuximab	3	Multiple sub-sites	178	137	14	31	Prospective cohort study	High	2
Gao 2018[24]	Fluorescent-labelled panitumumab	6	Multiple sub-sites	107	41	0	38	Non-randomised experimental study	Low	2
vanKeulen 2019 [53]	Fluorescent-labelled panitumumab	6	Oral cancer	108	37	2	13	Prospective cohort study	High	2
Voskuil 2020[26]	pH activatable nanoprobe-conjugated ICG	13	Multiple sub-sites	4	6	0	3	Prospective cohort study	Low	1
Voskuil 2020[25]	Fluorescent-labelled cetuximab	15	Multiple sub-sites	10	4	0	1	Non-randomised experimental study	Low	2

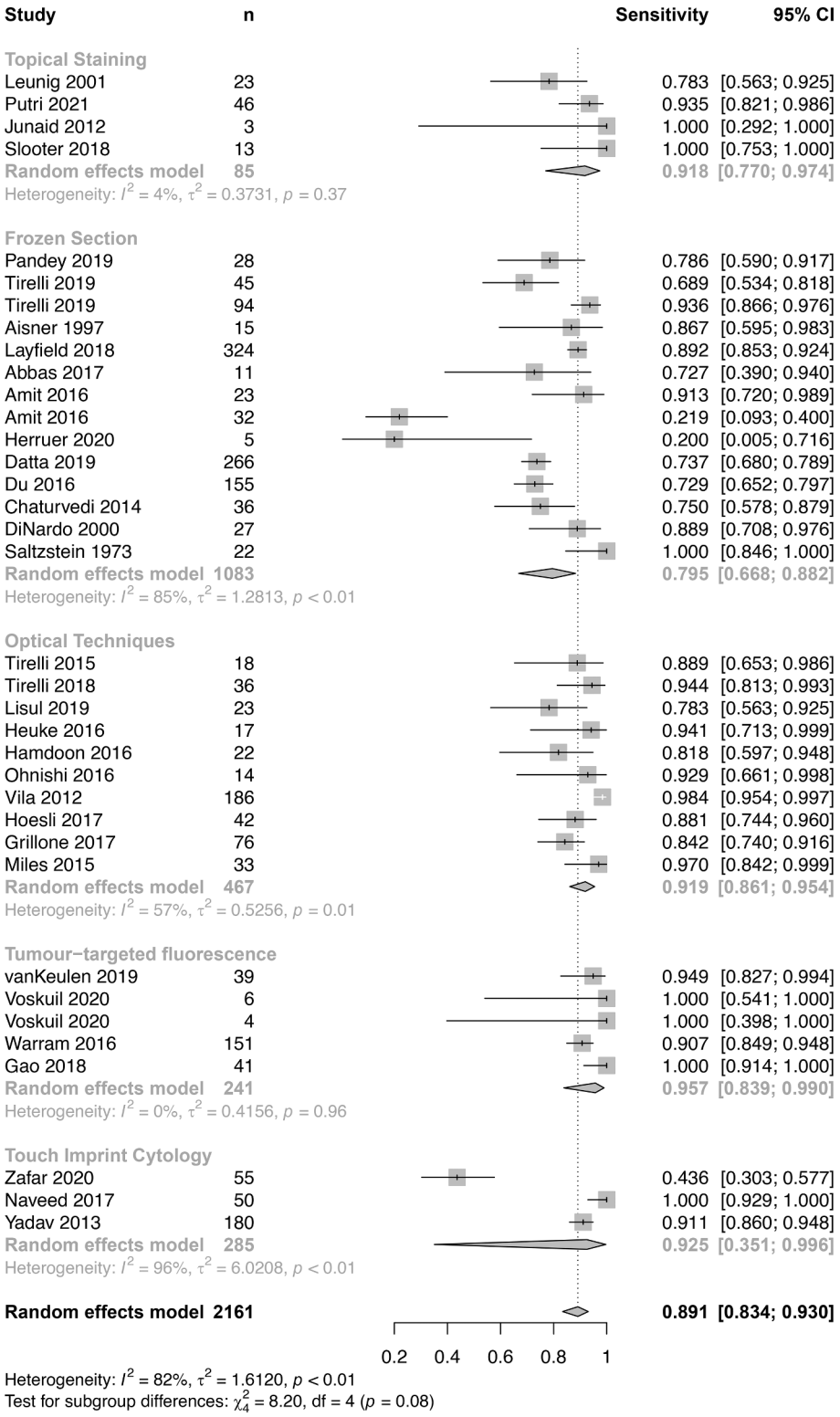

Figure 2. Forest plot showing sensitivity for each study, the pooled sensitivity for each group of studies (using the bivariate, random effects model) and for all studies. The Higgins I^2 score and p-value is shown for each group.

clinical practice.

Frozen section

Frozen section is an established technique for intraoperative margin assessment. In our meta-analysis, the specificity of frozen section was 0.991, the sensitivity was 0.795. The high specificity reflects the characteristic histopathological changes seen in samples containing malignant change, however frozen section has not become a routine part of head and neck resection surgery.

A key reason for the limited uptake of frozen section is that the major source of error is not accounted for by sensitivity and specificity metrics.

