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ORIGINAL ARTICLE – MELANOMA

Indocyanine Green Fluorescence Imaging with Lymphoscintigraphy for Sentinel Node Biopsy in Melanoma: Increasing the Sentinel Lymph Node-Positive Rate

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ABSTRACT

Introduction. The goal of this study was to analyze patients who underwent a sentinel lymph node biopsy (SLNB) in melanoma with the combination of radioisotope lymphoscintigraphy and indocyanine green (ICG) fluorescence imaging to compare our true positive (TP) rate, a means to perform immediate analysis of the SLNB, with that of the literature.

Methods. Consecutive cutaneous melanoma patients who underwent SLNB with lymphoscintigraphy and ICG-based fluorescence imaging by the senior author (BG) from 2012 to 2018 were prospectively enrolled. The average expected SLN-positive rate per *T* stage was calculated based on three studies and compared with our SLN-positive rate.

Results. Overall, 574 consecutive patients were analyzed. Average Breslow thickness was 1.9 mm. A total of 1754 sentinel nodes were sampled; 1497 were identified by gamma probe signaling and ICG, 241 were identified by gamma probe signaling only, and 16 were identified by ICG only. There were 123 (21.4%) patients with at least one positive SLN; 113 (91.9%) had at least one positive node identified with both gamma probe signaling and ICG, 8 (6.5%) had positive node(s) identified with gamma probe signaling only, and 2 (1.6%) had positive node(s) identified with ICG only. There was an overall 21.4% SLN-positive rate, with 8% T1, 18.5% T2, 41.1% T3, and 52.4% T4, which is higher than the predicted rates for each stage.

Conclusions. With the largest cohort of patients reported who underwent a melanoma SLNB with lymphoscintigraphy and ICG, we demonstrated that this technique results in higher SLN-positive rates than predicted. Patients are being followed but, given the TP data, knowledge of our results may foster the use of this modality to improve staging and treatment options.

In the management of cutaneous melanoma, a sentinel lymph node (SLN) biopsy (SLNB) permits highly accurate sampling of the lymph node basin with relatively limited morbidity. SLN status determines melanoma stage and is the best predictor of disease-free survival for melanoma. As such, information yielded from this procedure is indispensable for patient management decisions, including determination of adjuvant therapy eligibility.[1–5] To augment SLNB accuracy, various dyes and tracers can be utilized independently or in concert with one another to allow for SLN identification while minimizing disruption to the nearby lymphatics. Indocyanine green (ICG) fluorescence imaging has recently been utilized to aid SLNB in numerous cancer types, including melanoma; however, prospective studies with large patient populations are lacking.[6–13]

The surgical accuracy of SNLB can be assessed through the false negative rate (FNR), which compares the number of patients with false negative (FN) SLNs (FN, eventually become positive) with all patients with positive SLNs (true positives [TPs] and FNs); as such, FNR = FN/TP +

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FN.[14–20] Most melanoma studies do not report SLNB-positive rates by primary tumor pT stage; however, such data, pooled from numerous large cohort studies, can be extrapolated to predict the SLNB positivity rates for each pT stage. If a potential FN patient is converted to a TP patient, this should result in a lower FNR. While the surgical quality of the SNLB can be assessed through the FNR, a relatively lengthy follow-up time is required in this calculation, the time for FN patients to manifest with a clinically positive lymph node. However, immediate and pertinent analysis of surgical and pathological quality can be performed by examining the TP incidence of a cohort of patients with comparison to the predicted TP incidence.

Head and neck melanoma has historically been associated with high FNRs.[4,10,11] From an anatomical standpoint, there are more than 350 lymph nodes in the head and neck.[21] Studies have reported unpredictable and discordant lymphatic drainage in 27–84% of cases,[22–32] and lymphatic drainage can be bilateral and to multiple nodal levels.[22,33–36] Thus, if a cohort of melanoma patients had a high percentage of head and neck patients, due to their high FNR, it would be expected that the overall SLN-positive rate of the cohort should be lower.

The goal of this study was to analyze the largest reported cohort of patients undergoing an SLNB with radioisotope lymphoscintigraphy and ICG fluorescence. We have compared our SLN-positive rate utilizing this approach with the SLN-positive rate reported in the literature. We also performed subgroup analysis for head and neck melanoma patients due to this population's unique difficulty with SLNB. In this study, we demonstrate, for the first time, a higher than expected TP incidence with our SLNB technique, suggesting superior efficacy and reduced FNR.

