Added value of the hybrid tracer indocyanine green-99mTc-nanocolloid for sentinel node biopsy in a series of patients with different lymphatic drainage patterns

L. Frontado M, O.R. Brouwer, N.S. van den Berg, H.M. Mathéron, S. Vidal-Sicart, F.W.B. van Leeuwen, R.A. Valdés Olmos

Servicio de Medicina Nuclear, Hospital Dr. Peset, Valencia, Spain
Department of Nuclear Medicine, Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands
Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands
Servicio de Medicina Nuclear, Hospital Clinic, Barcelona, Spain

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A B S T R A C T
Introduction: Indocyanine green (ICG)-99mTc-nanocolloid is a novel hybrid fluorescent radioactive tracer for sentinel node (SN) biopsy. This study has aimed to evaluate the added value of this novel versatile tracer in a series of patients with different malignancies.

Material and methods: Twenty patients (with penile carcinoma, oral cavity tumors, melanoma) were consecutively included between March and May 2012. Planar lymphoscintigraphy was performed 15 min and 2 h after injection of ICG-99mTc-nanocolloid followed by SPECT/CT. Blue dye (1 ml) was injected in 14 patients in surgery room. Intraoperatively, SNs were localized using a gamma probe and visualized by optical SN-detection using blue dye and fluorescence imaging. Finally, a portable gamma camera was used to confirm complete SN removal.

Results: At least one SN was identified by SPECT/CT in all patients. All SNs (total 68, 100%) were excised using a combination of radio- and fluorescence guidance: 89.7% were intraoperatively localized with the gamma probe. The remaining SNs, located near the injection site, were localized using fluorescence imaging. During the surgery, 97% of the SNs were fluorescent while only 39.2% were stained blue. Ex vivo, all SNs were both radioactive and fluorescent. The SN was positive in 5 patients.

Conclusion: Synchronous radio- and fluorescence guided SN biopsy is feasible using ICG-99mTc-nanocolloid. This hybrid approach combines the beneficial properties of both modalities. Adding fluorescence imaging improves optical SN detection compared to blue dye. It has been shown to be especially useful in the localization of SNs near the injection site.

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Valor añadido del trazador híbrido verde de indocianina-99mTc-nanocoloide para la biopsia del ganglio centinela en una serie de pacientes con drenaje en diferentes territorios anatómicos

R E S U M E N
Introducción: El ICG (verde de indocianina)-99mTc-nanocoloide es un novedoso trazador híbrido radiactivo y fluorescente para la biopsia selectiva del ganglio centinela (BSGC). Nuestro objetivo fue demostrar el valor añadido de este trazador en una serie de pacientes con diferentes neoplasias.

Material y método: Se incluyeron consecutivamente 20 pacientes (con carcinoma de pene, cavidad oral y melanoma) entre Marzo-Mayo 2012. A todos se les realizó una gammagrafía planar a los 15 y 120 minutos tras la inyección de ICG-99mTc-nanocoloide, posteriormente un SPECT/TAC. En quirófano, se inyectó 1 ml de colorante vital (blue dye) a 14 pacientes. Durante la cirugía, los GCs fueron localizados utilizando la sonda gammadetectora, y visualizados por fluorescencia y blue dye. Finalmente, se confirmó la extirpación completa de los GCs con la gammacámara portátil.

Resultados: Mediante SPECT/TAC se identificó al menos un GC por paciente. Todos los GC (total: 68, 100%) fueron extirpados utilizando la combinación de guía radio-fluorescente: 89.7% se localizaron con la sonda gammadetectora. Los ganglios restantes, situados cerca del punto de inyección, fueron ubicados por fluorescencia. Durante la cirugía, 97% del total de GCs fueron fluorescentes y sólo el 39,2% azules. Ex vivo, todos los GC fueron radioactivos y fluorescentes. En 5 pacientes el GC fue metastásico.

