Integrating new imaging modalities in breast cancer management
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Simultaneous use of an $^{125}$I-seed to guide tumour excision and $^{99m}$Tc-nanocolloid for sentinel node biopsy in non-palpable breast-conserving surgery

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Abstract

Purpose
In the present study we describe patients with non-palpable breast lesions, in which an $^{125}\text{I}$-iodine (or “seed”) for excision of the primary tumour and $^{99m}\text{Tc}$-nanocolloid (or SNB) are used simultaneously. The purpose was to investigate any interference between $^{125}\text{I}$-seeds and $^{99m}\text{Tc}$-nanocolloid by an in vitro and in vivo analysis.

Methods
Contrast/interference-ratios between $^{125}\text{I}$ and $^{99m}\text{Tc}$ count-rates were determined in vitro using a realistic simulation model. Measurements were performed with 3 gamma-probes with different crystal materials. In 25 consecutive patients $^{99m}\text{Tc}$-nanocolloid was intratumourally administered at the site of an $^{125}\text{I}$-seed previously implanted. Respectively the $^{125}\text{I}$-setting and $^{99m}\text{Tc}$-setting of the gamma-probe guided the wide local excision and SNB and maximum counts-per-second (cps) were measured.

Results
In vitro the different probes varied in $^{125}\text{I}$- and $^{99m}\text{Tc}$-sensitivity. The contrast-ratio between $^{125}\text{I}$ and $^{99m}\text{Tc}$ in the $^{125}\text{I}$-channel was 4.6 for a 3-month-old $^{125}\text{I}$-seed using the most appropriate gamma-probe. In vivo the gamma-probe in the $^{125}\text{I}$-setting measured a median of 16300 cps at the tumour site compared to 4820 cps using the $^{99m}\text{Tc}$-setting. The $^{125}\text{I}$-seed could be well distinguished from the $^{99m}\text{Tc}$-nanocolloid in 92% of the patients and 96% required a single operation. The SNB was successful in all patients.

Conclusions
Simultaneous use of $^{125}\text{I}$-seeds and $^{99m}\text{Tc}$-nanocolloid is possible under well-standardised conditions. Non-palpable breast lesions can be safely excised using the $^{125}\text{I}$-seed in combination with a SN procedure. Use of $^{125}\text{I}$-seeds is a next step within fine-tuning breast-conserving surgery that should lead to further investigation to confirm its value.
Introduction

The challenge of nowadays widely performed breast-conserving surgery is to achieve tumour-free margins while excising no more breast tissue than necessary. An incomplete resection is a risk factor for local recurrence and mortality and should be avoided whenever possible.[1–3] This issue especially concerns excising non-palpable breast lesions where the surgeon is dependent on a localisation technique.

Different localisation techniques are being used. Wire localisation is one of the first used guiding methods.[4] The wire, if inserted correctly, should indicate the centre of the lesion, and consequently does not point out the margins of the tumour. Unfortunately, dislocation of the wire has been often described. Radioguided occult lesion localisation (ROLL), the ROLL-technique, is another popular technique [5–7] based on the injection of $^{99m}$Technetium-albumin macro-aggregates for allocation of the breast lesion. $^{99m}$Technetium-nanocolloid ($^{99m}$Tc-nanocolloid) is injected into the centre of the tumour to visualise both the lymphatic drainage and localise the breast lesion (Sentinel Node and Occult Lesion Localisation (SNOLL)).[8] Both the wide local excision and the sentinel node biopsy (SNB) are guided by a handheld gamma-probe. The accuracy of injecting the radiopharmaceutical in the middle of the lesion determines its success. The radioactive depot at the tumour-site often exceeds the amount of tissue necessary to remove.