METHODS

Study Design

After Institutional Review Board approval, consecutive primary cutaneous melanoma patients who underwent radioisotope lymphoscintigraphy (technetium-99 sulfur colloid) and ICG-based fluorescence imaging by the senior author (BG) from 2012 to 2018 were prospectively enrolled and included for analysis. Patients were selected for SLNB based on the guidelines established by the National Comprehensive Cancer Network (NCCN). Patients with a Breslow depth of < 1 mm were offered an SLNB due to mitosis, ulceration, or a positive deep margin on biopsy.

Variables analyzed included age, sex, Breslow thickness, mitotic index/mm^2, ulceration, tumor subtype, and location. The biopsy report and surgical pathology were reviewed and in cases of discordance, the higher grade of information was recorded. In cases where the original biopsy report was not present, the surgical report was used. Cutaneous melanoma staging was assigned based on the 8th edition of the American Joint Committee on Cancer (AJCC) staging system. Intraoperative results included SLN localization, number of SLNs harvested, and means of SLN identification.

Surgical Approach

All patients underwent preoperative lymphoscintigraphy. Filtered technetium-99 m sulfur radiocolloid (325-582uCi) was injected in two to four aliquots around the primary tumor site, and static and flow images were obtained. Patients also underwent single-photon emission computed tomographic/computed tomographics or standard lymphoscintigraphy to identify the affected regions. Accumulation of radiopharmaceutical foci identified by preoperative lymphoscintigraphy or single-photon emission computed tomographic/computed tomographics were marked. Intraoperatively, 0.1–0.3 mL (0.1 mL for head and neck, 0.2–0.3 mL elsewhere) of fluorescent ICG (Diagnostic Green, Farmington Hills, MI, USA) was injected intradermally at the primary tumor site. To dilute the ICG, 10 mL of sterile water was added to 25 mg of ICG. Thus, between 0.25 and 0.75 mg was injected per patient as we found this dose to demonstrate SLNs without resulting in background signaling.

The skin incision was made at the most radioactive site, as indicated by a handheld gamma probe (Neoprobe; Devicor Medical Products, Cincinnati, OH, USA), which quantifies the amount of radioactivity emitted by a lymph node after radiocolloid injection and/or ICG fluorescence. Low signal intensity makes incision placement and dissection design more challenging. In these instances, we observed that ICG fluorescence was critical to guide dissection and to appropriately identify the nodes in vivo. The study utilized SPY (Novadaq, San Jose, CA, USA), PDE (Mitaka, Denver, CO, USA), and Quest (Quest Medical Imaging, Akron, OH, USA) imaging systems as we identified advantages with improved technology. Although radioactivity aided in detecting the nodal basin, ICG fluorescence aided in identifying the actual lymph nodes. All lymph node basins emitting measurable radioactivity by handheld gamma probe signaling were examined. All lymph nodes detected by radioactivity (referred to in this article as 'gamma probe signaling') or ICG-based fluorescence were harvested as SLNs. Nodes that were within 10% of the most radioactive-emitting node were harvested regardless of ICG visibility. Fluorescence imaging was performed to visualize the nodes both in vivo and ex vivo, and gamma probe signaling counts were determined based

on signaling ex vivo once extirpated (electronic supplementary Video 1)

Determination of Expected Sentinel Lymph Node-Positive Rate

Three large studies were selected to determine the average expected SLN-positive rate after SLNB in melanoma for all body sites (Table 1).[16,37,38] The number of head and neck patients in each cohort was also recorded. Based on the pT stage and average SLNB positivity rate, the number of patients predicted to have a positive SLNB in our cohort was then calculated. To determine the expected SLN-positive rate by *T* stage, the positivity rate for each study by *T* stage was weighted and averaged with the other three studies.

RESULTS

The clinical features of our cohort are demonstrated in Table 2. Overall, 574 consecutive patients were analyzed. The average age was 61 years, with a range of 17–95 years, and there were 322 males and 252 females. Anatomic locations were distributed as follows: 126 head and neck, 160 trunk, 171 upper extremity, and 117 lower extremity melanomas. Histopathologic attributes of primary melanoma biopsies/excisions are depicted in Table 3. For some melanomas, there was more than one histologic subtype present, and, in these instances, all were recorded. The average Breslow thickness was 1.9 mm, with a range of 0.2–19 mm. There were 237 patients with a T1 melanoma, 178 with a T2 melanoma, 96 with a T3 melanoma, 62 with a T4 melanoma, and one patient with a Tx melanoma.