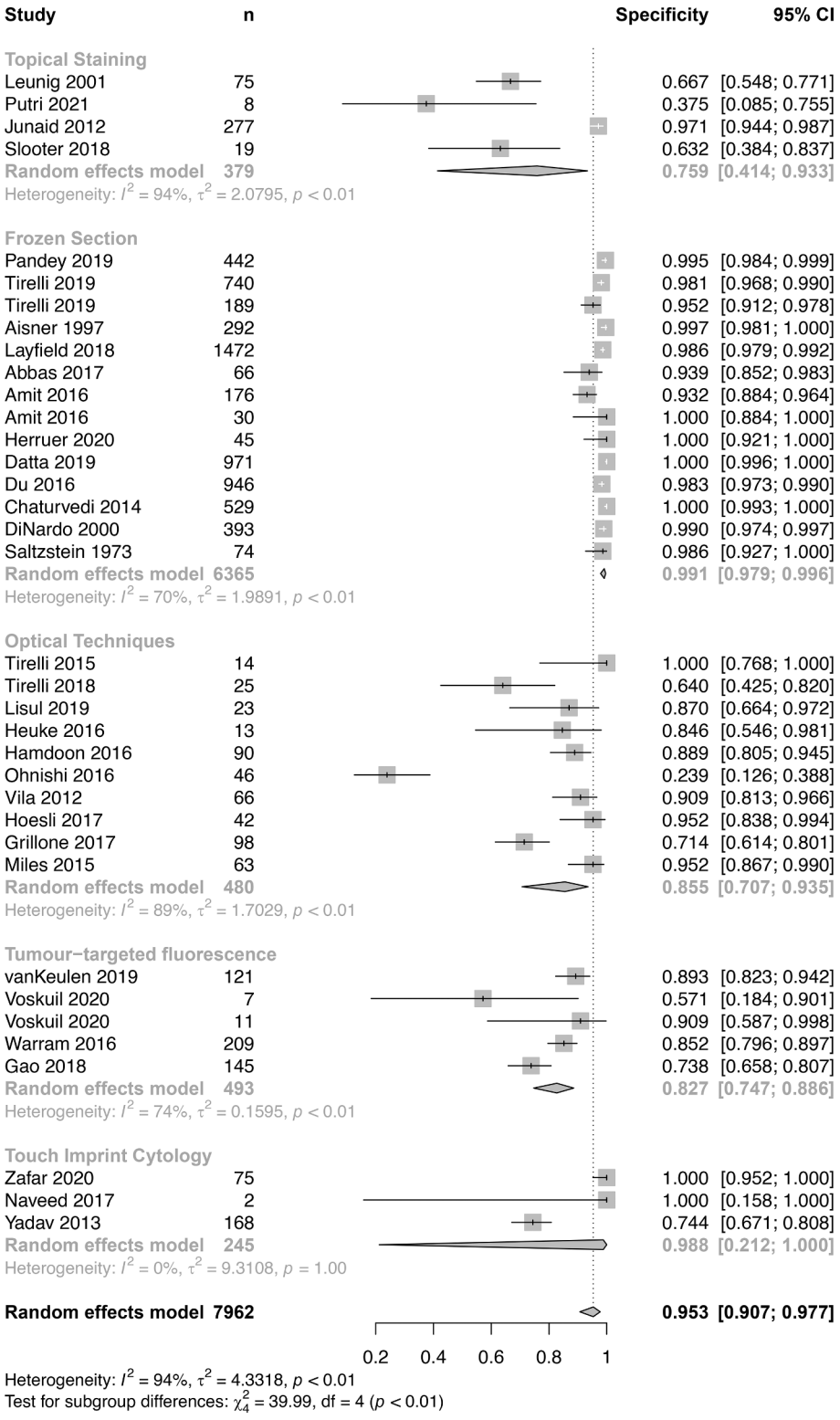

Figure 3. Forest plot showing specificity for each study, the pooled specificity for each group of studies (using the bivariate, random effects model) and for all studies. The Higgins I^2 score and p-value is shown for each group.

For any given tissue specimen submitted for analysis, errors of processing and interpretation are rare. Instead, most errors occur at the sampling stage. A common approach is for the surgeon to complete the tumour resection, then if concerns remain about risk of residual disease, they perform biopsies from the tumour bed for