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LYMPHATIC PATHWAYS OF THE UPPER MEDIAL QUADRANT OF THE BREAST IN HEALTHY WOMEN: RADIOTRACER STUDY OF THE SENTINEL LYMPH NODE

A. Tassenoy, P. van der Veen, A. Bossuyt, J. Lamote, P. Lievens

Departments of Rehabilitation Research (AT,PvdV,PL), Nuclear Medicine (AB), and Thoracic Surgery (JL), Vrije Universiteit, Brussels, Belgium

ABSTRACT

This study examined the lymphatic drainage after injection of a radiotracer into the upper medial quadrant of the breast in healthy women. Most studies of lymphatic pathways of the breast have been performed in patients with breast cancer and concentrate on the upper lateral quadrant of the breast because of the high incidence of carcinoma at this site. The lymphatic drainage pathways of the medial half of the breast, however, has been less studied. A radiotracer (Tc-99m human serum albumin nanocolloid or HSA) was injected intradermally into the upper medial quadrant of the right breast in 12 healthy women. Dermal markers were placed at the middle of the clavicle, the axilla and at the jugular incisura. Three minutes after injection a static image of the injection site was made with a scintillation camera (Multispect 2 Gamma Camera System) over 20 seconds. After nine minutes, local soft massage was instituted at the injection site for 6 minutes. Fifteen minutes after injection, a graphic scintigraphic image was made of both breasts and axillae over 22 minutes. After this interval, three or four static images were made for a few seconds to locate the sentinel lymph node as related to the injection site.

A sentinel lymph node (lymphatic pathway) in the axilla was visualized in 11 subjects (91.9%) and was undetected in one subject (8.3%). The radiotracer migrated in all patients (100%) towards the ipsilateral axilla. In 9 subjects, the sentinel lymph node was visualized 15 minutes after injection, whereas in 2 subjects it appeared within an hour.

Breast carcinoma is the second cause of death after lung cancer in women with most breast cancers found in the upper lateral quadrant. Current treatment consists of various combinations including surgery, irradiation, and chemotherapy. During the past 20 years, conservation techniques have evolved including modified radical mastectomy, partial mastectomy, and lumpectomy. Axillary lymph node dissection remains an integral part of "staging" while eradicating axillary metastases if present. Regional radiotherapy and cytotoxic drugs are also used to contain or eradicate local or systemic breast cancer.

An important complication after breast cancer treatment and particularly axillary staging and regional irradiation is early or late development of lymphedema of the arm as a consequence of structural damage to arm paraaxillary lymphatic pathways. The

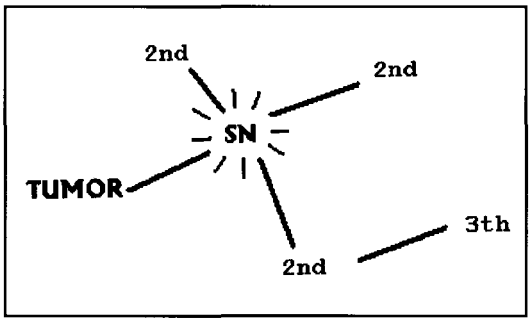

Fig. 1. The sentinel node.

incidence of arm lymphedema varies according to criteria used to define edema, cohort number of patients followed, the interval follow-up time, and edema measuring technique. A review by Petrek and Heelan (I) indicates an incidence of 6-30% with radiotherapy and chemotherapy considerably increasing the risk of arm lymphedema. An increased fluid volume of the arm is associated with both functional impairment and accompanying psychological side effects (2,3). Other potential complications of axillary dissection include dysesthesia from injury to the intercostal brachial nerve, wound infection, seroma formation, and lymphoceles.

Lymphatic mapping and sentinel lymph node (SLN) biopsy are recent innovations to minimize these sequelae and are rapidly changing the current management of breast cancer. SLN biopsy has the potential to identify those patients likely to benefit from full axillary dissection (that is, SLN positive for metastasis), while sparing SLN node negative-patients the morbidity of a radical dissection. Locally injected radiotracers and/or colored vital dyes are also used to localize the SLN.

