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Single modality indocyanine green is feasible for sentinel node detection in head and neck cutaneous melanoma: A prospective cohort study

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Summary Dual modality sentinel lymph node biopsy (SLNB) techniques are most commonly used in melanoma surgery, based on radiocolloid lymphoscintigraphy with either blue dye or indocyanine green (ICG) dye. No studies to date have analysed the use of ICG alone. This study examined the feasibility of ICG as a single modality for detecting sentinel nodes in cutaneous head and neck melanomas. A prospective cohort study was performed of all consecutive cutaneous head and neck melanomas patients between July 2023 and November 2024 undergoing SLNB at a supraregional skin cancer centre in the U.K. Three cohorts were formed: group A (ICG-only); group B (ICG and radiocolloid); and group C (radiocolloid and blue dye). 182 nodes were obtained from 67 patients. Where each technique was utilised, 100% of nodes were detected by ICG; 85% by radiocolloid; and 80% by blue dye. Radiocolloid failed to detect any nodes in three patients, and blue dye failed in four patients. In group B, ICG detected significantly more nodes than radiocolloid (p=0.0004); in group C, there was no difference between radiocolloid and blue dye (p=0.149). Twelve sentinel nodes were positive for melanoma: 100% were ICG positive, 88.9% were radiocolloid positive and 50% were blue dye positive. Single modality ICG is a feasible technique, with nodal detection and metastases detection rates comparable to dual modality. As an intraoperative surgeon-led technique, ICG-alone negates the need for preoperative lymphoscintigraphy with its associated radiation risk and nuclear medicine resource requirements.

Lymph node status is an important feature of staging cutaneous melanomas of the head and neck, as it guides treatment decisions and provides both diagnostic and prognostic information to patients.[1] The accurate detection of occult

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metastasis within lymph node basins has been a challenge that has only recently seen advancements in technique.

Snow (1892) first published the method of complete lymph node dissection (CLND) with haematoxylin and eosin staining, which resulted in the overtreatment of 80% of patients.[2] It took a century for the concept of targeted sampling of lymph node basins using the sentinel node status to become established, when Morton (1992) demonstrated that a sentinel node could be identified in 82% of lymph node basins using patent blue V and isosulfan blue dyes, with only 1% of metastases solely in the non-sentinel nodes.[3]

Blue dye as a single modality was used in the initial stages of the MSLT-1 trial to detect sentinel nodes,[4] until Bostick et al. (1997) proved the higher sensitivity in detecting sentinel nodes through dual modality blue dye with preoperative lymphoscintigraphy/intraoperative radiocolloid.[5] However, post-hoc analysis of the MSLT-1 population demonstrated that, even with the addition of lymphoscintigraphy, there was no sentinel node identified in 16% of cervical lymph node basins. Furthermore, there was an overall 6.3% false negative rate manifesting as regional nodal recurrence.[4]

Despite these issues with sensitivity and specificity, blue dye with lymphoscintigraphy has remained the gold standard of sentinel node detection. Protocol refinements over the years include same-day lymphoscintigraphy with computed tomography (CT) scan, i.e. SPECT-CT, which has been shown to increase the detection of metastatic nodes compared to planar lymphoscintigraphy.[6]

Over the last decade, the MSLT-II trial and new adjuvant therapies have changed the landscape for melanomas, with more importance on SLNB as a gateway to oncological therapies and fewer indications for invasive regional dissection.[7] Coinciding with advances in melanoma management has been the introduction of near-infrared technology to improve the accuracy of identifying sentinel nodes. Here, real-time indocyanine green (ICG) dye is used to trace lymphatic draining channels from a cutaneous melanoma site to its sentinel node(s).[8]

Numerous studies have demonstrated the superiority of dual modality ICG and radiocolloid over traditional blue dye and radiocolloid,[9,10] but there are no studies that have evaluated ICG as a single modality in detecting sentinel nodes. The benefits of validating ICG-alone as a non-inferior technique are clear: it presents a surgeon-led intraoperative technique, negates the risk of radiocolloid use, and is not dependent on the availability of radiocolloid tracers and lymphoscintigraphy.

