Feasibility of preoperative $^{125}$I-seed-guided tumoural tracer injection using freehand-SPECT for sentinel lymph node mapping in non-palpable breast cancer

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Abstract

Background
This study was designed to explore the feasibility of replacing the conventional peri/intratumoural ultrasound (US) guided $^{99m}$Technetium-albumin nanocolloid ($^{99m}$Tc-nanocolloid) administration by an injection of the same tracer guided by a freehand-SPECT device in patients with non-palpable breast cancer with an $^{125}$iodine- ($^{125}$I) seed as tumour marker scheduled for a Sentinel Lymph Node Biopsy (SLNB). This approach aimed to decrease the workload for the Radiology department, avoiding a second US-guided procedure.

Materials and Methods
In 10 patients the implanted $^{125}$I-seed was primarily localised using freehand-SPECT and subsequently verified by conventional US in order to inject the $^{99m}$Tc-nanocolloid. The following 34 patients were injected using only freehand-SPECT localisation. In these patients, additional SPECT/CT was acquired to measure the distance between the $^{99m}$Tc-nanocolloid-injection-depot and the $^{125}$I-seed. In retrospect, a group of 21 patients with US-guided $^{99m}$Tc-nanocolloid-administrations was included as a control group.

Results
The depth difference measured by US and freehand-SPECT in 10 patients was 1.6±1.6mm. In the following 36 $^{125}$I-seeds (34 patients) the average difference between the $^{125}$I-seed and the centre of the $^{99m}$Tc-nanocolloid-injection-depot was 10.9±6.8mm. In the retrospect study the average distance between the $^{125}$I-seed and the centre of the $^{99m}$Tc-nanocolloid-injection-depot as measured in SPECT/CT was 9.7mm±6.5mm and was not significantly different compared to the freehand-SPECT guided group (two-sample Student’s t-test, p: 0.52).

Conclusion
We conclude that using freehand-SPECT for $^{99m}$Tc-nanocolloid administration in patients with non-palpable breast cancer with previously implanted $^{125}$I-seed is feasible. This technique may improve daily clinical logistics, reducing the workload for the Radiology department.
Background

The use of mammographic screening in nationwide programs within western countries has led to an increase in the number of women with non-palpable breast cancer lesions. [1,2] Currently, more than 25% of the radiological suspicious breast lesions are considered clinical non-palpable. [3] Accordingly, in many patients accurate pre- and intraoperative localisation of these non-palpable lesions is important for adequate breast conserving surgery. At present four different techniques are used to localise the tumour prior to excision: wire, ultrasound (US), carbon and radioguided (i.e. guided by a radionuclide) localisation. [3–5] When lymph node involvement is undetermined these approaches are combined with a sentinel lymph node (SLN) procedure. [6,7]

At The Netherlands Cancer Institute, both radioguided occult lesion localisation (ROLL) in our institute with radioactive $^{99m}$Technetium-albumin nanocolloid ($^{99m}$Tc-nanocolloid) or radioguided seed localisation (RSL) are used for non-palpable breast tumour localisation during surgery. [8] In the case of RSL a 3.7 to 10.7 MBq $^{125}$iodine-seed ($^{125}$I) with a half-life time of 60 days is preoperatively implanted into the malignancy using US-guidance in most cases. When the tumour was only visible on mammography, placement of the $^{125}$-seed was performed under stereotactic guidance. In our institute, the location of the $^{125}$-seed is always confirmed by an additional mammogram at the day of implantation. Recent studies show advantages when looking at resection margins, duration of localisation and surgical excision time for RSL or ROLL over wire-based localisation. [3,9–11] At The Netherlands Cancer Institute RSL is a standard procedure and over a 1000 $^{125}$I-seeds have been implanted since 2008.

