Sentinel lymph node detection with large human serum albumin colloid particles in breast cancer

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Abstract. Detection of metastatic involvement of lymph nodes is essential for management and prognostic evaluation in breast cancer patients. The success of lymphatic mapping depends on identifying the sentinel lymph node(s) draining the primary tumour. However, when mapping is performed with a radiocolloidal agent, the number of hot lymph nodes varies with the agent and its size, among other factors. In this study, we evaluated prospectively the detection rate of sentinel lymph nodes in breast cancer when injecting large particles (100–600 nm) of human serum albumin colloids (Senti-Scint). In 128 consecutive breast cancer patients without palpable lymph nodes, pre-operative static lymphoscintigraphic mapping of the breast was performed after subcutaneous injection of 15 MBq of the radiocolloid. Lymphoscintigrahic results were compared with intra-operative surgical gamma detection probe and blue dye mapping data. Pre-operative lymphoscintigraphy and surgical gamma detection probe both correctly detected 203 sentinel lymph nodes in 122/128 patients (95%), while blue dye mapping showed only 183 sentinel lymph nodes in 82% of the patients. Only one or two sentinel lymph nodes were identified in each patient, which allowed the surgeon easily to find the sentinel lymph node(s) intra-operatively. In conclusion, lymphoscintigraphy with large particles of human serum albumin colloids is a helpful and reliable procedure for the surgical management of breast cancer.

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Introduction

The assessment of metastatic spread to lymph nodes in patients with breast cancer is of the utmost importance for selection of therapy and has prognostic implications [1]. Sentinel lymph node biopsy based on lymphoscintigraphic findings was introduced for the management of penile carcinoma by Cabanas in 1977 [2], and developed further for staging of melanoma by Morton in 1992 [3, 4]. The procedure is based on the concept that a primary tumour drains via efferent lymphatic channel(s) directly to the first lymph node, the so-called sentinel lymph node, from which further connections with second-echelon nodes exist. The sentinel lymph node is the first site of lymph node metastasis. The status of the sentinel lymph node, if negative for metastasis, was found to reflect the status of higher nodes in melanoma patients [5]. Lymphoscintigraphy helps in the localisation and resection of the sentinel lymph node(s) during surgery. Many surgeons use a surgical gamma probe as well as injection of blue dye for intra-operative mapping of the sentinel lymph node [6].

The requirements of a radiopharmaceutical for sentinel lymph node mapping include stable radiolabelling, rapid access to the lymphatic system from the injection site and no access to the bloodstream. The main focus of attention to date has been the optimisation of the technique for sentinel lymph node detection. The main variations among the different procedures concern the type of

radiopharmaceutical, the administered dose, the volume and route of injection of the radiopharmaceutical, and the size and number of colloid particles. These are non-specifically phagocytosed from lymphatic fluid by macrophages in lymph nodes. The rate of tracer transport through the lymphatic system is strongly related to the size of the colloid particles. The optimal particle size has not yet been established [7], but several investigations [8, 9] have suggested that colloids with diameters between 20 and 500 nm should be used. Retention of particles of this size in the sentinel lymph node is increased as compared with that of smaller particles, which are not phagocytosed in the sentinel lymph node and ascend along the rest of the lymphatic chain. Transport of smaller particles through the lymphatic system is faster than that of larger particles.

In this prospective study, we aimed to assess the value of lymphoscintigraphy with large particles of human serum albumin colloids for sentinel lymph node detection in breast cancer.

Materials and methods

Patient data. From among 384 patients prospectively investigated with lymphoscintigraphy for sentinel lymph node detection between June 1998 and June 2002, 128 consecutive female breast cancer patients who underwent excisional biopsy and histological evaluation of both the primary tumour and surgically removed lymph node(s) were enrolled in the study. Patient characteristics are summarised in Table 1. The follow-up time of the patients after the learning phase with the first 50 patients was 8–43 months (mean 22±7 months). The inclusion criteria were histologically confirmed breast cancer and absence of palpable lymph nodes. Exclusion criteria included infected or indurated areas, poorly healed scars, haematomas, multicentric primary disease, clinical suspicion of axillary metastasis, known metastatic disease, pre-operative chemotherapy and previous radiation therapy to the chest wall, which would preclude adequate flow of the colloid. A written patient informed consent was obtained before enrolment in the study. The study was conducted in accordance with the requirements of the local ethical committee. Each patient was followed up on a regular basis by clinical examination, determination of tumour marker levels (CEA and CA 15-3), mammography and ultrasound.

