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Original Article

Lymphatic flow is mostly preserved after sentinel lymph node biopsy in primary cutaneous malignant melanoma

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***Abbreviations*:** ICG, indocyanine green; NIR, near-infrared; PCMM, primary cutaneous malignant melanoma; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy.

ABSTRACT

Background: Knowledge of changes in lymphatic flow after sentinel lymph node biopsy (SLNB) is important for the development of strategies for postoperative adjuvant therapy in malignant melanoma.

Objectives: 41 patients (22 males and 19 females; average age: 67.0 ± 24.0 years) with primary cutaneous malignant melanoma (PCMM) participated in the present study. The primary tumor sites were the upper extremities (9 patients), the lower extremities (20 patients), the trunk (11 patients) and the scalp (1 patient). The tumor thicknesses of the PCMM lesions were from 0.5 mm to 9.0 mm (average: 3.3 ± 2.5 mm). All the participants underwent wide local excision and SLNB.

Methods: We studied lymphatic flow before and after SLNB by near-infrared (NIR) imaging in all 41 cases. In addition, we performed NIR imaging of lymphatic flow after the lymph node dissection in one case with sentinel lymph node (SLN) metastasis.

Results: Almost no changes in lymphatic flow were seen in 38 of the 41 patients (92.7%) after SLNB. Only in 3 patients (7.3%), one with SLN metastasis and the other two without SLN metastasis, was apparent alteration in the lymphatic flow observed after SLNB. Of the 16 patients without SLN metastasis, only 3 patients showed recurrence of the tumors. Interestingly, 1 of the 2 patients without SLN metastasis but with lymphatic flow alteration had recurrence (regional lymph node metastasis) of the melanoma, whereas only 2 of the 14 patients without SLN metastasis or lymphatic flow alteration had recurrence, 1 with regional lymph node metastasis and the other with distant lymph node metastasis. In 1 case, we re-examined the lymphatic flow after regional lymph node dissection and the lymphatic flow was found to be dramatically changed.

Conclusion: We clearly demonstrated that SLNB has only a minimal effect

on lymphatic flow. The present results suggest that SLNB does not increase the risk of local recurrence/in-transit metastasis and may support the efficacy of post-SLNB local adjuvant injection to prevent local recurrence and in-transit metastasis.

1. Introduction

Revealing the extent and patterns of changes in lymphatic flow after sentinel lymph node biopsy (SLNB) is essential for the development of strategies for postoperative adjuvant therapy of cutaneous malignant tumors, including primary cutaneous malignant melanoma (PCMM). It is apparent that SLNB results in less tissue damage than that for regional lymph node dissection [1]. However, information on lymphatic flow changes after SLNB has been limited.

Until recently, most imaging studies on human lymphatic flow have employed lymphoscintigraphy [2]. Studies by Celebioglu *et al.* [1] of lymph drainage in the arms after SLNB and after axillary lymph node dissection by lymphoscintigraphy revealed that lymph drainage was less affected by SLNB than by axillary lymph node dissection. However, lymphoscintigraphy is not sufficiently sensitive to demonstrate lymphatic flow alterations. Near-infrared (NIR) imaging methods have enabled us to investigate both the morphology of lymphatic vessels and lymphatic function [3]. In addition, it is possible to make repetitive imaging of the lymphatic system by NIR imaging, because NIR imaging is a low-invasive procedure with no risk from irradiation [4].

Here we studied the changes of lymphatic flow after SLNB by comparing the findings of pre- and post-SLNB NIR imaging of lymphatic flow in patients with PCMM. We clarified that lymphatic flow was not significantly affected by SLNB in most cases.

Local subcutaneous IFN-β injection around the surgical scar of the PCMM is widely used as postoperative adjuvant chemotherapy in Japan [5]. The

locally injected IFN-β is thought to be carried by the lymphatic flow tracing the lymph drainage from the primary lesion of PCMM, i.e., possible in-transit metastasis sites [6, 7]. If the lymphatic flow is altered significantly by SLNB, then the injected IFN-β cannot reach possible early metastatic lesions. In light of this, the present result is important because it indicates that locally injected IFN-β can be transported to possible in-transit metastasis sites by unaltered lymphatic flow.

2. Patients and Methods

2.1. The Patients

From June 2009 to July 2014, a total of 264 patients with PCMM were treated in the dermatological clinic of Nagoya University Hospital. For SLNB, patients with PCMM apparently <1.0 mm thick clinically were excluded. Patients with apparent regional lymph node involvement were excluded. Patients in whom apparent metastatic lesions were found by physical and imaging examinations were also excluded. Thus, SLNB was performed on 123 patients with PCMM. Of these, 41 patients (22 males and 19 females) agreed to participate in the present study after giving their fully informed consent. The participants were from 26 to 85 years of age (average: 67.0 ± 24.0 years of age). The sites of the primary lesions were the upper extremities (9 patients), the lower extremities (20 patients), the trunk (11 patients) and the scalp (1 patient). The tumor thicknesses of the primary lesions were 0.5 mm to 9.0 mm (average: 3.3 ± 2.5 mm).