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Introduction

Sentinel lymph node (SLN) biopsy is based on the theory that lymphatic extension in solid tumors is produced in a stepwise manner, and that the SLN presents a direct via of drainage from the primary tumor and is, therefore, the first to present metastatic involvement. At present, the use of SLN biopsy is frequent in breast carcinoma,1 melanoma2 and in carcinoma of the penis.3 Its importance lies in that it allows early patient staging by minimally invasive surgery and with lesser morbidity than lymphadenectomy.4

The standard procedure in most of the countries in Europe consists of the injection of a radiotracer (99mTc-nanocolloid) and the acquisition of dynamic planar and static images for the identification of the SLN. SPECT/CT is of great utility in this phase since it provides anatomical information which facilitates the surgical approach of the SLN and, in some cases, may detect lymph nodes not visible in the planar images.5

In superficial tumors intraoperative identification of the SLN is usually based on the combination of the acoustic signal of the gamma detector probe and the visual information provided by the perilesional injection of isosulfan blue (vital stain) minutes before initiating surgery. Both techniques have limitations since the gamma detector probe does not provide visual information, and in some anatomical zones it may be difficult to distinguish the radiation of the SLN from that of the injection point if the two are very close together. The vital stain is one of the most commonly used agents since it allows identification of the SLN through visualization of its afferent lymphatic vessels. However, its diffusion is rapid to second lymphatic level, and thus, lymph nodes which are not the SLN may be removed or some lymph nodes may have lost their staining at the time of surgery.6 Additionally, there is the risk of prolonged skin pigmentation,7 and it may affect the visualization of the surgical field, thereby limiting its use in some neoplasms.8,9

The portable gamma camera, Sentinella (Onconvision, Valencia, Spain), has recently been incorporated in radioguided surgery, providing real-time images of the radioactive lymph nodes with greater spatial resolution, albeit with limited anatomical information.10

With the aim of improving the localization of the SLN, fluorescence has been introduced in radioguided surgery since it provides real-time images of the SLN without affecting the surgical field. Indocyanine green (ICG) is a fluorescent agent with a short infrared wave that is currently used in the study of the lymphatic distribution of several tumors such as those of the breast, colon and the stomach.12,13 Although this agent has the advantage of being invisible to the human eye similar to vital staining, it has rapid migration in the lymphatic system toward the second level.14

The hybrid tracer, ICG-99mTc-nanocolloid, combines the advantages of both techniques allowing presurgical images of the SLN and increasing the time of permanence of ICG in the lymphatic system to obtain intraoperative images of the SLN and its anatomical relationships. It has recently been demonstrated that this hybrid tracer presents the same distribution as 99mTc-nanocolloid alone which means that the association with ICG does not affect its physicochemical characteristics.15

The aim of this study was to demonstrate the added value of the hybrid tracer ICG-99mTc-nanocolloid in selective biopsy of the SLN, describing a series of patients with different diseases (melanoma, carcinoma of the penis and squamous cell carcinoma of the oral cavity) and drainages in different anatomical zones as well as comparison with vital staining in the cases in which it was used.

Materials and methods

Patients

We included 20 patients (15 men and 5 women) with different diseases and with a mean age of 59 years (38–77 years) who were consecutively attended from March to May 2012 in the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (NKI-AVL) for selective biopsy of the SLN: 5 patients with carcinoma of the penis, 3 with squamous cells carcinoma of the oral cavity and 12 with melanoma (6 on the trunk, 5 on the head and neck and one in the lower right limb).

All the patients were clinically staged as N0 (with no clinical, echographic or cytologic evidence of lymph node metastasis) at the time of undergoing the SLN biopsy. The study protocol was authorized by the Medical Ethical Committee of the NKI-AVL, and informed consent was obtained from all the patients.

Tracer preparation

The 99mTc-nanocolloid was prepared aggregating 2 ml of pertechnetate (approximately 1400 MBq) to the commercial vial of nanocolloid containing 0.5 mg of albumin colloids (GE Healthcare, Eindhoven, The Netherlands). After 30 min of incubation at room temperature the solution of 99mTc-nanocolloid (pH 6–7) was exposed to air by the introduction of a needle to eliminate any excess reactive material.

A solution of ICG (ICG-Pulsion, Pulsion Medical Systems, Munich, Germany) was previously prepared dissolving 25 mg of solid ICG in 5 ml of sterile water (vial concentration: 5 mg/ml). At present, intravenous administration of ICG is authorized for diagnostic purposes at a maximum dose of 25 mg/kg. In this study we only used 0.25 mg which were finally aggregated to the 99mTc-nanocolloid and the control of quality of the compound was performed determining the color, the clarity and the pH (colorless, transparent, pH 6–7, respectively).

Presurgical procedure and image analysis

All the patients received a mean dose of 79.8 MBq (range from 63 to 90.5 MBq) in 0.4 ml. Injection was intradermic distributed in 3 or 4 points in the margins of the postresection scar of the primary lesion in cases of melanoma or in 3 points around the tumor in cases of squamous cell carcinoma of the head and neck and penile cancer. The injection was submucosal in the 3 patients with carcinoma of the oral cavity.

