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

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Comparing the hybrid fluorescent and radioactive tracer ICG-\(^{99m}\)Tc-nanocolloid with \(^{99m}\)Tc-nanocolloid for sentinel node identification: A validation study using lymphoscintigraphy and SPECT/CT

**Purpose:** To compare the lymphoscintigraphic drainage patterns of a novel hybrid sentinel node tracer consisting of the fluorescent dye indocyanine green (ICG) and \(^{99m}\)Tc-nanocolloid, to \(^{99m}\)Tc-nanocolloid alone, the current standard tracer in many European countries.

**Methods:** Twenty-five patients with either a melanoma in the head and neck region (\(n=10\)), a melanoma on the trunk (\(n=6\)), or penile carcinoma (\(n=9\)) who were scheduled for sentinel node (SN) biopsy were prospectively included. First, the standard \(^{99m}\)Tc-nanocolloid procedure was performed. After injection at the lesion site, lymphoscintigraphy was performed with a 10 minute dynamic study and static planar images at 10 minutes and 2 hours post injection, followed by SPECT/CT. The exact scintigraphic procedure was repeated after injection of hybrid ICG-\(^{99m}\)Tc-nanocolloid the same afternoon in 10 patients, or the next morning in 15 patients. The paired images of both injections were evaluated and count rates in the SNs were calculated and compared. SNs were surgically localized using blue dye, a gamma ray detection probe, a portable gamma camera and a fluorescence camera.

**Results:** Lymphatic drainage was visualized in all 25 patients using \(^{99m}\)Tc-nanocolloid leading to the identification of 66 SNs in total. These very SNs were also identified during the second scintigraphic procedure with ICG-\(^{99m}\)Tc-nanocolloid. Moreover, a high correlation between the radioactive counts rates in the SNs of both scintigraphic studies was observed (mean \(R^2 = 0.83\)). Intraoperatively (4-23 hours after the second injection), all preoperatively identified SNs could be localized using radio- and fluorescence guidance combined. In total, 95% of the SNs could be intraoperatively visualized by means of fluorescence imaging, whereas merely 54% stained blue. Ex vivo, all radioactive SNs were found to be fluorescent and vice versa. No adverse reactions were observed.

**Conclusion:** The lymphatic drainage pattern of ICG-\(^{99m}\)Tc-nanocolloid is identical to that of \(^{99m}\)Tc-nanocolloid. This observation, together with the added value of intraoperative fluorescence guidance, warrants wider evaluation of hybrid ICG-\(^{99m}\)Tc-nanocolloid as a tracer for SN procedures.
INTRODUCTION

Based on the hypothesis of sequential tumor spread, sentinel node (SN) biopsy is increasingly being used as a staging procedure for various malignancies.\(^1\) In the preoperative setting, the lymphatic drainage pattern can be gradually visualized using sequential lymphoscintigraphy, enabling identification of the first tumor draining (sentinel) lymph nodes.\(^8\) Single photon emission computed tomography with computed radiographic tomography (SPECT/CT) complements lymphoscintigraphy with three-dimensional anatomic data and can sometimes reveal additional SNs.\(^9\)-\(^11\) Intraoperative SN identification traditionally relies on the combination of acoustic signals generated by a gamma ray detection probe and optical SN visualization using a visible blue dye.\(^12\),\(^13\) Because the probe has a limited spatial resolution and SNs do not always stain blue, SNs can be difficult to localize in areas with a complex anatomy and when SNs are located close to the injection site.\(^14\),\(^15\) Although the incorporation of a portable gamma camera in the intraoperative procedure partially addresses these limitations by increasing the detection sensitivity and providing an intraoperative overview image of the radioactive SNs, this device does not depict the surrounding anatomical structures in the surgical field.\(^16\)-\(^18\)