Ultrasound can also guide the breast lesion excision.[9,10] Unfortunately, not all non-palpable lesions, especially DCIS, are visible on ultrasound.[10]

Radioactive seed localisation (RSL) is an alternative localisation method to the techniques mentioned above.[11–14] An $^{125}$Iodine ($^{125}$I)-seed is preoperatively implanted in the centre of the lesion and during the operation the gamma-probe guides the local excision using the $^{125}$I-setting. This technique has some of the same limitations as the ROLL-technique. The seed has to be placed correctly and there is, also, a lack of indication of the lesion margins. However, the seed is a focused point source and does not diffuse into its surrounding. This enables the conservation of healthier breast tissue. Moreover, $^{125}$I-seeds are radiographically detectable and one always knows the exact placement of the seed in relation to the tumour by using routinely performed mammography.[15] (Figure 1)

However, the simultaneous use of RSL for tumour excision and $^{99m}$Tc-nanocolloid for SNB has not yet been published and for this purpose it is essential to know what extent the radioactive $^{99m}$Tc-labelled radiopharmaceutical depot interferes with the activity
emitted by the $^{125}$I-seed. It is known that scattered radiation from $^{99m}$Tc (peak 140 KeV) will be detected in the energy-window of $^{125}$I (peak 30 keV). This is due to the Compton effect where photons scatter in the breast tissue and turn into photons with lower energy contents.\[16\]

In the present study variable $^{125}$I-activities, tracer protocols, and 3 gamma-probes are evaluated in an in vitro study to analyse the interference between the two isotopes and thereby to make sure the tumour is excised based on the activity of the seed and not by the depot of $^{99m}$Tc-nanocolloid. Hereafter this topic is described in patients with non-palpable breast lesions in which both an $^{125}$I-seed and an injection of $^{99m}$Tc-nanocolloid are placed in the centre of the lesion for combined RSL and SNB.

![Figure 1: Mammogram with an implanted $^{125}$I-seed.](image-url)
Methods

In vitro study

The in vitro experiments were conducted to study the sensitivity and distinctive capacity between $^{125}$I and $^{99m}$Tc. Three gamma-probes (A, B, and C)\(^1\) were compared based on sensitivity for $^{125}$I and $^{99m}$Tc. Contrast-ratios in the $^{125}$I-setting between $^{125}$I and $^{99m}$Tc (formula: $^{125}$I-cps/$^{99m}$Tc-cps) at a 2cm simulated lesion depth were determined. In our institute a two-day protocol is used for SNB and the first day maximal 140MBq $^{99m}$Tc-nanocolloid are intratumorally administered.[17] To simulate combination of RSL with SNB an $^{125}$I-seed of 3 months old a 2.9MBq seed was used together with approximately 8MBq $^{99m}$Tc taking into account only the physical decay of $^{99m}$Tc in a two-day protocol.

Gamma-probe A is equipped with a Cadmium Zinc Telluride crystal. The wireless Bluetooth probe was not suitable to specifically measure $^{125}$I in a combined RSL-SNB procedure; as it measures a window with the lower energy at the normal $^{125}$I-setting but without an upper boundary so that all $^{99m}$Tc-signal is present in the $^{125}$I-channel. (Figure 2) Hence, this probe is not used and just the wired probe is evaluated in the current study. This gamma-probe control unit is able to simultaneous display $^{99m}$Tc and $^{125}$I-counts.

Gamma-probe B is equipped with separate gamma-probes for $^{99m}$Tc (Cesium Iodine) and for $^{125}$I (Silicon pin-diode). This gamma-probe has no options for simultaneous measurements and moreover requires changing the gamma-probe when switching from one isotope to the other.

Gamma-probe C uses a Lutetium Yttrium Siliciodioxide crystal and has the feature to correct the counts of $^{99m}$Tc in the $^{125}$I-window based on the Compton scatter elsewhere. Before the start of the procedure the amount of correction required. This gamma-probe control unit is able to simultaneous display $^{99m}$Tc and $^{125}$I-counts, as well as the scatter corrected counts.