In all patients scheduled for tumour excision the procedure is combined with sentinel lymph node biopsy (SLNB) for regional staging of the disease. This staging is of great significance for the patient outcome, being a predictor of presence for further metastasis in the axillary basin. [12] Clinical protocols for this procedure may vary between institutes because the radiocolloid injection site for SLNB is still a matter of controversy. [13–17] At The Netherlands Cancer Institute the $^{99m}$Tc-nanocolloid for SLNB in non-palpable breast cancer is preferably administered intratumourally by US-guidance, although, in clinical practice it turns out to be either peri- or intratumoural. Peritumoural is defined as at least within a radius of 10mm to the tumour border. Intratumoural injections can sometimes result in resistance of the tumour tissue while administering the tracer, this is solved by small injection volumes (<0.2ml) and to slowly pull a little bit back while administering the tracer. This peri- or intratumoural
radiopharmacon administration will result in extra-axillary SLN's, which in our institute are included for diagnosis. [18] Prior to surgery the radiologist localises the $^{125}$I-seed by the ultrasonic reflection of the titanium capsule in order to place a needle into the tumour. Subsequently a nuclear physician injects the $^{99m}$Tc-nanocolloid. [19] This can be a challenging intervention due to difficulties in localising the $^{125}$I-seed in pathological and irregular breast tissue. Furthermore, the procedure requires two medical specialists (e.g. a radiologist and a nuclear physician) and a technologist. By avoiding this additional US-procedure the workload of the Radiology department will be decreased.

Recently a novel freehand-SPECT system (declipseSPECT, SurgicEye GmbH, Munich, Germany) for three dimensional (3D) radioguided imaging and navigation has been introduced. This device combines a conventional gamma probe with an optical tracking system. An algorithm links the measured counts from the location of the gamma probe in space and, accordingly, reconstructs a 3D visualisation. [20,21] The purpose of this study is to validate $^{125}$I-seed localisation guided by freehand-SPECT in patients with non-palpable breast cancer in order to facilitate $^{99m}$Tc-nanocolloid injections. Results of this study could also serve as a proof of concept for use of this specific radioguided navigation technique in other malignancies.

Materials and methods

Patient population

44 patients with a peri-/intratumoural $^{125}$I-seed (STM1251, Bard Brachytherapy, Inc. Carol Stream, IL, USA) and scheduled for an SLN-procedure were included. Patients were included in consecutive order and inclusion was based on availability of researchers, SPECT/CT-scanner and the freehand-SPECT device. The study protocol included a group of patients scheduled for both US and freehand-SPECT (group 1; n=10) followed by a second group of patients investigated with only the freehand-SPECT probe (group 2; n=34). Results of the second group were compared with a control group of patients who had received injections guided by US (group 3; n=21). Experienced radiologists measured the tumour size by means of MRI or mammography. The characteristics of the groups are as follow:

Group 1: In ten patients in the period October 2012 to December 2012 the location and depth of the $^{125}$I-seed was measured, using both freehand-SPECT and US. The $^{99m}$Tc-nanocolloid was injected exactly according to the standard protocol based on US-guided injections, which is the technique of choice at the Netherlands Cancer Institute.
The standard protocol means no additional SPECT/CT-scan to limit additional radiation exposure for patients.

**Group 2:** In the period from December 2012 to April 2013 34 patients were included. The location and depth of the $^{125}$I-seed was measured, using freehand-SPECT followed by a freehand-SPECT guided injection with $^{99m}$Tc-nanocolloid. These 34 patients received a SPECT/CT scan to measure the accuracy of the $^{99m}$Tc-nanocolloid injection location in relation to the $^{125}$I-seed.

**Group 3:** The control group was constituted retrospectively by 21 consecutive patients whom underwent US-guided $^{99m}$Tc-nanocolloid injection near the $^{125}$I-seed and a SPECT/CT in the period from April 2012 to March 2013. This means that only patients who had received a SPECT/CT in the context of a standard SLN-procedure (i.e. non-visualisation of lymphatic drainage or aberrant lymphatic drainage on the planar imaging) were included. This group is selected to study the US-guided injection depots of $^{99m}$Tc-nanocolloid by means of SPECT/CT without making additional SPECT/CT-scans.