This study examines the feasibility, accuracy and surgical technique of ICG as a single modality in detecting sentinel nodes in cutaneous head and neck melanomas, compared with dual modality radiocolloid lymphoscintigraphy with ICG and blue dye techniques.

Methods

A prospective cohort database was maintained of all consecutive patients diagnosed with cutaneous head and neck melanoma who underwent SLNB between July 2023 and November 2024 at a single supraregional skin cancer centre in the U.K. The study was registered with the Trust research department and adhered to the STROBE guidelines.[11]

Patients were excluded if they did not have a biopsy-proven cutaneous melanoma on the anatomical head or neck, or if their melanoma did not meet the National Institute for Health and Care Excellence (NICE) indications for SLNB in melanoma.[12] All operations were performed by head and neck plastic surgeons as day case general anaesthetic procedures. ICG dye was detected using the SPY Elite® (Stryker, Portage, MI, USA) near-infrared camera system. Preoperative SPECT-CT was performed as standard protocol for all head and neck melanoma SLNBs performed with dual modality in the Trust.

Data was collected on patient demographics; melanoma characteristics, including anatomical location, Breslow thickness, American Joint Committee on Cancer 8th edition clinical stage[13]; and the number and features of each sentinel node obtained, including location, detection technique, metastasis status. Data from preoperative SPECT-CT scan, operative records and intraoperative photographs were collected contemporaneously; histopathology results were collected prospectively when reported.

Three patient cohorts were formed for data analysis, based on the operative technique for sentinel node identification: group A patients underwent single modality ICG-only between April and November 2024; group B patients underwent dual modality radiocolloid and ICG between November 2023 and March 2024; and group C patients underwent dual modality radiocolloid and blue dye between July and November 2023. The introduction of dual modality ICG with radiocolloid lymphoscintigraphy was a planned evolution of SLNB technique in the unit, while the introduction of single modality ICG was in response to a planned national shortage of technetium-99 radiocolloid.

Group A: ICG-only

The ICG-only technique was modified over the study period to improve the ergonomics of its use and to establish standardised steps across patients; the current practice is outlined in Figure 1. Intraoperatively, 1 ml ICG dye (Verdye ™ 5 mg/1 ml, Diagnostic Green, Aschheim-Dornach, Germany) was injected around the primary melanoma site. The fluorescent ICG dye was traced transcutaneously through the lymphatic channels to the sentinel nodes by the operating surgeon using the greyscale mode on the handheld near-infrared camera. The skin incision was planned over or close to the identified sentinel nodes, using conventional surgical approaches where feasible. Dissection was guided by the near-infrared camera towards the fluorescent sentinel node(s), and ex vivo colour gradient and fluorescent mode photographs were obtained of each specimen. Figure 2 shows the intraoperative sequence as depicted on images on the near-infrared camera; the associated Supplementary Video demonstrates the technique in real-time. Nodal harvest was considered complete in an exposed lymph node basin when there were no further ICG-fluorescent nodes present.

Supplementary material related to this article can be found online at doi:10.1016/j.bjps.2025.05.002.

Preoperative in theatre

- Dilute Verdye™ 25mg (ICG) in 5mls of water for injection.
- Draw 1ml of Verdye™ (5mg/ml) into a 1ml insulin syringe with needle.
- Mark the margins of the melanoma wide local excision.
- Apply a clear film dressing over the melanoma biopsy scar and markings.
- Inject 1ml of ICG solution into the dermis of the melanoma biopsy scar through the clear film dressing at multiple points. The clear film prevents contamination of the skin with ICG fluorescent dye.
- With the clear film in place, press with gauze over the needle entry point only; do not wipe around with gauze as this will contaminate skin with ICG.
- Remove the clear film and do not touch injected area again until mapping is complete.
- Trace the ICG dye from the melanoma biopsy site to the lymph node basins using greyscale mode on the near-infrared handheld camera. Lymphatic drainage starts immediately after injection.
- For head and neck melanomas, it takes approximately 5 minutes to fully map the sentinel nodes due to the proximity of the lymph node basins.
- The sentinel node(s) are marked where a "confluence" of ICG appears on the camera, which is where the superficial lymphatics traverse deeper. There may be more than one confluence and therefore more than one sentinel node.
- Incision sites are marked where sentinel node(s) can most easily be explored, using conventional approaches where feasible.
- Photograph the mapping and markings for documentation.
- Once mapping is complete, inject the margins of your excision with local anaesthetic if required. There should be adequate time for the injected ICG dye to dissipate prior to skin preparation to avoid contamination, typically 5 minutes in the head and neck region.