frozen section analysis. [50] This risks sampling healthy tissue adjacent to an inconspicuous nidus of residual disease,[51] or miscommunication between surgical and pathological teams.[52] These errors do not affect the sensitivity and specificity of frozen section, but markedly reduce its utility as an IMA. Sampling errors can be reduced by taking a specimen-driven approach[19], but this substantially increases the time and cost per analysis.

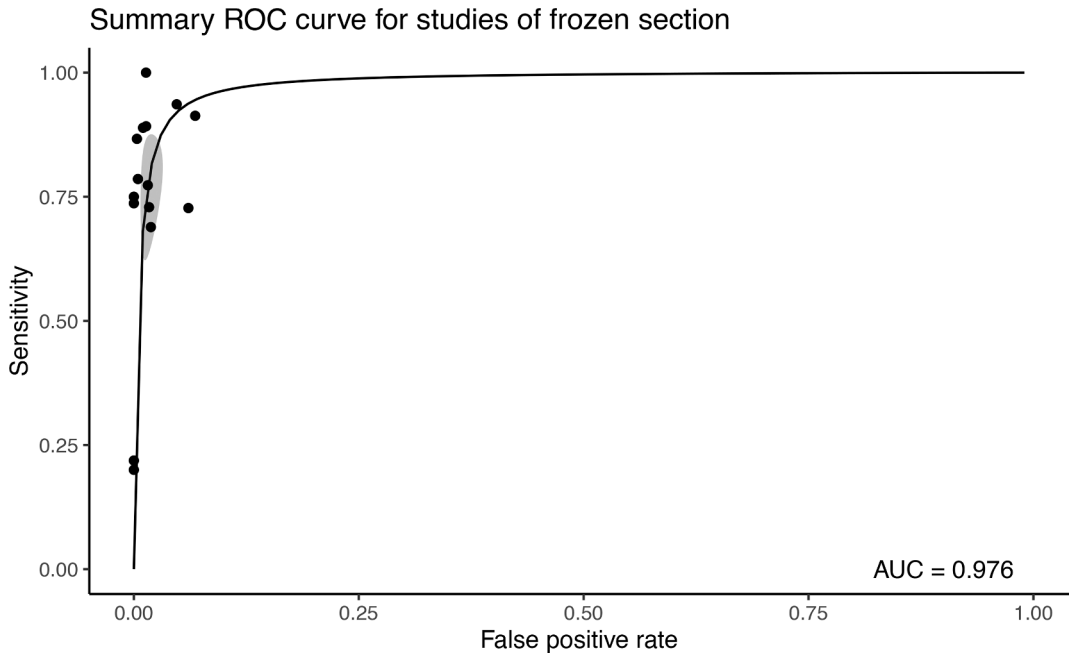

Figure 4. sROC curve for frozen section. Bivariate model. Grey area depicts the 95% confidence interval.