Radiopharmaceutical preparation (Senti-Scint, MEDI-Radiopharma, Budapest, Hungary). The labelling and quality control procedures were carried out according to the manufacturer's instructions. Radiochemical purity (%RCP) was determined by ascending instant thin-layer chromatography using silica gel-impregnated glass filter sheets (Gelman Sciences, Mich., USA). The %RCP limits for technetium-99m-labelled human serum albumin colloid were greater than 95%. Colloidal particle size was determined by the Dyna-Pro-LSR Particle Sizing Instrument (Protein Solution Inc. N.Y., USA) based on the principle of dynamic light scattering. Fifty-microlitre samples of ^{99m}Tc-human serum albumin colloid were measured at 20°C for 5 min. Graphical size analysis software allowed easy interpretation of size distribution, from 2 nm to 1 μm in radius. Before sample measurement, the accuracy of the particle sizing instrument was checked by use of a reference sample (New Duke Polystysrene Standard, MSTC-800; 5.5–6.5 nm).

Table 1. Clinical characteristics of 128 patients

Characteristic	Value
Age (years)	
Range	33–90
Mean	62.56±13.03
Postmenopausal	109 (85%)
Palpable tumour	92 (72%)
Tumour size (mm)	
Range	5–40
Mean	17.36±7.97
Localisation of tumour	
Inner upper quadrant	14 (10.9%)
Outer upper quadrant	76 (59.4%)
Inner lower quadrant	17 (13.2%)
Outer lower quadrant	6 (4.7%)
Nipple	15 (11.7%)
Tumour type	
Ductal carcinoma in situ	14 (10.9%)
Ductal invasive carcinoma	76 (59.4%)
Lobular invasive carcinoma	35 (27.3%)
Tubular invasive carcinoma	3 (2.4%)

Values are number of patients unless otherwise specified

Scanning protocol. Eighteen hours prior to surgery, 15 MBq of ^{99m}Tc-labelled human serum albumin colloid (Senti-Scint) in a volume of 0.4 ml was injected subcutaneously between the skin and the tumour. The injection site was chosen according to the mammographic and/or ultrasound findings. Immediately after the injection, the patient massaged the injection site for 5 min. Planar anterior images of the breast were obtained at 30 and 60 min and, if necessary, up to 4 h p.i. on a gamma camera (General Electrics, Helix, Haifa) with a low-energy, high-resolution collimator set for the 140-keV photopeak with a 20% window. Each static image was of 10 min duration. In the event of non-visualisation of the sentinel node in the anterior projections, additional lateral images of the breast and axilla were performed. After planar acquisition, the exact localisation of the sentinel node was sought using a gamma probe (Navigator, Waterdown, Mass, USA) with the patient lying supine and with the ipsilateral arm abducted to ensure the same anatomical localisation as during the surgery. The skin over the sentinel node was marked with a skin marker pre-operatively in the nuclear medicine department.

Three surgeons participated in all operations, two at the department of surgery and another at the department of gynaecology. During surgery, subareolar injection of blue dye (Lymphazurin 1%, USSC, Norwalk, Canada) was performed in each patient, to help localisation and removal of the sentinel lymph nodes. An intra-operative surgical gamma detection probe ensheathed in a sterile probe cover was also used. The lymph nodes with blue dye uptake and the radioactive lymph nodes were removed surgically. Thereafter mastectomy or lumpectomy was performed, followed

