Methodology and implications of lymphatic mapping and sentinel lymphadenectomy
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CHAPTER FOUR

Anatomy and Physiology of Lymphatic Drainage of the Breast from the Perspective of Sentinel Node Biopsy

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The introduction of lymphatic mapping with sentinel node biopsy has evoked a renewed interest in the anatomy and physiology of the lymphatic system of the breast. In an increasing number of hospitals, lymphatic mapping with sentinel node biopsy is an essential component of staging patients with breast cancer. Several aspects of mammary lymphatic drainage are unclear, causing significant differences in the technique of sentinel node biopsy among both nuclear medicine physicians and surgeons. The choice of a certain injection type and the time of scintigraphic imaging or surgery are based on theories about the structure of the lymphatic network, theories about particle uptake into lymph channels and about lymph flow. The purposes of this article are to review current knowledge on the anatomy and physiology of the lymphatic system of the breast, to translate this into implications for the clinical practice of lymphatic mapping, and to point out areas of controversy.

**General anatomy and physiology of the lymphatic system**

Lymph is absorbed from the interstitial space into blind-ending lymphatic capillaries. Lymphatic capillaries are 10 to 50 μm in diameter and consist of a single layer of endothelial cells with a discontinuous basement membrane. Overlapping interendothelial junctions function as valves with openings that are 10 to 25 nm wide, permitting the entrance of small particles. Pinocytosis may be responsible for the vesicular transport of larger particles through the endothelium. Collagen filaments anchored to the surrounding connective tissue prevent the collapse of lymphatic capillaries.

The filling of lymphatic capillaries can be explained by the osmotic pressure gradient and by fluctuating intraluminal pressures caused by contractions and forward flow of lymph. Lymph formation, active contractions and external pressures generate lymph flow. Peristalsis occurs at 10 to 15 contractions per minute by longitudinal and circular layers of smooth muscle in the media. Peristalsis is regulated by filling pressure, humoral mediators (serotonin, prostaglandins) and neural mechanisms. A transmural distending pressure of 2-4 cm H₂O is required for these contractions which spread at a velocity of 4-5 mm/s. The flow is unidirectional because of the lymphatic valves. Sustained external pressure reduces the flow speed and intermittent external pressure enhances it.

Lymphatic capillaries drain into collecting lymphatic vessels, which in turn drain into a lymph node. The afferent vessels drain into a marginal sinus and subsequently into medullary sinuses between the germinal centres. These centres contain large numbers of phagocytic cells that accumulate protein colloids, such as the radio labelled tracers, but not vital dyes. The plexus within the lymph node drains to the efferent lymphatic vessel, which joins the artery and vein in the hilum. Direct drainage of the marginal sinus into the efferent vessel also exists. Ludwig demonstrated two main types of relationship between lymph vessels and lymph nodes. In the first type, the lymph node receives lymph from the afferent duct, filters it and then discharges it into the efferent channel. In the other type, the lymphatic vessel runs through the lymph node or over its surface without
discharging its contents into that node (figure 1). This means that the first lymph node to which the afferent channel runs is not always directly at risk of harbouring tumour cells, which may be one of the explanations of a false negative sentinel node. The lymph of the entire body is collected in several large trunks that drain into the venous circulation. The lymph flow of the entire body amounts to 2 to 4 L/day in rest but varies with a diurnal rhythm and according to physiologic needs.\(^3,5\)

**Figure 1.** The different relationships between lymphatic vessels and lymph nodes according to Ludwig.\(^4\) Afferent lymphatic ducts on the left discharge their contents into the marginal sinus. One lymphatic duct runs through the node on the right and another over its surface bypassing the germinal centres (Drawing: PJ Tanis).

**Lymphatics of the breast**

The anatomy of the lymphatic system of the mammary gland has been studied for several centuries. The history of the lymphatic system of the breast has been described in detail by Haagensen.\(^6\) At the end of the eighteenth century, Cruikshank and Mascagni independently described two main lymphatic drainage routes of the breast: an external system and an internal system.\(^7,8\) The external route from the nipple, the integuments and the lactiferous tubules was shown to run to the axilla. The internal route from the dorsal part of the breast was thought to perforate the pectoral and intercostal muscles. Within in the intercostal spaces, these lymphatics were seen to subsequently join the plexus coming from the liver and the diaphragm and then run on each side of the internal mammary artery and veins.