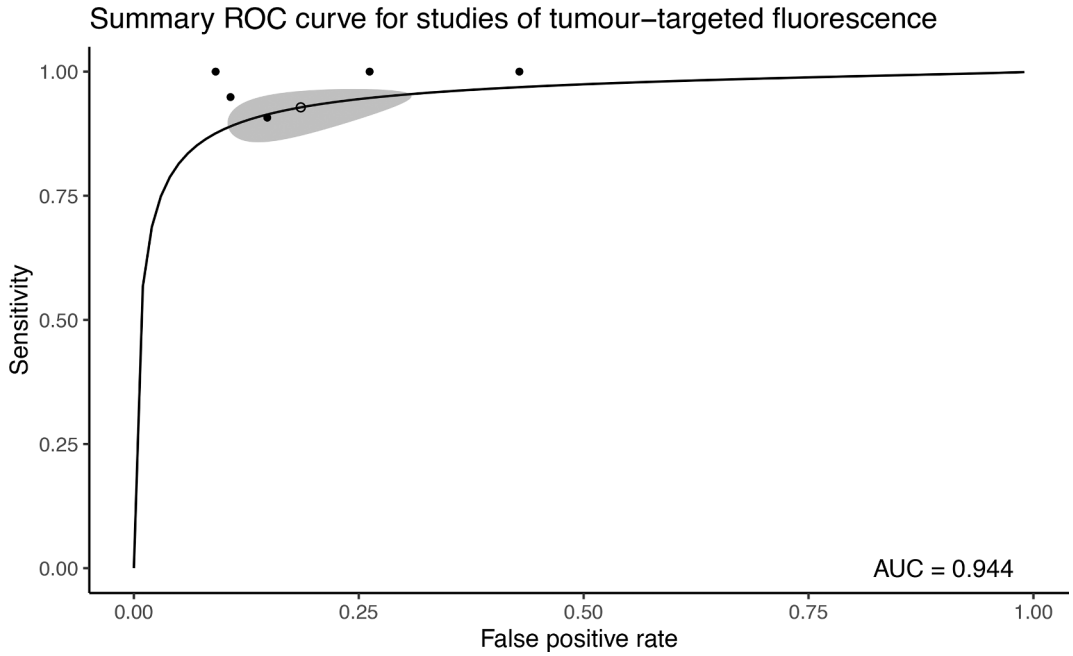

Figure 5. sROC curves for tumour-targeted fluorescence. Bivariate model. Grey area depicts the 95% confidence interval.

Frozen section requires specialised equipment, and for an experienced histopathologist to be able to be available for 20–30 min at short notice, with implications on their ability to do other routine pathological work for the health service.

Tumour-targeted fluorescence

Tumour-targeted fluorescence is a promising technique, using systemic administration of biocompatible fluorophores that preferentially accumulate in tumour tissue. Shining a light of appropriate wavelength on the area then elicits a fluorescent response allowing the tumour to be visualised in contrast to the background. In our meta-analysis, TTF techniques had excellent diagnostic accuracy, with overall sensitivity of 0.957 and specificity 0827. The AUC was 0.944. All the included studies were pilot or phase 1 studies, with limited patient recruitment, consistent with an emerging technique.

In addition to the excellent sensitivity, this approach commonly had a resolution that was able to identify small, otherwise undetectable satellite metastases[25]. It also allows detection of fluorescence signal through up to 6 mm of tissue, potentially allowing estimation of the proximity of the tumour to the resection margin, albeit with reduced specificity[53]. These results suggest that targeted fluorescence techniques may have a major role in the future of head and neck surgical oncology.