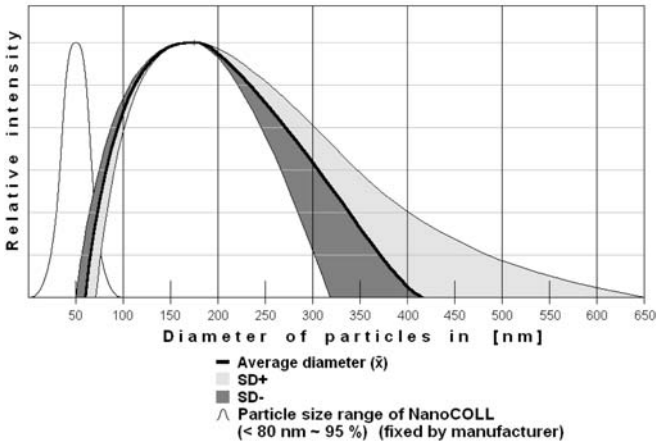

Fig. 1. Particle size distribution of ^{99m}Tc-human serum albumin colloid: Senti-Scint vs Nanocoll

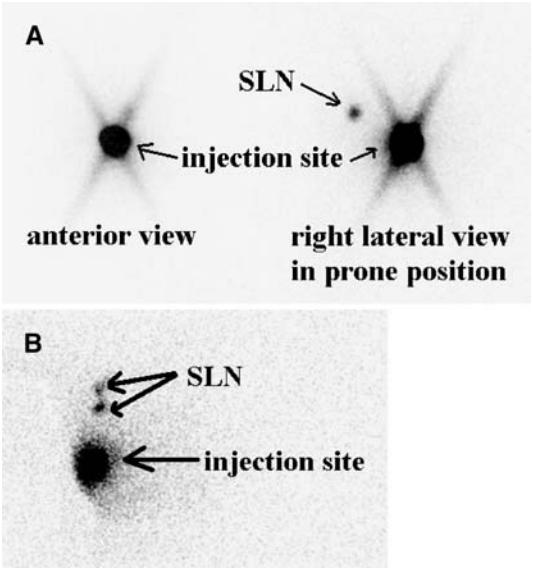

Fig. 2. A Tumour in the right outer upper quadrant; visualisation of the sentinel lymph node (*SLN*) in the lateral view. **B** Tumour in the right upper outer quadrant; visualisation of two sentinel lymph nodes (*SLN*) in the right axilla (anterior view)

by axillary lymph node dissection of levels 1 and 2 in the first 50 patients. The lymph nodes were sent to the department of pathology for rapid frozen multiple section. If the sentinel lymph node was positive for metastasis, axillary node dissection was performed immediately. A second surgical intervention was performed if a positive result was seen in the additional investigations with both haematoxylin and eosin (HE) and immunohistochemical cytokeratin antibodies (Cytokeratin AE1/AE3, IgG1-M3515, Dako, Calif., USA). The other extirpated non-sentinel lymph nodes were examined routinely with HE staining.

Results

The %RCP was 99.4%±0.3% for ^{99m}Tc-human serum albumin colloid (n=12). Evaluation of the particle size distribution of ^{99m}Tc-human serum albumin colloid used during this study (Fig. 1) showed that more than 90% of particles were in the range 100–600 nm (mean particle diameter 205 nm).

Pre-operative lymphoscintigraphic mapping (Fig. 2) and intra-operative gamma detection probe both showed 203 sentinel lymph nodes in 122 (95%) of the 128 patients studied. In only 12 cases were images up to 4 h required, and six of these cases remained negative up to 18 h after tracer application. Metastases were confirmed by histology in 38 cases, including five in which they were detected only by immunohistochemical cytokeratin antibodies. Only in a few cases (n=4) with a negative sentinel node was immunohistochemistry of non-sentinel nodes performed, which proved negative. One (axillary) sentinel lymph node with metastasis confirmed histologically by HE was neither visualised by pre-operative lymphoscintigraphy nor detected by blue dye uptake. The lymph node was palpated intra-operatively. This false negative sentinel lymph node on both lymphoscintigraphy and gamma detection probe was found in a patient with a surgery level 3 metastasis, whose axillary lymph node dissection of levels 1 and 2 was found to be