Anterior and lateral dynamic images were obtained during 10 min immediately after the administration of the radiotracer, and planar images were obtained at 15 min and at 2 h. Thereafter, a SPECT/CT (Symbia T, Siemens, Erlangen, Germany) was performed. In the lymphoscintigraphy the SLN was identified as lymph nodes presenting a direct via of drainage from the site of the injection6 or those which appeared as single lymph nodes in a drainage station.16 Lymph nodes appearing between the injection site and the first drainage lymph node as well as those of increasing intensity at a determined station were also considered as highly
probable of being SLN. Following fusion of the SPECT/CT images the lymph nodes defined as additional SLN were those localized in other stations and were superposed in the planar image. Finally, their projection was indicated on the skin with a waterproof marker and guided by a barium radioactive source with the aim of guiding the placement of the portable gamma camera during surgery.

In 12 patients the surgery was performed the same day as the injection of the tracer, and in the remaining 8 patients it was undertaken the following day, within a maximum interval of 26 h.

Surgical procedure

During the preparation of the patients in the operating room, intradermal administration of 1 ml of vital stain (Laboratoire Guerbet, Aulnay-sous-Bois, France) at the margins of the primary lesions was performed, except in patients with tumors of the oral cavity to avoid affecting the visualization of the surgical field and because of the risk of skin pigmentation in patients with melanoma of the head or neck. Injection of the vital stain was performed in 2 patients with melanoma because the lesions were on the scalp.

An image was obtained with the portable gamma camera (Sentinel, Oncovision, Valencia, Spain) to identify the SLM in the surgical bed and decide the incision site. On initiation of the surgery the gamma detector probe (Neoprobe, Johnson & Johnson Medical, Germany) was used for guidance and the images were obtained with a portable infrared ray camera (Photodynamic eye, Hamamatsu Photonics, Hamamatsu, Japan) for the localization and resection of the SLN. A visual examination was also made of the lymph nodes stained blue, and it was verified whether these coincided with those which were radiolabeled. After the excision of each lymph node a new image was obtained with the portable gamma camera to check with the image of preexcision and confirm the absence of activity in the surgical bed. Examination and resection of other lymph nodes in the same zone was performed in the cases with remnant activity (Fig. 1).

Immediately following surgery the lymph nodes extracted were analyzed with the gamma camera to verify their radioactivity and with the infrared ray camera to verify their fluorescence. All the lymph nodes were fixed in formalin, embedded in paraffin and cut into at least 6 levels of between 50 and 150 µm. The anatomopathological evaluation included staining with hematoxylin–eosin and immunohistochemical analysis (Anti-cytokeratin: CAM 5.2; Becton Dickinson, San José, CA, USA).

Results

Table 1 shows the characteristics of each patient as well as the preoperative findings. No local or systemic allergic reaction or secondary effects were observed after the administration of the hybrid tracer.

Preoperative image

A total of 48 SLN were visualized in the presurgical lymphoscintigraphy, with at least one per patient. In addition, the SPECT/CT visualized 7 lymph nodes that could not be identified with conventional lymphoscintigraphy (total: 55 SLN) (Fig. 2). In patient 1, the 2 lymph nodes visualized in the lymphoscintigraphy corresponded to small groups of lymph nodes in the SPECT/CT; all being at the left cervical level ii (Fig. 3). In patient 2, with melanoma in the medial parietal part of the scalp, the SPECT/CT visualized 2 SLN in the right retroauricular region which were poorly visible in the lymphoscintigraphy. In patient 14 (with carcinoma of the penis) the SPECT/CT confirmed that the focal deposits visualized in both groins by lymphoscintigraphy were due to 2 lymph nodes each. In patient 17 with carcinoma of the penis, a SLN was identified in each groin and with SPECT/CT another lymph node was found in the left groin and was also considered as a SLN.

Intraoperative findings

Table 2 shows the intraoperative findings.

During surgery a total of 68 SLN (mean of 3 per person) were removed. All SLN had been presurgically visualized by lymphoscintigraphy and SPECT/CT. All (100%) were localized using the combination of the radioactive signal and fluorescence (possible thanks to the hybrid tracer ICG-99mTc-nanocolloid); 89.7% (61 SLN) were localized with the gamma detector probe and 97% (66 SLN) were seen with the infrared ray camera: in 2 cases (patients 6 and 8) one lymph node did not show fluorescence in vivo, despite showing fluorescence ex vivo. In these patients the abundant subcutaneous or fat tissue around the lymph nodes may have attenuated the signal of the fluorescence in the surgical field.