Our study included two different techniques for localising the fluorescence within the tumour. The majority linked the fluorophore to

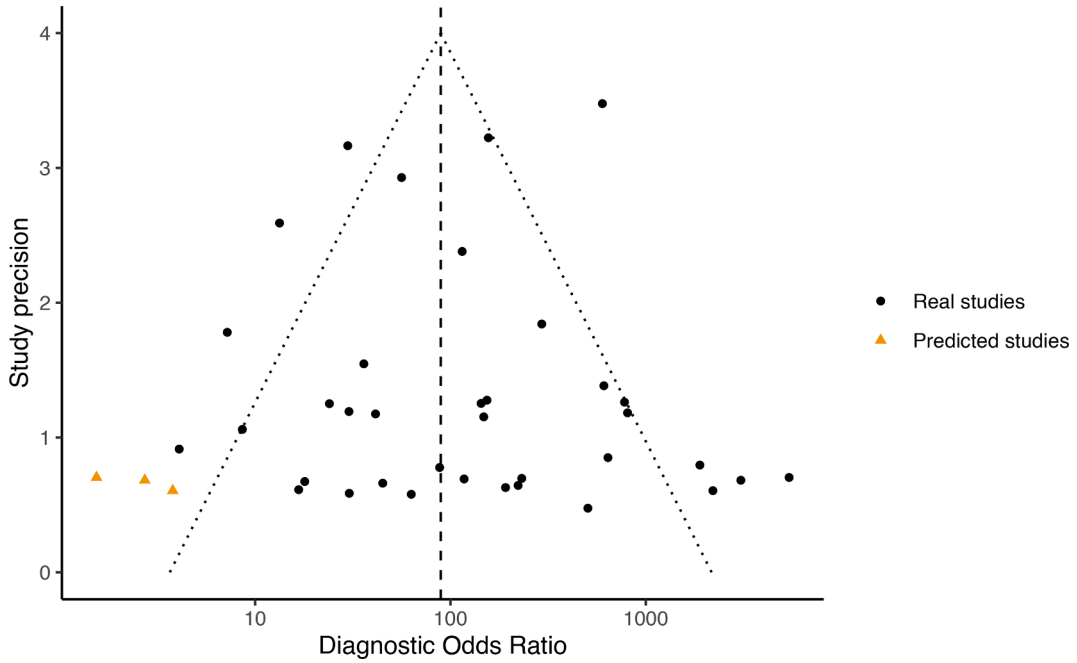

Figure 6. Funnel plot showing univariate effect size (Diagnostic Odds Ratio) plotted against the precision (1/Standard Error) for each study (dots). Three studies were predicted as being 'absent' due to publication bias ($K_0 = 3$) using the trim and fill method. They are represented in this figure as triangles, showing low diagnostic accuracy and low study precision.

monoclonal antibodies targeted at Epithelial Growth Factor Receptor (EGFR), a tyrosine kinase which is almost always overexpressed in HNSCC.[54] The fluorescent antibody binds to the surface of cells expressing EGFR, causing substantially increased fluorescent signal in tumour compared with adjacent normal tissue.

The other approach was *conditional quenching*, whereby the fluorophore is enveloped in a nanoparticle micelle that inhibits fluorescence.[26] When administered intravenously, the micelle remains stable until it reaches the hypoxic, low-pH tumour microenvironment. Here, the micelle irreversibly dissociates, releasing the fluorophore, resulting in tissue-specific fluorescence.

Each of these two approaches have their advantages and disadvantages.

The monoclonal antibodies used in these studies (cetuximab[25,55] and panitumumab[24,53]) are already in clinical use, with a well-established safety profile. Repurposing existing drugs provides greater confidence in the safety and side-effects profile, as well as speeding up development.[56].

Another advantage of monoclonal antibodies is that they can be combined with other labelling modalities. One possibility is the use of radiolabelling, allowing localisation of tumour deposits at depths greater than the 6 mm allowed by fluorescence, comparable to the process of sentinel node biopsy. This approach could not be used with nanoparticle micelles, as the quenching would have no effect on the transmission of gamma radiation, so signal would not be concentrated within the tumour. The use of monoclonal antibodies targeting EGFR are limited by the requirement for the tumour to *express* EGFR. This is usually significantly upregulated in HNSCC,[54] but can be suppressed in some tumour areas, particularly where the tumour microenvironment is hypoxic,[57] a common feature in HNSCC.[58].