The patient data (age, sex, sites of the primary lesions, tumor thickness, numbers of biopsied SLNs and SLNs with metastasis, lymphatic flow alterations, presence or absence of recurrence/metastasis and outcome (survival or decease) are summarized in Table 1. No patient had chemotherapy or immunotherapy prior to the operation.

All the participants underwent wide local excision of the primary lesions, and SLNB was performed subsequently or simultaneously using all three methods: visual dye, radioactive γ probe and NIR imaging. The NIR imaging method is described below. All the patients were locally injected with IFN-β as a postoperative adjuvant therapy.

This study was performed according to the principles expressed in *The Declaration of Helsinki* and the ethics policies of our institute, and it was approved by the Ethics Review Committee of the Nagoya University Graduate School of Medicine.

2.2. NIR imaging of lymphatic flow and SLN detection

The SLN detection methods are modified from those described elsewhere [8-12]. The agent for the NIR imaging was prepared as follows. Human serum albumin (5 mg), indocyanine green (ICG; Diano-green; Daichi Pharmaceutical, Tokyo, Japan)(0.6 mg) and Patent Blue (Wako Pure Chemical Industries, Ltd.) (6 mg) were dissolved in 1 mL of pure water. The ICG was visualized using a fluorescence imaging system (Photo Dynamic Eye; PDE, Hamamatsu Photonics, Japan) that includes a small charge-coupled-device camera with an integrated near-infrared (NIR) LED light source (energy: 4 mW; wavelength: 760 nm). An 820-nm band-path filter was employed to collect NIR radiation and to reject visible light. The fluorescence signals were sent to a digital video processor for display on a TV monitor. Videos were taken of the dye-injection process, the pulsing of collecting vessels and the overall lymphatic vessels.

2.3. Second-time NIR imaging of lymphatic flow after SLNB

The times between SLNB and the second NIR imaging were from 22 days to 786 days (average: 101.6 ± 137.3 days).

NIR imaging after SLNB was also performed using the same NIR imaging methods as those of the first-time NIR imaging before SLNB, described above.

2.4. Third-time NIR imaging of lymphatic flow after regional lymph node

dissection

In all 26 cases with SLN metastasis, regional lymph node dissection was performed. In one of these cases, (Patient 15), we repeated NIR imaging of lymphatic flow by the same methods used in the first SLNB after regional lymph node dissection.

2.5. Follow-up of the patients

All the patients were monitored postoperatively by means of clinical examinations, peripheral blood examinations and CT or PET/CT at least every 6 months. During the follow-up period, all the patients received postoperative adjuvant therapy of subcutaneous IFN-β injection around the surgical scar of the primary lesions, generally IFN-β (3×10^6 IU/body weight; Feron; Toray Industries, Inc.) local injection once a day for 10 consecutive days or once every week.

37 of the 41 patients (24 patients with SLN metastasis and 13 patients without SLN metastasis) were treated with postoperative chemotherapy of DAV regimen, as follows. Patients were administered DTIC (80–140 mg/m^2, 60-minute infusion once a day for 5 consecutive days), ACNU (50–100 mg/m^2, 30-minute infusion on day 1) and VCR (0.5–0.8 mg/m^2, 30-minute infusion on day 1) [5]. DAV therapy was done every 4 weeks in 3 cycles for stage II and in 5 cycles for stage III.

3. Results

3.1. SLN detection

In all 41 of the participating patients, SLNs were successfully detected by SLNB (from 1 to 6 SLNs; average: 2.6 ± 1.2 SLNs per case).

Histopathological observation revealed 25 of the 41 cases to show SLN metastasis (1-4 SLNs positive) and the other 16 patients to be negative for SLN metastasis (Table 1).

3.2. Lymphatic flow after SLNB

Of the 41 cases examined in the present study, almost no changes in lymphatic flow were seen in 38 patients (92.7%) after SLNB (Figs. 1 and 2). In only 3 patients (7.3%) was apparent alteration in the lymphatic flow observed after SLNB. In 1 of the 3 cases, SLN metastasis was positive; in the other 2 cases, SLN metastasis was not recognized. In all 3 cases, the lymphatic flow routes were altered by SLNB, although no lymphedema, congestion or backflow was seen in any case (Figs. 3 and 4). Considering the association of changes in lymphatic flow with SLN metastasis, 2 of the 16 patients (12.5%) without SLN metastasis showed altered lymphatic flow after SLNB. In contrast, 1 of the 25 patients (4.0 %) with SLN metastasis exhibited altered lymphatic flow after SLNB. The periods from the SLNBs to the second NIR imaging of the lymphatic flow were 181.3 ± 136.4 days in the cases with lymphatic flow alterations and 95.3 ± 133.5 days in the cases without lymphatic flow change.

3.3. Altered lymphatic flow after regional lymph node dissection

In 1 patient (Patient 15) who underwent regional lymph node dissection, we re-examined the lymphatic flow by NIR imaging after the regional lymph

node dissection. In this case, the PCMM lesion was on the right lower leg. At the first operation, total resection of the primary lesion was performed and 1 SLN was biopsied SLNB. Metastasis was confirmed histopathologically in the SLN, and afterward, right inguinal lymph node dissection was done. The lymphatic flow was dramatically changed after the regional lymph node dissection (Figure 5). The lymphatic flow was congested, leading to edema. The edema altered the lymphatic flow, resulting in “dermal backflow sign”.