Intraoperative

- Perform the SLNB before the wide local excision.
- Skin incision where marked.
- With the assistant holding the camera, dissect towards the confluence of bright light on the greyscale camera mode. Bipolar dissection to biopsy the lymph node can seal the lymphatic vessels to prevent bleed-through of ICG onto the surgical field.
- Confirm the presence of a lymph node in the biopsy using visual examination and colour gradient mode on the near-infrared camera, and document with photographs.
- Review the surgical field using the camera: if further bright confluence seen, repeat the steps to biopsy the bright lymph node. If surgical field is dark or only lymphatic vessels seen, no further dissection is required.
- Complete the above SLNB steps for all marked incision sites.
- Complete the wide local excision and any other operative steps.

Postoperative

- Send sentinel nodes separately in formalin to histopathology for analysis.

Figure 1 Pre-, intra-, and postoperative steps for the use of single modality ICG in head and neck melanoma.

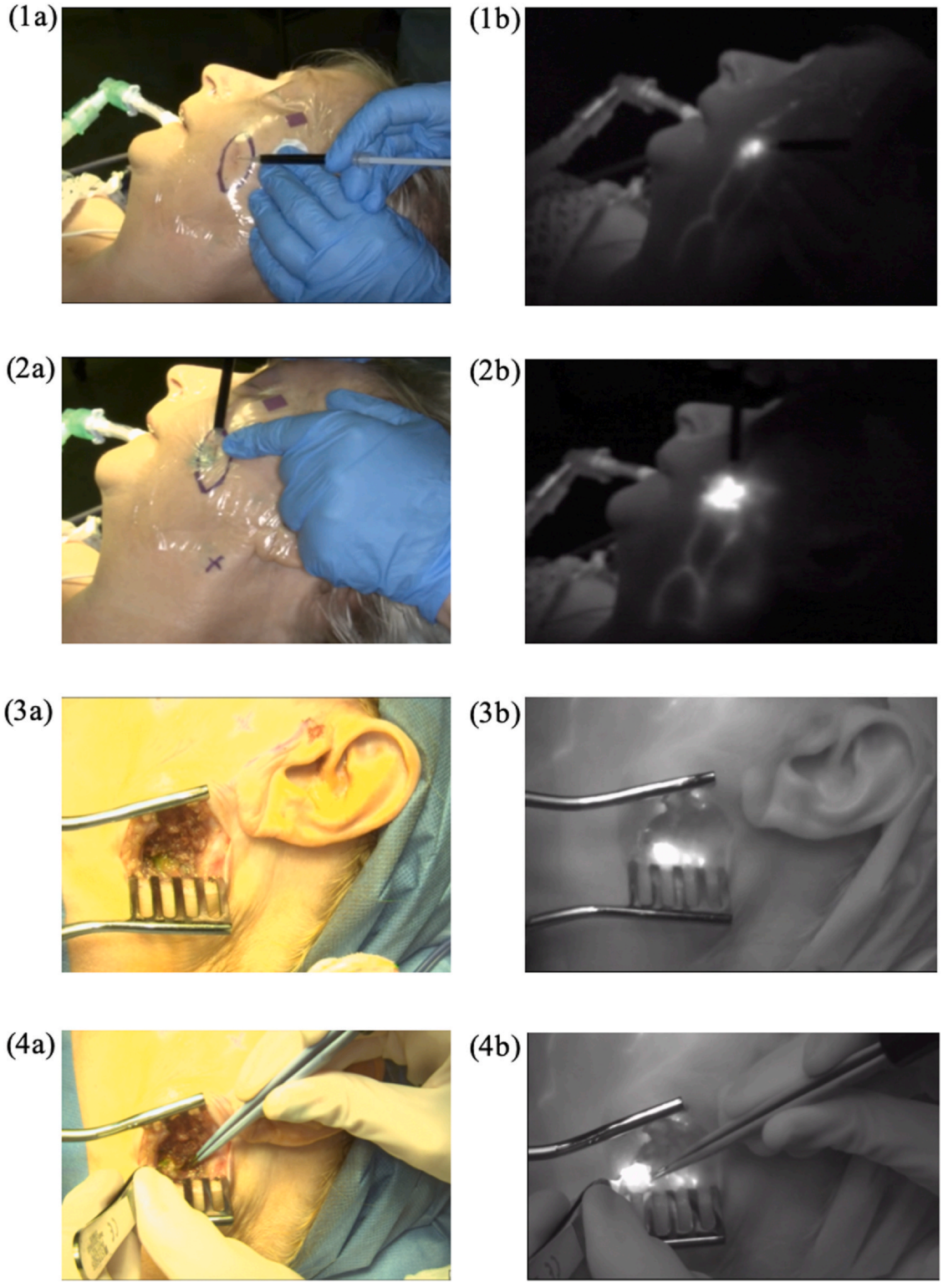

Figure 2 Intraoperative sequence of ICG-only sentinel node biopsy for melanoma of the left cheek, showing using (a) photos and (b) fluorescent near-infrared handheld camera images.[1] Injection of 1 ml ICG dye into dermis of melanoma scar[2]; Real-time drainage through lymphatic channels to sentinel node, marked "x" on photo and shown as fluorescent confluence on near-infrared[3] view of sentinel node after skin incision and dissection[4]; biopsy of sentinel node.

Group B: radiocolloid and ICG

Preoperative SPECT-CT scans were performed with technetium-99m radiocolloid on the day of surgery and reported by radiographers or radiologists. Intraoperatively, ICG dye was injected around the primary melanoma site as for Group A. Skin incisions for sentinel node harvest were planned based on both the SPECT-CT scan and the ICG dye locations. Dissection to an

identified sentinel node was guided by dual modality handheld gamma probe and near-infrared camera. Nodal harvest was considered complete in an exposed lymph node basing when the radioactive count was within 10% of the background count and there was no further ICG-fluorescent node present.