To obtain better insight in the contribution of scatter energy spectra of the two isotopes, energy spectra were determined using the NanoSPECT (Mediso Medical Imaging Systems, Budapest Hungary) with a general-purpose collimator.

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\(^1\) A is the Neoprobe® (Johnson & Johnson Medical B.V., Hamburg, Germany), B is the Crystal probe (Crystal Photonics GmbH, Berlin, Germany), and C is the Node Seeker (Intramedical Imaging Llc Hawthorne, Canada). The Neoprobe and wired probe were used for the in vivo experiments.
In vivo study
All patients with non-palpable invasive breast cancer or carcinoma in situ between February and June 2012 at the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital were eligible for this study and prospectively included (n=25). The tumour size was assessed with mammography, ultrasound and with contrast-enhanced MRI. Nodal status was determined pre-operatively by ultrasound-guided fine-needle aspiration to exclude patients with tumour-positive lymph nodes. All patients in the study underwent wide local excision plus SNB.

An $^{125}$I-radiolabelled (STM1251, Bard Brachytherapy, Inc., Carol Stream, IL, USA), with a half-life time of 59.6 days, size of 4.5mm$\times$0.8mm, and maximal apparent activity of 8.2MBq, was pre-operatively implanted in the centre of the lesion using ultrasound or stereotactic guidance. In general one $^{125}$I-seed was used to mark the tumour, except for two patients with multifocality in whom three $^{125}$I-seeds were inserted to mark all lesions. After placement of the $^{125}$I-seed, a mammography was performed to confirm correct placement.

On the day prior to breast surgery $^{99m}$Tc-nanocolloid (Nanocoll®, GE- Healthcare, Eindhoven, the Netherlands) was injected under ultrasound guidance near the $^{125}$I-seed to enable the SN procedure.[18] Lymphoscintigraphy was performed after ten minutes and three hours. A dual-head gamma camera equipped with low-energy high-resolution collimators (Symbia Siemens, Germany) was used. Both anterior and lateral images were routinely obtained with additional images if needed. The lateral views were made with the hanging breast technique to ensure an unobstructed view of the axilla.

Sentinel nodes were pursued in all regions indicated by lymphoscintigraphy. Lymph nodes directly draining from the injection site were considered as SNs. Gamma-probe A was used at the $^{99m}$Tc-setting to search for hot SNs and was used for the wide local excision using both the $^{99m}$Tc-and the $^{125}$I-setting. Maximum counts per second (cps) were measured with both settings at the tumour site, two centimetres away from the site, and after removal of the tumour. The axilla was carefully palpated and suspicious nodes were routinely removed.

Participating surgeons were asked to estimate the degree of difficulty to distinguish $^{125}$I-source from the $^{99m}$Tc-nanocolloid radioactivity after every operation.

The excision was performed from skin to the pectoral fascia. After surgery, both the removed tissue and the excision cavity in the breast were investigated with the gamma-probe to ensure removal of the $^{125}$I-seed. A specimen mammography was performed to
confirm the presence of the $^{125}$I-seed. The specimen was stored in a lead container posted with a caution sign for radioactive material before transport to the pathology department to follow the Dutch dose limits during transportation.

The pathologist extracted the $^{125}$I-seed from the specimen with a Scintillation Meter (Mini Instruments 5-40, Essec UK) that measures $^{99m}$Tc-nanocolloid and the $^{125}$I-seed simultaneously. Subsequently, the $^{125}$I-seed was stored at the Radionuclide Centre of the institution.

All harvested nodes were fixed in formalin, bisected, embedded in paraffin, and cut at a minimum of six levels at 50 to 150 µm intervals. Pathological evaluation included haematoxylin-eosin and immunohistochemical staining (CAM 5.2; Becton Dickinson, San Jose, CA, USA). Tumours were classified according the WHO-classification.