Near-infrared fluorescence imaging has the potential to address the drawbacks of radioguided SN detection by providing better spatial resolution and allowing for real-time optical detection of the SN within the surrounding anatomy.\(^19\)-\(^22\) Yet, because the signal penetration of fluorescent probes is limited by tissue attenuation, radio-guidance to the general area of interest is still indispensable.\(^23\) To combine the beneficial properties of both modalities, a hybrid tracer comprising the fluorescent dye indocyanine green (ICG) and the human serum albumin based radiocolloid \(^{99m}\)Tc-nanocolloid was developed.\(^24\),\(^25\) Recently, hybrid ICG-\(^{99m}\)Tc-nanocolloid was introduced for laparoscopic and open SN biopsies in patients with prostate cancer or head and neck melanoma.\(^23\),\(^26\),\(^27\)

Before this hybrid tracer can be used routinely, it is imperative to ensure that the addition of the fluorescent moieties does not alter the biological properties of the parental radiocolloid. Therefore, the main purpose of the present study was to assess the concordance between the lymphatic drainage pattern of \(^{99m}\)Tc-nanocolloid (the current standard in many European countries) and hybrid ICG-\(^{99m}\)Tc-nanocolloid, using lymphoscintigraphy and SPECT/CT. In addition, this study further evaluated the value of combined radio- and fluorescence guided SN biopsy in various malignancies with superficial lymphatic drainage to areas such as the groin, axilla, and neck.
MATERIAL AND METHODS

Patients
Twenty-five patients with either a melanoma in the head and neck region (n=10), a melanoma on the trunk (n=6), or penile carcinoma (n=9) who were scheduled for SN biopsy were prospectively enrolled in the study. Patient characteristics are listed in Table 1. The mean age of the patients was 54 years (range 26–75 years). All patients were clinically node-negative at the time of SN biopsy. The study protocol was approved by the medical ethical committee of our institution and all patients provided written informed consent.

Tracer preparation
$^{99m}$Tc-nanocolloid was prepared by adding 2 mL of pertechnetate in saline (approximately 1400 MBq) to a commercial vial of nanocolloid containing 0.5 mg of albumin colloid (GE Healthcare, Eindhoven, the Netherlands). After 30 minutes of incubation at room temperature, the $^{99m}$Tc-nanocolloid solution (pH 6–7) was exposed to air via a needle to get rid of any excess reactive elements. Subsequently, a 0.25-mg dose of ICG (ICG-Pulsion, Pulsion Medical Systems, Munich, Germany) was added to form hybrid ICG-$^{99m}$Tc-nanocolloid as described previously. Next, 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 preparations were performed under good manufacturing practices and with approval of The Netherlands Cancer Institute’s pharmacist.

Preoperative procedure and image analysis
To determine the concordance between the lymphatic drainage patterns of ICG-$^{99m}$Tc-nanocolloid and $^{99m}$Tc-nanocolloid, both tracers were injected in consecutive order in the same patients, and the lymphoscintigraphic findings of both tracers were directly compared. First, $^{99m}$Tc-nanocolloid was injected, in a volume of 0.4 mL containing 0.05 mg human serum albumin. Before tracer injection, the planned sites of injection were carefully marked with an indelible felt-tip pen (Fig. 1). In the melanoma patients, 4 injections were placed intradermally around the scar of the primary melanoma excision. For penile carcinoma, the same dosage was intradermally administered divided in 3 injections proximally around the tumor. The mean radioactivity dose of the first injection calculated on the basis of net administered doses was 71 MBq (range 54–88 MBq). Immediately after injection, anterior and lateral dynamic images were obtained with a dual-head gamma camera (Symbia T; Siemens, Erlangen, Germany) over 10 minutes to visualize the lymphatic flow and to identify lymph nodes on a direct lymphatic drainage pathway. Subsequently, static planar images were acquired at 15 minutes. Two hours after injection of $^{99m}$Tc-nanocolloid, delayed planar images were obtained to further differentiate first-echelon nodes from higher-echelon nodes and to identify SNs in other basins. In the same session, SPECT/CT (Symbia T; Siemens) was performed. The lymph
nodes draining directly from the injection site were classified as SNs. When there were multiple visible nodes without visible afferent vessels, the first node appearing in the basin was considered to be the SN.