In the 1830's, Sappey performed a more thorough study using mercury injection into the lymphatic channels.\(^9\) He concluded that most breast tissue drains centripetally into the subareolar plexus and then on to the axilla. These findings were later confirmed by Rouvière and Grant.\(^10,11\) Around the end of the nineteenth century and the beginning of the twentieth century, anatomists gained more knowledge of the mammary lymphatics using post-mortem injections of various tracer fluids. Evidence was presented that Sappey's concept is incomplete and that additional lymphatic routes exist.\(^6\)
A Dutch physician named Camper was the first to identify lymphatic drainage to lymph nodes along the internal mammary vessels in 1770. These nodes extend upwards from the fifth intercostal space to the retroclavicular glands. Injection studies with vital dyes showed that the internal mammary nodes receive their lymph from deep lymphatics. These lymphatics arise from the breast lobules, leave the posterior surface of the breast and pass through the pectoral and intercostal muscles to reach the internal mammary chain (figure 2).

Knowledge increased in the twentieth century by using new techniques such as autoradiography of surgical specimens with radioisotopes. In the 1950's, colloidal gold 198 with a particle size of about 5 nm was injected into the breast parenchyma. Turner-Warwick stated that ipsilateral axillary lymph nodes receive more than 75% of the lymph of the breast using this technique. Hultborn, Vendrell-Torné and Turner-Warwick confirmed that the ipsilateral internal mammary chain undoubtedly represents another important pathway of lymph drainage from both the lateral and medial halves of the breast.

Other less common drainage routes have been described. Lymphatics sometimes pass through lymph nodes on their way to the axilla or internal mammary chain, so-
called interval nodes (figure 2). These are the interpectoral nodes as described by Grossman and Rotter or lymph nodes in the breast parenchyma (intramammarian or paramammarian nodes) as observed by Cruikshank and Gerota. Mornard first described occasional direct drainage from the breast parenchyma to supraclavicular nodes. Retrosternal lymphatic drainage to the contralateral internal mammary chain occurs sporadically. Subcutaneous drainage to the contralateral axilla is unlikely to occur before the ipsilateral drainage is impaired by lymphatic obstruction caused by tumour growth, previous surgery, or irradiation. Blockage of normal lymph flow can also cause drainage in a retrograde direction to the liver via the internal mammary chain. The posterior intercostal lymph nodes have been shown to receive lymph from the breast in a small proportion of patients. Caplan described drainage to the anterior intercostal nodes.

**Course of lymph flow**

It is uniformly accepted that drainage from the breast can occur to lymph nodes at a number of different sites. There is also consensus that the axilla is the main basin for lymphatic drainage from the breast. However, no agreement exists about the course of lymph flow between the breast tissue and the nodal basins. Turner-Warwick suggested that the lymphatics run within the breast parenchyma and drain directly to the axilla. He stated that the importance of the subareolar plexus in the resting breast parenchyma had been overemphasised by Sappey and Rouvière and he indicated why earlier investigators were misled. Filling of the lactiferous system with tracer using random injections and observations in a lactating breast and infant cadavers had been confounding factors. Spratt stated that the lymphatics paralleling the lactiferous ducts are equivalent to the vertical lymphatics that connect the subepithelial and subdermal lymphatics. Their valve structure may be similar and lymph flow will be from superficial to deep. In a study of mastectomy specimens, we never found lymphatic channels from the tumour pass through the subareolar plexus before heading to the axilla. Our lymphoscintigraphy experience points in the same direction. Following injection of technetium-99m (99mTc) labelled nanocolloid into the breast carcinoma, a lymphatic channel is typically depicted running a direct course from the tumour to the axilla (Figure 3). Rarely, a curved lymphatic channel with an indirect course is visualised, but there is certainly no constant route via the subareolar plexus (Figure 4). Although Sappey’s view of drainage of the breast parenchyma through a subareolar plexus to the axilla is supported in the current literature, Turner-Warwick, Spratt and we believe that direct drainage from the breast to the axilla is the rule.