Storage of the $^{125}$I-seeds was organised under the surveillance of the department of Health, Safety and Environment. Radiation safety protocols and detailed documentation regarding the acquisition, handling and storage were required to limit the risk of damage or loss of the $^{125}$I-seeds.[19] The seeds arrived at the Department of Radiology in a single vial and were inserted into the tumour via an 18-gauge needle under ultrasound guidance. The low energy of the gamma radiation emitted by the $^{125}$I-seed ensures minimal radiation exposure to the staff, and makes a protecting lead apron unnecessary. There are living rules for radiation safety to protect children, for example for breast-feeding or work related to young children.

**Results**

*In vitro study (table 1)*

Gamma-probe A was had the best $^{125}$I-sensitivity and the best contrast-ratio between $^{125}$I and $^{99m}$Tc at a simulated tissue depth of 2 cm the contrast-ratio (using the $^{125}$I-setting): 4.6 for a 3 month-old $^{125}$I-seed. This means that a 6-month-old $^{125}$I-seed will result in approximately equal count-rates for $^{125}$I and $^{99m}$Tc at 2cm depth. Deeper located $^{125}$I-seeds will have even lower contrast-ratios due to the low energy content, and hence, relatively stronger attenuation (see also Figure 2). Gamma-probe B demonstrated count rates a factor 13 lower than gamma-probe A in the $^{99m}$Tc channel and 40 fold lower in the $^{125}$I channel. The contrast-ratio using the $^{125}$I-setting was 2.3 for a 3 month-old $^{125}$I-seed in combination with 7 MBq $^{99m}$Tc. Gamma-probe C had a three times lower $^{125}$I-sensitivity compared to gamma-probe A, a better sensitivity in the $^{99m}$Tc channel and a slightly lower contrast-ration if the scatter correction was not used.
Using the scatter correction the contrast-ratio could be improved considerably if the tuning of the correction was done in precisely the right geometry. However, when the probe has to be scanned over the patient (as is required in clinical use) the tuning turned out to depend so strongly on the relative positions of probe, isotopes and scattering tissue that the result was not reliable.

**Figure 2:** Three graphs with energy spectrums of $^{125}$I and $^{99m}$Tc. a) This graph demonstrates the relation between $^{99m}$Tc (red) and $^{125}$I (black) by displaying the counts. The black curve is the $^{125}$I in relation to $^{99m}$Tc in red when an $^{125}$I-seed of 3 MBq is used and 8 MBq $^{99m}$Tc at 2 cm breast tissue depth. b) In this graph the signal of 100 MBq $^{99m}$Tc (representing a one-day protocol in which the patient directly proceeds to surgery for example) (blue) has been added. c) This is the same image but zoomed into the area of interest, the curve of 100 MBq $^{99m}$Tc (blue) is clearly higher than the $^{125}$I-peak (black) and means that the RSL procedure will probably be impossible in a one-day procedure. The spectrums were obtained using the NanoSPECT (Mediso Medical Imaging Systems, Budapest Hungary) with a general-purpose collimator with the source at a depth of 2 cm in simulated breast tissue.

**In vivo study (table 2 and 3)**

A total of 25 patients with non-palpable breast lesions underwent wide local excision and SNB using both an $^{125}$I-seed and an injection of $^{99m}$Tc-nanocolloid in the centre of the lesion. The $^{125}$I-seeds were placed under ultrasound (22) or stereotactic guidance (3). All $^{125}$I-seeds were placed correctly, as confirmed by ultrasound and mammography. There were no difficulties or complications during or after placement of the $^{125}$I-seed. The $^{125}$I-seed was inserted a median period of 27 (6-47) days before the surgical treatment. At the date of implantation of the $^{125}$I-seed the median strength of the source was 5.0 MBq (2.6-8.4), which decayed to a median of 3.9 MBq (1.6-7.3) on the day of surgery.