The same afternoon (10 patients: 1-day protocol), or the next morning (15 patients: 2-day protocol), the complete scintigraphic sequence was repeated after injection of hybrid ICG-99mTc-nanocolloid. Shortly before the injection, a 5-minute static image was obtained as a point of reference for the second injection in 6 patients.

Hybrid tracer administration was then performed at the locations previously marked on the skin, by the same nuclear physician, using a similar tracer concentration (0.05 mg human serum albumin in 0.4 mL, mean 74 MBq, range 57–98 MBq). The mean interval between the 2 injections was 19 hours (median 21, range 2.5–24 hours, Table 1). Paired images of both injections were evaluated with regard to similarity of the depicted draining lymph node basins and the location and number of the SNs. Count rates (maximum counts per pixel) were measured from the planar anterior images at 2 hours after each injection and the reference images before the second injection using regions of interest drawn around the SN(s). Trendline-based linear regression correlations (Excel; Microsoft) were used to establish the correlation between the radioactive count rates of both scintigraphic studies in patients with more than 2 preoperatively identified SNs.

**Intraoperative procedure**

Shortly before surgery, 1.0 mL patent blue dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected in all patients except those with melanoma located on the face to prevent nonesthetic long-lasting skin marks by the blue dye. Next, a portable gamma camera (Sentinella; Oncovision, Valencia, Spain) was used to guide the skin incisions and to obtain a pre-excision overview image of the SNs. Intraoperatively, the SNs were firstly pursued using the acoustic guidance provided by a gamma ray detection probe (Neoprobe, Johnson & Johnson Medical, Hamburg, Germany). During this initial exploration, attempts were made to optically detect the SNs via the blue dye (by eye) and the hybrid tracer’s fluorescent component (ICG) using a hand-held near-infrared fluorescence camera (PDE; Hamamatsu). After excision of each SN, a post-excision image was acquired with the portable gamma camera to ensure its complete removal.

**Ex vivo analyses and histopathological examination**

Excised lymph nodes were postoperatively analyzed for the presence of a radioactive or fluorescent signal using the portable gamma camera and an ex vivo fluorescence camera system (IVIS 200; Caliper Lifesciences), respectively. All harvested nodes were fixed in formalin, bisected, embedded in paraffin, and cut at a minimum of 6 levels at 50 to 150-μm intervals. Pathological evaluation included haematoxylin-eosin and immunohistochemical staining (Anti-cytokeratin; CAM 5.2; Becton Dickinson).
RESULTS

Preoperative image analysis

Lymphoscintigraphy and SPECT/CT after injection of $^{99m}$Tc-nanocolloid showed at least 1 SN in all 25 patients. The conventional lymphoscintigrams depicted 65 SNs, of which 89% were visualized on the early static planar images. SPECT/CT showed the anatomic location of these nodes and revealed 1 additional SN that was not visualized on the conventional lymphoscintigrams. These 66 SNs were distributed over 51 nodal basins (Table 1; average 2.6 SNs per patient). In the 10 patients with a melanoma in the head and neck region, 25 SNs were observed in various neck lymph node basins ($n=17$), the parotid gland ($n=2$), the submandibular region ($n=2$), and the suboccipital region ($n=3$). The 6 patients with a melanoma on the trunk had 14 SNs in nine axillae and groin ($n=2$). In the 9 patients with penile carcinoma, 27 SNs were visualized in the groin ($n=16$).

The second scintigraphic study using ICG-$^{99m}$Tc-nanocolloid yielded an identical drainage pattern, with the same number of SNs in the corresponding nodal basins for all patients (Table 1; Figs. 1-3). A comparison of the radioactive count rates in the individual SNs at 2 hours after both injections revealed a high intensity correlation ($R^2$) of 0.85 ± 0.20 for the patients injected twice on the same day (1-day protocol) and 0.83 ± 0.19 for the patients within the 2-day protocol (Table 1). In the 1-day protocol, radioactive count rates measured 2 hours after the second injection (ICG-$^{99m}$Tc-nanocolloid) were higher than count rates measured 2 hours after the first injection ($^{99m}$Tc-nanocolloid) in 90% of the SNs. For the 2-day protocol, in which more of the original activity has decayed at the time of the second injection, this was 66%. Furthermore, in the 6 patients in whom a reference image had been acquired shortly before the second scintigraphic procedure (both 1- and 2-day protocols), an increased radioactive count rate was found in each SN 2 hours after the second injection (average 85%, range 47-96%). This indicates that in both the 1- and 2-day protocol, ICG-$^{99m}$Tc-nanocolloid drained to the very SNs identified during the first scintigraphic study (using $^{99m}$Tc-nanocolloid).