Of the 137 patients who had a T1a melanoma based on biopsy and were offered an SLNB, 55/137 (40%) biopsies showed > 0 mitoses/mm^2 and 80/137 (58.4%) had a positive deep margin. In some instances, the biopsy

TABLE 2 Patient demographics

All patients	574
Age, years [mean (min, max)]	61 (17, 95)
Sex	
Male	322 (56.1)
Female	252 (43.9)
Location of primary	
Head and neck	126 (22)
Trunk	160 (27.9)
Upper extremity	171 (29.8)
Lower extremity	117 (20.4)
Laterality	
Right	273 (47.6)
Left	265 (46.2)
Bilateral/midline	36 (6.3)

Data are expressed as *n* (%) unless otherwise specified

min minimum, *max* maximum

TABLE 1 Studies utilized to calculate the expected true positive rate of SLNB

	White et al.[37]	Testori et al.[16]	Chang et al.[38]	Weighted average
Total patients	3445	1270	3460	8175
T stage (based on Breslow)				
1 (< 1 mm)	735	358	Not included	1093
Positive SLNB	45 (6.1)	4 (1.1)		49 (4.5)
Negative SLNB	690 (93.9)	354 (98.9)		1044 (95.5)
2 (1.01–2 mm)	1421	482	1967	2449
Positive SLNB	166 (11.7)	63 (13.1)	241 (12.3)	304 (12.4)
Negative SLNB	1255 (88.3)	419 (86.9)	1726 (87.8)	2145 (87.6)
3 (2.01–4 mm)	845	301	1493	1794
Positive SLNB	214 (25.3)	95 (31.6)	343 (23)	438 (24.4)
Negative SLNB	631 (74.7)	206 (68.4)	1150 (77)	1356 (75.6)
4 (> 4 mm)	376	129	Not included	505
Positive SLNB	123 (32.7)	54 (41.9)		177 (35)
Negative SLNB	253 (67.3)	75 (58.1)		328 (65)
Head and neck patients	941 (27.3)	90 (7.1)	643 (18.6)	
Positive SLNB	111 (11.8)	16 (17.8)	76 (11.8)	
Negative SLNB	830 (88.1)	74 (82.2)	567 (88.1)	

Data are expressed as *n* (%)

SLNB sentinel lymph node biopsy

TABLE 3 Melanoma histopathologic features

Type of melanoma	
Superficial spreading	303
Lentigo maligna melanoma	21
Nodular	143
Nevoid	32
Spitzoid	17
Acral	18
Desmoplastic	10
Unknown/NOS/other	53
Breslow, mm [mean (min, max)]	1.9 (0.2, 19)
Ulceration	
Yes	140 (24.4)
No	432 (75.6)
Mitotic rate	
0	170 (29.6)
1–10	361 (62.9)
> 10	41 (7.1)
Unknown/other	2 (0.3)
pT stage	
1	237 (41.2)
2	178 (31)
3	96 (16.7)
4	62 (10.8)
X	1 (0.1)
Stage	
1a	216 (37.8)
1b	117 (20.3)
2a	59 (10.3)
2b	36 (6.3)
2c	19 (3.3)
3a	43 (7.5)
3b	27 (4.7)
3c	56 (9.8)
3d	1 (0.1)
T1a patients	
> 0 mitosis	55/137 (40.1)
Deep margin	80/137 (58.4)
None of the above	8/137 (5.8)
Change to higher than T1a after excision	5/137 (3.6)