3.4. Disease course during follow-up, patient outcomes

The mean follow-up period for all patients was 35.1 $\pm$ 20.0 months. 6 patients with SLN metastasis and 1 patient without SLN metastasis died from malignant melanoma. All the patients who showed changes in lymphatic flow after SLNB survived during the follow-up period.

Of the 16 cases without SLN metastasis, only 3 patients showed tumor recurrence. Of these 3 patients, 2 had regional lymph node metastasis and the 1 other showed distant lymph node metastasis. Interestingly, 1 of the 3 patients exhibited altered lymphatic flow after SLNB, although the other 2 showed preserved lymphatic flow. Considering the recurrence rates of patients with negative SLNB, 1 of the 2 patients (50%) who showed lymphatic flow alteration had recurrence (regional lymph node metastasis), although only 2 of the 14 patients (14%) without lymphatic flow alteration had recurrence (regional lymph node metastasis in 1 patient and distant lymph node metastasis in the other). The patient who showed no lymphatic flow alteration but had regional lymph node metastasis was affected by multiple distant metastases and died. The other 2 patients with recurrence after negative SLN metastasis are still alive.

In all 26 cases with positive SLN metastasis, regional lymph node dissection was performed. Of these 26 patients, 6 patients had recurrence of the tumor. In-transit metastasis was found in 5 patients whose lymphatic flow was preserved after SLNB. Distant metastasis was seen in 1 patient whose lymphatic flow was also preserved after SLNB. All 6 patients with recurrence died from the tumor.

4. Discussion

SLN detection and SLNB have long been performed by intraoperative visual dye detection (dye methods) using green or blue dyes such as indocyanine green and Patent Blue, and by preoperative lymphoscintigraphy and intraoperative γ probe/Geiger counter detection. Recently, NIR imaging methods have been established to investigate lymphatic flow [3]. NIR imaging can reveal the morphology of lymphatic vessels and lymphatic function with a single imaging modality [3]. Thus, nowadays, we usually perform SLNB by combining all three methods: visual dye, radioactive γ probe and NIR imaging.

NIR imaging is a low-invasive method that does not use any radioactive probes. Patients have no risk of exposure to radiation. Thus, by using NIR imaging methods, we can perform repetitive lymphatic vessel imaging and study lymphatic flow longitudinally after SLNB and regional lymph node dissection [4]. Innovations in NIR imaging methods enabled us to plan and perform our present study.

In 2004, Thomas and Clark [13] reviewed the literature of malignant melanoma cases with or without SLNB, focusing on the incidence of local/in-transit metastasis. They found that patients having SLNB had about twice the incidence of local/in-transit metastasis relative to the incidence for patients who underwent wide local excision of the tumor alone without SLNB [13]. Thomas and Clark [13] suggested that, in cases with congested lymphatic flow after the lymph node dissection, viable melanoma cells might be trapped in lymphatic capillaries, resulting in local/in-transit metastasis. Thus, they concluded that SLNB should not be performed outside of validation trials [13]. In contrast, Pawlik *et al.* [14] suggested

from the literature and their own multi-institution clinical experience that it is the tumor biology itself, rather than the SLNB surgical procedure, which determines the risk of in-transit metastasis; i.e., SLNB does not increase the risk of in-transit metastasis. In addition, other studies have also demonstrated that SLNB does not increase the risk of in-transit metastasis [15, 16]. In this context, Pawlik *et al.* [14] proposed that it was appropriate to offer SLNB to patients with intermediate-risk and high-risk primary cutaneous melanomas.

In the present study, the lymphatic flow was investigated before and after SLNB in 41 patients with PCMM. It was found that 38 of the 41 patients showed no alteration of lymphatic flow after SLNB, and only 3 patients had apparently changed lymphatic flow after SLNB. From these findings, we suggest that SLNB has only a minimal effect on lymphatic flow. Thus, we do not consider that SLNB procedures cause significant lymphatic flow congestion or increased risk for local recurrence/in-transit metastasis of PCMM. In light of this, we support the opinion of Pawlik *et al.* [14] that we should offer SLNB to patients with intermediate-risk and high-risk PCMM.

It might be surprising that ligation of the lymph channel at the SLN site did not lead to a change in the channels draining the primary tumor location. As to why the lymphatic flow was mostly unchanged after SLNB, we speculate that it was due to the rapid development of collateral channels to other neighboring lymph nodes close to the nodal area (i.e. not close to the primary site). In light of this, we hypothesize that lymphatic flow alterations were observed in the cases in which lymphatic flow was evaluated too early. We compared the periods from the SLNBs to the second NIR imaging of the lymphatic flow, between the cases with lymphatic flow alterations and the

cases without lymphatic flow change. However, there was no significant difference in the periods from the SLNBs to the second NIR imaging between the two patient groups.