In the 14 patients in whom the vital stain was used, 20 lymph nodes (39.2%) were stained blue (head and neck melanoma: 16.6%, melanoma of the thorax: 55.5%, melanoma of the lower limb: 33.3%, and penile cancer: 15.8%). In 3 patients (7, 15 and 16) no lymph node was found to be stained blue during surgery, although all the lymph nodes resected were radioactive and fluorescent.

Twenty lymph nodes were extirpated in addition to those visualized in the preoperative phase. This difference is explained in that if persistent activity was observed on acquisition of the post resection image with the SLN, the area was examined for the excision of other lymph nodes in the same area as the SLN (13 additional SLN). On the other hand, in some cases the anatomopathological
### Table 1
Characteristics of the patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Genre</th>
<th>Primary tumor</th>
<th>Localization</th>
<th>T injection/surgery (h)</th>
<th>Dose (MBq)</th>
<th>SLN 1st lympho.</th>
<th>SLN 2nd lympho.</th>
<th>SLN SPECT/CT</th>
<th>Localization in the SPECT/CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>M</td>
<td>Melanoma of the head</td>
<td>Pavilion left auricular</td>
<td>4</td>
<td>88.21</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>Nivel ii (parotidean cell) and level iii</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>M</td>
<td>Melanoma of the head</td>
<td>Scalp (left parietal)</td>
<td>5</td>
<td>71.64</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>Left retroauricular (4 SLN), Right retroauricular and left level v</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>F</td>
<td>Melanoma of the head</td>
<td>Scalp (right parietal)</td>
<td>4</td>
<td>70.58</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>Level ii A preauricular SLN and a SLN at level ii (parotidean cell)</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>M</td>
<td>Melanoma of the head</td>
<td>Right suprachilear</td>
<td>18</td>
<td>84.21</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Left retroauricular and left level v</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>M</td>
<td>Melanoma of the head</td>
<td>Right retroauricular</td>
<td>5</td>
<td>72.01</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>Right axilla</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>F</td>
<td>Melanoma of the thorax</td>
<td>Interscapular</td>
<td>21</td>
<td>73.62</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>Bilateral axillaries</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>F</td>
<td>Melanoma of the thorax</td>
<td>Right scapular</td>
<td>4.5</td>
<td>80.98</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Bilateral axilla and one subcapsular</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>M</td>
<td>Melanoma of the thorax</td>
<td>Right interscapular</td>
<td>25</td>
<td>78.13</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>Bilateral axilla and one subcapsular</td>
</tr>
<tr>
<td>9</td>
<td>77</td>
<td>M</td>
<td>Melanoma of the thorax</td>
<td>Left parascerular</td>
<td>26</td>
<td>76.55</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>Bilateral axilla and one subcapsular</td>
</tr>
<tr>
<td>10</td>
<td>59</td>
<td>M</td>
<td>Melanoma of the thorax</td>
<td>Left supraclavicular</td>
<td>3.5</td>
<td>70.08</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Bilateral axilla and one subcapsular</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
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<td>Melanoma of the thorax</td>
<td>Right interscapular</td>
<td>17.5</td>
<td>63.37</td>
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<td>12</td>
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<td>Melanoma of the lower limb</td>
<td>Anterior face of the right thigh</td>
<td>4.5</td>
<td>78.6</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>Bilateral axilla and one subcapsular</td>
</tr>
<tr>
<td>13</td>
<td>76</td>
<td>M</td>
<td>Carcinoma of the penis</td>
<td>Inferior face of the right thigh</td>
<td>23</td>
<td>81.74</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>Bilateral axilla and one subcapsular</td>
</tr>
<tr>
<td>14</td>
<td>38</td>
<td>M</td>
<td>Carcinoma of the penis</td>
<td></td>
<td>23</td>
<td>86.99</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>Bilateral inguinal (2 SLN on each side)</td>
</tr>
<tr>
<td>15</td>
<td>65</td>
<td>M</td>
<td>Carcinoma of the penis</td>
<td></td>
<td>24</td>
<td>90.57</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>Bilateral inguinal and one bilateral paravesical SLN</td>
</tr>
<tr>
<td>16</td>
<td>63</td>
<td>M</td>
<td>Carcinoma of the penis</td>
<td></td>
<td>6</td>
<td>82.79</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>Bilateral inguinal (2 SLN on each side)</td>
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<tr>
<td>17</td>
<td>75</td>
<td>M</td>
<td>Carcinoma of the penis</td>
<td></td>
<td>4.5</td>
<td>63.08</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>Bilateral inguinal (2 SLN on each side)</td>
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<tr>
<td>18</td>
<td>64</td>
<td>M</td>
<td>Carcinoma of the oral cavity</td>
<td>Tongue</td>
<td>5.5</td>
<td>89.11</td>
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<td>81.8</td>
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<td>86.5</td>
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<td>2</td>
<td>2</td>
<td>Bilateral inguinal (2 SLN on each side)</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td>48</td>
<td>55</td>
<td>204</td>
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</table>


