Innovating image-guided surgery: Introducing multimodal approaches for sentinel node detection

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Image-guided sentinel node biopsy in 104 melanoma patients; a hybrid approach

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Purpose: To explore the value of indocyanine green (ICG)-$^{99m}$Tc-nanocolloid for sentinel node (SN) identification in a cohort of melanoma patients and to compare this to optical SN identification with blue dye.

Methods: One-hundred-and-four patients with melanoma of the head and neck (n=53), trunk (n=33) or an extremity (n=18) were evaluated. Lymphoscintigraphy with subsequent SPECT/CT was performed after intradermal administration of ICG-$^{99m}$Tc-nanocolloid. The operation was performed 3–27 hours after tracer injection. Patent blue dye was injected prior to surgery, except in patients with a melanoma in the face (n=35). Intraoperatively, SNs were pursued via gamma ray tracing, followed by optical verification using fluorescence and/or blue dye. A portable gamma camera was used to confirm removal of all SNs.

Results: Preoperative imaging revealed at least one SN in all patients. Intraoperatively, an SN could only be localized using fluorescence imaging in 17 patients (16%). Overall, 98% of SNs could be intraoperatively visualized with the near-infrared fluorescence camera, whereas only 63% of SNs were blue (p<0.05).

Conclusions: ICG-$^{99m}$Tc-nanocolloid enabled preoperative lymphoscintigraphy and SPECT/CT imaging, together with intraoperative radio- and fluorescence-guided SN detection in all 104 patients. The addition of fluorescence imaging was particularly useful for the identification of SNs located near the injection site, located in a complex anatomical area, and for optical visualization of SNs that failed to accumulate blue dye.

# shared first authorship
**INTRODUCTION**

For melanoma, sentinel node (SN) biopsy has evolved into the routine procedure to determine the presence of lymph node metastasis allowing patients with nodal metastasis to be treated in a relatively early phase of their disease. A combination of radio-labeled colloid and a vital blue dye are commonly used to identify the SN. Radiolabeled colloids enable preoperative lymphoscintigraphy and intraoperative SN detection using a hand-held gamma ray detection probe (hereafter referred to as gamma probe). Vital blue dyes allow for optical detection of the SNs and their afferent lymphatic vessel during surgery. Although generally successful, this technique has limitations. A blue lymph vessel may be difficult to find and its dissection requires substantial expertise. Occasionally, blue dye causes an allergic reaction and it can stain the injection site for months. Also, SNs not always take up blue dye, particularly in the neck. As a result, the false-negative rate of SN biopsy in patients with melanoma is high.

Over the last decade several techniques have been introduced to improve SN identification. In the preoperative setting, single photon emission computed tomography with computed tomography (SPECT/CT) was shown to provide useful anatomical reference points regarding the anatomical context of the SNs enabling accurate planning of the operation. In the operating room, the introduction of a portable gamma camera was shown to be useful for visualization of the radioactive hotspots on-screen as an addition to the traditional acoustic guidance generated by a gamma probe. Near-infrared fluorescence imaging of indocyanine green (ICG) provides an alternative mode of intraoperative SN identification.

The hybrid tracer ICG-\(^{99m}\)Tc-nanocolloid was developed to combine the attractive migrational properties of radiolabeled colloids with the favorable optical imaging feature of ICG. With this hybrid tracer being both radioactive and fluorescent, one single compound allows for: I) conventional lymphoscintigraphy and SPECT/CT imaging. II) During the operation, SNs can be radiotraced using a gamma probe. III) In addition, SNs can be optically detected via fluorescence imaging. The feasibility of this hybrid approach was demonstrated in several pilot studies. The present study evaluates the value of ICG-\(^{99m}\)Tc-nanocolloid for SN biopsy in a large population of patients with melanoma. Secondly, a comparison between fluorescence and blue dye-staining was made after SN identification.
Methods

Patients

Between March 2010 and March 2013, a total of 104 patients with melanoma of the head and neck (n=53), trunk (n=33) or an extremity (n=18) were studied. The first 13 patients were also included in previous feasibility trials.12,13 Patients were prospectively enrolled after a diagnostic excision of a melanoma with a Breslow thickness of at least 1.0 mm (average 2.7 mm). All clinically node-negative patients were scheduled for SN biopsy and re-excision of the biopsy wound. The study protocol was approved by the Institutional Review Board (N09DRF, NL 26699.031.09), and all patients were included after giving informed consent.