The median dosage of $^{99m}$Tc-nanocolloid was 115 MBq (45-142) and the time between injection and operation was 23.9 hours (18.3-28.6). The median remaining activity (decay corrected injected activity) of $^{99m}$Tc-nanocolloid at the time of operation was 7.2 MBq (1.7-13.9).
At the time of operation the median of the ratio between the remaining strength of the $^{125}\text{I}$-seed and the remaining $^{99m}\text{Tc}$-nanocolloid activity per patient was 0.56 (range 0.22-3.0). For 90% of the patients the ratio was higher than 0.3.

Table 1: In vitro measurements to determine the sensitivity and contrast ratios between $^{125}\text{I}$ and $^{99m}\text{Tc}$. For combined RSL-SLNB procedures the contrast ratio between $^{99m}\text{Tc}$ and $^{125}\text{I}$ in the $^{125}\text{I}$-channel is important ($^{125}\text{I}$-cps/$^{99m}\text{Tc}$-cps).

<table>
<thead>
<tr>
<th></th>
<th>$^{125}\text{I}$-channel</th>
<th>$^{125}\text{I}$-channel</th>
<th>$^{125}\text{I}$-channel</th>
<th>$^{99m}\text{Tc}$-channel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity $^{125}\text{I}$ (cps/MBq at 10cm in air)</td>
<td>Sensitivity $^{125}\text{I}$ (cps/MBq at 2 and 4 cm source depth in simulated tissue)</td>
<td>Contrast ratio ($^{2.9}\text{MBq}$ $^{125}\text{I}$ and $^{7}\text{MBq}$ $^{99m}\text{Tc}$ at 2cm depth)</td>
<td>Sensitivity $^{99m}\text{Tc}$ (cps/MBq at 10cm in air)</td>
</tr>
<tr>
<td>Gamma-probe A</td>
<td>317</td>
<td>2500 - 460</td>
<td>4.6</td>
<td>135</td>
</tr>
<tr>
<td>Gamma-probe B</td>
<td>8</td>
<td>68 - 7</td>
<td>2.3</td>
<td>10</td>
</tr>
<tr>
<td>Gamma-probe C</td>
<td>100</td>
<td>540 - 100</td>
<td>3</td>
<td>288</td>
</tr>
</tbody>
</table>

Surgery
The wide local excision was performed at the point of maximum cps of the $^{125}\text{I}$-seed. The median cps at the tumour site measured by the gamma-probe in the $^{125}\text{I}$-setting was 16300 compared to 4820 cps using the $^{99m}\text{Tc}$-setting. At a point 20mm away from the tumour site this was a median of 148 and 20 cps respectively. After removing the specimen, the median residual cps was 17 for the $^{125}\text{I}$-setting and 30 using the $^{99m}\text{Tc}$-setting. In 5 patients more than 5% of the highest cps of the $^{99m}\text{Tc}$ was still measured in the breast after excision.

Concerning evaluation of the degree of difficulty of the procedure the surgeons performing the procedures concluded that the $^{125}\text{I}$-source could be distinguished from the $^{99m}\text{Tc}$-nanocolloid radioactivity without difficulty in 23 patients (92%). In 2 patients this difference could be made, with more difficulty.

In 19 patients, lymphatic drainage was depicted towards the ipsilateral axilla, in 4 patients both axillary and extra-axillary SNs were found and 2 patients had intramammary drainage only.

Pathology
Most patients were diagnosed with an invasive ductal carcinoma or ductal carcinoma in situ. The median tumour size was 10mm (4-55mm). In 19 patients, the wide local excision resulted in tumour-free margins. In 4 patients, the removed specimen had a focal deposit of carcinoma in situ at the resection border and in 1 patient the invasive
tumour was not completely excised. Only the latter patient underwent a re-resection and in the other 96% of the patients only one operation was performed. The median number of harvested SNs was 1.0 (range 1-5), and all were tumour-negative. The pathologist could find the $^{125}$I-seed in all cases using the scintillation detector.