results demonstrated more than one lymph node in the piece sent as a single SLN (7 additional SLN).

In 1 patient with carcinoma of the penis only one lymph node was localized by fluorescence due to the presence of little radioactivity, possibly because the surgery was performed 24 h after the injection (patient 15). In patients 4 and 5, both with melanoma on the head and the SLN at cervical level ii, the point of injection was very close to the SLN and was therefore not possible to locate with the probe. It was visualized with the portable gamma camera to make the incision, although without anatomical references, thereby making fluorescence very useful in the definitive localization of these SLN (Fig. 4).

In the ex vivo verification all the lymph nodes were radioactive and fluorescent. The definitive anatomopathology was positive for metastasis in 5 patients (patients 3, 4, 5, 11 and 20). Patients 4 and 5, both with melanoma on the head, presented one metastatic lymph node each at the right cervical level ii. This lymph node was, moreover, that which was localized thanks to fluorescence due to its proximity to the injection point.

Discussion

Review of this series of patients provides an in depth description of the technique of preparation and administration of a novel hybrid tracer developed in the NKI-AVL, combining fluorescence (ICG) with the radioactive signal ($^{99m}$Tc-nanocolloid). This hybrid tracer has shown to be effective in the selective biopsy of the SLN in several diseases such as head and neck tumors and melanoma.\textsuperscript{17,18} Its utility has also been demonstrated in the laparoscopic approach to the SLN in carcinoma of the prostate.\textsuperscript{19}

In the present study 97% of the SLN showed fluorescence in vivo. On comparison with the vital stain we found that our finding of only 39.2% of lymph nodes stained blue is similar to the results of other studies and confirms the contention that it should not be

Fig. 2. Injection of ICG-$^{99m}$Tc-nanocolloid allows localization of the SLN in different malignant tumors using scintigraphy and SPECT/CT: (A1) scintigraphy showed 2 SLN in patient 20 with carcinoma of the oral cavity (arrows). (A2) The SPECT/CT images reconstructed in 3D demonstrated that the 2 SLN were located at cervical levels ib and iii, respectively (arrows). (B1) Early scintigraphy showed right axillary drainage posterior to the injection of the hybrid tracer in a patient with melanoma in the interscapular region (arrows). (B2) In the SPECT/CT one of the lymph nodes was observed not to be axillary but rather corresponded to a lymph node in transit in the subscapular region (arrows). (C1) Early scintigraphy of patient 12 with melanoma in the inferior right extremity (T) in which 2 SLN were identified (arrows) by their early appearance, and a third SLN (more cranial arrow) was identified due to visualization of the lymphatic duct. (C2) The SPECT/CT confirmed the 3 SLN and moreover, demonstrated a second lymphatic level in the iliac region (arrow).

Fig. 3. Additional SLN in the SPECT/CT of patient 1: (A and B) scintigraphy of a patient with head/neck melanoma in which 2 left laterocervical SLN were visualized (arrows). (C and D) Images reconstructed in 3D and axial slices of the SPECT/CT showing a deposit of the radiotracer at cervical level ii and another at level iii. (E) In the corresponding axial slice of the CT these deposits were confirmed to correspond to 4 SLN (arrows).
Table 2
Intraoperative findings.