We often offer local adjuvant injection to patients with intermediate-risk and high-risk PCMM after wide local excision. Specifically, shortly after wide local excision, patients receive postoperative adjuvant therapy of subcutaneous IFN-β injection around the surgical scar of the primary lesion, generally IFN-β (3×10^6 IU/body weight) local injection once a day for 10 consecutive days or once every week [5]. Afterward, during the follow-up period, local subcutaneous IFN-β injection around the surgical scar of the primary lesion at a dose of 3×10^6 IU/day every 3-4 weeks for 2-3 years [5]. Local adjuvant injection treatments are based on the concept that, after wide local excision, residual melanoma cells may be trapped in lymphatic capillaries, resulting in local recurrence and in-transit metastasis, and that locally injected adjuvants can reach residual sites of such melanoma cells via original lymphatic flow, producing anti-tumor effects against the melanoma cells [6, 7]. If the lymphatic flow route had been altered significantly after SLNB, then the locally injected adjuvant could not have reached the residual melanoma cells in the lymphatic capillaries. In light of this, our present findings are very important, because they clearly indicate that the lymphatic flow routes are not altered significantly after SLNB procedures in PCMM patients. Based on the assumption that lymphatic flow is preserved in most cases after SLNB, we can expect postoperative local adjuvant injection to be effective in preventing local recurrence and in-transit metastasis in patients with PCMM who undergo wide local excision and SLNB. We suggest that it is appropriate to offer local adjuvant injection treatment to patients with intermediate-risk and high-risk PCMM who have undergone wide local

excision and SLNB, especially cases with negative SLNB, in whom no regional dissection was performed.

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Figure legends

Fig. 1. Lymphatic flow imaging in a representative case, Patient 34, shows no alteration of lymphatic flow after SLNB. The patient was a 62-year-old female with PCMM on the left wrist. Total resection surgery of the primary tumor and SLNB were performed. The primary tumor was 5 mm thick, and SLN metastasis was confirmed. A comparison of the lymphatic flow routes from the primary tumor site to the SLN before SLNB (the top fluorescence image and illustration with dotted lines) versus after SLNB (the bottom fluorescence image and illustration with dotted lines) shows no apparent change.

Fig. 2. Lymphatic flow imaging in a representative case, Patient 35, without any alteration of lymphatic flow after SLNB. The patient was a 78-year-old male with PCMM on the right sole. The thickness of the primary lesion was 4.2 mm, and SLN metastasis was seen. No apparent change is observed between the lymphatic flow from the primary tumor site on the sole to inguinal SLNs before (the top fluorescence image and illustration with dotted lines) and after (the bottom fluorescence image and illustration with dotted lines) the SLNB.

Fig. 3. Lymphatic flow alteration in a representative case, Patient 10, shows lymphatic flow changes after SLNB. The patient was a 37-year-old man with PCMM on the right lumbar area. The primary tumor was 1.5 mm thick. No SLN metastasis was detected. The lymphatic flow routes from the primary tumor site to the regional lymph nodes drastically changed, as seen in a comparison of the routes before SLNB (the top fluorescence image and illustration with dotted red lines) versus after SLNB (the bottom

fluorescence image and illustration with black lines), although no apparent lymphatic congestion or backflow was observed after SLNB.

Fig. 4. Altered lymphatic flow in a representative case, Patient 3. The patient, a 51-year-old female, had PCMM on the right thigh. The primary lesion was 3.6 mm thick, and SLN metastasis was seen in 1 of the 3 SLNs. A comparison of the lymphatic flow routes before SLNB (the top fluorescence image and illustration with blue lines) versus after SLNB (the bottom fluorescence image and illustration with yellow lines) shows apparent flow route changes. No apparent lymphatic congestion or backflow was seen, even after SLNB.

Fig. 5. Dramatically altered lymphatic flow showing "dermal backflow sign" in Patient 15. The patient was a 60-year-old female with PCMM on the right lower leg. The primary lesion was 4 mm thick. 1 SLN was biopsied, and metastasis was confirmed in the SLN. After the right inguinal lymph node dissection, severe lymphatic congestion and dermal backflow of the dye was observed.