Group C: radiocolloid and blue dye

Preoperative SPECT-CT scan was performed as described for Group B. Intraoperatively, a 1 ml intradermal injection of Patent Blue V dye was administered around the primary melanoma scar in four injections. Skin incisions were planned based on the SPECT-CT scan alone. Dissection to the sentinel node was guided by handheld gamma probe, and visual blue colouration was used to detect the sentinel nodes. Nodal harvest was considered complete in an exposed lymph node basin when the radioactive count was within 10% of the background count and there was no further visually blue node present.

Statistical analyses were performed using SPSS software. Continuous data were described using median and percentiles in non-parametric distribution of data, and mean and standard deviation in parametric distribution of data. The Mann-Whitney U and Kruskall-Wallis tests were used to compare continuous variables between groups. Chi-squared (x^2) test was used to compare categorical variables, with Fisher's exact test used for smaller numbers.

Results

Sixty-seven consecutive patients underwent sentinel lymph node biopsy for cutaneous head and neck melanoma over the study period (Table 1). Median age was 68 years (range, 23 to 92 years). Male patients represented 64% of the total cohort, with no significant difference across groups ($p = 0.463$). The most common melanoma site was the cheek (31%), followed by the ear (24%), scalp (3.4%), forehead (12%) and neck (9.0%). Melanoma site was non-significant across groups ($p = 0.671$), but in group C melanomas on the ear (31%) and neck (12.5%) were more represented. Median Breslow thickness was 1.7 mm (range, 0.8 to 14 mm), with no significant difference across groups ($p = 0.581$). In all groups, the most common Breslow thickness was between > 1.0 mm and 2.0 mm, followed by > 2.0 to 4.0 mm in group A and > 4.0 mm in groups B and C. Median follow up was 8.3 months (range, 1.8 to 17 months), with significant variation between all three groups due to the use of different modalities during different time periods ($p < 0.0001$).

Sentinel node detection and biopsy

182 sentinel nodes were obtained from 67 patients.