<table>
<thead>
<tr>
<th>Patient</th>
<th>SLN resected</th>
<th>Localized with probe</th>
<th>Blue</th>
<th>Fluorescent in vivo</th>
<th>Fluorescent ex vivo</th>
<th>SLN (+) for metastasis</th>
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X: vital stain not administered in patients with tumors of the oral cavity and in those with melanoma of the head and neck.

used only for the biopsy of the SLN, but rather should, preferentially, be combined with another more sensitive technique. On the other hand, it has been seen that drainage of the vital stain is not always visible in neoplasms of the head and neck, and its use is also limited by the risk of skin pigmentation and it may affect the margins of resection. In this study drainage was not observed in 2 patients with carcinoma of the penis. It remains to be established whether the application of vital stain in this type of neoplasm is useful. On comparing its sensitivity with that of the radiotracer, it has been reported in the literature that not all the radioactive lymph nodes are also blue (84%) and that the greatest sensitivity (99.8%) is obtained with the combination of the two techniques.

With respect to the $^{99m}$Tc-nanocolloid, the hybrid tracer has the advantage of allowing real time vision of each lymph node and its anatomical relationships. Moreover, the SLN, which is near the injection point, may be easily localized. This is especially useful in head and neck neoplasms in which the injection site and the SLN are usually very close, and the activity of the first make localization of the second difficult with the gamma detector probe and even with the portable gamma camera. This was evident in 2 patients with head and neck neoplasm in whom fluorescence allowed localization of 2 SLN located very close to the injection point (level ii) and which posteriorly were the only SLN positive for metastasis.

In regard to fluorescence, the use of ICG as a single tracer has been proposed for selective biopsy of the SLN. However, it should be taken into account that one of its disadvantages is its rapid passage through the lymphatic system (similar to the vital stain) as well as the fact that no presurgical image of the drainage site of the SLN is provided. In this aspect, lymphoscintigraphy is essential to determine the drainage map of the SLN, to detect anomalous drainages and know its precise localization prior to surgery. Another point that should be taken into account is that the tissue adjacent to the fluorescent lymph node may attenuate the signal, making its visualization difficult. This has been reported in angiographic studies with ICG in which the signal of the fluorescent vessel was completely attenuated by blood clots and other adjacent tissue. In the present study this attenuation was observed during surgery in 2 patients. In one of these patients the SLN was located only because of its radioactive activity, despite confirming that it was also fluorescent ex vivo.

The hybrid tracer (ICG-$^{99m}$Tc-nanocolloid) used in this study combined the advantages of both techniques allowing the lymphatic drainage of the patient to be individualized through attainment of presurgical planar and the SPECT/CT images with the possibility of using the portable infrared ray camera to visualize the SLN up to the day after the injection of the radiotracer. This was due to persistence of the ICG in the lymph nodes because of its combination with the $^{99m}$Tc-nanocolloid as shown in this study (up to 26 h). The production and implementation costs of this hybrid tracer are not as elevated since several doses of

**Fig. 4.** Added value of the fluorescent image in a patient with a SLN localized near the injection site: (A) delayed scintigraphy and reconstruction in 3D of the SPECT/CT images. (B) Following injection of the hybrid tracer around a melanoma in the retroauricular region (T) in which drainage was observed toward a SLN located at cervical level i and another SLN in the parotid gland. The latter was very difficult to distinguish from the injection point in the presurgical images (upper arrow); (C and D) During surgery the fluorescence allowed visualization and extirpation of the SLN (D, arrow) located near the injection point (T) where the elevated radiation impeded its localization with the gamma detector probe.
ICG-$^{99m}$Tc-nanocolloid may be obtained from one vial of ICG. A large variety of infrared ray cameras are currently marketed, being especially designed for application in surgery. The first models were very costly, but at present their price may be similar to that of a gamma detector probe.\textsuperscript{54}

**Conclusions**

The hybrid tracer ICG-$^{99m}$Tc-nanocolloid combines the advantages of the radioguide with those of fluorescence during surgery and presents the same biodistribution as the $^{99m}$Tc-nanocolloid without additional secondary effects.

The ICG is invisible to the human eye and provides real time images with anatomical relationships but without staining the surgical margins. However, it is visible at only a few centimeters of distance. For this reason the radioguide is indispensable for establishing the drainage map of the patient for presurgical identification of the SLN and to guide the placement of the fluorescence camera during surgery.

The advantages of combining fluorescence with the radiocolloid were more evident in the surgery of small anatomical regions with vital structures nearby in which the SLN is usually very close to the point of injection but also in those neoplasms with drainages in variable territories and in which the drainage of the vital stain is not always visualized.

**Conflict of interests**

The authors declare no conflict of interests.

**References**