Intraoperative results

On average, SN biopsy started 6 hours after injection of the hybrid tracer (range 4–23 hours, median 5 hours, Table 2). Image acquisition using the portable gamma camera ranged between 20 seconds and 1 minute. Near-infrared fluorescence imaging using the hand-held fluorescence camera was performed in real-time. Intraoperatively, all scintigraphically visualized SNs could be localized and excised using radio- and fluorescence guidance combined. In 6 patients, post-excision imaging with the portable gamma camera identified considerable residual radioactivity at the original location of the SN, resulting in the pursuit and removal of 13 additional SNs. Of the total of 79 SNs, 74 (94%) could be localized using the gamma ray detection probe. The remaining 5 nodes were identified using the fluorescence
camera (Table 2). Three of these SNs concerned melanomas in the temporal region and were located in front of the ear. Although these SNs could be distinguished using the portable gamma camera, the overwhelming radioactive signal from the nearby injection site hampered probe guidance. Two SNs in the inguinal region in penile carcinoma patients were difficult to localize using the probe and portable gamma camera because of the low radioactive count rate in the SNs compared with the high background.

A total of 75 SNs (95%) could be visualized by fluorescence imaging after initial surgical exploration (exemplified in Fig. 4), whereas only 54% of the SNs were stained when patent blue was used (21 patients, Table 2). The 4 SNs that eluded fluorescence imaging were probably not sufficiently exposed to enable detection of the fluorescent signal. Nevertheless, a fluorescent signal could be visualized in all radioactive SNs (including the above 4) ex vivo using the more sensitive IVIS fluorescence imaging system. In addition, none of the excised nodes exclusively contained fluorescence. This underlines the stability of the ICG-99mTc-nanocolloid complex.

Histopathological examination revealed metastases in 6 excised SNs in 4 patients (Table 2). The use of ICG-99mTc-nanocolloid was not associated with adverse reactions.

**DISCUSSION**

This study showed that the hybrid radioactive and fluorescent tracer ICG-99mTc-nanocolloid has the same lymphatic drainage pattern as 99mTc-nanocolloid, the current standard radiopharmaceutical in most European countries. Lymphoscintigraphy and SPECT/CT after injection of ICG-99mTc-nanocolloid did not reveal any SNs at other locations, and all preoperatively identified SNs were found to contain ICG after excision. This confirms that ICG-99mTc-nanocolloid drains to the same SNs as 99mTc-nanocolloid and accumulates in the SNs accordingly. The high correlation between count rates measured in the SNs on the lymphoscintigrams 2 hours after each tracer injection in both the 1-day and 2-day protocol further substantiates these findings. Combined with the absence of adverse reactions, these findings validate the use of ICG-99mTc-nanocolloid as a tracer for preoperative lymphatic mapping and SN identification.

The reproducibility of lymphoscintigraphy with 99mTc-nanocolloid when performed twice has been studied by us in the past for penile carcinoma and melanoma. Although our present results are in agreement with the 100% reproducibility rate found for penile carcinoma, a discordance in lymphoscintigraphy results after the second scintigraphic study could have been anticipated for melanoma, as Kapteijn et al. found a reproducibility rate of 88% for lymphoscintigraphy repeated after 2-4 weeks in melanoma patients. A possible explanation for the high reproducibility observed in the current study might lie in the significantly shorter interval between both injections. The placement of the second injection at the exact same locations
previously marked on the skin is also likely to have had a positive influence on the high reproducibility rate found in the current study (Fig. 1).