Twenty-nine patients were included in group A. A total of 71 nodes were detected and biopsied, with a mean of 2.45 ± 1.33 nodes obtained per patient (Table 2). 100% (71 out of 71) nodes that were detected by ICG were surgically obtained, and all patients had at least one node obtained for biopsy (Table 3).

Twenty-two patients were included in group B. Preoperative SPECT-CT mapped a total of 66 nodes (mean 3.0 ± 1.51 nodes), with 61 nodes subsequently surgically biopsied (mean 2.77 ± 1.47 nodes). 34% (21 out of 61) nodes obtained did not anatomically correspond with the preoperative SPECT-CT images or report. Out of the total 61 biopsied nodes, intraoperative localisation of nodes was significantly higher by ICG (100%, 61 out of 61 nodes) than

Table 1 Patient demographics and melanoma characteristics.

	Total		Group A		Group B		Group C		
	No.	%	No.	%	No.	%	No.	%	*p*
Sex	67		29		22		16		0.463[a]
Female	24	35.8	8	27.6	9	40.9	7	56.3	
Male	43	64.2	21	72.4	13	59.1	9	43.7	
Anatomical location	67								0.671[b]
Cheek	21	31.3	11	37.9	7	31.8	3	18.8	
Ear	16	23.9	8	27.6	3	13.6	5	31.3	
Scalp	9	13.4	3	10.3	4	18.2	2	12.5	
Forehead	8	11.9	2	6.9	2	9.1	4	18.8	
Neck	6	9.0	2	6.9	2	9.1	2	12.5	
Temple	2	3.0	1	3.4	1	4.5	0	0	
Eyelid	2	3.0	0	0	2	9.1	0	0	
Postauricular	1	1.5	0	0	1	4.5	0	0	
Upper lip	1	1.5	1	3.4	0	0	0	0	
Nose	1	1.5	1	3.4	0	0	0	0	
Breslow thickness (mm)									0.581[b]
0.8 - 1.0	6	9.0	4	13.8	2	9.1	2	12.5	
> 1.0 - 2.0	29	43.3	10	34.5	10	45.5	9	56.3	
> 2.0 - 4.0	14	20.9	9	31.0	4	18.2	1	6.3	
> 4.0	16	23.9	6	20.7	6	27.3	4	25	

[a] x^2 test.
[b] Fisher's exact test.

Table 2 In each group, mean number of sentinel nodes (a) mapped by lymphoscintigraphy and (b) surgically biopsied.

	Group A	Group B	Group C	*p*
Mean number of nodes mapped by lymphoscintigraphy (mean ± SD)	-	3.0 ± 1.51	2.25 ± 0.90	0.0989[a]
Mean number of nodes surgically biopsied (mean ± SD)	2.45 ± 1.33	2.77 ± 1.47	3.06 ± 1.39	0.499[b]

SD; standard deviation.
[a] Mann Whitney U.
[b] Kruskall-Wallis test.

Table 3 Number of (a) sentinel nodes detected and (b) metastatic nodes detected, by sentinel node biopsy technique.

	ICG	Gamma probe	Patent V blue dye	*p*
Number of nodes detected				
Total	132 (100%)	93 (84.5%)	39 (79.6%)	< 0.0001[a,b]
Group A	71 (100%)	-	-	
Group B	61 (100%)	48 (78.7%)	-	0.0004[a,b]
Group C	-	45 (91.8%)	39 (79.6%)	0.149[a]
Number of metastatic nodes				
Total	6 (100%)	8 (88.9%)	3 (50%)	
Group A	3 (100%)	-	-	
Group B	3 (100%)	2 (66.7%)	-	
Group C	-	6 (100%)	3 (50%)	

ICG: indocyanine green.
[a] x^2 test.
[b] Denotes significance.

by gamma probe (79%, 48 out of 61 nodes), p=0.0004. Radiocolloid failed in two patients; one patient had no nodes preoperatively mapped on SPECT-CT, while one patient had no signal on intraoperative gamma probe.

Sixteen patients in group C had a total of 36 nodes mapped by preoperative SPECT-CT (mean 2.25 ± 0.90), with subsequently 49 nodes surgically biopsied (mean 3.06 ± 1.39). 43% (21 out of 49) nodes obtained did not anatomically correspond with the preoperative SPECT-CT images or report. Gamma probe intraoperatively localised 92% (45 out of 49) of the biopsied nodes, while blue dye localised 80% (39 out of 49) of the biopsied nodes; this was not statistically significant ($p = 0.149$). Blue dye failed to identify any nodes in four patients, while intraoperative gamma probe did not pick up a signal in one patient.

