THE USE OF LYMPHOSCINTIGRAPHY TO LOCALISE THE SENTINEL LYMPH NODE/S IN EARLY BREAST CARCINOMA USING THE SINGLE INTRATUMORAL INJECTION TECHNIQUE.

BY
NISAAR AHMED KOROWLAY
MBChB (NATAL)

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CONTENTS i

ACKNOWLEDGEMENTS vii

DECLARATION x

ABSTRACT xi

INTRODUCTION 1

AIM OF STUDY 5

LITERATURE REVIEW 6

Normal Lymphatics 6

Historical Background 6

Organisation of the Lymphatic System 6

Breast Lymphatics 8

Introduction 8

Historical Background 9

Distribution of Breast Lymphatics 10

Lymph nodes 11

Introduction 11

Regional Axillary Lymph Node Basin 13

Breast Carcinoma 16

Epidemiology 16

Classification 16

Clinical Course	17
The Sentinel Lymph Node	19
Introduction	19
The History of the Sentinel Lymph Node	20
The Sentinel Lymph Node Concept	20
The Sentinel Lymph Node Definition	21
The Axilla	22
Internal Mammary Nodes	23
Intramammary Nodes	24
Radiopharmaceuticals in SLN detection	25
^{99m}Tc antimony trisulphide colloid	25
^{99m}Tc albumin-based colloid	26
^{99m}Tc nanocolloid	26
^{99m}Tc microaggregated albumin	27
^{99m}Tc macroaggregated albumin	27
^{99m}Tc sulphur colloid	27
^{99m}Tc human serum albumin	28
Other Agents	28
Radiation Dosimetry and Protection	29
Injection Techniques to Identify the SLN	32
Subareolar and Periareolar Techniques	33
Peritumoral Technique	34
Intratumoral Technique	34
Subdermal or Intradermal Technique	35
Imaging Technique	36
Preoperative Lymphoscintigraphy	36
The purpose of Dynamic Imaging	36
The purpose of Static Imaging	36
Causes for Non-visualisation of SLN	37

Cause for Delayed Visualisation of SLN 37
The Handheld Gamma Probe 38
Vital Blue Dyes in SLN Localisation 40
The Role of Pathology 42
Serial Sectioning 42
Immunohistochemical Staining 43
Reverse Transcriptase Polymerase Chain Reaction 44
Clinical Significance of Micrometastases 44
Reliability of Intraoperative SLN Analyses 46
Intraoperative Frozen Section 46
Significance Of Non-Sentinel Lymph Nodes 48
Reasons for false-negative SLN biopsy 49

PATIENTS AND METHODS 50
Patient Criteria 50
Inclusions 50
Exclusions 50
Study Protocol 50
Ethical and Legal Considerations 51
Procedure and Technique 51
Injection technique 51
Imaging Protocol 52
Dynamic phase 52
Static phase 53
Skin markings 54
Processed Data 55
Visual Assessment 56
Reporting 56

Surgery 58

RESULTS 59

Patient and Tumour Characteristics 59

Lymphoscintigraphy 60

Lymphatic Channels 60

Sentinel Lymph Nodes 61

Number of SLN seen 61

Times SLN first seen 61

Sites of SLN 62

2nd Echelon Nodes 62

Surgical and Histological Findings 62

DISCUSSION 65

CONCLUSION 71

REFERENCES 72

LIST OF FIGURES

Figure 1	Lymphatic drainage of the breast, anterior view.	11
Figure 2	Normal lymph node architecture.	12
	A. Schematic diagram of a lymph node.	12
	B. Low power view of a lymph node.	12
Figure 3	Schematic drawing illustrating the major lymph node groups associated with lymphatic drainage of the breast.	15
Figure 4	A sentinel node is defined as a lymph node that receives drainage directly from the primary tumour.	21
Figure 5	Demonstration of the injection technique.	51
Figure 6	Patient positioned between the detector head and the transmission source for dynamic and lateral static acquisitions.	52
Figure 7	Oblique image showing nodal clusters.	53
Figure 8	C-Trak handheld gamma probe.	54
Figure 9	Complete study: processed images.	55
Figure 10	Example of scintiscans.	56
	A. Single channel with a single SLN in left axilla.	56
	B. Two SLNs seen in left axilla simultaneously without channels.	56
	C. Single SLN in the IMN without a channel.	56
Figure 11	Primary tumour distribution in various quadrants.	58

LIST OF TABLES

Table 1	Ten-year survival rates of nodal negative, nodal positive and of patients with supraclavicular lymph node metastases or distant disease.	18
Table 2	Type of radiopharmaceuticals and particle size.	28
Table 3	Effective dose of Radiological and Nuclear medicine procedures.	30
Table 4	The advantage of touch imprint cytology vs frozen section.	46
Table 5	Patient and tumour characteristics.	59
Table 6	Number of nodes seen at preoperative lymphoscintigraphy.	60
Table 7	Histological findings of SLN versus the remaining axillary nodes.	62

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DECLARATION

I, Nisaar Ahmed Korowlay, **declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise), and that neither the whole work nor any part has been, is being, or is to be submitted for another degree in this or any other university.**

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ABSTRACT

Greater public awareness and early screening has led to patients presenting with smaller breast tumours.

Knowledge about axillary node metastases is essential to accurately stage early breast carcinoma and plays an important role in determining prognosis and post-operative management.

The standard method of evaluating the axilla has been a complete axillary lymph node dissection. This can result in significant patient morbidity, particularly lymphoedema and neurological disturbances in the ipsilateral arm.

Sentinel lymph node (SLN) biopsy is being used increasingly for staging early breast carcinoma in place of complete axillary lymph node dissection. The optimal method to identify the SLN and has not been clearly elucidated in the literature.

A number of techniques have been proposed for identifying SLN/s. The main debate centres on whether to use a blue dye or radiopharmaceutical method either singly or in combination. The different injection techniques described in the literature reliably identify the "true" SLN/s in the axilla. It can thus be hypothesised that predefined SLNs representative of the entire breast, can be found irrespective of the injection site of the radiopharmaceutical or blue dye.

With any new technique some degree of learning is required in order to perform it accurately in a reproducible manner with a minimum failure rate

and complications. To validate the new technique, a study consisting of the removal of the SLN together with a complete axillary lymph node clearance is required. This allows the nuclear medicine physician and the surgeon to determine the pathological status of the identified sentinel lymph node and the rest of the axillary lymph nodes in order to assess the false-negative rate of the sentinel lymph node.

AIM

To determine if the single intratumoral technique with preoperative lymphoscintigraphy is a valid and reliable method to identify the SLN/s.

PATIENTS AND METHODS

Patients with a clinical stage I (1-2 cm) or a stage II (>2cm - 5 cm) breast carcinoma and non-palpable axillary lymph nodes were included.

The technique for SLN identification consisted of:

1. A single intratumoral injection of ^{99m}Tc-nanocolloid.
2. Preoperative lymphoscintigraphy.
3. Intraoperative removal of the SLN guided by the handheld gamma probe and methylene blue dye within 36 hours.

The first 36 procedures included a SLN biopsy followed by complete axillary lymph node clearance. The SLN/s together with the remaining axillary lymph nodes was sent for histological analysis. This was done to assess the accuracy of the intratumoral injection technique in identifying the true SLN and to predict the status of the rest of the axillary lymph nodes.

Based on the experience gained and the high identification rate of the true SLN from the first 36 procedures, the subsequent surgical management of the axilla changed as a complete axillary lymph node clearance was done only if the SLN was positive at frozen section.

RESULTS

A total of 102 patients were analysed and 103 SLN procedures were performed as one patient had bilateral synchronous breast carcinoma. The median age of the patients was 52 yrs (range 30-77 yr).

The first 36 procedures had a SLN identification rate of 89% as only in 32 procedures was the SLN identified. There was a false-negative rate of 5% in 28 SLN biopsies when compared to 28 complete axillary lymph node clearances.

The overall SLN identification rate was 96% (99/103). Ninety percent of the SLNs were visualised within two hours by lymphoscintigraphy.

In 97% (96/99) of the procedures, the SLN was located in the axilla irrespective of the location of the tumour while in 3% (3/99), it was noted only in the internal mammary node (IMN) chain. The axilla was the only site in 73/96 (76%) procedures while in 23 it occurred in the axilla as well as other sites. Of these 12/23 were in the IMN chain, 10/23 were in the intramammary area and in 1/23 the SLNs were seen in the IMN and intramammary sites.

CONCLUSION

A single intratumoral injection technique with preoperative lymphoscintigraphy is a valid and reliable method for identifying SLNs.

INTRODUCTION

Breast carcinoma is a worldwide problem with over one million new cases diagnosed each year[1]. In South Africa it is the second commonest carcinoma in women[2].

Greater public awareness and early screening has led to patients presenting with smaller breast tumours. Knowledge about axillary node metastases is essential to accurately stage early breast carcinoma and plays an important role in determining prognosis and post-operative management. The incidence of axillary nodal involvement in early breast carcinoma ranges between 20 – 40%. Clinical examination is a poor determinant of nodal involvement and histological examination is thus essential to identify the latter[3].