Adjuvant treatment consisted of radiotherapy alone (n=16) or in combination with hormonal treatment (n=6) and also chemotherapy (n=2). One patient received no further therapy. (Table 3)

**Table 2:** Counts at and around the tumour site.

<table>
<thead>
<tr>
<th></th>
<th>$^{125}$I-setting</th>
<th>$^{99m}$Tc-setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Cps at site of the $^{125}$I-seed</td>
<td>17734</td>
<td>16300</td>
</tr>
<tr>
<td>Cps 20mm next to source site</td>
<td>476</td>
<td>148</td>
</tr>
<tr>
<td>Specimen cps</td>
<td>25237</td>
<td>24500</td>
</tr>
<tr>
<td>Residual cps</td>
<td>96</td>
<td>17</td>
</tr>
</tbody>
</table>

**Table 3:** Operative and postoperative characteristics.

<table>
<thead>
<tr>
<th></th>
<th>N (range/%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sentinel node biopsy</td>
<td></td>
</tr>
<tr>
<td>Median harvested SNs</td>
<td>1.0 (1-5)</td>
</tr>
<tr>
<td>Number of tumour positive SNs</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Wide local excision</td>
<td></td>
</tr>
<tr>
<td>Tumour size</td>
<td>10mm (4-55mm)</td>
</tr>
<tr>
<td>Radical excision breast lesion</td>
<td>24 (96%)</td>
</tr>
<tr>
<td>Re-excision</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Pathology breast lesion</td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>14</td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td>8</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Lobular carcinoma in situ</td>
<td>1</td>
</tr>
<tr>
<td>Invasive papillary cancer</td>
<td>1</td>
</tr>
<tr>
<td>Adjuvant treatment</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>16</td>
</tr>
<tr>
<td>Radiotherapy and hormonal treatment</td>
<td>6</td>
</tr>
<tr>
<td>Radiotherapy, hormonal treatment and chemotherapy</td>
<td>2</td>
</tr>
</tbody>
</table>

*SN= sentinel nodes, mm= millimeter, N= number
Discussion
In this article, we have shown that RSL to excise the primary tumour and SNB by means of a radiopharmaceutical injection can, under certain restrictions, successfully be combined.

Evaluation of different gamma-probes features
Gamma-probes were specifically evaluated for the combined RSL-SNB procedures. Only the wired probe of gamma-probe A was suited for the combined RSL-SNB procedure and was evaluated both in vitro and in vivo. Gamma-probe B was not used for intraoperative evaluation because of the lower sensitivity and the need to change the complete probe when switching between $^{125}$I and $^{99m}$Tc. The feature of gamma-probe C, using an estimation of the Compton scatter to correct the $^{125}$I-signal was a priori considered promising and would hopefully compensate for lower sensitivity for $^{125}$I than gamma-probe A. However the amount of scatter correction depended on the geometry (relative positions of probe, isotopes and scattering tissue) to be useful. Because of the low sensitivity for $^{125}$I, gamma-probe C was not used for clinical procedures even though the sensitivity for $^{99m}$Tc was better than that of the other evaluated gamma-probes.

In vivo
The wide local excision and SNB could be performed in all patients. In almost all patients it was easier to depict the primary tumour site guided by the $^{125}$I-seed than by the $^{99m}$Tc-nanocolloid activity, because of the highly focused source of the radioactivity and therefore larger count rates. At a point 20mm away from the tumour site and after removing the specimen, median counts were generally low for both settings. The excess counts in the $^{125}$I-setting compared to the $^{99m}$Tc-setting are due to scatter of $^{99m}$Tc-radiation from the surrounding tissue. The residual counts after the $^{125}$I source had been removed are also due to this $^{99m}$Tc-scatter. The counts at the primary tumour site were considerably higher using the $^{125}$I-setting to detect the $^{125}$I-seed than the counts measured by the $^{99m}$Tc-setting, this confirms that the $^{125}$I-seed acts more as a point source than $^{99m}$Tc-nanocolloid.
Moreover, in general the $^{125}$I-seed and $^{99m}$Tc-nanocolloid did not interfere. In 92% of the patients the distinction was evident but in two patients this took more time and this was probably caused by a poor contrast-ratio between the two radionuclides. One patient had to undergo a re-resection due to tumour-positive margins. This patient had
multifocal breast cancer that appeared to be larger than estimated on preoperative MRI. One of the four patients with residual focal tumour had a large deposit of 55mm of ductal carcinoma in situ (DCIS). The recurrence rate in a mean follow-up of 33 months was 0.9% and 3% respectively.