Where each technique was utilised, 100% of nodes were detected by ICG; 85% by radiocolloid; and 80% by blue dye. Radiocolloid failed in three out of 58 patients (5.1%) and blue dye failed in four out of 16 patients (25%). A total of 38% nodes (42 out of 110 nodes) that were biopsied were not identified on preoperative SPECT-CT lymph node mapping.

Nodal metastasis detection

In total, twelve nodes in ten patients were identified as positive for melanoma metastasis.

Group A detected three metastatic nodes in three patients using ICG-only, which represent 4.2% of the 71 nodes biopsied. A median of 2.0 (range, 1 to 2) non-sentinel negative nodes were obtained alongside the positive node.

Similarly, group B had three metastatic nodes detected in three patients, which represent 4.9% of the 61 nodes biopsied. A median of 2.0 (range, 1 to 2) non-sentinel negative nodes were obtained. Preoperative SPECT-CT did not detect one positive node obtained. Intraoperatively, all three metastatic nodes were identified by ICG, while gamma probe identified two out of three metastatic nodes.

Group C had six metastatic nodes detected in four patients, which represent 12.2% of the 49 nodes biopsied. A median of 2.0 (range, 0 to 5) non-sentinel negative nodes were obtained. Preoperative SPECT-CT did not detect two positive nodes obtained in two patients. Intraoperatively, all six metastatic nodes were detected by radiocolloid, while three out of six were identified by blue dye.

Overall, ICG detected 100%; radiocolloid detected 89%; and blue dye 50% of nodal metastases in this cohort. Three out of 42 (7.1%) nodes that were not highlighted on preoperative SPECT-CT were positive for metastatic disease; in two patients, they were the only positive node present.

False positive biopsies

8.1% of sentinel node biopsy specimens did not reveal a lymph node on histopathology assessment: 8.9% (seven out of 78 specimens) in group A, 13% (nine out of 70 specimens) in group B, and 0% (0 out of 49 specimens) in group C. In group A, four out of seven false-positive specimens were in the first ten patients, while the last ten patients had no false-positive specimens. In group B, seven out of nine false-positive specimens were identified by both ICG and gamma probe; two specimens were identified only by ICG.

Discussion

This study demonstrates the logistical feasibility of ICG as a single modality to detect sentinel nodes in cutaneous head

and neck melanomas, in the only prospective cohort study to analyse ICG as a standalone technique.

Single modality ICG did not significantly deviate in the mean number of lymph nodes surgically obtained during a sentinel node biopsy compared to dual modality techniques. As a technique, ICG was the only modality to intraoperatively detect 100% of biopsied sentinel nodes and 100% of nodal metastases, with significantly fewer sentinel nodes and fewer metastatic nodes detected by gamma probe and by blue dye. In our cohort, ICG was the only technique that did not fail to detect any sentinel nodes in a patient.

These results are comparable to a recent meta-analysis of nine studies by Hotchkies et al. in 2024,[10] where it was shown that ICG had a sensitivity of 95%, which was significantly superior to blue dye (48%) and comparable to radiocolloid (91%) in head and neck melanomas. This meta-analysis, along with the meta-analysis by Patel et al. in 2021[14] have proven the inferiority of blue dye compared to ICG and radiocolloid, and its use in modern practice should be limited to instances where ICG is not available. The superiority of ICG or radiocolloid is more ambiguous, with equivocal data in the literature as presented by the meta-analysis of seven studies by Wölffer et al. in 2024,[9] where it was noted that prospective studies favoured radiocolloid and retrospective studies favoured ICG, albeit with significant heterogeneity in study populations and length of follow-up.