The standard method of evaluating the axilla has been a complete axillary lymph node dissection. This can result in significant patient morbidity, particularly lymphoedema and neurological disturbances in the ipsilateral arm[3]. Sentinel lymph node (SLN) biopsy has revolutionised the investigation and management of axillary nodes. Identification and meticulous histological examination of the sentinel lymph nodes allows for accurate prediction of the tumour status of the regional axillary basin and avoids the morbidity and expense of complete axillary dissection in node negative patients[4,5,6].

A number of techniques have been proposed as the optimal method for identifying SLN/s. The main debate centres on whether to use a blue dye or radiopharmaceutical method either singly or in combination.

The different injection techniques described in the literature all reliably identify the "true" SLN/s in the axilla. It can thus be hypothesised that predefined SLNs representative of the entire breast, can be found, irrespective of the injection site of the radiopharmaceutical or blue dye[7,8].

When a new technique is introduced in a unit, some degree of learning is required in order to perform it accurately in a reproducible manner with a minimum failure rate and complications. To validate the new technique, a study consisting of the removal of the SLN together with a complete axillary lymph node clearance is required. This allows the nuclear medicine physician and the surgeon to see whether the pathological status of the sentinel lymph node identified, correctly reflects the status of the rest of the axillary lymph nodes in order to assess the false-negative rate.

According to the American Society of Breast Surgeons, a validation study of at least 30 or more cases should be done in order to attain a SLN identification rate of 85% and a false-negative rate of less than or equal to 5%[9,10,11].

SLN biopsy in breast carcinoma was studied at Groote Schuur Hospital in 1995-1996. Disappointing results with the blue dye method alone led to the technique being abandoned[12]. In April 2000, Amersham International (UK) donated an intraoperative handheld gamma probe to the Nuclear Medicine Department. To initiate the use of lymphoscintigraphy and SLN biopsy, Amersham International (UK) brought out Professor C.A. Hoefnagel, a nuclear medicine physician, and Dr E.J. Th. Rutgers, a surgical oncologist, both from the Netherlands Cancer Institute, to demonstrate the intratumoral injection technique.

At the Netherlands Cancer Institute[13], this technique was initiated in breast carcinoma shortly after Morton originally described lymphatic mapping in melanoma. They felt it was best to inject the radiopharmaceutical directly into the primary tumour so that the radiopharmaceutical would follow exactly the same route as the disseminating tumour cells. A concern was that the injection of the radiopharmaceutical distant to the tumour might increase the risk of crossing a lymphatic watershed and thus visualise a node that was not the true sentinel lymph node[13]. Therefore, in order to preserve the normal physiology of lymph drainage, a small volume of only 0.2 ml was injected into the tumour[13].

Criticism of the intratumoral injection technique centres on the belief that injection into a tumour causes tumour cells to spread along the needle track with the release of additional tumour cells into the circulation. However, surgeons and pathologists routinely perform fine needle aspiration cytology or tru-cut needle biopsy to confirm the diagnosis of breast carcinoma with no apparent adverse events[14].
The average breast tumour is present for 5-10 years before it is clinically detected. During this period tumour cells continuously detach from the primary tumour. The rate of tumour cell shedding has been measured in breast carcinomas of rats and was found to be 3.2 million cells per 24 hours per gram of tissue[13]. Tumour cells that are shed from a solid tumour as a result of manipulation are transient and therefore unlikely to have a measurable impact on the prognosis[13].

The intratumoral injection of the radiopharmaceutical reliably migrates to regional lymph node basins. This was confirmed in studies done at the Netherlands Cancer Institute where in 516/531 (97%) patients, a SLN was identified[13]. The advantages of the technique are that more than 95% of the

injected radioactivity remains at the injection site and on average only 0.16% of the injected dose ends up in the SLN. The small volume injected will also easily be removed by a lumpectomy[13].

It is against this background that we designed the present study to identify the SLN. The radiopharmaceutical was injected intratumorally, followed by preoperative lymphoscintigraphy aided by the handheld gamma probe and blue dye intraoperatively.

AIM OF STUDY

To determine if the single intratumoral technique with preoperative lymphoscintigraphy is a valid and reliable method to identify the SLN/s.

LITERATURE REVIEW

NORMAL LYMPHATICS

HISTORICAL BACKGROUND

In '*On the Glands*', one of history's earliest known medical texts; Hippocrates described, "white blood in nodes". Aristotle noted fibres containing colourless fluid located between blood vessels and nodes[15]. In 1627, Gasparo Aselli[15] first recognised the lymphatic system. In the mid 1600, Swammerdam[15], first discovered the presence of valves in the collecting lymphatic channels. By the early nineteenth century, the anatomy of the lymphatic system was almost completely characterised. However, the embryological origin is still controversial i.e., whether the lymphatic channels arise from veins or de novo from lymphangioblasts or both[15].

The lymphatics function as a draining system by regulating tissue fluid haemostasis by returning macromolecules (proteins) to the blood circulation. Via this route about 25-50% of the total circulating plasma protein enters the blood circulation per day. It also serves as a major route for dissemination of tumour cells, the transport of bacteria and immune cells to distant sites on entering the bloodstream[15].

ORGANISATION OF THE LYMPHATIC SYSTEM

There are 5 main categories in the lymphatic system: the capillaries, collecting channels, lymph nodes, trunks and ducts. Their sizes range from 10μm to 2mm in diameter. Lymph is formed when interstitial fluid is absorbed into blind ending lymphatic capillaries (initial lymphatics), which are 10-60 μm in diameter and are significantly larger than arteriovenous capillaries (8μm). Initial lymphatics consist of a single layer of endothelial

cells with a discontinuous basement membrane that overlaps and serves as valves, which are approximately 10-25nm wide. This membrane is attached to the surrounding tissues by means of anchoring collagen filaments that prevent the collapse of the lymphatic capillaries. The basement membrane mainly consists of type IV collagen and does not contain heparin sulphate, proteoglycan or fibronectin that is found in vascular capillary membrane. The osmotic pressure gradient and fluctuating intraluminal pressures caused by contraction and the forward flow of lymph at the interstitial lymphatic interface, explain the filling of lymphatic capillaries[15,16].

Following lymph formation, lymph drains from the lymphatic capillaries to the collecting lymphatics. These have mainly bicuspid valves but may be unicuspid or tricuspid with smooth muscles in their walls. This is unlike the lymphatic capillaries, which are devoid of valves and smooth muscles[15,16]. Segments of collecting lymphatics between valves are called lymphangions, which serve as contractile components contracting in a peristaltic fashion propelling the lymph into the next component at regular intervals to prevent backflow of lymph. This is also aided by systemic forces such as respiration, blood pressure and skeletal muscle movement[15,16]. Lymph flow and lymphatic contractility increases in response to tissue oedema (oedema safety factor), warm baths, exposure to cold (ice packs) and intermittent external pressure. Sustained external pressure however, reduces lymph flow. All collecting channels pass through lymph nodes, which are capsular and organised in clusters throughout the lymphatic system varying in size from 1-10mm in diameter[15,16].

The lymphatic trunks are the largest channels that drain lymph from the final set of lymph nodes into ducts with the exception of lymphatics from the intestinal, hepatic and lumbar areas that drain directly into a sac-like structure; the distal end of the thoracic duct (cisterna chyli), which lies on the 1st and 2nd lumbar vertebrae between the aorta and the right crus of the diaphragm. The thoracic duct opens near the union of the internal jugular and subclavian veins and is responsible for draining most of the body, while the right lymphatic duct, which enters the right brachiocephalic vein, drains mostly the right upper quadrant of the body[17].

The normal lymph flow is 2-4 litres per day at rest and varies according to a diurnal rhythm and physiological needs. It normally contains clotting factors, protein, water insoluble fats and lymphocytes. The protein composition of lymph is equivalent to interstitial fluid, which in turn is similar but less concentrated than that of blood plasma. However, the lymph protein content varies with the region from which the lymph is drained. Intestinal lymph has a high fat content so that the lymph is turbid and is often called chyle[18].

BREAST LYMPHATICS

INTRODUCTION

Knowledge of the lymphatic pathways from the breast is paramount to the understanding of the natural course, staging and the treatment of breast carcinoma[19]. Following the introduction of sentinel lymph node biopsy in early breast carcinoma there has been a renewed interest in the anatomy and physiology of the lymphatics of the breast[20]. Currently, different injection sites, timing of scintigraphic imaging and surgery are based on

theories relating to the structure of lymphatic channels, particle uptake into lymphatic channels and lymph flow[20].