Data are expressed as *n* (%) or *n/N* (%) unless otherwise specified

NOS not otherwise specified, *min* minimum, *max* maximum

demonstrated both mitoses and a positive deep margin. There were 8 (5.8%) patients with a T1a melanoma on biopsy who did not have any of the aforementioned biopsy characteristics. Of the entire cohort of 137 patients who had a T1a melanoma based on biopsy, 5 (3.6%) were upstaged to a higher pT stage after the wide excision and SLNB procedure (Table 3). Among 574 consecutive patients, a total of 1754 sentinel nodes were sampled. 1497/1754 (85.3%) SLNs were identified by gamma probe signaling/ICG, 241 (14%) by gamma probe signaling only, and 16 (0.9%) by ICG only. Of these 1497 SLNs identified by gamma probe signaling/ICG, 13 (0.8%) were identified ex vivo and were < 10% of the highest gamma probe signaling reading (Table 4). Of the 574 patients who underwent SLNB, there were 123 (21.4%) patients with at least one positive SLN. For these 123 patients, 113 (91.9%) had at least one positive node identified with both gamma probe signaling and ICG. However, 8/123 patients (6.5%) had positive node(s) identified with gamma probe signaling only, and 2/123 (1.6%) had positive node(s) identified with ICG only (Table 4). In the 123 patients with at least one positive SLN, there were 159 positive nodes (9.1% of all nodes). 145/159 lymph nodes (91.1%) were found by gamma probe signaling/ICG, 12 (7.5%) by gamma probe signaling only, 2 (1.3%) by ICG only, and 1 (0.6%) by ICG and gamma probe signaling ex vivo < 10% of the highest gamma probe signaling reading. For the two nodes that were identified by ICG only, and the 12 nodes in eight patients that were identified by gamma probe signaling only, those were the only positive nodes in these patients.

In all surgeries, the gamma probe signaling reading of each SLN was recorded to permit comparison with the pathologic results. In the 123 patients with at least one positive lymph node, 64 had more than one lymph node identified with gamma probe signaling. When comparing the gamma probe signaling reading for the 64 TP patients with more than one node identified by gamma probe signaling, in 39 (60.9%) patients the node with the highest gamma probe signaling reading was the positive node, in 24 (37.5%) patients the node with the highest gamma probe signaling reading was not the positive node, and in one (1.6%) patient the positive and negative nodes had equal gamma probe signaling readings (Table 4). The amount of metastatic melanoma was quantified in 93 patients. The average deposit was 1.8 mm, with a range of 0.001–18.2 mm. For the remaining patients, the amount of metastatic melanoma was subjectively reported and ranged from ‘single cell’ to ‘multiple foci’ (Table 4). Of the 123 TP patients, there were 28 head and neck melanomas, 35 trunk melanomas, 27 upper extremity melanomas, and 33 lower extremity melanomas. Overall, by pT stage, there was an SLNB positivity rate of 8%, 18.5%, 41.1%, and 52.5% for *T* stages 1–4, respectively. TP patient and melanoma characteristics are reported in Table 5.

There were 126 (21.9%) patients with a melanoma of the head and neck. The average Breslow thickness of this cohort was 2.3 mm, with a range of 0.27–19 mm. There were 46 T1 patients, 43 T2 patients, 18 T3 patients, and 19 T4 patients. Complete patient and melanoma

TABLE 4 Sentinel lymph node data

No. of sentinel lymph nodes	1754
Found with gamma and ICG	1497 (85.3)
Found with gamma < 10% hottest node and ICG	13 (7.4)
Found with gamma	241 (14)
Found with ICG	16 (0.9)
Found with ICG only or gamma < 10% hottest node and ICG	29 (16.5)
Positive node detection per patient	123 patients (24.1% overall patients)
At least one positive node found with gamma and ICG	113 (91.9)
Positive nodes found with gamma only	8 (6.5)
Positive nodes found with ICG only	2 (1.6)
No. of positive nodes	159 (9.1% of all nodes)
Found with gamma and ICG	145 (91.1)
Found with gamma < 10% hottest node and ICG	1 (0.6)
Found with gamma	12 (7.5)
Found with ICG	2 (1.3)
Found with ICG only or gamma < 10% hottest node and ICG	3 (1.9)
Was the positive node the hottest node for patients with more than one node identified with gamma?	64 patients
Yes	39 (60.9)
No	24 (37.5)
Equal	1 (1.6)
Metastatic melanoma in the node	
Measured [mean (min, max)]	93 patients [1.8 (0.001, 18.2) mm]
Few/foci/isolated/rare/scattered	16
Single cell/cluster	7
Micrometastasis	4
Multiple foci	3

Data are expressed as *n* (%) unless otherwise specified

ICG indocyanine green

characteristics for this cohort are reported in Table 6. Of the 126 head and neck patients, there were 28 TP patients (22%). TP patient and melanoma characteristics are reported in Table 7.