To our knowledge, all published studies have analysed the different SLNB techniques by using double or triple modality in the same patient, without the use of ICG as a single modality. In single modality ICG, the lack of preoperative SPECT-CT mapping means that surgical incisions and dissection are guided solely by real-time lymphatic drainage using near-infrared camera, which is a different technique to assessing multiple modalities in the same patient. The only study in the literature to blind surgeons to the preoperative SPECT-CT during the use of ICG is a nonrandomised phase II trial by de Carvalho et al.,[15] which found that 90.9% of sentinel nodes were identified by ICG, but only 70% of cases had a sentinel node identified by ICG without assistance from radiocolloid or blue dye. The difference may be down to the study's technical differences in ICG-led sentinel node biopsy and the inclusion of all anatomical sites, as melanoma in the head and neck has faster ICG drainage to the lymph node basins and tracing the ICG dye through the skin may be easier than truncal or limb melanomas.

Challenges of detecting sentinel nodes in the head and neck region include its complex anatomy, variable drainage patterns and proximity of the injection site to the lymph node basins that may mask nearby nodes. To this end, SPECT-CT has considerably refined planar lymphoscintigraphy by improving sentinel node detection rates, the retrieval of positive nodes and guiding the surgeon in planning surgical incisions, particularly in the supraclavicular and periparotid regions.[6,16,17] However, in our study over one in three (38%) nodes that were biopsied and three out of the 12 metastatic nodes were not identified on preoperative SPECT-CT. This may be due to the rapid diffusion of radiocolloid tracer to non-sentinel nodes in the head and neck, which was identified as a principal concern leading to a 20% false negative with lymphoscintigraphy in a systematic review by de Rosa et al.[18]. By contrast, mapping using near-infrared camera/ICG allows real-time transcutaneous tracing of lymphatic drainage from the primary melanoma site to the sentinel node basins, which ensures the accurate placement of incisions prior to tracer diffusion.

The authors noted a learning curve in using standalone ICG as demonstrated by the improving false positive rate between the first and last ten ICG-only SLNBs, which echoes the experiences of the first papers published on dual modality radiocolloid/blue dye technique. This is exemplified by analysis of the MSLT-1 trial, which showed that in high volume units the first 25 cases had a false negative – i.e. nodal recurrence on long term follow up – rate of 10%, while the second 25 cases had a false negative rate of 5.2%.[4] Our experience of performing 29 pre-planned and, due to two cases of radiocolloid failure in group B, 31 overall ICG-alone SLNBs, has been instrumental in establishing a consistent technique across patients (Figure 1).

This study's limitations include its single-centre setting, relatively small sample size to calculate high-powered statistics and the lack of long-term follow up to establish true false negative and sensitivity rates based on nodal recurrence. The standardised ICG-only technique developed in this study for the head and neck lymph node basins may not easily translate to truncal and limb melanomas, as the latter may be more difficult to trace transcutaneously in higher BMI patients ($> 30\,kg/m^2$) and may require longer intraoperative mapping time due to longer lymphatic drainage distances.[19] With longer follow-up and larger study size, these results may form the basis of future diagnostic non-inferiority studies for ICG-only and prompt technique-based studies in truncal and limb melanomas.

The benefits of standalone ICG are numerous. The properties of ICG mean that it can be visualised transcutaneously in real time up to a depth of 0.5 to 1.0 cm for up to 10 h, without the use of radiation or staining tissues not tattoo tissues, and has a lower risk of severe anaphylaxis (0.05%) compared with patent blue (0.3%) and isosulfan blue (1.1%).[20]

As an intraoperative surgeon-led mapping technique, standalone ICG may be the only the option of performing SLNBs globally during periods of radioactive tracer shortage; in hospitals without a nuclear medicine department; or where the cost of lymphoscintigraphy is prohibitive.

Conclusion

Our study uniquely evaluates ICG as a standalone technique in sentinel lymph node biopsy of cutaneous melanomas of the head and neck. It demonstrates that ICG-alone is feasible with comparable detection of sentinel and metastatic nodes, without the need for preoperative SPECT-CT. Longer follow up and a larger cohort of ICG-only SLNBs would allow definitive comment on the true false negative rates based on nodal recurrence.

Ethical approval

This study has been registered with the NHS Trust research department. In accordance with our institution's policies, formal ethical approval was not required.

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Conflict of interest

AHM has performed paid consultancy for Stryker. All remaining authors have declared no conflicts of interest and no funding or payment was received for this study.

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