The SLN is defined as the first lymph node depicted by the radiopharmaceutical or blue dye *(Fig. 1)*. The physiologic concept behind SLN biopsy is based on the idea that tumor spreads via lymphatics in a sequential pattern. If there is no metastasis in the SLN, then the risk of other lymph nodes being involved with metastasis is highly remote.

Since introduction of mammography, small breast cancers are found with greater frequency. When the diameter of the tumor is <1cm, metastases in axillary lymph nodes are found in only 10 to 20% (4,5). Thus, 80 to 90% of patients undergo radical staging that is therapeutically unnecessary with potential for complications including arm lymphedema. In larger tumors, axillary lymph nodes are involved in more than 70% of patients and complete axillary dissection remains necessary in these individuals.

Two methods of lymphatic mapping are used to identify SLN. The dye method employs isosulfan blue dye injected into the breast near and around the tumor. A small incision is then made below the hairline in the axilla and one visually searches for a blue-stained lymph node. The radiotracer approach involves injection of a radiopharma-ceutical (usually a Tc-99m labeled colloidal particle) followed by scintillation counter imaging and ultimately by an intraoperative hand-held probe for localizing lymph node containing radioactivity.

The current study aimed to visualize the lymphatic pathways and SLN after injection of a radiotracer into the upper *medial* quadrant of the breast in healthy women. Most previous studies have been performed in patients with breast cancer or have concentrated on the upper *lateral* quadrant of the breast with the high incidence of breast carcinoma in this area.

MATERIALS AND METHODS

The radiotracer (99 technetium labeled human serum albumin or HSA) was injected intradermally into the upper medial quadrant of the right breast while the subject was supine. The same experienced nurse performed the injection and the imaging procedure in each subject.

The radiopharmaceutical HSA (type Sorin Nanocoll, by Nycomed Amersham Sorin, Italy) was used with an injection volume of 0.4 ml and a dose of 0.5 mCi. The mean diameter of the particles was 90 nm.

Fig. 2. Massage of the injection site.

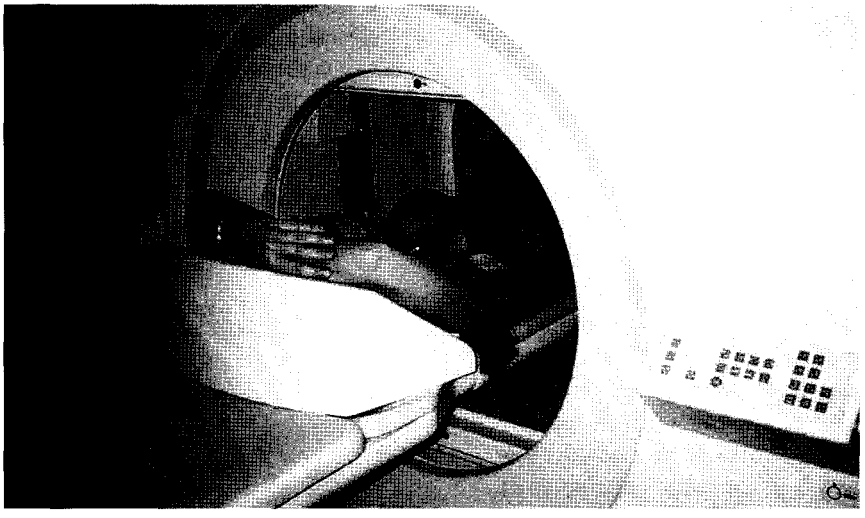

Fig. 3. Positioning in the gamma camera.

After the injection, the subject was placed under the scintillation camera without moving the arms. Three minutes after injection, a static image was made of the injection site for 20 seconds. The subject moved as little as possible to avoid muscle contraction and to minimize disturbance of the image.

After nine minutes, a local soft massage *(Fig. 2)* was performed at the injection site for six minutes to facilitate uptake into regional lymphatics (7). Fifteen minutes after injection, a dynamic image was made of both breasts and axillae for 22 minutes to detect the presence of the SLN *(Fig. 3)*. The subject was now lying supine with her arms above the head.