Figure 1

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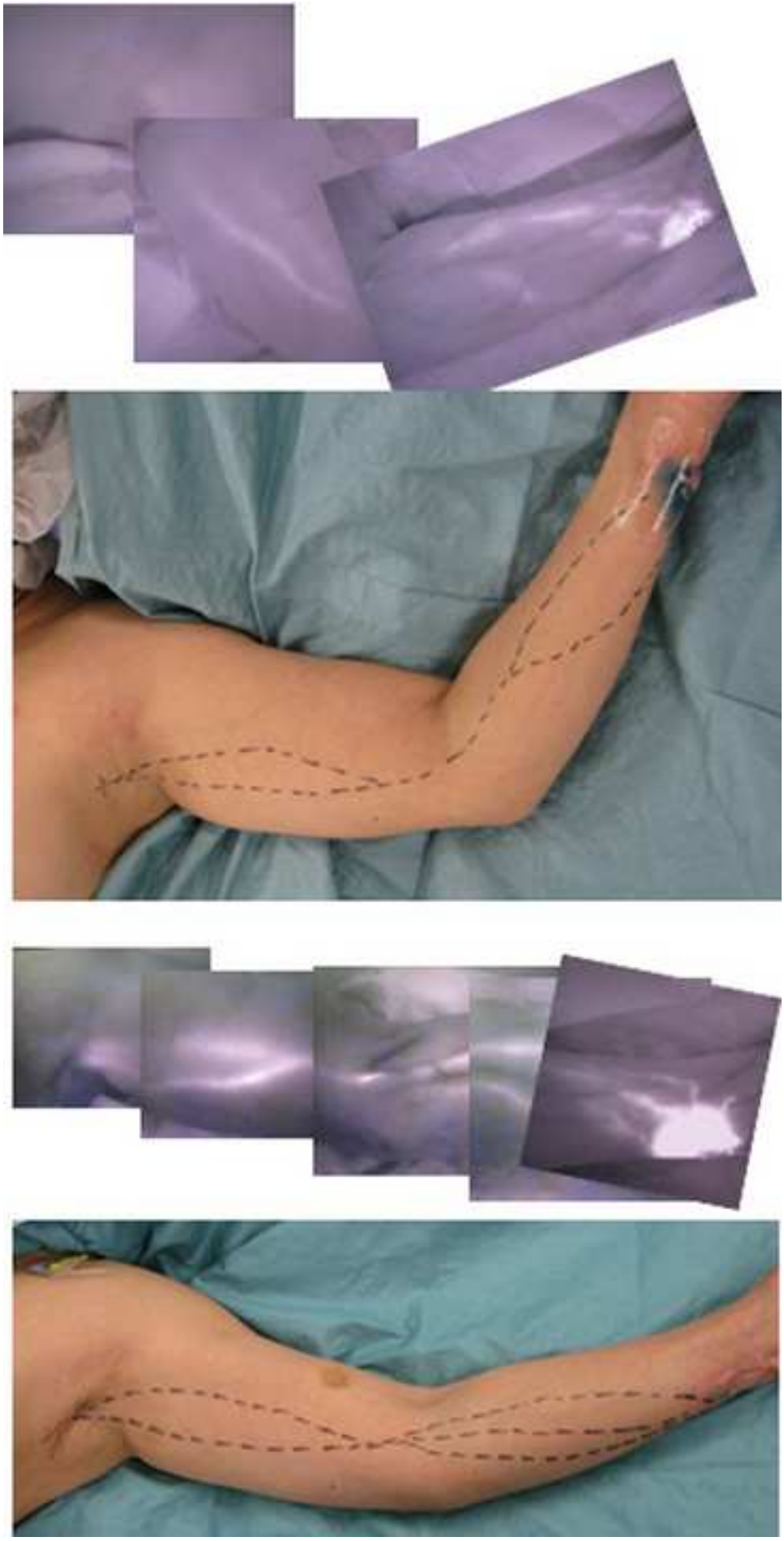

Figure 2

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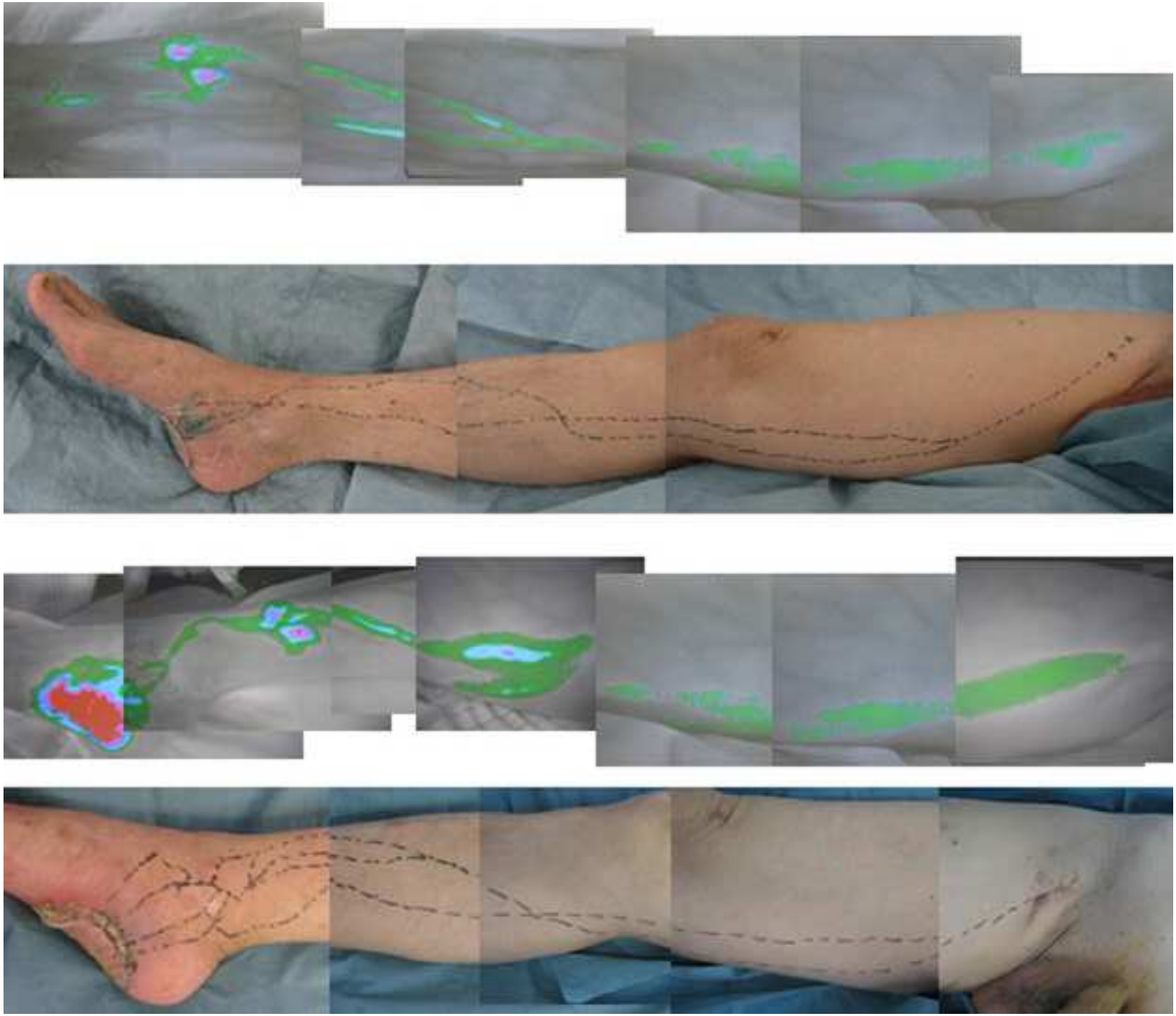