Tracer preparation

After preparation of \(^{99m}\)Tc-nanocolloid with a final pH of 6-7 (GE Healthcare, Eindhoven, The Netherlands), hybrid ICG-\(^{99m}\)Tc-nanocolloid was formed by adding 0.25 mg of ICG (ICG-Pulsion, Pulsion Medical Systems, Munich, Germany) to the \(^{99m}\)Tc-nanocolloid solution as previously described.12-14 Subsequently, approximately 90 MBq ± 10% was subtracted from the vial containing the ICG-\(^{99m}\)Tc-nanocolloid solution. Saline was then added to reach a total volume of 0.4 ml in the syringe. All procedures were performed under good manufacturing practice (GMP-z) and under supervision of the institution’s pharmacist.

Preoperative procedure

An average of 77 MBq (range 54-113 MBq) ICG-\(^{99m}\)Tc-nanocolloid was injected intradermally in 4 deposits around the excisional biopsy scar (total volume 0.4 ml). Anterior and lateral dynamic lymphoscintigraphy was performed using a dual-head gamma camera (Symbia T, Siemens, Erlangen, Germany) during the first 10 minutes after injection to visualize the draining lymphatic vessel(s) and the first draining lymph nodes. Static planar gamma camera images were acquired 15 minutes and two hours post-injection. SPECT and CT imaging (Symbia T) was performed 2 hours post-injection. After correction for scatter and tissue attenuation, the SPECT was fused with the low dose CT (40 mAs).

Lymph nodes draining from the site of injection through an own lymphatic vessel were identified as SNs.16,17 SNs were anatomically localized using multiplanar reconstruction which enabled comparison of fused SPECT/CT images with concomitant CT. Additionally 3D SPECT/CT display of SNs in relation to the anatomical structures was accomplished using volume rendering.

Surgical procedure

In the 1-day protocol, the surgical procedure started 5 hours after injection on average (range 3-9.5 hours; 58 patients), whereas in the 2-day protocol this was 21 hours (range 18-27 hours; 46 patients).

Immediately before the operation a mean volume of 1.0 ml of patent blue dye was injected intradermally at the SN location to confirm SN location and to visualize the lymphatic vessel(s) for easier identification.18,19
(Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected intradermally around the original melanoma site in the patients with melanoma located outside the facial area (n=69). Immediately preceding the incision, an overview image of the hotspots was acquired using a portable gamma camera (Sentinella, Oncovision, Valencia, Spain) as a reference image. Image acquisition times ranged from 15 seconds to 1 minute in accordance with previous reports. During surgical exploration, SNs were initially pursued with a gamma probe (Neoprobe, Johnson & Johnson Medical, Hamburg, Germany). Alternating attempts were then made to optically visualize the SNs through fluorescence imaging using a handheld near-infrared fluorescence camera (PhotoDynamic Eye; Hamamatsu Photonics, Hamamatsu, Japan) and/or visual detection of the blue dye. Fluorescence imaging required the lights in the operating room to be dimmed to minimize the background signal, whereupon reflection of the excitation light was used to visualize the surrounding tissues. After excision of a SN, a second portable gamma camera image was acquired to verify SN removal. If residual radioactivity was observed at the site of a previously excised SN, it was considered part of a cluster of multiple adjacent SNs and also harvested.

**Pathology and ex vivo analyses**

All SNs were fixed in formalin, bisected, embedded in paraffin, and cut at a minimum of six levels at 50-150 μm intervals. Histopathologic evaluation included a hematoxylin and eosin, S-100 (cat. no. Z0311; DAKO, Heverlee, Belgium) and MART-1 (cat. no. M7196; DAKO) staining.

**Statistical analysis**

Statistical evaluation of the difference between the number of fluorescent and blue nodes was performed for each subgroup (head and neck, trunk, and extremity) and for all groups together using a 2-sample test for equality of proportions with continuity correction. A p-value of <0.05 was considered significant.