The hypoxic tumour microenvironment and associated low pH is, however, the central premise behind the nanoprobe quenching approach to targeted fluorescence. This has the further advantage of being 'tumour agnostic': applicable to any rapidly growing solid tumour.[26] Questions remain over whether it would be able to detect a relatively well vascularised satellite lesion distinct from the main tumour mass: further work is needed.

A common weakness of both TTF approaches is that it is difficult to use *in situ* as the operation is progressing. The signal *in vivo* is 87% lower compared with imaging of the resected specimen. Repeated interruption to surgical workflow with the need to dim operative lights to take fluorescence imaging made this approach inaccurate and impractical [59]. The authors now routinely take the resection specimen to an analysis box on a back table in theatre for analysis.[24] This loses some of the immediacy of real-time feedback, but remains much faster than frozen section.

A recent meta-analysis looking exclusively at TTF found sensitivity of 0.917 and specificity of 0.719, comparable to our findings.[60] This study had different eligibility criteria and outcomes, and had only one study in common with our analysis, making the similarity of the diagnostic metrics more striking.

The DOR of TTF was 66.4, compared with 309.8 for frozen section. This difference is particularly notable given the comparable sensitivity, specificity, and AUC for these groups. This is likely to reflect the effect of defect-driven frozen section, in which samples are selected based on clinical suspicion of positive margins, increasing the proportion of positive test results. The DOR is a helpful univariate measure for summarising test performance, however it does not factor in the prior probability of a disease positive result, nor does it consider the relative importance of false negative and false positive results.[61] In this instance, the high DOR for frozen section masks its poor sensitivity, making it ill-suited for the purpose of detecting occult positive margins.

Touch imprint cytology

The pooled sensitivity of touch imprint cytology was 0.925, the specificity was 0.988, and there was significant heterogeneity within the three studies included.[23,62,63] The simplicity and speed of this technique is compelling, particularly in resource constrained environments as minimal complex equipment is required.[23] This is reflected in the included papers, which were all from low- and middle-income countries. However, it is a highly technique sensitive approach and has been shown to have poor results in well differentiated tumours;[64] it is hard to see it having a role as the gold standard of care.

Tissue staining techniques

Four studies of tissue staining techniques contributed to a pooled sensitivity of 0.918, with specificity 0.759. Two studies evaluated stains which have been in clinical use for some time: Toluidine blue[22] and Lugol's Iodine.[65] Toluidine blue is a tissue dye, often used in examination of fixed tissue slides, that stains nucleic acids blue. The high abundance of nucleic acids in rapidly multiplying malignant tissue means that it stains more intensely than adjacent normal tissue.[66] Lugol's iodine stains starches that are present in normal tissue and absent in dysplastic or malignant tissue.[66] Although not universally adopted some units use it regularly. This systematic review highlights a need for the publication of further research into its diagnostic accuracy to support its continued use: it has been investigated in a clinical trial [20], but as of the time of writing the results are unavailable. Neither of these techniques can distinguish between malignant and dysplastic tissue. This risks overtreatment because of false positives: dysplastic tissue being characterised as invasive cancer.

The other two studies employed a fluorescent dye that was activated in the presence of tumour. One used a precursor fluorophore activated by gamma-glutamyltranspeptidase (GGT), an enzyme that is overexpressed in HNSCC.[67] The other used 5-aminolevulinic acid (5-ALA), which when applied to tissues leads to preferential accumulation of fluorescent protoporphyrin IX in tumour cells.[68].

A weakness of all staining techniques is that they are only of use at the start of the procedure. They interact unpredictably with blood and other fluids in the operating field, making it difficult to discriminate tumour from normal tissue at the deep margin.