The expected number of SLNB-positive patients, and specifically head and neck patients, for each *T* stage in our cohort, based on rates previously published, are reported in Table 8. The expected TP SLNB rates based on the literature were 4.5%, 12.4%, 24.4%, and 35% for pT1–4, respectively. Using these expected SLN-positive rates, for our patient cohort it was predicted that there would be 11, 22, 23, and 22 patients in the pT1–4 cohorts. This was then compared with our actual number of TP patients. For all patients, there was an overall 21.4% SLN-positive rate, with 8% T1, 18.5% T2, 41.1% T3, and 52.4% T4. This gave a range of increased percentage of TP patients over expected ranging from 49.2 to 77.8% for each stage. For the head and neck population, there was an overall 22% SLN-positive rate in this cohort, with 10.9% T1, 16.3% T2, 87.5% T3, and 47.4% T4. This gave a range of increase in the number of TP patients over expected ranging from 23.9 to 258.6% for each stage.

DISCUSSION

For cutaneous melanoma, SLN status has been shown to be the best predictor of disease-free survival,[1–5] and identifying the correct sentinel node is crucial to ensuring appropriate adjuvant work-up and treatment. Having at least one positive SLN increases melanoma stage from Ia or Ib to IIIa or higher. This increase in stage prompts more strict surveillance, allows for potential adjuvant therapy or clinical trial eligibility, and initiates a complete metastatic work-up that has the potential to reveal occult stage IV disease. Indeed, while the majority of melanoma-related deaths occur in patients with stage I and II disease,[39] this is the same population of patients most at risk for understaging and most lacking in options for adjuvant treatment.

TABLE 5 Patient and melanoma characteristics for patients with a positive SLNB

No. of true positive patients (% of the total cohort)	123 (21.4)
Age, years [mean (min, max)]	60.9 (21, 95)
Sex (% of the TP cohort)	
Male	72 (58.5)
Female	51 (41.5)
Location of the primary (% of the TP cohort, % of that location cohort)	
Head and neck	28 (22.8, 22.2)
Trunk	35 (28.5, 21.9)
Upper extremity	27 (22, 15.8)
Lower extremity	33 (26.7, 28.2)
Laterality (% of the TP cohort)	
Right	61 (49.6)
Left	49 (39.8)
Bilateral/midline	13 (10.6)
Type of melanoma (% of the original cohort)	
Superficial spreading	40 (13.3)
Lentigo maligna melanoma	3 (14.3)
Nodular	59 (41.2)
Nevoid	4 (12.5)
Spitzoid	5 (29.4)
Acral	6 (33.3)
Unknown/NOS/other	14 (26.4)
Breslow thickness, mm [mean (min, max)]	3.4 (0.33, 19)
Ulceration (% of the TP cohort)	
Yes	64 (52)
No	59 (48)
Mitotic rate (% of the TP cohort)	
0	16 (13)
1–10	84 (68.3)
> 10	21 (17)
Unknown/other	2 (1.6)
T stage based on biopsy (% of the TP cohort)	
T1a	8 (6.5)
T1b	14 (11.3)
T2a	26 (21.1)
T2b	11 (8.9)
T3a	14 (11.4)
T3b	26 (21.1)
T4a	7 (5.1)
T4b	16 (13)
Unknown	1 (0.8)
Final *T* stage (no. of patients, % of the TP cohort, % of that total cohort)	
T1	19 (15.4, 8)
T2	33 (26.8, 18.5)
T3	39 (31.7, 40.6)
T4	32 (26, 51.6)
pTNM (% of the TP cohort)	
T1aN1a	3 (2.4)
T1aN2a	2 (1.6)
T1bN1a	9 (7.3)
T1bN2a	3 (2.4)
T2aN1a	21 (17)
T2aN2a	6 (4.9)
T2bN1a	7 (5.7)
T2bN2a	1 (0.8)
T3aN1a	11 (8.9)
T3aN2a	3 (2.4)
T3bN1a	17 (13.8)
T3bN2a	5 (4.1)
T3bN3a	3 (2.4)
T4aN1a	6 (4.9)
T4aN2a	1 (0.8)
T4bN1a	16 (13)
T4bN2a	7 (5.7)
T4bN3a	2 (1.6)
Stage (% of the total cohort)	
3a	41 (33.3)
3b	27 (22)
3c	54 (43.9)
3d	1 (0.8)

SLNB sentinel lymph node biopsy, *TP* true positive, *min* minimum, *max* maximum, *NOS* not otherwise specified