In recent years, the fluorescent dye ICG has been evaluated as a single agent for intraoperative lymphatic mapping and SN identification. Like vital blue dyes, ICG is a small-particle organic dye and migrates quickly through the lymphatic system, resulting in a relatively short detection window after injection and the necessity for careful timing to intraoperatively identify the SNs. In practice, this may require the use of 50-fold higher ICG dosages than the one used in the current study. Moreover, rather than visualizing lymph flow with fluorescence imaging, the hybrid approach individually illuminates the very nodes identified on lymphoscintigrams and SPECT/CT. The high rate of fluorescent SNs visualized in the operating room (95%) compared with the relatively low percentage of SNs that were stained blue (54%), underline how the fluorescent extension provided by ICG-\(^{99m}\)Tc-nanocolloid can improve intraoperative visualization of the SNs. On top of this, the time window for fluorescent detection of the SNs was extended up to 23 hours post-injection in the current study. The improved tissue penetration that fluorescence imaging offers over vital blue dyes and the high resolution that can be obtained compared with gamma-tracing modalities help enhance optical identification of the SNs. This advantage proved to be especially helpful when high radioactive background signals impeded SN localization using the probe, in accordance with previous findings in patients with head and neck melanoma or prostate cancer.

In the present study, the application of hybrid ICG-\(^{99m}\)Tc-nanocolloid was successfully extended to anatomic areas such as the axilla and the groin. This success encourages further extension of this technique to other areas where radioguided surgery can be challenging. The introduction of hybrid tracers also poses new technological challenges for manufacturers of imaging systems. In the current study, separate devices for radioguided and optical SN detection were used. In the future, the development of hybrid devices combining the 2 techniques may further improve the logistics in daily clinical practice.

**CONCLUSION**

The lymphatic drainage pattern of hybrid ICG-\(^{99m}\)Tc-nanocolloid is identical to that of \(^{99m}\)Tc-nanocolloid, and no adverse reactions were observed. These findings, together with the added value of intraoperative fluorescence guidance, warrant further evaluation of hybrid ICG-\(^{99m}\)Tc-nanocolloid as a tracer for SN procedures.
This research is supported, in part, by a KWF-translational research award (Grant No. PGF 2009-4344) and a NWO VIDI-Grant (STW BGT 11271). We would like to thank the melanoma surgeons, head and neck surgeons, urologists, operation department practitioners, and the technicians of the nuclear medicine department for their contribution. We also gratefully acknowledge Professor Theo J.M. Ruers for kindly providing the fluorescence camera system.

FIGURES

FIGURE 1. Comparison of the lymphatic drainage pattern of 99mTc-nanocolloid and ICG-99mTc-nanocolloid in a patient with a melanoma on the right posterior flank. (A) Firstly 99mTc-nanocolloid was injected after the planned sites of injection were carefully marked with an indelible felt-tip pen. (B) Planar lymphoscintigram two hours post injection shows 4 deposits at the injection site (1) and 2 SNs (SN), 1 in each axilla. (C) Axial SPECT/CT image revealing the anatomic information for both SNs. (D) Same patient was injected with ICG-99mTc-nanocolloid 24 hours later at previously marked locations. (E) Planar lymphoscintigram 2 hours post injection reveals the same 2 axillary SNs. (F) Axial SPECT/CT image confirms that both SNs are located at exactly the same locations compared to the first scintigraphic study using 99mTc-nanocolloid.