**The standard clinical SLN-protocol**

All patients undergoing a SLNB get a $^{99m}$Tc-nanocolloid (GE Healthcare, Eindhoven, The Netherlands) injection of 140 MBq in 0.2 ml one day prior to surgery. Five-minute static scintigraphic images are acquired from anterior and lateral 5-30 minutes, and 2-3 hours post-injection. In case of non-visualisation or aberrant lymphatic drainage an additional SPECT/CT scan is obtained. All SPECT/CT data are acquired using a hybrid camera (Symbia-T; Siemens, Erlangen, Germany). The dual-head SPECT (128x128 matrix, 40 frames, 30s/frame) is performed using 9° angular steps in a 30s time frame. For CT (130kV, 40mA, B30s kernel), 5mm slices are obtained. Both attenuation and scatter correction are applied.
Figure 1: (a) Data acquisition using the freehand-SPECT device, radioactivity is measured with the probe from multiple directions. P is the probe and in yellow the detection beam of the probe. The orange cloud is an accumulation of the area where is measured. (b) The localisation of the $^{125}$I-seed after reconstruction. The $^{125}$I-seed reconstruction is projected over the optical image in purple. T is the patient tracker. (c) 3D visualisation of the distance and direction of the probetip to the $^{125}$I-seed. (d) Injection of $^{99m}$Tc-nanocolloid guided by freehand-SPECT. I is the tracer injection localisation.

Freehand-SPECT acquisition and reconstruction
This method was based on combining counts measured with a conventional gamma probe with data of the location and orientation of the gamma probe using a reflective reference target attached to a specific site on the probe. Through a calibration procedure the relation between the gamma probe tip and the reference target was determined. [22] To acquire an accurate 3D volume reconstruction from the count data, a surface scan was made by hovering the probe over the area of interest in three different orientations (e.g. x, y, z planes). The system requires at least 1500 measurements to accurately create a 3D visualisation; in our protocol we adopted thus
a minimum of 2000 measurements in three or more directions. This planar surface scanning takes about 2 minutes and the reconstruction of the volume takes another 20 seconds. After the reconstruction the optical camera of the used system was combined with a radioactivity map. (Figure 1b) The window level was adjusted by using the touchscreen to set a visualisation threshold similar to the ones used in conventional nuclear medicine until the number of hotspots equals the number of $^{125}$I-seeds in-situ. The 3D window enabled the best navigation to the $^{125}$I-seed. (Figure 1c)

For patients of group 1, the perpendicular distance from the skin to the $^{125}$I-seed was determined with the freehand-SPECT and the most intensive focus in the 3D reconstruction was marked on the skin of the patient. Next, the radiologist, who was blinded for the depth information, localised the $^{125}$I-seed with US and measured the perpendicular depth from the marked place on the skin to the $^{125}$I-seed after which he injected the $^{99m}$Tc-nanocolloid close to the $^{125}$I-seed. To avoid breast tissue deformations it is important that the patient does not change position during the freehand-SPECT and US-measurements.

For patients of group 2 the perpendicular depth to the $^{125}$I-seed, which was used for US-guided injections as well, was measured per patient and the optimal injection location was marked on the skin. The needle was injected to the depth indicated by the freehand-SPECT. (Figure 1c) Three hours after injection of $^{99m}$Tc-nanocolloid a SPECT/CT scan was obtained. Verification of the $^{99m}$Tc-nanocolloid injection relative to the $^{125}$I-seed was performed by comparing the $^{99m}$Tc-nanocolloid-depot on the SPECT images to the location of the $^{125}$I-seed on the CT-scan. The distance from the $^{125}$I-seed to the centre of the activity depot in the axial plane was measured. All distances on SPECT/CT were once more determined by a second independent blinded observer to study the limits of agreement.