HISTORICAL BACKGROUND

The anatomy of breast lymphatics has been studied for several centuries. At the end of the eighteenth century, Cruikshank and Mascagni[20] independently described 2 main lymphatic draining routes i.e. the external and internal system. The external route drains the skin, nipple and lactiferous ducts to the axilla. The internal route draining the dorsal part of the breast was thought to perforate the pectoral and intercostal muscles. Within the intercostal spaces these lymphatics were noted to join the plexus from the liver and the diaphragm and follow the internal mammary channels on either side. In 1770 Campher[20], a Dutch physician was the first to identify lymphatic drainage to lymph nodes along the internal mammary channels. These nodes extended from the 5th intercostal space to the retroclavicular nodes. Vital dye studies showed that the deep lymphatics drained into the internal mammary nodes. These deep lymphatics arise from breast lobules; leave the dorsal surface of the breast by piercing the pectoral and the intercostal muscles to reach the internal mammary chain[20]. In 1830, Sappey[20] using mercury injections into lymphatic channels concluded that most of the breast tissue drains centripetally into the subareolar plexus and then to the axilla. Rouviere[20] and Grant & Associates[20] later confirmed these findings. At the end of the 19th and the beginning of the 20th century, anatomists refuted this concept and stated that additional lymphatic routes existed. In the 1950's, Turner-Warwick[21], injected colloidal gold (^{198}Au), with a particle size of 5nm, into the breast and concluded that more than 75% of the breast drains to the ipsilateral axillary lymph nodes. The remainder drains into the ipsilateral internal

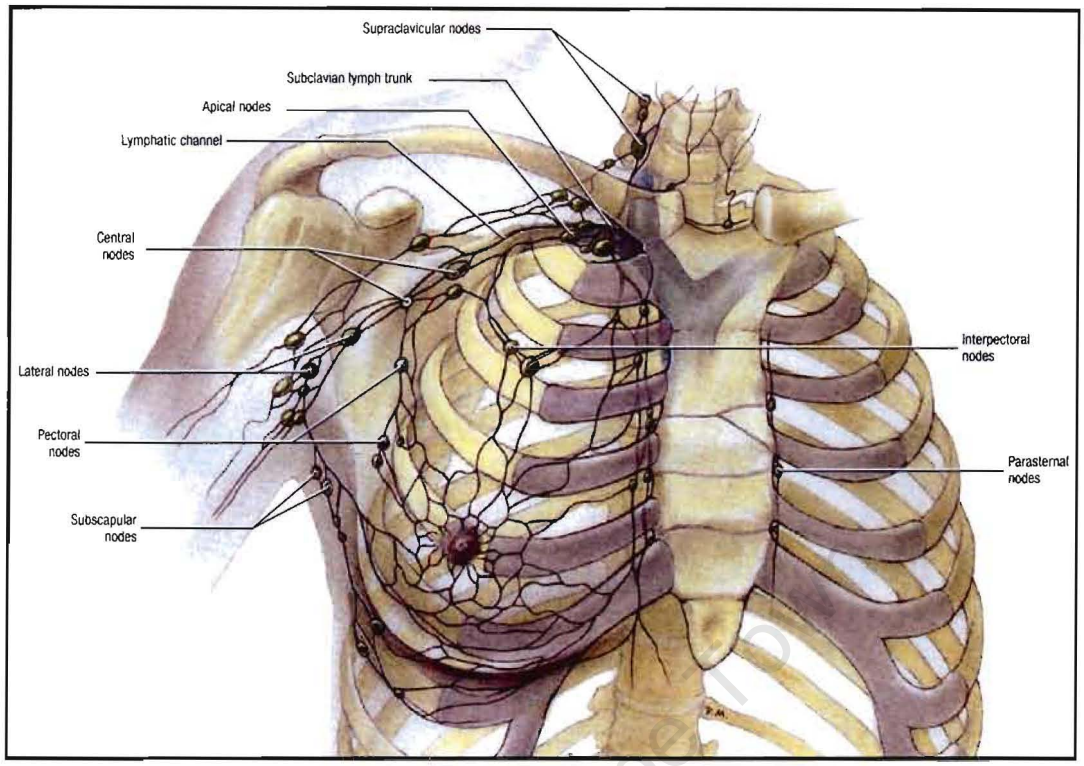
mammary chain from both the medial and lateral quadrants of the breast[21]. Hultborn et al[22] and Vendrell-Torné et al[23] and Uren et al[24] confirmed this.

Less common drainage routes are lymphatics passing through so-called interval nodes, the interpectoral and intramammary nodes, on the way to the axilla and internal mammary chain. The interpectoral (Rotter's) node[19,25] is found between the pectoralis major and minor muscle. The intramammary nodes are situated in the breast parenchyma[20]. Occasionally drainage from the breast goes directly to supraclavicular nodes, as well as sporadic drainage to the contralateral internal mammary chain, which occurs if ipsilateral lymph drainage is impaired by tumour growth, previous surgery or radiotherapy. Blockage of normal lymph flow can also cause retrograde drainage to the liver via the internal mammary chain. Drainage to posterior intercostal lymph nodes and anterior intercostal nodes has also been described[20].

DISTRIBUTION OF BREAST LYMPHATICS

It is generally accepted that drainage from the breast can occur to lymph nodes at a number of different sites. Both the axilla and the internal mammary chain receive lymph from all quadrants of the breast.
The breast receives its blood supply from 3 main vessels - mainly the axillary and internal mammary vessels with a minor contribution from the lateral perforating branches of the intercostal vessels. The lymphatic drainage in these 3 directions is approximately proportional to the blood supply so that more than 75% of the total lymph drains to the axilla and the remainder into the internal mammary chain[21].

FIGURE 1



Lymphatic drainage of the breast, anterior view. (From Agur AMR, Lee MJ, Grant's Atlas of Anatomy. 10th Edition, Philadelphia, Lippincott Williams & Wilkins 1999.)

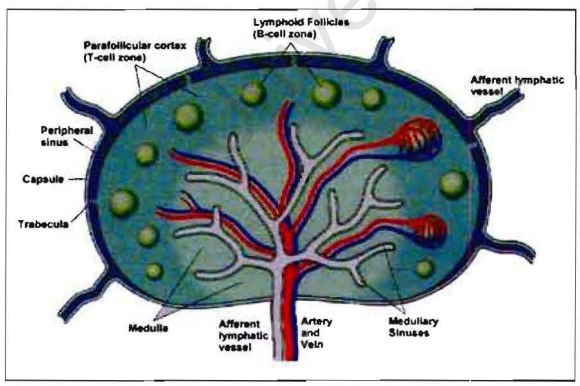
LYMPH NODES

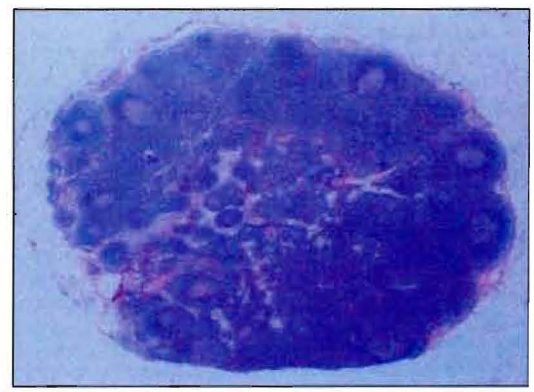
INTRODUCTION

Lymphocytes and monocytes not only circulate in blood and lymph but also accumulate in discrete organised masses forming the lymphoreticular system. The main components of this system include lymph nodes, thymus, spleen, tonsils, adenoids and Peyer's patches with less accumulation occurring in the bone marrow, lungs and GI tract. Lymph nodes are the most widely distributed and easily accessible component of the lymphoid tissue and are therefore frequently examined for diagnosis of lymphoreticular disorders[16,26]. Lymph nodes are discrete structures surrounded by a capsule, composed of connective tissues and a few elastic fibrils. The capsule is perforated by multiple afferent lymphatics on

its convex aspect that empty into a fenestrated peripheral subcapsular sinus. Lymph then moves through the node via the cortical and medullary sinuses, exiting through a single efferent lymphatic at the hilum on its concave aspect. Blood vessels only enter and leave the lymph node via the hilum *(Figure 2A)*. The cortex contains spherical aggregates of lymphoid cells forming the primary follicle representing the B - lymphocyte area. Between the primary follicles is the paracortex rich in T – lymphocytes. Deep to the cortex is the medulla containing numerous plasma cells and relatively few lymphocytes *(Figure 2B)*. Lymph nodes function as barriers, filters and reservoirs and are constantly reacting to multiple stimuli. Thus, enlarged lymph nodes in adults are almost never 'normal'. Drainage of tumour cell debris, tumour antigens or both induces change in the lymph nodes. Therefore, histological evaluation of an enlarged node in patients with carcinoma is necessary as it may be due to reactive hyperplasia or growth of tumour cell Lymph node/s of normal size may also be infiltrated[16,26].

FIGURE 2

A. 

B. 

Normal lymph node architecture

A. Schematic diagram of a lymph node. B. Low power view of a lymph node. (From Cotran RS, Kumar V, Collins T, Robbins Pathologic Basis of Disease. 6th edition, Philadelphia, WB Saunders Company 1999)

REGIONAL AXILLARY LYMPH NODE BASIN

The boundaries of the lymphatic drainage of the axilla are not well demarcated. There are considerable variations in the position of the groups of regional nodes. Grossman in 1896[19] was the first to define the number and position of axillary nodes. He found 12-36 nodes in the axilla and divided them into 4 groups. Rouvière's classification of axillary nodes into 6 groups is currently used[19,25].