**RESULTS**

**Preoperative results**

A schematic overview of the procedure is depicted in Fig. 1. Preoperative SN mapping results for each subgroup (head and neck, trunk, and extremity) are specified in Table 1. The combination of lymphoscintigraphy and SPECT/CT revealed at least one SN in 103 of the 104 patients (99%) with a total of 245 SNs. Neither lymphoscintigraphy nor SPECT/CT depicted a SN in the remaining patient presenting with a pre-auricular melanoma. In this case, the portable gamma camera located the SN close to the injection site as demonstrated in Fig. 2. A total of 246 SNs were preoperatively identified (average 2.4 SNs per patient, range 1-6). No adverse reactions to ICG-99mTc-nanocolloid were observed.
Intraoperative results

Intraoperative SN biopsy results are specified in Table 2. All but 4 preoperatively identified SNs could be intraoperatively localized using a combination of radio- and fluorescence guidance and blue dye detection (Fig. 3). In 9 patients, fluorescence allowed the surgeon to localize the SN in the parotid area or close to the injection site; where radioactivity-based detection of the SNs was hampered. In 14 patients (26%) with drainage to the head and neck, fluorescence imaging was particularly useful for depth estimation and “pinpointing” of the SN. Fluorescence imaging also facilitated the identification when low radioactive counts were present in the SN. For example, in one patient with a truncal melanoma and a patient with a melanoma on a lower extremity, a SN was difficult to find using the probe because of a low initial radioactive count rate due to radioactive decay (the operation started 21 and 20 hours after injection, respectively). In these patients, fluorescence imaging enabled localization of the SNs after which the SNs could be excised. Similarly, when SNs had failed to take up blue dye, fluorescence imaging allowed optical verification of the location of the SN.

In 33 patients, 59 additional SNs were excised. In retrospect, re-analysis of the CT and the fused SPECT/CT images revealed the presence a cluster of nodes. Examples are illustrated in Fig. 4. A total of 301 SNs was ultimately harvested (average 2.9 SNs; range 1-9). Ninety-five percent of these nodes could be localized using the gamma probe. Ninety-eight percent of the SNs could be intraoperatively visualized using the fluorescence camera (Figure 3), whereas only 63% of the SNs were blue in the 69 patients in whom blue dye was used (p<0.05).

Pathological analysis revealed a total of 310 excised SNs. SNs metastases were found in 26 patients (25%). Of the 39 tumor-positive SNs 13% contained isolated tumor cells, 51% micrometastases (diameter 0.2-2 mm), and 36% macrometastases (diameter >2.5 mm).

**DISCUSSION**

With this study in a cohort of 104 melanoma patients, we strengthen previous reports indicating that the hybrid tracer approach using ICG-\(^{99m}\)Tc-nanocolloid adds specific fluorescence guidance to the otherwise standard SN biopsy procedure. Intraoperative fluorescence imaging was found to be especially valuable in cases with intricate anatomy, for example for the detection of intra-parotid SNs. Through fluorescence imaging optical SN identification was facilitated, even when the SNs were located in close vicinity to the injection site; an indication where SN identification via conventional gamma tracing is notoriously difficult. Fluorescence-based SN identification significantly outperformed blue dye-based SN identification, 98% vs. 63%, respectively (p<0.05, Table 2). Combining the fluorescent and radioactive signature in one compound (ICG-\(^{99m}\)Tc-nanocolloid) prevents discrepancies in drainage patterns which may occur after separate injections of e.g. radiocolloid and blue dye. Although blue dye may potentially also allow the
identification of SNs that failed to accumulated radiocolloid, in this study no SNs were found that were only blue.

The difference in the optical detection mechanisms for blue dye and ICG may explain the superior efficiency found for ICG-99mTc-nanocolloid. Blue dye is visible only when the SN or lymphatic vessel is already (partially) exposed.\textsuperscript{18} The generation of a near-infrared fluorescence signal is a process in which the dye is excited by light with a near-infrared wavelength (±780 nm) whereafter near-infrared emission light is produced. This emission signal is of a higher wavelength (±820 nm) and can be detected with a near-infrared fluorescence camera. Since the latter takes place in the tissue transparence window, it has a superior tissue penetration going as deep as 0.5-1.0 cm.\textsuperscript{11,19} The value of the superior tissue penetration is demonstrated by the several cases where near-infrared fluorescence imaging allowed (transcutaneous) detection of the SN, before visual identification using blue dye was possible (Fig. 3). Unfortunately, the fluorescence camera used in the current study makes it difficult to follow lymphatic vessels, though not impossible. Nevertheless, camera systems with more a powerful excitation light source, e.g. the (mini-)FLARE system\textsuperscript{20} may allow the real-time detection of the draining lymphatic vessels thereby further reducing the need for blue dye.