Statistics for data analysis

Continuous variables were represented by mean ± standard deviation (SD). Differences between the measured depths of the $^{125}$I-seed by freehand-SPECT and US are evaluated by Bland-Altman graphs. The limits of agreement between the different observers were also evaluated by Bland-Altman graphs. This results in a mean difference and the 95% confidence interval (95% CI). [23]
Results

Group 1: US-validation
The characteristics of all 10 patients are outlined in Table 1. The lesions were found on various locations in the breast. The 10 perpendicular measurements with US and freehand-SPECT of the $^{125}$I-seed resulted in absolute variations in the range of 0 to 5mm. The average difference in depth was $0.05 \pm 2.4$mm (range -3.5-5mm), and the absolute average was $1.6mm \pm 1.6mm$, (range: 0-5mm). These data are displayed in a Bland-Altman plot, which visualises the mean and the 1.96 times the standard deviation ranges. [24] (Figure 2)

Group 2: SPECT/CT-validation
The characteristics of all 34 patients are outlined in Table 1. Patients had either one or two $^{125}$I-seeds implanted and the tumours were located on various locations within the breast. The average distance form the centre of the $^{99m}$Tc-nanocolloid-depot to the $^{125}$I-seed on SPECT/CT was $10.9 \pm 6.8$mm (range: 0-29mm).

Retrospective analysis of the data showed a possible relation between the number of measurements made by the freehand-SPECT and the distance between the $^{99m}$Tc-nanocolloid-injection-depot and the $^{125}$I-seed. In ten injections we noticed that the number of measurements was more than the protocolled 2000-2500 but was 3000-3500 measurements at least. We evaluated the differences to study whether higher number of measurements will result in more accurate injections. The ten injections with more measurements resulted, after measuring the distance between the $^{99m}$Tc-nanocolloid-injection-depot and the $^{125}$I-seed, in a mean distance of $10.0mm$ instead of $11.2mm$ in the other 26 injections.

Group 3: control group
In the retrospectively selected US-guided $^{99m}$Tc-nanocolloid injections (patient group 3) the distance from the depot to the $^{125}$I-seed showed was $9.7mm \pm 6.5mm$ (range 2-30mm) on SPECT/CT.

Comparing the distance from the depot to the $^{125}$I-seed in the freehand-SPECT (group 2) and US-guided injections (group 3) revealed no significant difference (two-sample Student’s t-test, p: 0.52). This means there is no difference in accuracy for US-guided and freehand-SPECT guided injections. The mean difference between the two observers in this setting was $0.5mm$ (95% CI: 2.9 to -3.5mm), for freehand-SPECT guided injections $0.1mm$ (95% CI: -3.0mm to 3.1mm) and for the US-guided injections
0.9mm (95% CI: -2.2mm to 4.0mm). (Figure 2, 3) There are images included in this work that illustrate the location of the $^{125}$I-seed and the $^{99m}$Tc-nanocolloid-depot by a fusion of the SPECT signal and the CT-scan. (Figure 4)

In total 65 peri-/intratumoural $^{99m}$Tc-nanocolloid injections were included in this study for analysis. The overall SN identification rate was 1.2 SN per patient and 56/65 had SN visualisation on either lymphoscintigraphy or SPECT/CT. The intraoperative SN identification rate was higher thanks to prolongation of the time interval (allowing further lymph drainage) and the use of blue dye.