1. LATERAL / AXILLARY VEIN GROUP
   Consists of 4-6 nodes situated medial or posterior to the axillary vein. It receives most of the lymph drainage from the upper extremity.
2. EXTERNAL MAMMARY / ANTERIOR / PECTORAL GROUP
   Consists of 5-6 nodes and is located along the lower/lateral border of the pectoralis minor muscle contiguous with the lateral thoracic vessel. It receives lymphatic drainage from the lateral aspect of the breast.
3. SCAPULAR / POSTERIOR / SUBSCAPULAR GROUP
   Consists of 5-7 nodes located on the posterior wall of the axilla, along the lateral border of the scapula and are contiguous with the subscapular vessel. This group principally receives lymph from the lower neck, posterior trunk and posterior shoulder.
4. CENTRAL GROUP
   Consists of 3-4 nodes imbedded in a pad of fat immediately posterior to the pectoralis minor muscle. The nodes in this group are the largest and are most easily palpated in the axilla. The clinical status is usually based on this group of nodes[19]. This group receives lymph from the aforementioned groups but it can also receive lymph directly from the breast.

5. APICAL / SUBCLAVICULAR GROUP

   Consists of 6-12 nodes located medial and superior to the upper or medial border of the pectoralis minor muscle. This group receives lymph from all the axillary nodes and unites with the efferent vessels from the apical group to form the subclavian trunk[25]. This trunk on the right drains directly into the subclavian vein or joins the right jugular trunk. On the left, it usually drains into the thoracic duct[27].

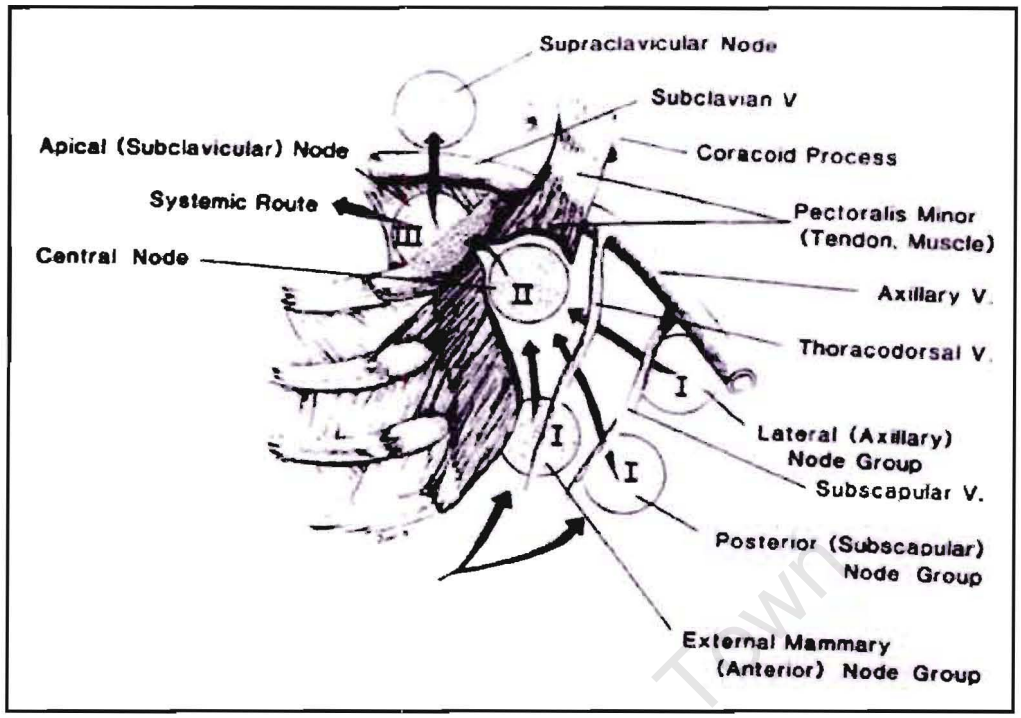
6. INTERPECTORAL / ROTTER'S GROUP

   First described by Grossman in 1896[19] and later by Rotter in 1899[19]. It consists of 1-4 nodes lying between the pectoralis major and minor muscles along the pectoral branches of the thoracoacromial vessels. Lymph from this group passes directly into the central or apical group.

Surgeons and Pathologists often define metastatic axillary lymph node involvement into 3 levels in relation to the pectoralis minor muscle. This was described in 1955 by Berg, a pathologist[19].

Level I is lateral to the pectoralis minor muscle and includes the external mammary, lateral and scapular groups. Level II is behind the pectoralis minor muscle and it includes the central and interpectoral groups, whilst Level III is medial and superior to the pectoralis minor muscle and includes the apical group[25,27].

FIGURE 3



Schematic drawing illustrating the major lymph node groups associated with lymphatic drainage of the breast. The Roman numerals indicate three levels or groups of lymph nodes that are defined by the location relative to the pectoralis minor muscle. (From Schwartz SI, Shires GT, Spencer FC, Daly JN, Fischer JE, Galloway AC, Principle of Surgery. 7th edition, New York, McGraw-Hill 1999.)

The Internal mammary nodes (IMNs) are a pair of longitudinally linked chains of nodes on either side of the sternum, medial to the internal thoracic / mammary vessels. There are usually 4-5 nodes in total on each side found mainly in the first to third intercostal space, and the fifth or sixth intercostal space[28,29,30].

BREAST CARCINOMA

EPIDEMIOLOGY

Worldwide, breast carcinoma accounts for 32% of all female carcinomas and is responsible for 19% of premature deaths in the 40-50 yr age group[26]. This is exceeded only by lung carcinoma[25,26]. Breast carcinoma is the second commonest female carcinoma after cervical carcinoma in Africa[2].

In South Africa during 1996 and 1997, breast carcinoma was the second leading cause of carcinoma in females. During this period, a total of 4460 and 4789 new female breast carcinoma cases were reported. This constituted an average of 17% of all female carcinomas per year[2]. In 1996 and 1997, the age adjusted rate for all females ranged between 27.8 per 100 000 and 29.0 per 100 000 respectively. From 1995 to 1997, the lifetime risk of developing breast carcinoma in South Africa increased from 1 in 36 to 1 in 31[2]. The incidence of breast carcinoma among South African Asian females is high and compares with those reported in England, Wales & USA[2]. The incidence in South African black females is similar to those reported in other African countries[2].

CLASSIFICATION

Breast carcinomas are all derived from epithelial cells that line the terminal duct lobular unit. This is contrary to previous belief that ductal carcinoma arises from ducts and lobular carcinomas from lobules[1,26].

Breast carcinomas are divided into non-invasive / in situ, which accounts for 15-30% of the total in the Western world. The proportion of in situ carcinoma detected is dependent on the number of women undergoing

mammographic screening and ranges from less than 5% in unscreened women to 15-30% in women who have been screened[26].
Invasive carcinoma accounts for 75-85% of the total. Ductal carcinoma (no special type) accounts for the majority of invasive carcinomas (79%)[26].

CLINICAL COURSE

A breast carcinoma is usually detected as a solitary painless lump in the breast on palpation or routine mammogram[26]. It usually takes 100 days for a tumour to double in size so that there is a latent period of more than 8 yrs for a single neoplastic cell to grow to a 1-2cm clinically detectable mass (10^9 cells)[25]. During this latent period approximately one third will be noted to have axillary involvement. Mammographically detected invasive carcinomas are on average smaller than 1cm in size and less than one fifth will have axillary metastases[26].

A number of factors influence the clinical course and prognosis of breast carcinoma such as; tumour size, histological subtype, tumour grade, the presence of angiogenesis, lymphovascular invasion, oestrogen and progesterone receptors. The single most important prognostic factor, however, is the axillary lymph node status[26].

TABLE 1

Ten-year survival rates of nodal negative, nodal positive and of patients with supraclavicular lymph node metastases or distant disease [31].

Stage of Disease	Involvement of the axillary nodes (no. of involved nodes)	10 – year survival rates (%)
N_0	0	65 – 75
N+	1 – 3	45 – 65
N+	4 – 9	25 – 30
N+	> 10	< 20
Supraclavicular node involvement (M1)		< 5
M1		< 5

N_0 = Node negative

N+ = Node positive

M1 = Distant metastasis

THE SENTINEL LYMPH NODE (SLN)

INTRODUCTION

The management of breast carcinoma has evolved towards individualising treatment, minimising morbidity and maximising cure rates[4]. It is useful to know the lymphatic drainage pattern of a malignant tumour for staging, determining prognosis and optimising appropriate therapeutic management. The widespread use of early mammographic screening and enhanced public awareness has resulted in approximately 33% of patients presenting with smaller tumours of less than 1cm in developed countries[4]. Smaller tumours are associated with less likelihood of axillary lymph node involvement. 70-80% of clinical Stage I (≤ 2cm) or II (> 2cm - 5cm) lesions are found to have no histological evidence of metastatic involvement[32,33]. Although level I and II axillary lymph node dissection has been the standard of care in the initial staging of early breast carcinoma, it adds no additional survival benefits and is associated with morbidity[4,5,6,34]. In patients with early breast carcinoma, often only a single node is found to contain tumour cells[4].