The intraoperative detection of additional SNs is not uncommon, but it is striking that 20% additional SNs were intraoperatively identified by improving the identification technique(s) through the combined use of a portable gamma camera and fluorescence imaging. This finding indicates that thorough confirmation of SN removal, e.g. by using a portable gamma camera, remains of importance. Retrospective analyses suggest that more careful evaluation of the CT scan in combination with the fused SPECT/CT scan may help to better predict the number of SNs in a certain area. Not surprisingly, the largest number of additionally identified SNs was found in the area with the most complex anatomy, namely the head and neck area (Table 2).
**CONCLUSION**

In conclusion, the hybrid tracer ICG-$^{99m}$Tc-nanocolloid enabled preoperative imaging and intraoperative radio- plus fluorescence-guided SN biopsy in all 104 melanoma patients. The fluorescent moiety was found to be of added value for the detection of SNs close to the injection site, SNs located in a complex anatomy, and SNs that failed to accumulate patent blue dye.

**ACKNOWLEDGEMENTS**

This work was partially supported by a Dutch Cancer Society translational research award (Grant No. PGF 2009–4344) and an NWO-STW-VIDI grant (Grant No. STW BGT11272). We gratefully acknowledge the entire surgical staff, pathology department, hospital pharmacy, and technical support of the nuclear medicine department for their contribution.
Figure 1. Schematic overview of the SN biopsy procedure for melanoma. The hybrid tracer ICG-99mTc-nanocolloid is injected in four deposits (total volume 0.4 ml) intradermally surrounding the melanoma scar (A). Immediately after tracer injection, dynamic lymphoscintigraphy is performed followed by static images at fifteen minutes and two hours post-injection. Thereafter a SPECT/CT scan is acquired (B). Prior to the start of the operation, a volume of approximately 1.0 ml patent blue dye is injected after which the injection site is massaged (C). Intraoperatively, SNs are detected using a combination of radioguidance, fluorescence imaging and blue dye detection (D).

Figure 2. Identification of a SN located in close proximity to the injection site not seen with conventional lymphoscintigraphy. Preoperative lymphoscintigraphy (A and B) did not reveal any SN. However, with a portable gamma camera (C) it was possible to distinguish a SN directly next to the injection site (D; right panel; G; arrow). The signal of the SN could not be distinguished using the conventional gamma probe (E), but with the handheld near-infrared fluorescence camera (F) the SN could be visualized (G; arrow). After removal of the SN, another portable gamma camera image was taken (H) confirming that indeed the SN was removed. The left panel shows the location of the SN before excision (arrow) whereas this spot is no longer visible after SN excision (right panel).
Figure 3. SN identification via fluorescence imaging. Occasionally, when SNs are located superficially, SNs can be seen through the skin (A). Detection of a SN when no blue dye was used (B), when only a blue vessel was found running to the SN (C) or when the lymphatic vessel and SN had stained blue (D).
Figure 4. Preoperative SN mapping. Conventional lymphoscintigrams (A panels) and SPECT/CT images (B panels) showing the SNs with regard to the injection site (IS). Intraoperatively, additional SNs were removed in several patients. Retrospective analysis revealed these SNs being part of clusters (C and D panels).
### Table 1: Preoperative results per melanoma site