After undergoing an SLNB, with appropriate length of follow-up, patients can be characterized into one of three categories. TP, meaning an SLN with disease was found at the time of biopsy, true negative (TN), meaning the SLN was free of disease and the patient did not recur in the basin, or FN, meaning that the SLN was free of disease but the patient recurred in the nodal basin, with or without a local or in-transient recurrence. Presumably, FN patients should have been TP patients at the time of biopsy. Incorrect classification of such patients could be due to the correct SLN not being selected, disease not identified on pathology, or quiescent disease such as dermal lymphatic disease with delayed spread to the lymph node.[40] If a potential FN patient is converted to a TP, this should yield a low FNR and high TP incidence. Thus, the surgical quality of the SNLB can be assessed through the FNR as it is essentially a comparison of the TP and FN rates. However, in order to calculate the FNR, adequate follow-up is required, typically at least 2 years, the mean time to recurrence for melanoma.[41,42] Immediate and pertinent

TABLE 6 Head and neck melanoma and patient characteristics

All patients	126
Age, years [mean (min, max)]	64.4 (18, 95)
Sex	
Male	99 (78.6)
Female	27 (21.4)
Laterality	
Right	65 (51.2)
Left	49 (38.9)
Bilateral/midline	12 (9.5)
Type of melanoma	
Superficial spreading	53
Lentigo maligna melanoma	13
Nodular	31
Nevoid	11
Spitzoid	0
Acral	0
Desmoplastic	4
Unknown/NOS/other	8
Breslow, mm [mean (min, max)]	2.33 (0.27, 19)
Ulceration	
Yes	29 (23)
No	97 (77)
Mitotic rate	
0	31 (24.6)
1–10	78 (61.9)
> 10	11 (8.7)
Unknown/other	6 (4.8)
T stage	
1	46 (36.5)
2	43 (34.1)
3	18 (14.3)
4	19 (15.1)
Stage	
1a	39 (31)
1b	34 (27)
2a	7 (5.6)
2b	13 (10.3)
2c	5 (4)
3a	9 (7.1)
3b	8 (6.3)
3c	11 (8.7)

Data are expressed as *n* (%) unless otherwise specified

min minimum, *max* maximum, *NOS* not otherwise specified

analysis can be performed by examining the TP incidence and following the same cohort of patients, to eventually calculate the FNR.

To extrapolate the predicted SLN-positive rate of our patient cohort, we employed three large studies, each with thousands of patients, where the correlation between *T* stage and SLNB positivity rate, a data point not commonly reported, was delineated.[16,37,38] This information was utilized to predict the expected number of TP patients in our cohort. In our cohort of 574 patients, it was extrapolated that there should be 77 TP patients. However, 123 patients (21.4%) in our cohort had a positive SLNB, with a range of SLNB positivity by *T* stage of 8–52.4%, increasing with increasing *T* stage. Compared with the expected rate of TP patients, there was a range of 49.7–77.8% increase per *T* stage with our SLNB technique. Although our mean Breslow depth was 2 mm, by stratifying our results by pT stage we can ensure that our high TP is not merely due to a high-risk melanoma cohort. Thus, this suggests that with our technique utilizing two means of SLNB localization, there may be improved efficacy.

Prior results from our institution, as well as from others, have demonstrated the utility that ICG-based technology can have in identifying SLNs in cutaneous and conjunctival melanoma, as well as Merkel cell carcinoma (MCC).[8–11,43] Studies performed at our institution have demonstrated that ICG in combination with gamma probe signaling is more sensitive and specific than blue dye with gamma probe signaling, and results in a lower FNR than previously published, even in patients with head and neck melanoma.[9–11] Another prospective study with 80 patients analyzed the feasibility and benefit of utilizing ICG fluorescence in combination with gamma probe signaling for melanoma SLNB. They demonstrated that visualization of the SLN with fluorescence was successful in only 21% of patients prior to incision, but after skin incision it was successful in 96% of nodes. The SNLB positivity rate in this study was 18% and all nodes were detected by both modalities. However, this study did not perform subanalysis by pT stage.[12] Another prospective study of 87 patients utilized triple detection with fluorescence, radioisotope lymphoscintigraphy and blue dye for melanoma SNLB. Similar to our previous study,[9] these researchers found that while ICG/fluorescence could not detect the nodal basin preoperatively, intraoperatively it was equivalent to gamma probe signaling and superior to blue dye.[13] In this study, 91.9% of patients had at least one SN sampled that was identified by both gamma probe signaling and ICG, 6.5% by gamma probe signaling only, and 1.6% by ICG only. However, two positive nodes were found by ICG only, 12 were found with gamma probe signaling only, and one was found with ICG and gamma probe signaling ex vivo < 10% of the highest gamma probe signaling reading, the cut-off typically employed. For the two patients with a positive SLN identified by ICG only, this was their only pathologically positive SN, and for eight patients with a positive SLN identified by gamma probe signaling only, this was their only positive SN. Thus, ICG may be able to identify nodes that would have been