**Clinical implications**

**Tracer uptake and lymph flow**

The structure of the lymphatic system has implications for the choice of labelled colloid. Colloids with a small particle size (eg, antimony trisulfide, 3 to 12 nm) can rapidly pass the openings of the interendothelial junctions (10 to 25 nm) and often
visualise the lymphatic channels leading directly to the sentinel node. A disadvantage of these small particles is that phagocytic cells in a sentinel node often cannot trap them all, so that some of the tracer moves on to lodge in secondary nodes. Larger particles (e.g. unfiltered sulfur colloid, 50-1000 nm) enter the lymphatic channels more slowly through pinocytosis. The channel is visualised less often but the tracer travels on to secondary nodes less frequently. Even larger particles do not migrate from the injection site. The optimum size is probably between 10 and 100 nm.\textsuperscript{27,28}

Figure 3. The lymphatic route between the tumour and the lymph node has a direct course in most patients. A: Lymphoscintigraphy using \textsuperscript{99m}Tc-nanocolloid with direct drainage from the tumour (T) in the upper medial quadrant to an axillary lymph node. B: Three separate lymphatics to three axillary sentinel nodes, each running on a different level, are visualised in a patient with a tumour in the lower lateral quadrant. Another sentinel node is situated in the breast tissue just lateral from the tumour (arrow). C and D: Anterior and right lateral view of three lymphatic ducts from a central tumour to an internal mammary chain node (arrow) in the fourth intercostal space and to two axillary sentinel nodes.

Figure 4. In some patients, an indirect drainage pattern is visualised with a highly variable course. A and B: An anterior and left lateral view show one lymphatic vessel (open arrow) originating at the medial-anterior surface of a tumour (T) in the subareolar area and running with an S-shaped course to an axillary lymph node. Another lymphatic channel (solid arrow) arises from the posterior surface of the tumour, runs to the deep part of the breast and drains into an internal mammary chain node. C and D: Anterior and left lateral view of a lymphatic channel (arrow) running from the medial-posterior surface of a lower-lateral quadrant tumour to a sentinel node in the axilla.
The timing of scintigraphy must be chosen carefully, because lymphatic flow and absorption of tracer are highly variable. Early static images at one hour after injection fail to identify sentinel nodes in patients with a slow tracer uptake and flow. Late images 18 to 24 hours after injection depict all radioactive nodes, but discrimination between first- and second-tier nodes is more difficult at this time because there is no visualisation of lymphatic channels. The sequential pattern of filling of the lymphatic ducts and stepwise uptake of tracer in the first- and second-echelon nodes can be visualised with multiple scintigraphic examinations between a few minutes and a few hours after injection as used by Sandrucci, Veronesi, Schneebaum, Canavese and Doting.29-33

Knowledge of the physiology makes it clear that lymph flow is guaranteed by a delicate balance between pressures inside and outside the lymphatic vessel. This has repercussions for the optimum volume of tracers. The volumes of radioisotope injection described in literature range from 0.2 to 16 ml (tables 1 and 2). These numbers differ a factor of 80, and this illustrates that we do not know the optimum volume. The volumes of blue dye injection have a smaller range of 0.5 to 7.5 ml (table 2). Investigators who use a small volume argue that they do not want to disturb the physiology of lymph flow and that they want to avoid the risk of visualising non-sentinel nodes. A small tracer volume does not disturb the pressure equilibrium and results in 85 to 91% visualisation as shown by Mertz, Sandrucci, Reuhl, Uren and Doting (tables 1 and 2). The sentinel node was visualised in 75 of our last 76 patients (99%) using a small 0.2 ml volume of the tracer.

Table 1. Sentinel node biopsy techniques in breast cancer. Studies using radioactive isotope.

<table>
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<tr>
<th>Author</th>
<th>N</th>
<th>Type of colloid</th>
<th>Volume (ml)</th>
<th>Dose (mCi)</th>
<th>Injection site</th>
<th>Scintigraphy visualisation (%)</th>
<th>Drainage IMC (%)</th>
<th>Identification rate (%)</th>
<th>False-negative rate (%)</th>
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IMC=internal mammary chain, SC=sulfur colloid, SCf=filtered sulfur colloid, DX=dextran, NC=nanocolloid, LS=lymphoscint, HA=human albumin colloid, PT=peritumoural, IT=intratumoural, SD=subdermal, SA=subareolar, ND=not done, NS=not stated, †=also drainage to supraclavicular nodes (2%), *=also drainage to interpectoral (0.7%), intramammary (0.2%) and supraclavicular sentinel nodes (0.2%), †=also drainage to intramammary nodes (4%)
Table 2. Sentinel node biopsy techniques in breast cancer. Studies using radioactive isotope and blue dye.