The SLN concept has revolutionised and individualised the surgical management of regional axillary nodes. The procedure accurately stages patients allowing for correct prediction of the tumour status in the remaining axillary nodes whilst avoiding the morbidity and expense associated with a complete axillary lymph node dissection[4]. SLN mapping may also detect drainage to extra-axillary sites not routinely evaluated during a standard axillary lymph node dissection[35].

THE HISTORY OF THE SENTINEL LYMPH NODE

The SLN concept is not new. In 1907, Jamieson and Dobson[36] describe the spreading of neoplastic cells to the so-called primary gland. In 1960, Gould et al[36] coined the term "sentinel node" in a patient with carcinoma of the parotid gland. In 1963, Oliver Cope[32,37], in thyroid carcinoma, referred to the "delphian node" as the node that can "foretell the nature of the disease process" affecting a nearby organ. Roman Cabanas, in 1977[38], introduced the concept of the sentinel lymph node in the management of penile carcinoma. In 1992, Morton[39,40] used this principle in the treatment of skin melanoma and using a dye-guided surgical procedure, removed the first stained lymph node and coined the definition of the sentinel lymph node. In 1994, Giuliano[41] used this concept in early, node negative breast carcinomas by locating and removing the sentinel lymph node also using a dye-guided surgical procedure. Krag and Alex[42] localised the sentinel lymph node using a radiopharmaceutical and a handheld gamma probe. In 1996, Albertini et al[43] identified the sentinel lymph node using the combination of blue dye and radiopharmaceutical. Turner et al[44,45] provided histological evidence for the sentinel lymph node hypothesis. In 103 patients with breast carcinoma, cytokeratin immunohistochemical (IHC) staining was used in sentinel and non-sentinel lymph nodes that were negative on hematoxylin & eosin stain (H&E). The authors concluded that if the sentinel lymph node is tumour free by H&E and IHC staining, the probability of non-sentinel lymph node involvement is less than 0.1%[44,45].

THE SENTINEL LYMPH NODE CONCEPT

The sentinel lymph node concept is based on two basic principles:

1) The existence of an orderly and sequential pattern of lymphatic flow from the primary tumour by single or multiple lymphatic channels draining directly to the first (sentinel) lymph node/s within a particular

regional lymph node basin[46,47].

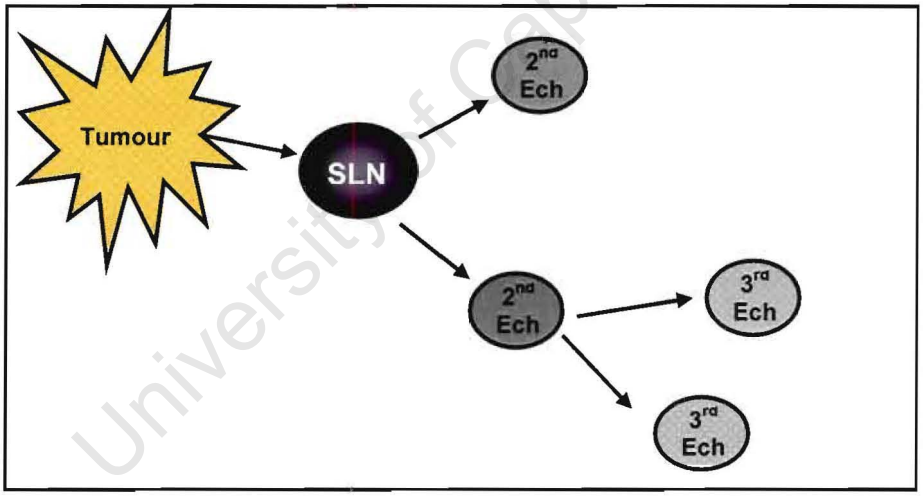
The histopathological analysis of the sentinel lymph node/s must reflect the tumour status of the entire regional basin[48].

2) The sentinel lymph node must function as an effective barrier and filter to retain the earliest metastatic tumour cells[46,47].

Underlying this hypothesis is the assumption that the SLN is identified, correctly located and removed and this is followed by meticulous pathological analysis[46,49].

THE SENTINEL LYMPH NODE DEFINITION

FIGURE 4



A sentinel lymph node is defined as a lymph node that receives drainage directly from the primary tumour. A second echelon node receives drainage from a sentinel node. A third echelon node receives drainage from a second echelon node, and so on. (From Eur J Nucl Med 1999; 26 Suppl: S11-S16.)

The dictionary defines 'sentinel' as a guard, watchdog or protector[50].

A SLN is defined as the first lymph node to receive lymphatic drainage from the tumour. The identification varies depending on which technique is used[34]. When a radiopharmaceutical is used with preoperative lymphoscintigraphy the first node/s seen on scintigraphy is called the SLN.

Depending on the number of channels emerging from the primary tumour, a single or multiple nodes will be regarded as sentinel lymph node/s. Nodes seen after the SLNs are defined as 2nd echelon nodes. During surgery, the intraoperative handheld gamma probe identifies radioactive nodes but cannot readily distinguish between the sentinel and 2nd echelon lymph nodes. Some investigators regard all radioactive nodes as sentinel lymph nodes while others regard nodes with the highest count rate as the sentinel lymph nodes. Correlation between good quality lymphoscintigraphy and surgical findings is frequently helpful[34]. Intraoperative blue dye similarly, cannot distinguish between the sentinel and 2nd echelon lymph node/s. Most investigators regard all blue nodes or blue and radioactive nodes as sentinel lymph nodes. The various techniques used to define and find the sentinel lymph node, make comparison of studies with different protocols difficult[34].

THE AXILLA

The management of the axilla in breast carcinoma patients has been a subject of intense debate and controversy[32]. Despite a tendency toward a conservative approach of primary breast carcinoma surgery, axillary lymph node dissection has remained an integral part of breast carcinoma management for more than a century. Axillary lymph node dissection results in effective regional control with a 1-2% recurrence rate and gives significant staging information but does not confer long-term survival benefit[32,45,51]. The extent of the axillary lymph node dissection depends on the level of dissection and the number of nodes removed[52]. A standard level I and II axillary lymph node dissection is commonly performed as only 3% of patients will have involvement of level III nodes without positive nodes in levels I and II[52]. Veronesi et al[31], based on a mathematical model

of 1 446 complete axillary lymph node dissections, also showed that a minimum of 10 level I nodes need to be examined to make sure that 90% of the axilla is pN_0 (pathologically no nodal metastases).

INTERNAL MAMMARY NODES (IMNs)

Nodes in the internal mammary chain are best identified by intraparenchymal injection of the radiopharmaceutical. The IMN is an important pathway of lymphatic drainage of both the medial and lateral aspects of the breast. However, the clinical practicality and relevance of IMN sentinel lymph node biopsy is a matter of controversy, as extra axillary nodes are not taken into consideration for the management of breast carcinoma unless there is locally advanced disease[28]. Whereas others believe it is clinically relevant as metastases exclusive to the IMN occur in 5-10% of patients and have the same prognostic significance as axillary nodal metastases. Removal of these nodes is considered necessary to obtain complete and correct staging[29,53,54,55]. Most IMN metastases are located in the first and second intercostal spaces[28,56]. The most common complication of the IMN biopsy is a pneumothorax[28]. Preoperative lymphoscintigraphy and intraoperative handheld gamma probe are the best method for the detection of IMNs. A major limitation of IMN SLN biopsy is the lack of confirmation of the true false-negative rate as a complete IMN dissection is rarely performed[28].

INTRAMAMMARY NODES

By definition, intramammary nodes are completely surrounded by breast tissue and histologically they can be differentiated from low lying axillary and deep pectoral lymph nodes[57]. This differentiation can be particularly difficult on mammography[57]. Intramammary nodes can be found in virtually any quadrant of the breast[58]. The combined technique of preoperative lymphoscintigraphy, intraoperative blue dye and handheld gamma probe for SLN identification has increased the detection of extra axillary nodes[59]. Extra axillary nodes are predominantly found in the IMN chain but also in the inter-pectoral region and within the breast parenchyma (intramammary). The frequency of finding intramammary lymph nodes both positive and negative has great variability and probably depends on the degree of vigilance and technique[60]. Intramammary nodes are present in 28-47% of breast carcinomas and may have metastatic involvement in up to 9.8% of cases[57]. The relevance of intramammary node metastases is difficult to assess due to low reported incidences. Once a positive intramammary node has been found, there is no consensus in the literature about what to do with the axilla[60]. Egan and McSweeney[61] address the issue of involved intramammary node and the effect on prognosis and treatment. Despite the small numbers in their study, they concluded that involved intramammary nodes in an otherwise pathologically node negative patient has a similar prognosis as patients with involved axillary nodes. If both intramammary and axillary nodes were involved there was no worsening of prognosis. Intramammary node involvement appears to have the same significance as metastatic axillary nodes where prognosis and treatment are of concern[57].