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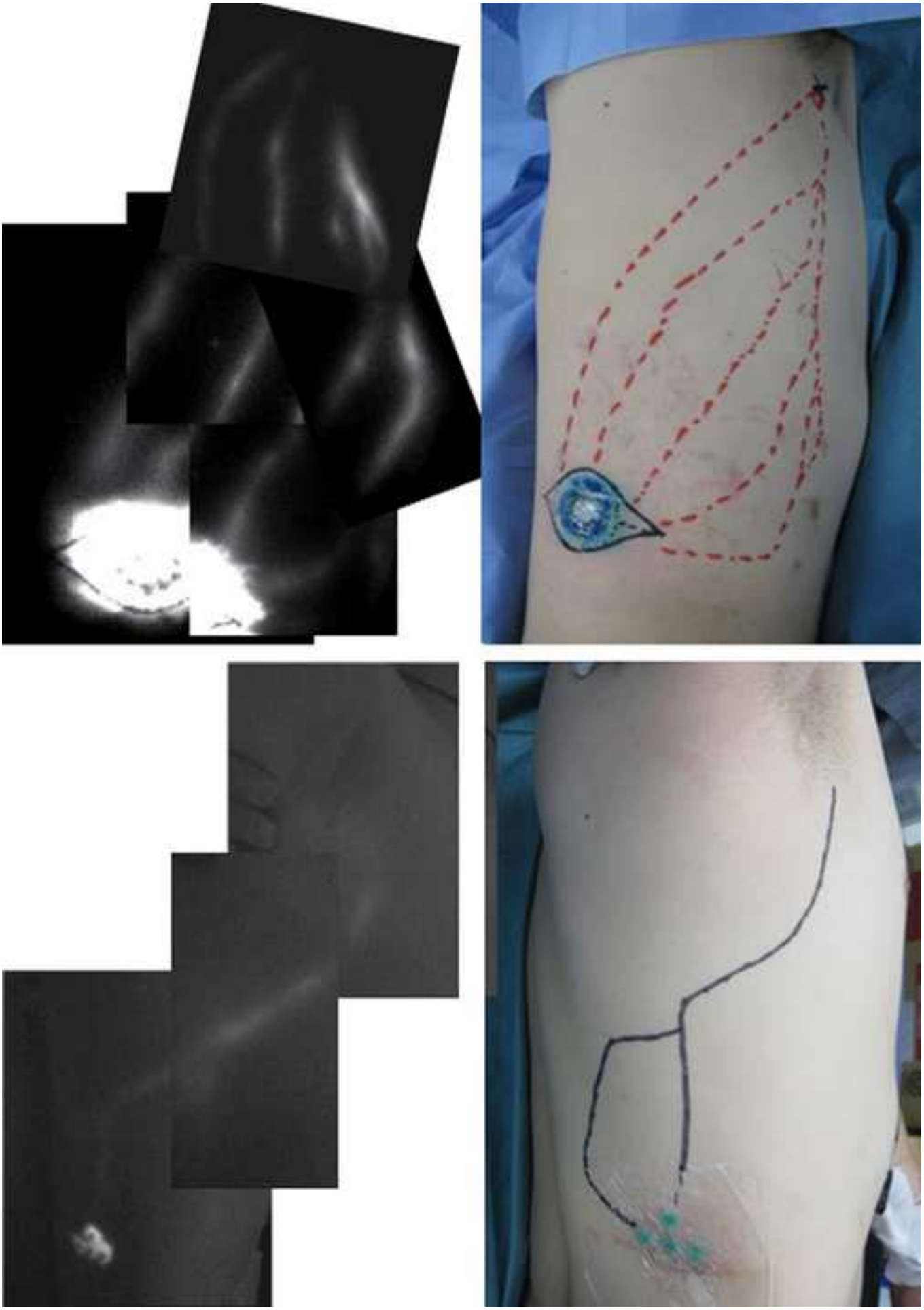