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Vol=volume, lnj=injection, IMC=internal mammary chain, SC=sulfur colloid, SCf=filtered sulfur colloid, RC=rhenium colloid, NC=nanocolloid, HA=human albumin colloid, TC=tin colloid, MS=microcolloid sulfide, IS=isosulfan blue, PB=patent blue, MB=methylene blue, IC=indigo carmine, PT=peritumoural, IT=intratumoural, SD=subdermal, ND=not done, NS=not stated, *=colloid and dye together, †=also drainage to supravacuolar sentinel nodes (2%), §=also to supravacuolar sentinel node (2%), $=also intramammary (3%) and interpectoral (2%)

Investigators using the larger volumes do want to change the physiology; they intend to increase the lymph flow and thus increase the chance of visualising a lymph node. Krag and associates reported a higher identification rate when the volume was increased from less than 3 ml to more than 8 ml, but this study was not carried out in a randomised fashion, and results are likely to improve with increasing experience no matter what technique is used. Schmidt identified the sentinel node in 90% of the patients with the combination of a high volume (16.0 ml), filtered sulfur colloid and massaging of the injection site (table 2). From a physiological point of view, high volumes may result in sustained external pressure exceeding the transmural distending pressure required for uptake of the tracer and lymph flow. On the other hand, the anchoring filaments pulling on the duct cells as a result of interstitial fluid expansion may widen the clefts between ductal cells, facilitating the entry of particles. A disadvantage of a large volume is an increased diffusion zone at the injection site, which hamper scintigraphy and probe detection of nodes nearby. Although tracer volume is a subject of controversy, detection rates seem good with both small and larger volumes of tracer fluid. The amount of radioactivity that is accumulated in a lymph node does not only depend on particle size and possibly the injected volume but also on a number of other tracer parameters like radioactivity dose, the number of particles, their surface characteristics and stability. Other factors can influence the pattern and speed of
lymph drainage. Valdés Olmos found that the age of the patient was a significant factor for sentinel node identification. Humoral mediators and neural mechanisms play a role but these factors are beyond our control. Anaesthetic drugs may hamper the uptake of blue dye. Halothane has been shown to decrease the lymph flow rate by 25% to 59%. Hydration of the patient may be a factor. Patients typically come to the operating room in a poorly hydrated state. It is conceivable that administration of ample fluids before the tracer is injected increases the likelihood of finding a sentinel node. Gentle massaging of the injection site is definitely an important manoeuvre, because intermittent external pressure stimulates lymph flow.

Injection site

As mentioned previously, different routes of tracer administration are being used in the sentinel node procedure for breast cancer. The injection can be periareolar, subareolar, intradermal or subcutaneous over the primary tumour site, peritumoural and intratumoural (tables 1 and 2). The first four injection types are based on the hypothesis that the breast and the overlying skin share the same lymphatic drainage because the mammary gland is embryologically derived from the ectoderm. This was suggested in a study that demonstrated a 100% concordance between intradermal patent blue injection and peritumoural radioactive tracer injection. Anatomic studies have shown that the density of lymphatics is greater in the skin than in breast parenchyma. This means that tracers are cleared more rapidly from the skin than from parenchyma. Lymphatic channels are visualised almost without exception following an intradermal injection, whereas this happens in 40% of our patients following intraparenchymal administration. Visible lymphatic channels allow one to better distinguish first-echelon nodes from higher-echelon nodes and this is a definite advantage of the intradermal injection technique. Another advantage is that one can choose the injection site anywhere in the skin of the breast so that interference of scattered radiation with imaging or probe detection is kept to a minimum and lymphatic mapping in non-palpable lesions is made easier. On the other hand, it may be presumptuous to rely on the connections between collecting lymphatic vessels from the skin and those originating at the tumour site and assume that there is no lymphatic watershed in between.

An increasingly popular technique is subdermal or subcutaneous injection over the primary tumour. This approach does not provide the certainty that the identified lymph node is indeed the node that receives drainage from the primary tumour and this approach is also hampered by the absence of a dense lymphatic network like the one that is present in the skin. Despite these theoretical shortcomings, this technique has provided good identification results of lymphatic mapping. Subareolar injection, based on Sappey’s concept, has also shown good results. Canavese and associates compared subdermal injection of radioisotope over the tumour with subdermal or intraparenchymal injection away from the primary tumour. Because of a high percentage of mismatches, they concluded that there is not a sentinel node in the axillary basin that indiscriminately drains the entire breast. Other authors such as Borgstein, Roumen, Mertz, Klimberg and Linehan tried to determine the reliability of injection sites away from the primary tumour for axillary staging. Often-used criteria for judging such comparative studies are...
identification rates and concordance with a ‘gold standard’ (peritumoural injection). These are questionable criteria. Identification rate is multifactorially defined, as already mentioned. Concordance when radioisotope and blue dye are injected at different sites does not necessarily signify that the hypothesis is correct. Such a result also depends on the different physiological behaviour of the two tracers or a difference in injection technique by nuclear medicine physician and surgeon. Many sentinel nodes are either blue or radioactive even when both tracers are administered at the same site. Variation in lymphatic flow can be one of the explanations of discordance after repeated radioisotope injection, as shown by reproducibility studies in melanoma. Even the identification of the only tumour-positive node with intradermal injection as described in a few patients by Borgstein, Linehan, Roumen, Bourgeois and their colleagues is not the decisive evidence of the accuracy of the technique. Hill described that a positive sentinel node was only blue or radioactive with peritumoural injection of both blue dye and radioisotope. The main point is that the sensitivity has not been firmly established in all these studies. Confirmatory axillary dissection was not performed in all patients and non-sentinel node evaluation was insufficient by modern standards lacking step-sectioning and immunohistochemistry staining.