RADIOPHARMACEUTICALS IN SLN DETECTION

The radiopharmaceutical must be able to rapidly flow through the lymphatic channels, localise and be retained in the sentinel lymph node by mechanical trapping or phagocytosis and must deliver as low a radiation dose as possible. The ability of the radiopharmaceutical to meet these criteria is dependent on the size and the number of particles that are administered[62]. Particle size of a colloid can be determined by electron microscopy, photon spectroscopy and ultracentrifugation. It should be noted that most particles are not uniformly shaped[63]. The number of injected colloidal particles influences the rate of outflow from the injection site and phagocytosis within the lymph nodes. The optimal particle size identified from animal studies for lymphatic drainage is estimated to be less than 100 nm[62]. Larger particles (500-2000 nm) remain trapped at the injection site and are unsatisfactory. Small particles (< 4-5 nm) penetrate blood vessel capillary membranes and therefore do not migrate through lymphatic channels[63].

There is no consensus on the radiopharmaceutical of choice for lymphoscintigraphy and SLN identification. Currently, the most widely used agents are all particulate in nature and are all retained in the lymph nodes. Choice of radiopharmaceutical varies internationally; in Europe; ^{99m}Tc nanocolloid, in Australia; ^{99m}Tc antimony trisulphide colloid and in America; ^{99m}Tc sulphur colloid[62,63].

A. ^{99m}Tc antimony trisulphide colloid [^{99m}Tc-ATC]

The first ^{99m}Tc radiopharmaceutical developed for lymphoscintigraphy was ^{99m}Tc-ATC. This colloid has a high percentage of uniformly sized particles (3-30 nm) that permits studies of a very high quality. The ^{99m}Tc-labelling of ATC has been proposed to occur on the surface of the particles with the

final particle size determined by the size of the antimony colloid used[62,63]. The agent was developed when lymphoscintigraphy and the sentinel lymph node concept were not widely recognised. As a result, it was never developed worldwide and its use remains restricted to Australia and New Zealand. Uren et al[63]. used ^{99m}Tc-ATC to locate SLN in 34 patients with breast carcinoma. Images were acquired immediately and at 2.5 hours after 4 peritumoral injections of 2.5-7MBq of ^{99m}Tc-ATC were administered. Successful lymphatic drainage patterns were identified in all but three patients. Drainage to the ipsilateral axilla occurred in 85% of patients where a single SLN was seen in all cases. Adverse reactions to ^{99m}Tc-ATC have been reported[63].

^{99m}Tc albumin-based colloids

Three types of albumin-based ^{99m}Tc colloid radiopharmaceutical agents have been studied viz., nanocolloid, microaggregated albumin and macroaggregated albumin[62].

1. ^{99m}Tc nanocolloid

Biodistribution of nanocolloid depends on its particle size. More than 95% are smaller than 80nm, less than 4% are between 80-100 nm and only 1% is more than 100 nm. It is licensed in Europe for lymphoscintigraphy and bone marrow scintigraphy. It is available in a form of a 'shake and mix' kit and trades under different names such as Microlite, Ciscolloid, Sorin etc., depending on the manufacturer. The kit contains human albumin nanocolloid particles, stannous chloride, glucose, poloxamer 238, sodium phosphate and sodium phytate. The human serum albumin is obtained from donor blood, which tested negative for hepatitis B surface antigen and antibodies for HIV and the hepatitis C virus. During the reconstitution of ^{99m}Tc nanocolloid it is critical to exclude oxygen from the vial to avoid the

formation of stannous technetium colloid, which will not bind to the albumin[62,63].

2. ^{99m}Tc microaggregated albumin

It is licensed for liver and spleen scintigraphy. It has a particle size of 200-3000 nm with 90% less than 1000 nm in size. This is not ideal for lymphoscintigraphy although Paganelli et al[62,63] have reported good results.

3. ^{99m}Tc macroaggregated albumin [MAA]

Its particle size ranges from 10 000 to 90 000 nm with very poor migration from the injection site to nodes therefore making it unsuitable for lymphoscintigraphy[62].

B. ^{99m}Tc sulphur colloid

There are 2 methods to prepare ^{99m}Tc sulphur colloid. Initially, sulphur colloid was prepared by percolating hydrogen sulphide gas through an acidic pertechnetate solution. This resulted in very small particles. Nowadays, ^{99m}Tc sulphur colloid preparation is formed by the reaction of thiosulphate under acidic conditions. The acidified pertechnetate solution is added to thiosulphate in the presence of a stabilising agent. This can be gelatin, polygeline or mannitol. Essentially, all the pertechnetate is incorporated in the sulphur colloid. Heating the solution for 5-10 min at 100 ^{0}C enhances colloid formation. The particle size ranges from 100-1000 nm[63]. However, lymphoscintigraphy requires a much smaller particle size. This is obtained by reducing the heating time of the colloid from 5-10 to 3 minutes, as well as adding pertechnetate from a generator that has not been eluted for at least 72 hours, as this eluate would have a higher concentration of ^{99}Tc pertechnetate[62]. By filtering this preparation through either a 0.1 or 0.2 μm membrane filter most of the particle size produced is less than 30 nm[62].

C. ^{99m}Tc human serum albumin [HSA]

This agent has a small particle size of 2-3 nm. It is not particulate and there is minimal retention of the agent within the lymph nodes[62,63].

D. OTHER AGENTS

There are a number of other agents that have been used or are being developed for lymphoscintigraphy including ^{197}Hg sulphide colloid, ^{67}Ga citrate and monoclonal antibodies labelled with ^{111}In, ^{131}I and ^{125}I. Although several of these agents have been used with varying degrees of success, the use of ^{99m}Tc agents is more common owing to its availability and radionuclide properties.

Other ^{99m}Tc labelled radiopharmaceuticals have been evaluated as potential lymphoscintigraphic agents such as hydroxyethyl starch, dextran, stannous phytate etc. These agents are only experimental at present[62].

TABLE 2

Type of radiopharmaceuticals and particle size[64].

Type of radiopharmaceuticals	Size range (nm)
^{99m}Tc human serum albumin	2-3
^{99m}Tc dextran	2-3
^{99m}Tc antimony trisulphide colloid	3-30
^{198}Au colloid	5-30
^{99m}Tc filtered sulphur colloid	15-50
^{99m}Tc nanocolloid	5-80
^{99m}Tc sulphur colloid	100-400
^{99m}Tc stannous fluoride	50-600
^{99m}Tc stannous phytate	200-1000
^{99m}Tc microcolloidal albumin	200-3000

RADIATION DOSIMETRY AND PROTECTION

The main radiation consideration is the absorbed radiation dose to the patient, the cumulative radiation exposure to staff (medical, technical & theatre) involved with SLN biopsy and possible contamination of the surroundings. The radiopharmaceutical uptake in the SLN is only a small fraction of the administered dose. At the Netherlands cancer Institute, the activity in 103 SLNs was measured approximately 23.5 hours after 74 MBq of ^{99m}Tc-nanocolloid was injected intratumorally in 51 patients with breast carcinoma. The mean uptake in the SLNs was 6.5 kBq (range 0.03-102 kBq), which is 0.16% (range 0.001-2.5%) of the injected radiopharmaceutical[64]. These measurements reflect spontaneous lymphatic drainage i.e., without any massaging of the injection site. Bass et al[64] showed that massaging of the injection site improved the blue staining of SLNs but the radiopharmaceutical uptake was not significantly affected. The greatest absorbed radiation dose is by far at the injection site i.e., the local skin, the primary tumour or peritumoral bed, assuming that most of the radiopharmaceutical will remain there for total decay. In the case of the intraparenchymal (intratumoral or peritumoral) or subdermal injections above the tumour, the injection site will be of least concern, as it will be routinely resected. Radiation dose to the lymph nodes was calculated to be in the order of 47mGy[64]. The radiation exposure to the total body, bone marrow or ovaries is insignificant and roughly ten times less than that of a routine bone scintiscan and much less than a number of radiological procedures[64].

TABLE 3

Effective dose of Radiological and Nuclear medicine procedures[65].