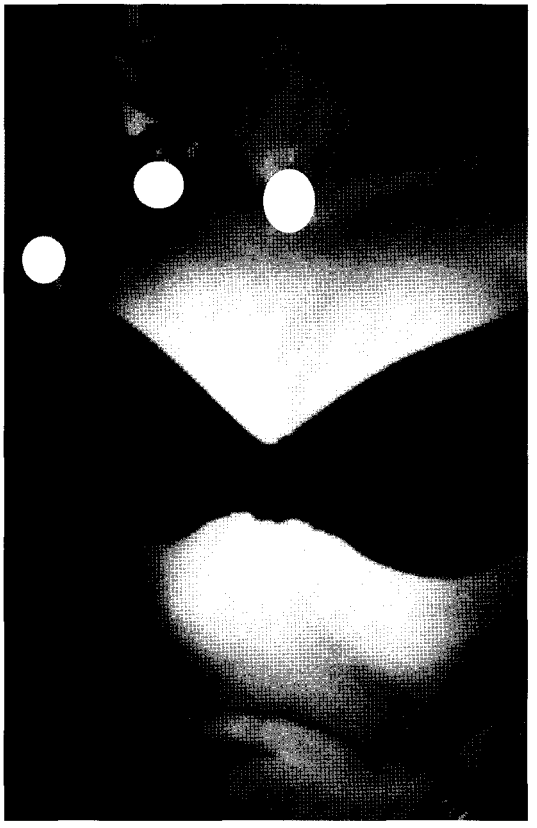

Fig. 4. Localization of dermal marks.

A Multispect 2 Gamma Camera System (Siemens) with a low energy collimator was applied to visualize the lymphatics and SLN. This camera has two heads each rotating 180° around the subject. Over the 22 minutes, the camera changes its position 45 times, each time making images over 30 seconds. At the end, the camera is at the same position as at the beginning. All tomographic images were analyzed with a Power Macintosh 8100/110 Icon of Apple. Images were reconstructed with the program OSEM.

After this interval, three or four lateral or anterior static images were made with and without marks (adhesive tapes with a small radioactive content) to locate the SLN as related to the injection site. Markers were placed on the skin in the middle of the axilla, midclavicular, and in the jugular incisura *(Fig. 4).*

RESULTS

Twelve healthy female volunteers (mean age 22.2 years; mean height 165 cm, and mean weight 60.0 kg) entered the study after informed consent. Ethical approval for the study was obtained from the Ethics Committee (OG16-20001014D) of the Vrije Universiteit Brussel.

Static Image

Location of the SLN related to the markers was obtained from the static images as shown in *Fig. 5.* If a second lymph node was visualized, one had to distinguish between this second node and the SLN. The computer calculates the mean "counts" or radioactive particles in a similar area of the node. The node with the highest radioactivity is deemed the SLN, even if not necessarily closest to the injection site. (8)

Axillary lymph nodes are typically subdivided into levels I, II and III to locate the SLN. Level I axillary nodes are situated between the latisimus dorsi muscle and the lateral margin of the pectoralis minor muscle; level II are situated between the medial and lateral margin of the pectoralis minor muscle;

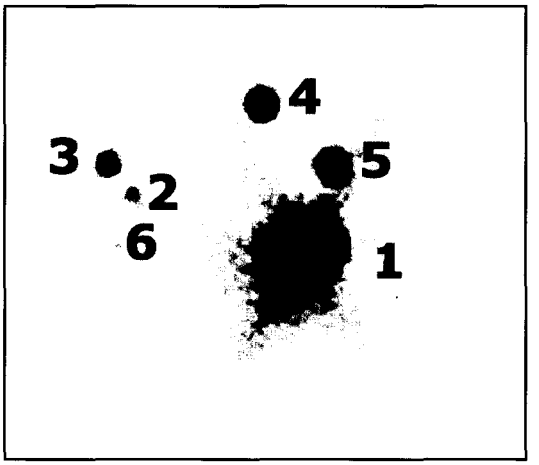

Fig. 5. Static image with (1) injection site; (2) sentinel lymph node; (3) axillary mark; (4) midclavicular mark; (5) suprasternal mark; (6) second lymph node.