The implication of the injection site for identification of sentinel nodes outside the axilla seems to be clearer. Drainage to internal mammary nodes is rarely seen after intradermal or subdermal injection of radioisotope in breast cancer patients. Studies from the European Institute of Oncology in Milan nicely illustrate the difference in visualisation of sentinel nodes outside the axilla after subdermal and peritumoural injection. Veronesi and Zurrida from that institution found drainage to internal mammary nodes after replacing routine subdermal injection by peritumoural injection for deep lying tumours. Apparently, intradermal or subcutaneous injections visualise the superficial lymphatic system running towards the axilla but not the deep lymphatics that run to the internal mammary, interpectoral or intramammary nodes. Internal mammary sentinel node identification after peritumoural or intratumoural injection occurs in up to 35% of the patients. Interpectoral and supraclavicular sentinel nodes are less frequently seen (in about 2%) but only after intraparenchymal tracer administration. Intramammary nodes were seen in 21 of 305 patients (7%) according to our own experience with intralesionl tracer administration, and in 4% by Rull and colleagues with peritumoural injection. Sentinel nodes in all these locations can be harvested and may contain relevant staging information.

**Summary and conclusions**

Knowledge of the anatomy and physiology of the lymphatic system are helpful when considering a particular sentinel node biopsy technique. The delicate balance between internal and external pressures in a lymphatic channel can be influenced by the injection volume and by massage in a negative or positive way. The narrow openings in the interendothelial junctions determine the speed of clearance of particles with a certain size, and this has implications for the timing of lympho-
scintigraphy and surgery. Tracer uptake and lymph flow are highly variable and depend on a number of factors, some of which are beyond our control.

The lymphatic anatomy is not completely understood despite numerous studies since the end of the eighteenth century. Several topics have been elucidated in more recent studies and through experience with sentinel node biopsy. First, although axillary drainage is the principle lymphatic path of the breast, any drainage pattern from any quadrant of the breast can occur. Secondly, most lymph from the breast flows to the nodal basins with a direct course, not passing through the subareolar plexus. Another relevant point is that gentle massaging encourages lymph flow and facilitates sentinel node detection.

What problems do we still face in clinical practice? The optimum size and number of labelled colloid particles remain to be established. The optimum volume of the tracer also remains to be determined. The main controversy concerns the injection site. Although the intradermal injection technique has attractive practical features, there is currently insufficient certainty that drainage of tracer injected anywhere in or underneath the skin of the breast reflects drainage from the cancer. Connections between collecting lymphatic vessels from the tumour site and the collecting vessels from the skin and subdermal lymphatics can explain the concordance between intraparenchymal and superficial injections in most patients.

To determine the technique that yields the best sentinel node identification rate with the least possible false negative rate would require a large randomised trial with all patients undergoing a completion lymph node dissection and evaluation of all other axillary lymph nodes using serial sections and immunohistochemistry. Current knowledge about sensitivity is based on examination of the other axillary nodes with hematoxylin and eosin staining and not with immunohistochemistry, with the exception of two studies. In addition, a complete level I to III dissection may not have been done in all patients and it is not certain that pathologists removed and examined all the nodes from the specimens. The proposed study seems impossible now that routine axillary node dissection has been abandoned by the larger centres around the world.

Choosing the most attractive approach comes down to determining the aim of lymphatic mapping. A superficial injection technique may be adequate when the purpose is to spare patients without lymph node metastases in the axilla an unnecessary axillary node dissection. An intraparenchymal injection technique should be used when the additional purpose is to determine the stage as accurately as possible and, therefore, identify sentinel nodes elsewhere as well.

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