INVESTIGATION	EFFECTIVE DOSE (mSv)
SLN study	***0.32***
^{99m}Tc bone scan	3
^{99m}Tc perfusion lung scan	1
^{123}I thyroid scan	4
^{99m}Tc scintimammography	11
Chest X-ray	0.02
Mammography	0.4
Plain Abdominal X-ray	1
Lumbar spine X-ray	1.3
Intravenous urography	2.5
Barium meal	3
Brain CT	2.3
Abdominal CT	10
Chest CT	8

Waddington et al[65], measuring radiation doses to patient and surgical staff during surgery at 24hr after a subdermal injection of ^{99m}Tc colloidal albumin, found the effective dose to the patient to be 0.32 mSv, which is comparable to mammography. The whole body dose to surgical staff was less than 2mSv i.e., less than 0.2% of the annual dose limit. Surgical swabs may contain up to 10% of the injected dose[64,65]. The exposure to the pathologist from the breast histological specimen (activity <3.7 kBq) and the SLN (activity <0.2 kBq) is low. Persijn[64] also concluded that the radiation exposure to patient and personnel is low and he extrapolated that a surgeon operating 3-6 hrs after injection of 75 MBq can perform at least 2000 SLN biopsies per year before reaching the annual dose limit to the hands as the general public (50mSv/yr).

At the Netherlands Cancer Institute, Hoefnagel et al[64] calculated the radiation exposure and dose to those personnel closely involved in SLN biopsy. At the time of injection the nuclear medicine physician, injecting a radiation dose of 80MBq of ^{99m}Tc colloid with an unshielded syringe held between the fingers for approximately 2 minutes, will receive the highest dose i.e. 2.45 mSv to the hands and 0.06 mSv to the body. During the dynamic phase (up to 30 minutes), the nuclear medicine technologist standing more than 2 metres away will receive 0.23 mSv. At delayed 4 hr imaging, the nuclear medicine technologist will receive 0.145 mSv. The nuclear medicine physician marking the sentinel lymph node at 4 hrs or later, will receive a dose to the hands of 19.2 mSv. During surgery between 4 and 24 hrs, the surgeon will receive 115 mSv to the hands and 1.16 mSv to the body assuming that the procedure takes one hour and the radioactive specimen is handled with surgical instruments most of the time. During surgery after 24 hrs, the same procedure can be carried out with a ten times lower radiation dose to the surgeon's hands (11.5 mSv) and body (0.11 mSv). During pathological assessment of the tumour, assuming that this is done after fixation and only takes 30 minutes, the remaining activity will only be 0.0195 MBq so that the radiation dose to the pathologist is negligible i.e., 0.0225 mSv to the hands and 0.0002 mSv to the body. The total radiation exposure to the nuclear medicine technologist at 2 metres including attendance at surgery is 0.42 – 0.68 mSv depending on the time of surgery[64]. An additional radiation exposure to the patient and nuclear medicine technologist is from the Cobalt-57 or Technetium-99m flood source, which is used as a transmission source during imaging to outline the body contour. The radiation dose from the Cobalt-57 or Technetium-99m flood source of equal activity (370 MBq), is 70μSv to the patient[66,67]. There is an additional radiation dose of 52.5μSv to the nuclear medicine technologist from the preparation of the Technetium-99m flood source[66].

INJECTION TECHNIQUES TO IDENTIFY THE SLN

The injection technique is important as the success of the SLN biopsy is determined by its identification rate and false negative rate.
The SLN identification rate is defined by the percentage of procedures in which the SLNs have been successfully localised and removed[68].
The false-negative rate is the frequency of the SLN being pathologically negative while other axillary lymph nodes harbour metastases[9,68].

Several radiopharmaceutical injection sites for the identification of the SLN have been documented. These include the peritumoral, intratumoral, subdermal or intradermal above the tumour, subareolar or periareolar with radiopharmaceutical volumes ranging from 0.2 to 16 mls[20] and doses from 37-370 MBq[36].

Variables such as the injection site, volume and dose have their advantages and disadvantages. Those who use a small volume prefer not to disturb the normal physiology of lymph flow and avoid the risk of visualising non-sentinel lymph nodes. Those who use a large volume seek to alter the normal physiology, thereby increasing the chance of visualising a lymph node[34]. Surprisingly, the different injection sites reliably identify the "true" SLN in the axilla[7,8].

From the above it can be hypothesised that predefined SLNs representative of the entire breast can be localised irrespective of the site of the radiopharmaceutical or vital dye injection[8]. The functional lymphatic anatomical basis for this hypothesis revolves around the breast lymph flow concept proposed by Grant et al in 1953[8] and Halsell et al in 1963[8]. They showed, using the vital dye staining and radiography that all lymphatic

channels of the skin and the underlying breast parenchyma communicated with the subareolar lymph complexes[8]. In 2000, Borgstein et al also stated that the breast functions as a single biological unit with preferential lymph drainage pathways from all quadrants draining essentially towards the same axillary SLN[69].

SUBAREOLAR AND PERIAREOLAR TECHNIQUES

ADVANTAGES

1. It is easy to teach (minimal training), practical, reliable and reproducible[8,70,71,72].
2. It is away from the axilla and primary tumour. This allows detection of the sentinel lymph node that is in close proximity to the tumour by avoiding the shine-through effect from the injection site[8,70,71,72].
3. It avoids the theoretical possibility of seeding tumour cells along the needle track[8,70,71,72].
4. It avoids the difficulties relating to the size of the tumour, non-palpable tumours, sonographically ill-defined tumours and previous manipulation of the primary tumour[73].
5. It requires a small radiopharmaceutical dose and volume with less radiation exposure to patient and hospital staff[36].
6. It allows rapid visualisation of SLN[8,70,71,72].

DISADVANTAGES

1. Non-visualisation of internal mammary lymph nodes[74,75].
2. The visualised node may not be the first node that drains the tumour as the risk of crossing the lymphatic watershed is increased[74]

PERITUMORAL TECHNIQUE

ADVANTAGES

1. Allows visualisation of extra-axillary lymph nodes[74].
2. Depicts the drainage pattern from the tumour to the visualised lymph node/s[74].

DISADVANTAGES

1. The blossoming effect from multiple deposits around the tumour may obscure visualisation of intramammary lymph nodes and axillary lymph nodes situated in close proximity to the injection site. This can lead to incorrect identification of the SLN[74].
2. May increase the variability of results due to the different lymphatic drainage patterns from the 4 quadrants of the breast[74].
3. Less frequent visualisation of lymphatic channels leading to a SLN[74].
4. Delayed visualisation of SLN in patients with large breasts and those who are postmenopausal[74].
5. Technically difficult to inject multiple deposits uniformly around the tumour[74].

INTRATUMORAL TECHNIQUE

ADVANTAGES

1. When inserting the needle into the tumour, the resistance of the tumour is felt, this leads to a more accurate and focal deposition of the tracer ensuring the precise mapping of the actual drainage pattern of the primary tumour[13,74].
2. On average, a mere 0.16% of the injected dose ends in the SLN while 95% of the dose remains at the injection site[13,74].
3. It allows visualisation of extra-axillary lymph nodes[13,74].

DISADVANTAGES

1. Theoretically, tumour cells can spread along the needle track[36,46].
2. Immediate leakage of the tracer can occur from tumour into the peritumoral space due to the relatively high interstitial and intercellular pressures[36,46].
3. The tumour may intrinsically be devoid of organised lymphatic channels resulting in poor and slow drainage[36,46].
4. Lymph nodes closely situated to the tumour are obscured during imaging by the scatter or blossoming effect from the activity at the injection site[36,46].
5. Visualisation of the SLN is variable and can be detected early or late[36,46].

SUBDERMAL OR INTRADERMAL TECHNIQUE

Due to the embryological origin in the ectoderm, the lymphatic drainage from the skin overlying the breast is richer than the drainage from a tumour[3].

ADVANTAGES

1. Allows visualisation of lymphatic channels and early detection of the SLN within 15-30 minutes post injection[3].
2. Allows the use of a smaller dose and volume[3].
3. Less blossoming effect and radiation exposure[3].
4. Easy to perform[3].

DISADVANTAGE

1. Non-visualisation of internal mammary nodes[3].

IMAGING TECHNIQUE

PREOPERATIVE LYMPHOSCINTIGRAPHY

The use of radiopharmaceuticals enables preoperative visualisation of the SLN. Preoperative scintigraphy consists of a dynamic phase followed by delayed static images.

THE PURPOSE OF DYNAMIC IMAGING

1. To determine the number of nodes in the same or a different regional drainage basin that is on a direct drainage pathway from the primary tumour[76].
2. To differentiate the true sentinel lymph node from second echelon nodes[76].
3. To determine the exact location of the sentinel lymph node/s thereby minimizing the extent of surgical dissection[76].
4. To enable easier identification of the true sentinel lymph node/s on delayed static images[76].
5. To identify drainage to extra-axillary nodes[76].

Preoperative lymphoscintigraphy increases the likelihood of finding all true sentinel lymph nodes[10,48,77,78,79,80]. The nuclear medicine physician provides the "road map" to guide the surgeon[34].

THE PURPOSE OF STATIC IMAGING

Early images [30min – 2hr)

1. Where there is non-visualisation of SLN on dynamic imaging[76,81].
2. To confirm the radiopharmaceutical activity seen on the dynamic images and to distinguish activity in a lymphatic channel from nodal activity[76,81].

3. To identify additional uptake in echelon nodes[81].
4. To ensure accurate counting statistics of SLN counts with the handheld gamma probe.