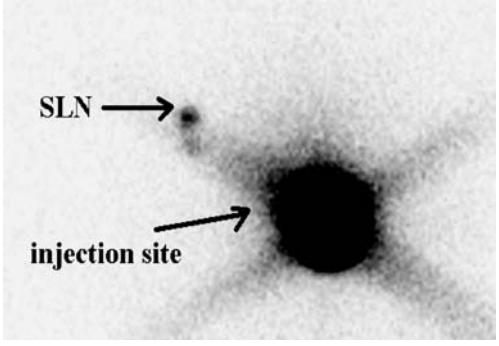

Fig. 3. Tumour in the left lower inner quadrant. A parasternal sentinel lymph node (*SLN*) is visualised on the left side, with faint tracer uptake caudal to it (probably a second lymph node). There is no lymph transport to the left axilla (anterior view)

negative for lymph node metastasis. Lymph drainage to the internal mammary nodes was detected in five patients (Fig. 3). One intramammary sentinel lymph node was observed (Fig. 4). Axillary dissection of levels 1 and 2 and dissection of the intramammary lymph nodes was performed in all of these patients, and additional dissection of internal mammary lymph nodes was performed in two of them. In none of the cases did we observe passage of the radiopharmaceutical across the middline, with visualisation of contralateral lymph nodes. Lymphoscintigraphy was well tolerated by all patients. Blue dye mapping identified 183 sentinel lymph nodes in 105 patients (82%); in two cases the sentinel node was identified only by blue dye. To date, no tumour recurrence has been detected after surgery in any of the 128 patients.

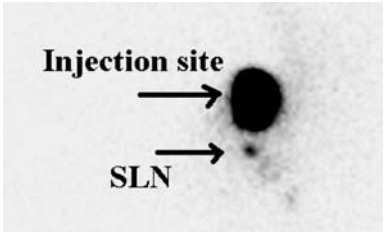

Fig. 4. Tumour in the left upper outer quadrant; visualisation of an intramammary sentinel lymph node (*SLN*) (left lateral view in the prone position)

Discussion

In the last few years, sentinel lymph node biopsy has become an accepted technique that enables avoidance of unnecessary axillary lymph node dissection in most breast cancer patients [10, 11]. Lymphoscintigraphy helps to identify patients with lymphatic drainage to sites other than the axilla [12], thereby allowing more accurate staging and treatment of this subgroup of patients. The potential of sentinel lymph node mapping to allow more accurate tumour staging, with reduction of the morbidity and expense associated with a complete axillary dissection, has stimulated the widespread investigation of different sentinel lymph node sampling techniques. The success of lymphatic mapping depends on identifying the direct drainage pathway of the primary tumour. "Skip metastases", as found in one patient of our study with involvement of level 3 lymph nodes and negative level 1 and 2 lymph nodes, are quite rare and occur in less than 3% of all patients with positive lymph nodes [1, 13]. Any node with a direct connection with the tumour must be regarded as a sentinel lymph node. Great individual variations in lymphatic drainage are observed. Furthermore, when lymphatic mapping is performed with a colloid, the number of hot lymph nodes varies with the agent as well as with the interval between the injection of the radiopharmaceutical and the surgical procedure. Therefore the surgeon may not be able to determine the true sentinel lymph node among the hot nodes that are intra-operatively detected by the surgical gamma detection probe.

Lymphatic mapping with radiocolloidal agents will not realise its full potential until it has been standardised. In a previous study it was suggested that sentinel node detection is more easily achieved when large microcolloids are used [14]. However, there still seems to be a need to identify the ideal colloid and particle size for sentinel lymph node detection, particularly in breast cancer. The disadvantage of injecting smaller tracers is that more secondary lymph nodes are visualised, as more particles flow through the lymphatic chain. Sequential imaging can help to differentiate between sentinel and secondary lymph nodes [15], but the surgical verification remains difficult. In our study, pre-operative lymphoscintigraphy and intra-operative gamma detection probe after subcutaneous injection of large particles of radiocolloids correctly identified one or two sentinel lymph nodes in 95% of the patients, even though the tumours were non-palpable and in some cases were as small as 5 mm. By contrast, blue dye mapping showed only 183 sentinel lymph nodes in 82% of the patients. One limitation of this study is that during the learning phase, immunohistochemistry was not performed in all non-sentinel nodes and some metastases may therefore have passed undetected. In our opinion, one of the key elements in the successful imaging of the lymph nodes is massage of the breast after tracer injection, as described previously [16]. Our rate of detection of sentinel lymph nodes is similar to that reported by De Cicco et al. [17], who found that sentinel node identification was more accurate when 200–1,000 nm radiocolloid was injected subdermally in a relatively small injection volume of 0.4 ml.