Table 1: Patient information for US-validation (n = 10), patient information for SPECT/CT validation (n = 34) and retrospect US-guided injections (n = 21)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1, US-validation (n = 10) (SD, range)</th>
<th>Group 2, SPECT validation (n = 34) (SD, range)</th>
<th>Group 3, Retrospect US-guided injections (N = 21) (SD, Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (years)</td>
<td>51 (8.4, 42-66)</td>
<td>61.3 (12.1, 26-89)</td>
<td>59 (10.6, 42-86)</td>
</tr>
<tr>
<td>Tumour size (mm)</td>
<td>11.5 (3.1, 9-20)</td>
<td>17.1 (13.8, 3-60)</td>
<td>18.4 (13.1, 8-55)</td>
</tr>
<tr>
<td>Tumour type</td>
<td>3xDCIS, 6xIDC, 1xILC</td>
<td>12xDCIS, 1xLCIS, 14xDCIS, 4xIDC, 3xunknown</td>
<td>14xDCIS, 4xLCIS, 3xunknown</td>
</tr>
<tr>
<td>Number of $^{125}$I-seeds</td>
<td>1</td>
<td>30x1, 4x2 seeds</td>
<td>1</td>
</tr>
<tr>
<td>Location of $^{125}$I-seeds</td>
<td>3 medial, 6 lateral, 1 central</td>
<td>7 medial, 22 lateral, 5 central</td>
<td>5 medial, 13 lateral, 3 central</td>
</tr>
<tr>
<td>Days after $^{125}$I-seed implantation</td>
<td>30 (13, 12-56)</td>
<td>33.5 (23.3, 10-118)</td>
<td>Not measured</td>
</tr>
<tr>
<td>$^{125}$I-seed depth (by US) (mm)</td>
<td>11.6 (6.4, 5-23)</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>$^{125}$I-seed depth (by freehand-SPECT) (mm)</td>
<td>11.5 (6.6, 5-25)</td>
<td>15.3 (6.7, 8-35)</td>
<td>Not measured</td>
</tr>
<tr>
<td>Difference in localisation or location (mm)</td>
<td>Mean difference: 0.05 (2.4, -3.5-5)</td>
<td>Absolute mean difference: 1.6 (1.6, 0-5)</td>
<td>10.9 (6.8, 0-29) (CT compared with SPECT)</td>
</tr>
<tr>
<td>Irradical procedures</td>
<td>1/10 (focal irradical)</td>
<td>9/34 (focal irradical or irradical)</td>
<td>5/21 (focal irradical or irradical)</td>
</tr>
</tbody>
</table>
Figure 2: Bland-Altman analysis for the distances in depth measured with the US-probe and with the freehand-SPECT. The analysis indicates the average of the measurements. The upper and lower dotted lines represent the Bland-Altman limits the 95% confidence interval.

Figure 3: Bland-Altman analysis for the interobserver agreement between freehand-SPECT guided and US-guided injections. The analysis indicates the average of the measurements. The upper and lower dotted lines represent the Bland-Altman limits the 95% confidence interval.

Discussion
This study demonstrates that peri-/intratumoural $^{99m}$Tc-nanocolloid injections using a freehand-SPECT device are feasible in patients with non-palpable breast cancer marked with a $^{125}$I-seed. The freehand-SPECT is able to localize the $^{125}$I-seed and obtains navigation parameters for subsequent SLN-procedure related tracer injection. The manufacturer specified a spatial resolution of 5mm for the freehand-SPECT, suggesting that this device was appropriate for the intervention described in our study. [25] Our results confirmed this by showing a mean difference of 1.6 ± 1.6mm (range: 0-5mm) compared to the conventional US-technique. Additionally, it was concluded that the
concordance of freehand-SPECT guided administrations compared to US-guided administrations validated by means of SPECT/CT imaging was clinically acceptable for the approach that we pursue. This study was not designed to study a learning curve, we also did not find a learning curve in this limited number of cases. This might be the result of varying observers. However, to use the freehand-SPECT device a training period is required. The results of this study and the benefits of using this technique seem to support the use of freehand-SPECT for $^{125}$I-seed-guided radiocolloid injections in patients scheduled for SLNB and thereby enhance the logistics and workload for Nuclear Medicine and Radiology departments.

*Image-guided injections*

For SLNB, US-guided injections are commonly used to deliver $^{99m}$Tc-nanocolloid into or in the vicinity of the tumour. [19] In cases where the $^{125}$I-seed is not identifiable a stereotaxic procedure is performed. The US-guided injections and the stereotaxic procedures have certain drawbacks; first of all the planning is more complicated because there are two departments involved and the time per procedure is variable (15 to 45 minutes). Furthermore the localisation of the $^{125}$I-seed may be time-consuming and requires a radiologist. The injection using freehand-SPECT is straightforward and, as described in the present study, clinically applicable. The procedure can be performed at the Nuclear Medicine department, which does increase the flexibility in planning. In our experience the procedure never exceeded 20 minutes taking in average 10-15 minutes. A second benefit is that this procedure may avoid potential pitfalls in misjudging the identity of the $^{125}$I-seed and thereby an incorrect injection location of $^{99m}$Tc-nanocolloid in patients with other types of markers in situ or calcifications in the breast. These other non-radioactive markers do not affect the freehand-SPECT technique.