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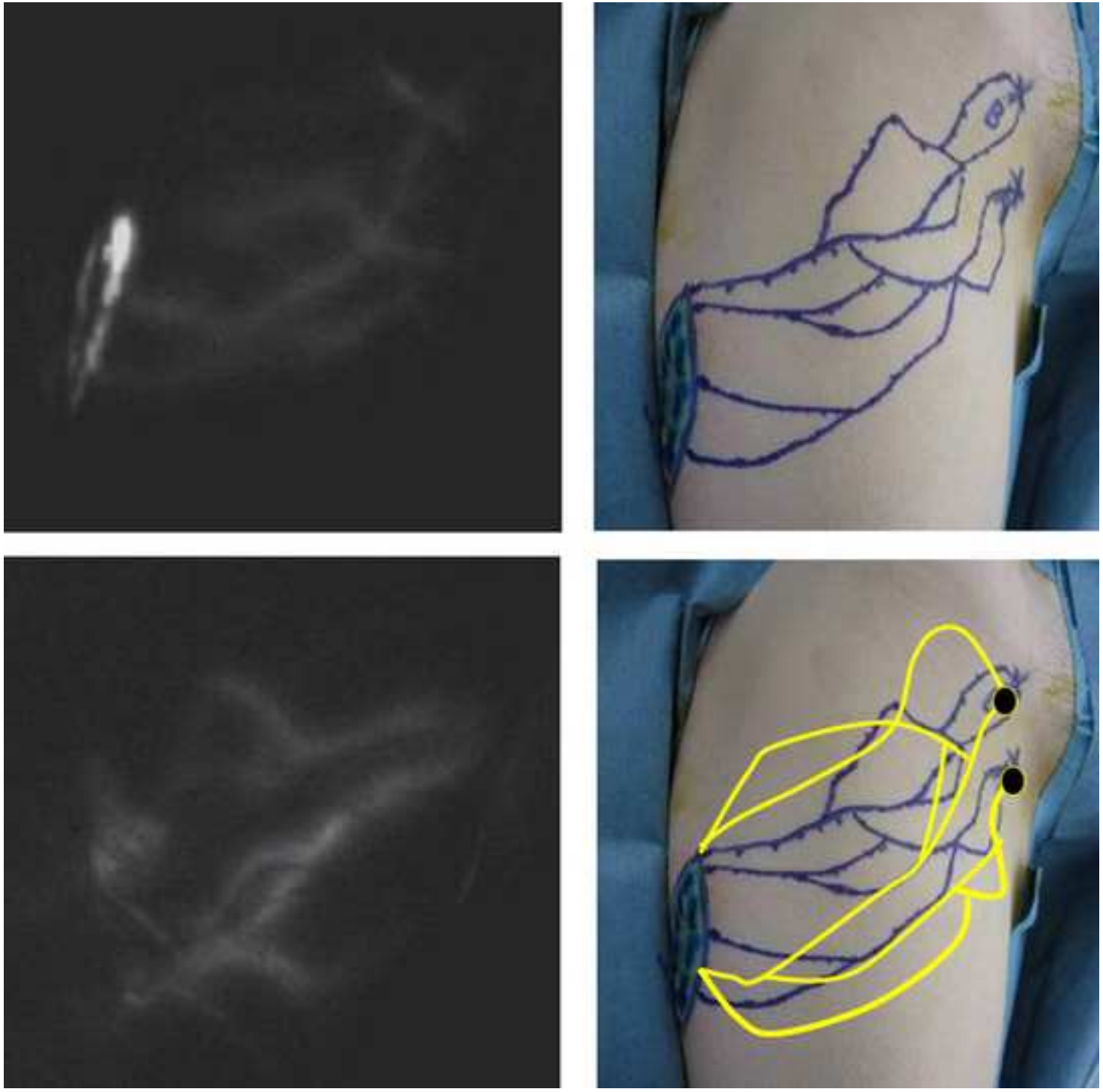