Optical techniques

Optical techniques as grouped here all measure the signal returned when target tissue is illuminated with electromagnetic radiation, usually visible light. In our meta-analysis they had a pooled sensitivity of 0.919, and a specificity of 0.855. There was significant heterogeneity within and between studies.

The included studies used a wide range of techniques. Some optical techniques enhanced the visual contrast between diseased and healthy tissue during direct inspection of tissue by the surgeon. Narrow band imaging[69,70] uses dichromatic visible light to highlight abnormal tumour interpapillary capillary loop patterns in the mucosa. Tissue autofluorescence[28] uses monochromatic light to activate fluorescence in normal tissue. Through an optical filter, normal tissue fluoresces bright green, but abnormal mucosa loses this property and appears dark. Both techniques are commonly used in the outpatient and pre-operative diagnostic settings,[66] however, there appears to be less support for their use as IMA devices. This may be due to practical concerns: the presence of blood in the field obscuring tissue and limiting the utility of the technique once dissection has started.

Other techniques offered ways to evaluate minimally processed tissue samples, by analysing the images or spectra returned from the tissue using a variety of modalities. Optical Coherence Tomography (OCT) uses low-coherence light to capture micrometre resolution, cross-sectional images of tissue,[71] similar to the views provided by ultrasound imaging. High resolution microendoscopy (HRME)[30,72] uses a fibre probe in theatre to generate high-resolution, real time dynamic microscopic imaging of resection specimen tissue architecture. Pathological and normal tissue can be distinguished following minimal training. Elastic scattering spectroscopy,[73] coherent Raman scattering spectroscopy,[74] optomagnetic imaging spectroscopy[75] and multimodal nonlinear microscopy[76] all used similar electromagnetic spectral analysis to evaluate ex vivo tissue samples.

All of these optical approaches show promise, but it is difficult to make concrete recommendations based on this meta-analysis as the studies, techniques and results were all so heterogeneous. Additionally, many of the included studies in this group were assessed as being of poor quality and/or having a high risk of bias. Clearly, more research is needed before any of them can be recommended for routine use as an IMA.

Safety

The risk of adverse events associated with IMA techniques needs careful consideration. Frozen section is long-established, and involves analysis of tissue remote from the patient, as does touch imprint cytology, minimising the risk to the patient. Optical techniques involve illumination of tissues and evaluation of the ensuing spectra data: there are no significant safety concerns.

Tissue staining techniques involve the application of agents to mucosa, and so there is the potential for toxic or allergic responses. Iodine [77], toluidine blue [78] and 5-ALA[79] are all safe when administered correctly, with low anaphylaxis risk. gGlu-HMRG has been evaluated as safe based on studies in mice:[80] to our knowledge it has not undergone dedicated safety analysis in humans,[81] though the lower doses associated with topical application are likely to mitigate any risks.

Tumour-targeted fluorescence techniques require intravenous administration of complex, biologically active molecules, presenting potential safety concerns that must be thoroughly investigated. ICG itself has a long track record of safety for injectable use,[82] but the delivery mechanisms require careful scrutiny. Cetuximab and panitumumab are both FDA approved for treatment of cancer, with skin rashes and other dermatological complications representing the main adverse events over a course of treatment, [83,84] although serious infusion reactions have been reported with cetuximab[83] as, unlike panitumumab, it is not a fully humanised monoclonal antibody.[24] Note that patients undergoing TTF during surgery would receive only a single dose, rather than a full course of treatment, and small safety studies of cetuximab[85] and panitumumab[24] showed no adverse events of grade 2 or higher. Although based on even newer technology, the study of pH-activatable nanoprobes included in this systematic review had safety as a primary outcome, and reported no adverse events. [26].