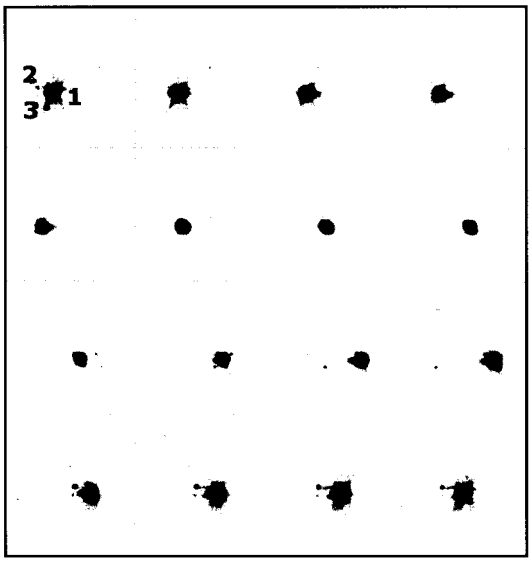

Fig. 6. Tomographic image with (1) injection site); (2) sentinel lymph node; (3) second lymph node.

and level III are situated between the medial margin of the pectoralis minor muscle and the axillary vein.

SPECT-Image

The volume-rendered SPECT (simple photon emission computed tomography) image *(Fig. 6)* represents a rotating three-dimensional image of the SLN over time. The first image is taken at the beginning of tomography, the last at the end when the camera has returned to its original position.

The SLN was successfully identified in 11 subjects (91.9%), and was not visualized in one subject (8.3%). The radiotracer migrated to the axilla in all patients (100%). No migration of the tracer occurred towards the parasternal lymph nodes *(Table 1)*. In 9 women, the SLN was visualized within 15 minutes after injection. In 2 subjects, the SLN node appeared later *(Table 2)*. In 5 subjects, a second lymph node was visualized *(Table 3)*.

TABLE 1
Anatomic Distribution of Sentinel Nodes in 12 Women

	#	%
Axillary, level I	0	(0)
Axillary, level II	10	(83.3)
Axillary, level III	1	(8.3)
Parasternal	0	(0)
Subclavicular	0	(0)
No sentinel node found	1	(8.3)

TABLE 2
Time of Appearance of the Sentinel Nodes in 12 Women

	#	%
Before 15 minutes	9	(75)
After 15 minutes	2	(16.6)
No sentinel node found	1	(8.3)

TABLE 3
Visualization of a Second Lymph Node in 12 Women

	#	%
Present	5	(41.6)
Absent	2	(58.3)

DISCUSSION

Several groups have studied lymphatic drainage pattern in patients with carcinoma of the breast. Although a high percentage of lymph drains towards the axilla, in some drainage also occurs to internal mammary nodes or other extra-axillary sites. Byrd et al (9) found that the internal mammary chain was involved in 17% and based on quadrant

location of the carcinoma was upper outer quadrant 10%, lower outer quadrant 27%, upper inner quadrant 17%, lower inner quadrant 25%, and central 29%. Uren et al (10) found that most breast cancer patients (93%) have lymphatic drainage toward the axilla, but in 56% drainage occurred to lymph nodes outside the axilla [internal mammary chain (45%), supraclavicular (13%), interpectoral and intramammary interval nodes (12%)]. Other studies (11-14) confirm the existence of drainage pathways other than to the axilla.

Examining the various publications, it seems clear, however, that no standardized technique exists to locate the SLN.

The radiotracer, Tc-99m-nanocolloid (diameter of 90 nm), is used in most studies to depict the SLN node in patients with breast cancer (15-17) and was successfully identified in ±89.4% of patients. We were successful in 11 of 12 healthy subjects (91.7%). As previously reported, the percentage of "non-found" SLN is between 5 and 30%, with a mean of 8%. In our study, the SLN was not found in 1 of 12 subjects or 8.3%. Tc-99m-HSA was used in other studies (14,15,17) but 95% of the particles are smaller than 80nm diameter. This latter colloid is probably absorbed faster and is transported through the lymphatic pathways with a prolonged retention in the SLN with less radiotracer gaining access to higher level nodes.

The behavior of particles injected interstitially depends on the diameter of particles. Large particles (500μm) have a slower rate of clearance from the interstitial space and stay longer at the injection site (17,18). Small particles (4 nm) migrate quickly so that only a fraction is trapped in the SLN with "secondary" lymph nodes often reached (12). They also penetrate venous capillaries to gain access directly to the bloodstream and are therefore unavailable for migration through lymphatic vessels. Blood capillary uptake also potentially adds undesirable blood background counts to an image and probe detection of an SLN (18). Particles smaller than 100nm satisfy the requirement of rapid migration into lymphatics, transport with lymph fluid and are large enough so they do not leak from lymphatics and do not pass into blood vessels directly (18). Colloid substances are commonly used as they are absorbed exclusively by the lymphatic system.