It is concluded that lymphoscintigraphy with large colloid particles of 100–600 nm is a reliable procedure that assists in the surgical management of breast cancer. Pre-operative lymphoscintigraphy and surgical gamma detection probe showed a similarly high sensitivity for sentinel lymph node detection, which exceeded that of blue dye mapping only. Furthermore, in accordance with the findings of Paganelli [14, 18], the results of this study suggest that large colloidal particles will allow the surgeon easily to find the sentinel lymph node intra-operatively, because only one or two sentinel lymph nodes are identified in the majority of patients.

References

1. Veronesi U, Rilke F, Luini A, et al. Distribution of axillary node metastases by level of invasion: an analysis of 539 cases. *Cancer* 1987; 59:682–687.
2. Cabanas RM. An approach for the treatment of penile carcinoma. *Cancer* 1977; 39:456–466.
3. Morton D, Wen D, Cochran A. Management of early stage melanoma by intraoperative lymphatic mapping and selective lympadenectomy: an alternative to routine elective lymphadenectomy or "watch and wait". *Surg Oncol Clin North Ann* 1992; 1:247–259.
4. Morton D, Wen D, Wong J, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992; 127:392–399.
5. Reintgen DS, Cruse CW, Bermann C, et al. An orderly progression of melanoma nodal metastases. *Ann Surg* 1994; 220:759.
6. Rutgers EJTh, Muller SH, Hoefnagel CA. The use of intraoperative probes in surgical oncology. In: Murray IPC, Ell PJ, eds. *Nuclear medicine in clinical diagnosis and treatment, 2nd edn.* London: Churchill Livingstone; 1998:1025–1036.
7. Tanis PJ, van Sandick JW, Nieweg OE, et al. The hidden sentinel node in breast cancer. *Eur J Nucl Med* 2002; 29:305–311.

8. Eshima D, Fauconnier T, Eshima L, Thornback JR. Radiopharmaceuticals for lymphoscintigraphy: including dosimetry and radiation considerations. *Semin Nucl Med* 2000; 30:25–32.
9. Wilher AJ, Mijnhout S, Franssen EJF. Radiopharmaceuticals in sentinel lymph-node detection. An overview. *Eur J Nucl Med* 1999; 26 Suppl:36–42.
10. Krag D, Weaver D, Ashikagat T, et al. The sentinel node in breast cancer – a multicenter validation study. *N Engl J Med* 1998; 339:941–946.
11. Tafra L, Lannin DR, Swanson MS, et al. Multicenter trial of sentinel node biopsy for breast cancer using both technetium sulfur colloid and isosulfan blue dye. *Ann Surg* 2001; 233:51–59.
12. Keshtgar MRS, Ell PJ. Sentinel lymph node detection and imaging. *Eur J Nucl Med* 1999; 26:57–67.
13. Van Lancker M, Goor C, Sacre C, et al. Patterns of axillary lymph node metastasis in breast cancer. *Am J Clin Oncol* 1995; 18:267–272.
14. Paganelli G, De Cicco C, Cremonesi M, et al. Optimized sentinel node scintigraphy in breast cancer. *Q J Nucl Med* 1998; 42:49–53.
15. Valdés Olmos R, Hoefnagel C, Nieweg O. Optimized mammary lymphoscintigraphy using larger colloid particles [reply]. *J Nucl Med* 2001; 42:826.
16. Alazraki NA, Styblo T, Grant S, et al. Breast cancer sentinel lymph node lymphoscintigraphy: comparison of subdermal and peritumoral injections. *J Nucl Med* 1999; 40:59.
17. De Cicco C, Cremonesi M, Luini A, et al. Lymphoscintigraphy and radioguided biopsy of the sentinel axillary node in breast cancer. *J Nucl Med* 1998; 39:2080–2084.
18. Paganelli G. Sentinel node biopsy: the role of nuclear medicine in conservative surgery of breast cancer. *Eur J Nucl Med* 1998; 25:99–100.