The first prospectively randomised controlled trial published on non-palpable breast lesions randomised to either RSL or WL described 97 patients. Treatment with RSL had a significant lower re-excision rate than with WL and the amount of breast tissue excised using RSL was (not significantly) less than harvested during WL-procedures. Two more recent studies from Lovrics et al. and Murphy et al. demonstrated similar rates for positive margins and the volume excised in RSL or WL. Noteworthy, the rates of tumour-free margins achieved by RSL are not due to larger specimen sizes and thereby underline the importance of a point source as tumour marker. An earlier report from our institute demonstrated minimal migration for seeds that stayed on average for 59.5 days in the breast lesion (3–136 days).

Another important aspect to discuss is the variety of injection methods for SN procedures used in different centres. We used an intra- or peri-tumoural injection but elsewhere different injection methods (e.g. subdermal, periareolar) are used. The injection location changes the contrast-ratio either positively (by creating more distance between the radioisotopes) or negatively because the gamma-probe will be closer to the shallow $^{99m}$Tc than to the deeper low energy $^{125}$I-seed.

Based on in vitro measurements and our surgical experiences we propose minimal requirements for simultaneous use of $^{125}$I and $^{99m}$Tc in RSL-SNB procedures. It is important to note that the strength of the $^{125}$I-seed must be sufficiently high during operation to prevent interference from scattered low energy $^{99m}$Tc-radiation. Reducing the amount of $^{99m}$Tc-nanocolloid will decrease the success rate of the SN procedure, which is not recommended. RSL-SNB procedures should always be performed with a two-day SNB protocol to allow enough activity in the SN and have a sufficient contrast-ratio between the isotopes. (Table 4, Figure 2). We recommend that the $^{125}$I-seed should be chosen such that the ratio of the remaining strength of the $^{125}$I-seed (in MBq) and the remaining $^{99m}$Tc-nanocolloid activity (in MBq) is larger than 0.3 and to use to $^{125}$I-seeds of at least 1.0 MBq to achieve sufficient contrast-ratios between $^{125}$I and $^{99m}$Tc.

It is important that the probe should be used at the proper count range (audiosensitivity) such as 50.000 or 10.000 instead of the 100 setting.
The studies described above have already established the safety and advantages of using RSL when excising non-palpable breast lesions. Our in vivo results, although in a modest number of patients, show that the $^{125}$I-seed and $^{99m}$Tc-nanocolloid can be used simultaneously provided that the $^{125}$I-source is sufficiently active, the gamma-probe is suited for the specific application, and the user is aware of the interference. Therefore we conclude that non-palpable lesions can be safely excised with the radioactivity emitted by the $^{125}$I-seed, even in an area of $^{99m}$Tc-radioactivity. We feel that this simultaneous use is a next step within fine-tuning breast-conserving surgery and should lead to further investigation to confirm its value.

**Table 4:** Recommendations for combined RSL-SNB procedures

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Minimal activity (MBq)</th>
<th>Maximal activity (MBq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I-seed</td>
<td>&gt;1 (at the moment of surgery)</td>
<td>&lt;10 (more is not needed for accurate RSL procedures)</td>
</tr>
<tr>
<td>$^{99m}$Tc-nanocolloid</td>
<td>±8 (at the moment of surgery)</td>
<td>±8 (at the moment of surgery)</td>
</tr>
</tbody>
</table>
References