<table>
<thead>
<tr>
<th></th>
<th>Head and Neck</th>
<th>Trunk</th>
<th>Extremity</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td># Patients</td>
<td>53</td>
<td>33</td>
<td>18</td>
<td>104</td>
</tr>
<tr>
<td>Preoperative SN mapping</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Early lymphoscintigraphy</td>
<td>113 (82%)</td>
<td>62 (82%)</td>
<td>28 (85%)</td>
<td>203 (83%)</td>
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<td>Late lymphoscintigraphy</td>
<td>127 (93%)</td>
<td>73 (96%)</td>
<td>32 (97%)</td>
<td>232 (94%)</td>
</tr>
<tr>
<td>SPECT/CT</td>
<td>136 (99%)</td>
<td>76 (100%)</td>
<td>33 (100%)</td>
<td>245 (99%)</td>
</tr>
<tr>
<td>Portable gamma camera</td>
<td>1 (1%)</td>
<td>-</td>
<td>-</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Total # SNs (av; range)</td>
<td>137 (2.6; 1-6)</td>
<td>76 (2.3; 1-4)</td>
<td>33 (1.8; 1-3)</td>
<td>246 (2.4; 1-6)</td>
</tr>
<tr>
<td># SN basins (av; range)</td>
<td>111 (2.1; 1-5)</td>
<td>53 (1.6; 1-4)</td>
<td>19 (1.1; 1-2)</td>
<td>183 (1.8; 1-6)</td>
</tr>
<tr>
<td>Basins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suboccipital</td>
<td>7%</td>
<td>2%</td>
<td>5%</td>
<td>14%</td>
</tr>
<tr>
<td>Temporal</td>
<td>1%</td>
<td>4%</td>
<td>21%</td>
<td>26%</td>
</tr>
<tr>
<td>Parotid</td>
<td>7%</td>
<td>4%</td>
<td>2%</td>
<td>13%</td>
</tr>
<tr>
<td>Auricular</td>
<td>16%</td>
<td>75%</td>
<td>74%</td>
<td>96%</td>
</tr>
<tr>
<td>Neck</td>
<td>67%</td>
<td>2%</td>
<td>11%</td>
<td>80%</td>
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<tr>
<td>Supraclavicular</td>
<td>2%</td>
<td>1%</td>
<td>1%</td>
<td>4%</td>
</tr>
<tr>
<td>Neat:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suboccipital:</td>
<td></td>
<td></td>
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<tr>
<td>Temporal:</td>
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<td>4%</td>
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<td>32%</td>
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<tr>
<td>Neck:</td>
<td>1%</td>
<td>4%</td>
<td>2%</td>
<td>7%</td>
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<tr>
<td>Suboccipital:</td>
<td>7%</td>
<td>4%</td>
<td>2%</td>
<td>13%</td>
</tr>
<tr>
<td>Supraclavicular:</td>
<td>2%</td>
<td>75%</td>
<td>74%</td>
<td>96%</td>
</tr>
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</table>
| # = number; SN = sentinel node; av = average.

### Table 2: Intraoperative results

<table>
<thead>
<tr>
<th></th>
<th>Head and Neck</th>
<th>Axillar</th>
<th>Inguinal</th>
<th>Abberant%</th>
<th>Other@</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td># Patients</td>
<td>54</td>
<td>36</td>
<td>18</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td># Basins</td>
<td>110</td>
<td>44</td>
<td>20</td>
<td>4</td>
<td>5</td>
<td>183</td>
</tr>
<tr>
<td># SNs not excised (# patients)</td>
<td>3 (2)</td>
<td>1(1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4 (3)</td>
</tr>
<tr>
<td># Additional excised SNs (# patients)</td>
<td>46 (24)</td>
<td>10 (6)</td>
<td>2 (2)</td>
<td>-</td>
<td>1 (1)</td>
<td>59 (33)</td>
</tr>
<tr>
<td>Total # excised SNs</td>
<td>179</td>
<td>74</td>
<td>38</td>
<td>4</td>
<td>6</td>
<td>301</td>
</tr>
<tr>
<td>Intraoperative SN detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Radio tracing</td>
<td>165 (92%)</td>
<td>73 (99%)</td>
<td>38 (100%)</td>
<td>4 (100%)</td>
<td>6 (100%)</td>
<td>286 (95%)</td>
</tr>
<tr>
<td>Fluorescence guidance</td>
<td>176 (98%)</td>
<td>71 (96%)</td>
<td>38 (100%)</td>
<td>4 (100%)</td>
<td>6 (100%)</td>
<td>295 (98%)</td>
</tr>
<tr>
<td>Blue dye visualization</td>
<td>23 (37%)*</td>
<td>55 (74%)</td>
<td>35 (92%)</td>
<td>1 (25%)</td>
<td>2 (50%)*</td>
<td>116 (63%)</td>
</tr>
</tbody>
</table>
| * In the 19 patients in which blue dye was used, 63 SNs were excised. & In the two patients in which blue dye was used, a total of 4 SNs was excised.
| % Abberant: Intermediate trunk, pectoral or scapular area. @ Other: Supraclavicular and epitrochlear basin.
| # = number; SN = sentinel node.
REFERENCES