The radiocolloid injection site for SLNB is still a matter of controversy. [13,14,16,17] The freehand-SPECT method as described in this study is only of clinical relevance for tumour-related tracer administration. For injections in the vicinity of the tumour this technique is sufficient. However, for injections in small lesions this technique requires more precision. This could be acquired with an optically tracked needle integrated in the freehand-SPECT system. There are prototypes of needles or catheters with optical tracking systems, enabling exact needle tip localisation and thereby possibly more accurate injections. [26] For the 36 freehand-SPECT guided injections we used 15, 25, 35mm needles and the depth was determined on the basis of depth estimation.
Optimisation of freehand-SPECT

There are several possibilities to explain the observed distance deviation between the $^{99m}$Tc-nanocolloid-injection-depot and the $^{125}$I-seed on SPECT/CT. First, the use of older (weaker in radioactivity) $^{125}$I-seeds may give significantly less signal, which influences the image quality. Another explanation could be the fact that the freehand-SPECT device indicates a depth and a direction, which is marked on the skin. The nuclear physician had to inject exactly similar to this direction or else larger deviations in deeper injections would logically be the result. Further analysis of the relation of depth and inaccuracy hinted to a relation where an increase in depth results in more inaccurate injections (correlation of 0.58). (Figure 5) When only the $^{125}$I-seeds with a depth of < 26mm are taken into consideration (25/36 $^{125}$I-seeds) the average distance between the $^{99m}$Tc-nanocolloid-injection-depot and the $^{125}$I-seed is 8.2mm (SD: 5.1mm, Range: 0-20mm).

This is less than the average distance measured on the SPECT/CT scans for all US-guided injections (group 2).

The retrospect evaluation of the accuracy in 10 injections with higher number of measurements demonstrated a mean distance of 10.0mm instead of 11.2mm in the first 26 injections, this suggests a favourable relation to obtain more measurements. With these small numbers this is not a significant conclusion. Nevertheless, we recommend using higher numbers of measurements, because more data for the calculations would logically result in more accurate reconstructions and could for example compensate the weak signal of older $^{125}$I-seeds. An additional source of error in the evaluation might be the registration between CT and SPECT and the slice thickness of the CT images. These factors can have both a positive and a negative impact on the evaluation, but have to be considered when looking at the standard deviation of the results.

In the present study freehand-SPECT reconstruction was based on settings used for standard intraoperative procedures. In theory, it is possible to increase the number of iterations or reduce the voxel size. The standard number of iterations for reconstruction is 20; experimental settings where the number of iterations rises up to 100 iterations can result in more accurate localisations but may drastically increase the reconstruction time. The voxel size is also variable; this can be reduced from 5mm voxels to 2mm voxels. Experimental setups will be required in the future to evaluate which are the optimal settings for specific applications. This study also demonstrates potential use of freehand-SPECT for intraoperative $^{125}$I-seed localisation since accurate navigation to the radioactive tumour marker is enabled. Furthermore, the margins of
breast cancer specimens relative to the $^{125}$I-seed could be ex vivo determined as predictor for surgical margins. A prospective study to investigate this assumption is currently in preparation.

**Figure 4:** Axial SPECT/CT-images. (a) The low-dose CT. (b) The SPECT and CT image fused. (c) Close up of the low-dose CT. (d) The SPECT and CT image fused with the measurement of distance from centre of activity $^{99m}$Tc-nanocolloid to the $^{125}$I-seed, the measured distance is 8mm. The green lines indicate the same position in the different images.
Conclusion

Peri-/intratumoural $^{99m}$Tc-nanocolloid injection for SLN-mapping using freehand-SPECT in patients with non-palpable breast tumours and implanted $^{125}$I-seeds for tumour excision are feasible. This approach may become a reliable alternative for US-guided $^{99m}$Tc-nanocolloid injections, alleviating daily/clinical logistics in both the Nuclear Medicine and Radiology departments.

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References