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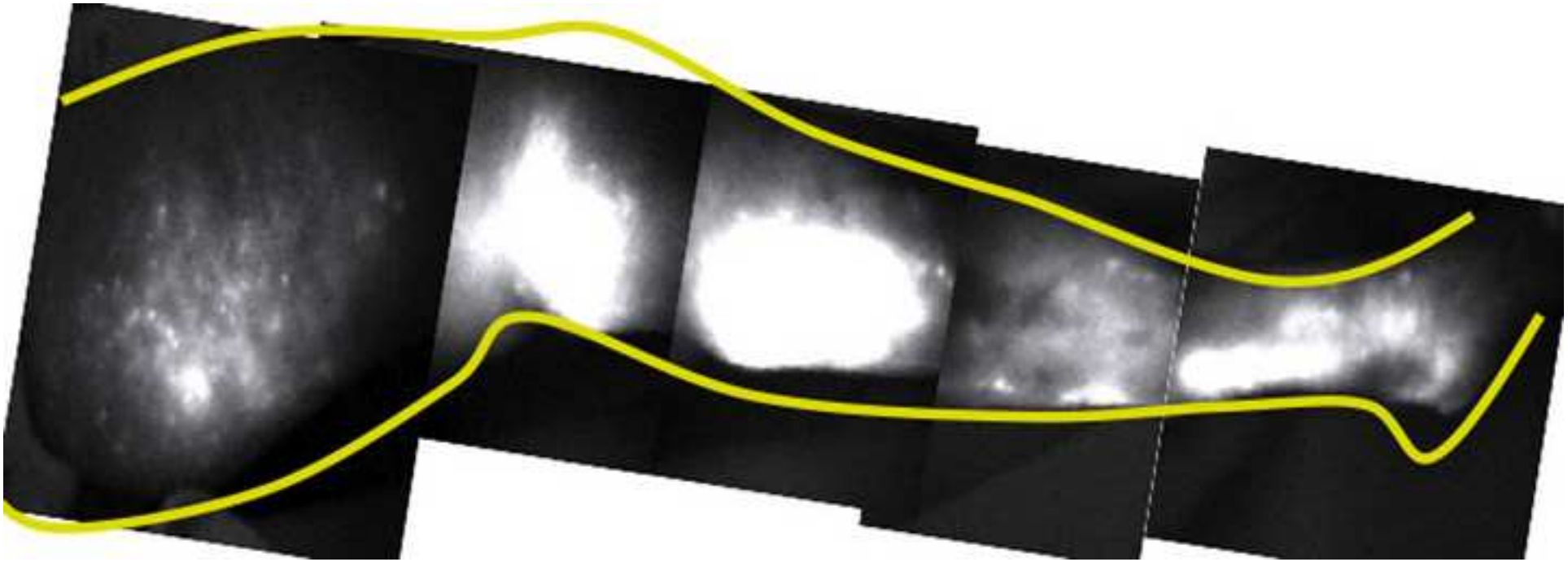

Table 1

Table 1. Clinical data of PCMM patients included in the present study.

Patient No.	Age	Sex	Primary site	Tumor thickness (mm)	Number of biopsied SLNs	Number of SLNs with metastasis	Lymphatic flow alteration after SLNB	Recurrence/metastasis	Survival outcome
1	60	F	Rt. thigh	3.8	3	3	–	–	survival
2	63	F	Rt. upper arm	1.7	1	0	–	–	survival
3	51	F	Rt. thigh	3.6	3	1	+	–	survival
4	79	M	Lt. abdomen	9	1	1	–	In-transit metastasis -> lung metastasis	decease
5	63	M	Rt. I toe	0.8	1	0	–	–	survival
6	43	F	Rt. thigh	1.4	3	1	–	–	survival
7	68	F	Rt. ? toe	1	2	0	–	–	survival
8	64	M	Genitalia	9	3	1	–	In-transit metastasis -> multiple metastases	decease
9	69	M	Lt. sole	2	3	0	–	-	survival
10	37	M	Rt. waist	1.5	2	0	+	Regional LN metastasis	survival
11	53	M	Rt. sole	1.4	3	0	–	–	survival
12	73	M	Rt. chest	4	3	0	–	–	survival
13	41	F	Rt. forehead	5	3	0	–	Regional LN metastasis -> multiple metastases	decease
14	26	F	Rt. lower leg	3.6	2	1	–	In-transit metastasis -> multiple metastases	decease
15	60	F	Rt. lower leg	4	1	1	–	In-transit metastasis -> multiple metastases	decease
16	71	F	Lt. thumb	3	2	0	–	–	survival
17	62	M	Rt. upper arm	1.3	5	0	+	–	survival
18	75	F	Lt. thumb	7	1	0	–	–	survival
19	38	F	Lt. chest	1.1	1	0	–	–	survival
20	44	M	Rt. index finger	2.5	4	2	–	–	survival
21	66	M	Rt. V toe	2.1	4	2	–	–	survival
22	63	M	Rt. chest	3.5	1	1	–	–	survival
23	27	M	Rt. forearm	3.5	1	1	–	–	survival
24	58	F	Lt. foot	6	1	1	–	In-transit metastasis -> multiple metastases	decease
25	64	F	Rt. hand	3	3	3	–	–	survival
26	85	F	Lt. sole	3	1	1	–	–	survival
27	54	M	Rt. waist	3	1	0	–	Distant LN metastasis	survival
28	81	M	Rt. sole	4	1	1	–	–	survival
29	65	M	Rt. sole	1	6	4	–	–	survival
30	78	M	Lt. buttock	5	1	1	–	–	survival
31	62	M	Lt. sole	1.8	3	1	–	–	survival
32	65	F	Lt. sole	8.5	3	2	–	Multiple metastases	decease
33	69	F	Lt. sole	3	3	2	–	–	survival
34	62	F	Lt. wrist	5	1	1	–	–	survival
35	78	M	Rt. sole	4.2	2	2	–	–	survival
36	75	M	Rt. middle finger	9	2	2	–	–	survival
37	47	M	Lt. I toe	9	2	2	–	–	survival
38	66	M	Lt. shoulder	2.3	1	1	–	–	survival
39	75	F	Lt. lower leg	4.2	2	0	–	–	
40	64	F	Lt. I toe	0.5	2	0	–	–	
41	62	M	Lt. back	0.75	3	0	–	–	