TABLE 7 True positive head and neck melanoma and patient characteristics with percentages, compared with the overall head and neck cohort

All patients	28 (22% of head and neck patients)
Age, years [mean (min, max)]	62 (21, 95)
Laterality	
Right	14 (50)
Left	10 (35.7)
Bilateral/midline	4 (14.3)
Type of melanoma	
Superficial spreading	9
Lentigo	2
Nodular	10
Nevoid	1
Spitzoid	0
Acral	0
Desmoplastic	1
Unknown/NOS/other	6
Breslow, mm [mean (min, max)]	4.4 (0.5, 19)
Ulceration	
Yes	19 (67.9)
No	9 (32.1)
Mitotic rate	
0	4 (14.3)
1–10	21 (75)
> 10	3 (10.7)
Unknown/other	
T stage	
1	5 (17.9)
2	7 (25)
3	7 (25)
4	9 (32.1)
Stage	
3a	9 (32.1)
3b	8 (28.6)
3c	11 (39.3)
Positivity rate by *T* stage compared with the overall head and neck cohort	
1	5/46 (10.9)
2	7/43 (16.3)
3	7/8 (87.5)
4	9/19 (47.4)

Data are expressed as *n* (%) or *n/N* (%) unless otherwise specified

min minimum, *max* maximum

otherwise missed by gamma probe signaling, and vice versa. The data suggest the importance of utilizing two modalities to augment SLN identification; missing the correct positive node would have prevented appropriate staging and management of the implicated patients.

While gamma probe signaling was critical in identifying SLNs, and ICG only detected a minority of nodes independently, the utility of ICG extends beyond this objective measure. ICG allowed us to locate nodes more easily than if gamma probe signaling or blue dye were utilized independently or in concert with one another. Especially in the head and neck region, or in individuals with large amounts of subcutaneous fat, ICG allowed us to visualize lymphatics and nodes that may have otherwise been missed. Thus, while gamma probe signaling assisted in locating the general region of the SLN, ICG assisted with precise localization and extirpation without damaging nearby lymphatic channels. With the software that now allows for heat gradient mapping with ICG, we can trace lymphatic channels to deeper nodes which can then be confirmed with gamma probe signaling. While these nodes would be classified as ICG/gamma probe signaling positive, ICG was critical in these instances. Due to the ease of lymphatic channel visualization with ICG, nodal extirpation is precise and minimizes damage to nearby lymphatic channels to limit lymphatic compromise. As we are a teaching institution, using ICG also enables teaching of residents due to its superior visualization of both the nodes and lymphatics; however, these factors are impossible to objectively quantify.

With the publication of MSLT-II,[44] there has been a transition away from performing complete lymph node dissections (CLNDs) in response to a positive SLNB. However, AJCC version 8 staging is based on the number of lymph nodes involved by tumor: 0 (N0), 1 (N1a), 2–3 (N2a) and 4 + (N3a). As such, if a patient undergoes an SLNB and has less than two positive nodes and does not undergo a CLND, he/she may be understaged. Understaging will negatively impact adjuvant therapy options and postoperative surveillance. As an example, a patient with a T1b melanoma and one positive SLN would be stage IIIa, where the role of adjuvant therapy is controversial. However, a patient with T1b melanoma and four positive SLNs would be stage IIIc, where the role of adjuvant therapy is accepted. In our experience, utilizing ICG allows the selection of lymph nodes without disturbing local lymphatics due to its real-time depiction of lymph nodes and lymphatic drainage. Thus, more than one SLN can be removed without concern for disrupting postoperative lymphatics.