The role of IMAs

In this study, we have comprehensively and robustly evaluated the diagnostic accuracy of techniques used to identify tumour tissue at resection margins. However, diagnostic metrics should be considered in the broader context of clinical practice. Improved diagnostic accuracy does not necessarily result in improved rates of negative margins or improved survival outcomes. This is illustrated by the importance of sampling error in frozen section. 14 to 22% of patients may have a positive margin in the final specimen despite a negative intraoperative frozen section result.[51,86] The high specificity of frozen section means that it can accurately identify a negative margin, ensuring a correct diagnosis *for that sample* if there are no technical failures during sample processing. The disappointing sensitivity of frozen section is likely because it is very technique sensitive and evaluating the faces of the specimen likely results in fewer sampling errors than a defect-driven approach [19]. Further research evaluating the accuracy of specimen-driven frozen section will likely demonstrate a better sensitivity [19,42,51,87,88].

Accordingly, an ideal IMA technique would provide results for the entirety of the specimen and/or the resection defect with high resolution whilst retaining excellent sensitivity and specificity. Targeted fluorescence could present a powerful tool to overcome the risk of sampling error by providing a comprehensive view of the sample on a theatre back table, and spatially resolved data on the risk of positive or close margins. This could also work synergistically with other IMA techniques, guiding frozen section sampling, or as a complement to more advanced techniques. Possibilities could include any of the techniques presented in this study, or emerging technology such as the iKnife, which is able to

provide near-instant tissue classification using mass spectrometry analysis of surgical aerosol, either during dissection or directed sampling, and has shown great promise in early studies.[89].

However, resection with unexpected positive margins may reflect a fundamentally aggressive tumour phenotype that would recur whether or not IMAs facilitate resection with clear margins.[7] The revision of positive margins to negative, guided by frozen section, is a major risk factor for local recurrence,[90] and where multiple resections are required to achieve clear margins, disease-free and overall-survival is significantly worse.[91].

Limitations

The findings of this systematic review are limited by the heterogeneity of the included studies, particularly those in the optical and frozen section subgroups. In addition to the variety of techniques included within these broader categories, there was considerable methodological heterogeneity too, with studies variably reporting per-sample or per-patient diagnostic accuracy. This heterogeneity limits the generalisability of the results. Furthermore, many studies were excluded from this systematic review and meta-analysis because the authors reported combined diagnostic accuracy results for *all* head and neck cancers, including salivary, thyroid, and non-squamous mucosal disease. Despite their close anatomical and surgical relationship, the tumour biology and oncological outcomes are vastly different.

Although we have assessed the risk of publication bias as low ($k_0 = 3$), full text screening was notable for the high number of studies (57) that did not provide raw diagnostic data. This represents a lost opportunity for synthesis of otherwise useful work into our current understanding of intraoperative diagnostics. For this reason, along with previous authors,[43,92] we call for future publications of diagnostic studies in head and neck cancer to provide raw diagnostic data - ideally in the form of a confusion matrix - with clear reporting of how the index and reference tests were performed.

Future perspectives

The results we have presented here reflect only a small proportion of the ongoing work in head and neck cancer and surgical oncology more broadly to bring meaningful intraoperative diagnostic capability to surgeons. As research continues, new techniques will undoubtedly be developed and tested, but also the use cases for the technology will become more refined. It may well be that intravenous fluorescent agents present no patient benefit for shallow T1 lateral tongue tumours, but offer significantly improved oncological outcomes for advanced T4 tumours with neck metastases. Staining and optical techniques may well be more beneficial in smaller tumours and offer possibilities of more conservative resection with confidence of clear margins. Furthermore, it may be that a combination of approaches offers the greatest benefit. Concerns regarding sampling error with frozen section could be overcome by localisation of small deposits of tumour using tumour targeted fluorescence to guide sample collection.

Conclusion

Intraoperative margin assessment techniques must be accurate, rapid, easily interpreted, and cost-effective to become a routine part of head and neck ablative surgery, and to reduce the burden of morbidity and mortality associated with positive margins. This meta-analysis has identified that frozen section is well-established but lacking in sensitivity. Tumour-targeted fluorescence techniques have excellent sensitivity and practical applicability. However, they require the injection of a systemic agent. Emerging techniques, such as intraoperative mass spectrometry with the iKnife, must present robust evidence of comparable accuracy before considering clinical adoption.

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Data access

The data collected during the systematic review are available on request.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2023.106419.

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