Vendrell-Torné et al (6) found after injection of a radiotracer into the upper medial quadrant of the breast, lymphatic pathways drained towards the axilla in 38%, towards the internal mammary lymph nodes alone in 6%, in 50% both towards parasternal and axillary lymph nodes and in 6%, a combination of a parasternal-axillary and supraclavicular pathways was detected. In our study of 12 healthy women, lymphatic pathway were only towards the axilla when the radiotracer was injected into the upper medial aspect of the breast. Vendrell-Torné et al. (6) used Au^{198}-colloids (diameter 35 na°) to identify the SLN in 250 healthy women. Perhaps the difference in radiotracer accounts for the difference in findings.

The dose of tracer used in patients with breast cancer has varied between 7 and 370 MBq, while the injected volume has varied between 0.2 and 4.0 ml (17). Small amounts of nanocolloids (17) minimally disturb lymph flow and minimize the risk of visualizing non-sentinel lymph nodes. Large amounts of tracer (15,17) tend to increase lymph flow with a greater likelihood of visualizing an SLN. We injected a volume of 0.4 ml, which agrees with most current investigators.

With breast cancer patients, the aim is to study lymphatic drainage from the tumor. It seems logical, accordingly, to inject the tracer as close as possible to the tumor. Three injection sites have been used: peritumoral, subdermal, and intratumoral. The dermal contains an abundance of lymphatics compared with the subcutaneous level which is sparsely populated by lymph vessels (19). Injection directly into the tumor is not favored because intratumoral tissue has high interstitial and intracellular pressure and

leakage of the tracer from the tumor can theoretically result in spreading of tumor cells along the needle tract (18).

Injection into overlying skin increases the chance to detect lymphatics and the SLN (15,17). According to Paganelli et al (20), a subdermal injection is most suitable because of fast detection of the SLN and patient comfort. Paganelli et al (20) and Nieweg et al (17) had a success rate of 98% in depicting the SLN.

There is still major disagreement about the optimal timing for imaging. Borgstein et al (15) suggest that lymph nodes do not entrap colloids until 2 hours after injection. Except for the non-visualization, the SLN was visualized within an hour in all our subjects. In 9 of 11 subjects, the SLN appeared within 15 minutes after injection. This observation is supported by Paganelli et al (20) who noted that in 90% of patients the SLN was depicted in < 15 minutes. There are also conflicting data on the optimal timing to start imaging. We began tomography 15 minutes after injection whereas some (13-15) prefer to image at 2 and 18 hours after injection; still others (16) favor 30 minutes after injection.

It is unclear why in one healthy person, no SLN was identified in our study. With breast cancer, draining lymphatic pathways can be plugged by tumorous tissues obliterating the SLN (17) but this explanation is insufficient for our young healthy subject.

In one of eleven subjects the SLN was found at level III of the axilla while the other ten were found at level II. Occasionally, metastases of breast cancer are found in higher lymph node levels of the axilla, whereas the lower nodal level is free of metastases (so called "skip" metastases). Further investigation is needed to determine whether this phenomenon is caused by tumor cells bypassing metastatic lower nodes to reach higher lymph nodes or simply drains towards the higher levels.

CONCLUSIONS

We found the SLN in the axilla from injection of a radiotracer into upper medial quadrant of the breast is 100% in the axillary lymph nodes when the tracer was injected intradermally in healthy women. Studies on the localization of the SLN show that the tumors situated in the upper medial quadrant drain with a high frequency to the internal mammary chain and subclavicular lymph nodes. When an SLN is found at these sites, the surgeon should also search for sentinel nodes in the axilla.

The fact that breast cancer patients occasionally demonstrate SLN in the internal mammary chain and elsewhere suggests that lymphangiogenesis (like hemangiogenesis) occurs with cancer to account for drainage to other nodal basins.

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Dr. A. Tassenoy
Department of Rehabilitation Research
Laarbeeklaan 103
1090 Brussels, Belgium