The range of Breslow thickness for patients with a positive SLNB was 0.33–19 mm. Eight T1a patients and 14 T1b patients had a positive SLNB; however, as we restaged all patients based on AJCC version 8, some of these patients would have been stage T1b, not T1a, or vice versa, at the time of wide local excision and SLNB. Stage T1 patients in our cohort, with an SLNB positivity rate of 8%, had the largest percentage increase (77.8%) of SLN-

TABLE 8 Expected true positive rate of SLNB applied to our data

T stage	No. of patients in our cohort	Predicted true positive SLNB rate, based on the literature (%)	Predicted no. of true positive patients in our cohort	Actual no. of true positive patients in our cohort [*n* (%)]	Percentage increase over expected in our cohort compared with the literature
1	237	4.5	10.7	19 (8)	77.8
2	178	12.4	22.1	33 (18.5)	49.2
3	96	24.4	23.4	39 (41.1)	68.4
4	62	35	21.7	32 (52.4)	49.7
			77.4	123 (21.4)	
Head and neck only					
1	46	4.5	2.1	5/46 (10.9)	128
2	43	12.4	5.3	7/43 (16.3)	23.9
3	8	24.4	2	7/8 (87.5)	258.6
4	19	35	6.7	9/19 (47.4)	35.4
				28 (22)	

SLNB sentinel lymph node biopsy

positive rate compared with the expected SLN-positive rate based on the literature. SLNBs are typically universally recommended for patients with a Breslow depth of 1 mm, and thus at least a T2a melanoma. The T1a and T1b cohorts are patient populations in which the role for SLNBs has been controversial. SLNB may be offered based on mitoses, ulceration, or positive deep margins, as mitotic rate and ulceration have been identified as independent risk factors for SLN positivity by both our group and others.[45–48] In this cohort, patients with a depth of < 1 mm were offered an SLNB due to mitosis, ulceration, or a positive deep margin on biopsy, and SLNB was proceeded with based on patient preference. Based on our previously reported finding that the presence of mitoses in thin melanoma increases SLNB positivity,[48] as well as the data observed in the current study, at our institution an SLNB is offered to patients with dermal mitoses or ulceration, regardless of Breslow thickness. In this patient population, careful counseling is performed to communicate that while SLNB in these very thin melanomas is not part of the NCCN guidelines, the node may demonstrate disease based on the melanoma pathological features.

The SLN-positive rate for SLNB in our head and neck cohort was 22%. The average Breslow thickness of head and neck melanoma patients with a positive node was 4.4 mm, but patients in this cohort with a Breslow thickness as thin as 0.5 mm had positive SLNB. When compared with the predicted number of TP patients based on literature for all sites, the percentage increase over the expected number of TPs ranged from 23.9 to 258.6%. Our data can also be compared with a large cohort study of 7266 head and neck melanoma patients where the SLNB positivity rate for T1 was 2.6%, T2/3 was 7.1%, and T4 was 11%, which was lower than our cohort for each stage.[49] Our cohort included a large subset of head and neck melanoma patients (21.9%) that was greater than two of three studies on which the predicted SLN-positive rate was modeled (7.1% and 18.6%)[16,38] and that was comparable with the third study (27.3%).[37] As this subgroup of patients is known to have a high FNR,[10,11] it would be expected that our high proportion of head and neck patients should drive our overall SLN-positive rate lower. However, despite this substantial patient cohort, our overall SLN-positive rate of 22% was still higher than expected, giving further support to the efficacy of our technique. Future studies will determine and report the effect our dual SLN detection technique has on the FNR, after sufficient time has passed.

Limitations

Our study has certain limitations. Although the study prospectively enrolled patients, it was a single-institution study. Furthermore, as the same personnel did not necessarily perform the nuclear medicine studies and pathological analyses for all patients and specimens, a user-dependent bias may have been introduced. ICG-based fluorescence imaging can aid in the identification of SLNs, however this application is a novel and off-label approach still being investigated for efficacy and efficiency.

CONCLUSIONS

Our group has previously demonstrated, in a much smaller cohort study, that the utilization of ICG in SLNB results in a low FNR for patients with melanoma of all body sites. We now demonstrate, with the largest cohort of patients reported who underwent a melanoma SLNB with

ICG-based technology, that this technique results in higher SLN-positive rates than previously predicted, theoretically reducing the future FNR. These patients reflect our learning curve cases and technology changes, but our results were still positive. Given the large cohort, we are prospectively following these patients and will report our FNR findings in the future when we have at least a 2-year average follow-up. We will also analyze local and distant disease-free survival to determine if selecting the proper SLN has a survival benefit. However, given the TP data, we are compelled to report our results early in order to foster the use of a modality that may improve staging and treatment options for select patients.

AUTHOR CONTRIBUTIONS Study design was undertaken by BG. All authors contributed to data collection and analysis, and writing of the manuscript.

CONFLICTS OF INTEREST Brian Gastman is a consultant for Quest Diagnostics.

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