FIGURE 2. Comparison of the lymphatic drainage pattern of 99mTc-nanocolloid and ICG-99mTc-nanocolloid in a patient with a melanoma on the parietal scalp. (A) Planar lymphoscintigram 2 hours after injection of 99mTc-nanocolloid showing 2 SNs (arrows) in the left neck region with second-echelon activity in caudal direction. (B) 3D volume-rendered SPECT/CT image revealing the 2 SNs (arrows) in level V of the left side of the neck and a second-echelon node located more caudally. (C) 3D volume-rendered SPECT/CT image reveling a SN in level V on the right side of the neck (which was also visible on the anterior planar image, not shown). (D) Planar lymphoscintigram 2 hours after injection of ICG-99mTc-nanocolloid (23 hours after the 99mTc-nanocolloid injection) showing the same 2 SNs on the left side, with increased higher-echelon activity. (E) 3D volume-rendered SPECT/CT image showing the same SNs at the same location compared to the first scintigraphic study, with more notable higher-echelon activity. (F) Injection of ICG-99mTc-nanocolloid also led to the identification of the same SN on the right side, as seen on the 3D volume-rendered SPECT/CT image.
FIGURE 3. Comparison of the lymphatic drainage pattern of 99mTc-nanocolloid and ICG-99mTc-nanocolloid in a patient with penile carcinoma. (A) Planar lymphoscintigram 2 hours after injection of 99mTc-nanocolloid showing drainage to 3 SNs in the left inguinal region (arrows). (B) The next morning, the residual image before ICG-99mTc-nanocolloid injection shows decreased activity in the 3 nodes due to radioactive decay. (C) Planar lymphoscintigram 2 hours after injection of ICG-99mTc-nanocolloid shows drainage to the same 3 SNs in the left inguinal region (later confirmed by SPECT/CT).

FIGURE 4. Combined intraoperative radio- and fluorescence-guided SN biopsy in a head and neck melanoma patient. (A) After initial exploration guided by the gamma probe and portable gamma camera, the hand-held fluorescence camera is used to visualize the SN. (B) Near-infrared fluorescence image shows the exact location and margins of an infraauricular SN. (C) The portable gamma camera is used to make a pre- and post-excision image of the radioactive SNs. (D) By comparing the pre-excision (left) and post-excision (right) image on the screen of the portable gamma camera, the surgeon can confirm successful removal of each SN (arrow) in the operating room.
Table 1. Patient characteristics and lymphoscintigraphy results

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<td>72</td>
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<td>65</td>
<td>M</td>
<td>Penile carcinoma</td>
<td>2.5</td>
<td>83</td>
<td>2</td>
<td>2</td>
<td>74</td>
<td>2</td>
<td>2</td>
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</tr>
</tbody>
</table>

* = Correlation between count rates measured two hours after each injection could only be calculated in patients with > 2 SNs. Study 1 = First examination, using 99mTc-nanocolloid; study 2 = Second examination, using ICG-99mTc-nanocolloid; R² = correlation of sentinel node count rates in both studies
Table 2. Intraoperative sentinel node and pathology results

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Patients (n)</th>
<th>SNs preoperatively identified with SPECT/CT (n)</th>
<th>Injection 2* to operation (hours)</th>
<th>Additional SNs found with portable gamma camera (n)</th>
<th>Excised SNs (n)</th>
<th>SNs localized with probe</th>
<th>Blue SNs</th>
<th>intraoperatively fluorescent SNs</th>
<th>SN metastases (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma, trunk</td>
<td>6</td>
<td>14</td>
<td>4 – 23</td>
<td>1</td>
<td>15</td>
<td>100% (15/15)</td>
<td>74% (11/15)</td>
<td>93% (14/15)</td>
<td>1</td>
</tr>
<tr>
<td>Melanoma, head/neck</td>
<td>10</td>
<td>25</td>
<td>4 – 6</td>
<td>2</td>
<td>27</td>
<td>89% (24/27)</td>
<td>37% (7/19)**</td>
<td>93% (25/27)</td>
<td>0</td>
</tr>
<tr>
<td>Penile carcinoma</td>
<td>9</td>
<td>27</td>
<td>4 – 7.5</td>
<td>10</td>
<td>37</td>
<td>95% (35/37)</td>
<td>54% (20/37)</td>
<td>97% (36/37)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>66</td>
<td>4 – 23</td>
<td>13</td>
<td>79</td>
<td>94%</td>
<td>54%</td>
<td>95%</td>
<td>6</td>
</tr>
</tbody>
</table>

* Injection of hybrid ICG-99mTc-nanocolloid
** Patent blue was injected in 6 patients with head and neck melanoma outside facial area (19 sentinel nodes)
REFERENCES