Late images [4 – 24hr]

1. Where there is non-visualisation of SLN on dynamic and early static images[81].
2. Where there is poor visualisation of SLN on dynamic and early static images[81].

CAUSES OF NON-VISUALISATION OF SLN

1. Significant tumour infiltration[80].
2. Age of patient (≥ 50 yrs) due to[82,83]:
   a. Decrease in tissue turgor with resultant decrease in the intra-lymphatic hydrostatic pressure that drives the tracer into the node;
   b. Nodal tissue is replaced by fat, decreasing the phagocytic activity of reticuloendothelial cells in the lymph node thereby reducing the ability to concentrate the tracer even if it is delivered successfully. However, this is disputed by Hughes et al[84] and Derossis et al[85].
3. Increase in tumour grade may cause non-visualisation[80,86].

CAUSE FOR DELAYED VISUALISATION OF SLN

Haigh et al[87] found that the tracer transit time is slower in women with large breasts than those with small breasts.

THE HANDHELD GAMMA PROBE

The first use of the handheld gamma probe in a surgical procedure was in 1949 by Selverstone et al[88] where it was used to define an astrocytoma using a Geiger Müller counter after IV injection of ^{32}P, a beta emitter. During the subsequent years various probe designs were developed but there was minimal clinical application for its use. Since 1990 the usefulness of the handheld gamma probe has significantly increased with the acceptance of the SLN concept in melanoma and breast carcinomas[88].

The use of the handheld gamma probe preoperatively helps the surgeon to locate the precise position of the underlying sentinel lymph node before an incision is made. Intraoperatively, it guides the surgeon with an auditory signal to the SLN and to the possible presence of residual lymph nodes. Intraoperative mapping without preoperative lymphoscintigraphy may not be optimum as the first (SLN) node may be indistinguishable from other hot nodes detected by the handheld gamma probe. Furthermore, the detection of drainage to unexpected drainage basins is difficult to determine with the handheld gamma probe alone, unless preoperative lymphoscintigraphy has been done[78]. Radioactive counts are measured in the regional lymphatic drainage basin using the handheld gamma probe before, during and after the SLN has been excised[78]. Once the SLN is excised, the maximum count rate (ex vivo) in counts per second (cps), of the excised tissue is ascertained and recorded. The ex vivo count rate is usually 2-3 times higher than the in vivo count rate as there is no attenuation of the excised tissue to reduce the count rate. A background reading is made of the SLN bed to ensure that the entire SLN is removed. To ensure that there are no residual radioactive nodes, a background reading of less than 10 cps is

usually acceptable[78,88]. The SLN should at least contain ten times the count of the background[78].

The handheld gamma probe consists of a detector, collimator, digital or analog display with an audio-signal detector and power supplies. At present, commercially available handheld gamma probes have either a Sodium iodide [NaI] or Cesium iodide [CsI] scintillation crystal or Cadmium Zinc Tellerium [CdZnTe] or CdZn semi-conductor detectors[88,89].
The scintillation detector based handheld gamma probes, using a NaI or CsI crystal, connected to a photomultiplier tube by a fibre optic cable have the advantages of reliability, relative low cost and high sensitivity especially for medium to high-energy photons. However, the disadvantages include poor energy resolution, poor scatter rejection and bulkiness[89].
The C-Trak handheld gamma probe consists of a CsI crystal mounted in a tip of stainless steel tube, which is angled for better manoeuvrability. The crystal is inside a tungsten collimator so that it is shielded from external radiation striking the side of the tube.
Semi-conductor probes are compact, have excellent energy resolution and good scatter rejection. Their main disadvantage is lower sensitivity.
Intraoperative handheld gamma probes for sentinel lymph node detection require excellent spatial resolution to allow precise localisation of the small target (SLN)[89].

VITAL BLUE DYES IN SLN LOCALISATION

The various dyes used as potential lymphatic mapping agents include methylene blue, isosulfan blue (lymphazurin 1%) and patent blue V[78]. Methylene blue, due to its smaller molecular weight (319.9) and the lack of sulphonic acid groups in its structure, does not bind to plasma proteins. It will, therefore, rapidly diffuse into the surrounding tissues either with minimal or no staining of the SLN[90,91]. In contrast, patent blue V and isosulfan blue with large molecular weights enter the lymphatics and are retained in the lymph nodes with minimal diffusion into the surrounding tissue[91].

However, Simmons et al[92] and Blessing et al[91] reported that methylene blue dye has a similar localisation rate to isosulfan blue. Methylene blue dye is used at many institutions to localise the SLN in breast carcinomas because of its greater availability, lower cost and decreased risk of adverse side effects when compared to isosulfan blue[92]. In a few cases, the use of isosulfan blue has resulted in serious anaphylactic reaction[91,93].

Stradling et al[92] however, reported that 5 patients developed skin lesions (variety of forms) secondary to only the intradermal injection of methylene blue dye. None of these lesions required debridement or other interventions as all responded to topical creams[92].

The blue dye is injected through a needle at the time of surgery at various sites (peritumoral, intratumoral, subdermal / intradermal above the tumour, subareolar / periareolar) using .a volume ranging from 0.5 to 3mls. The breast is gently massaged to move the blue dye along the lymphatic channels. The interval between dye injection and skin incision depends on the location of the primary breast tumour and varies from 5 to 10 minutes[78]. By using the blue dye technique alone, one cannot distinguish between the sentinel and second echelon nodes nor can one detect unexpected

drainage sites or the location of the SLN so that the surgical incisions tend to be larger. The combination of using blue dye with a radiopharmaceutical is more reliable as it provides a visual guide and an auditory signal during SLN dissection.

The literature reviewed by Hoefnagel et al[64] revealed that the combined use of lymphoscintigraphy, the handheld gamma probe and blue dye yielded the best results with a detection rate varying from 93-100%, blue dye only 66-82% and the handheld gamma probe only 84-93%. According to Nieweg et al[77], the detection rate for lymphoscintigraphy, handheld gamma probe and blue dye was 93.8%, blue dye only 76.3%, handheld gamma probe only 91.5%, lymphoscintigraphy and handheld gamma probe 87.8%, blue dye and handheld gamma probe 91.3%. According to Kraft et al[94], the detection rate for lymphoscintigraphy, handheld gamma probe and blue dye was 98.4%, blue dye only 74.4% and handheld gamma probe only, 88.7%. The false-negative detection rate was the lowest with the combined technique varying between 1-5% compared to 11-13% with only the handheld gamma probe and 11-17% with only the blue dye[64].

THE ROLE OF PATHOLOGY

It is of critical importance that the node examined is truly the sentinel lymph node. Currently, to determine that the node removed is the SLN depends on the information from preoperative lymphoscintigraphic identification by the nuclear medicine physician and the intraoperative localisation by the surgeon using the handheld gamma probe and blue dye. The ability to detect metastatic tumour in the axillary lymph node is directly related to the extent of the axillary lymph node dissection, the methods used for histopathological analysis and the experience of the pathologist[95]. Traditional staging of the ipsilateral axilla included a complete axillary lymph node dissection and a single random H&E staining for histopathological analysis. Unfortunately, 30% of the patients who were considered to have negative nodes developed recurrent disease. Not surprisingly as serial sectioning and immunohistochemical (IHC) staining could detect occult metastases in as many as 25% of "negative" axillary lymph nodes. These findings suggested that either the tissue being sampled or the means of disease detection was inadequate[96].

SERIAL SECTIONING

In 1948, Saphir and Amromin[97] first suggested that a standard pathological examination consisting of bisecting the node and examining each side with H&E stain was inadequate for the consistent detection of axillary lymph node metastases. They hypothesised that the examination of serial sections taken systematically through the entire lymph node would increase the detection rate of metastatic deposits compared with the examination of a single random section stained with H&E. While no standard definition of serial sectioning exists, a variety of protocols have demonstrated that evaluating a lymph node with serial sections increases

the metastatic tumour detection rate from 7 to 33% when compared to a single H&E section. Routine serial section on all lymph nodes from a complete axillary lymph node dissection is cost prohibitive, labour intensive and time consuming[97].

In a report from the Ludwig breast cancer study group, lymph nodes taken from 921 patients were sectioned at 6 levels after a negative H&E stain of a single section. The examination of the serial sections identified occult metastatic disease in 83 (9%) patients. At 6 years, both the disease free survival rate (DFS) 53% vs. 71%, and the overall survival rate (OS) 70% vs. 86%, were worse in those patients with occult micrometastatic disease when compared to those without occult disease[97].

De Mascarel et al[97] examined nodes from 785 patients with an average of three H&E sections per node, also showed that the 10 year DFS and OS rates were worse for those patients with micrometastases compared to those without micrometastatic disease[97].

IMMUNOHISTOCHEMICAL STAINING

IHC staining of axillary nodes against the epithelial membrane antigen, mucin or cytokeratin antibodies has been shown to enhance the tumour detection rate. However, pathologists must be aware that lymph nodes may contain benign epithelial inclusions or cytokeratin immuno-reactive mesenchymal cells. Many retrospective studies have shown an increased tumour detection rate of 10-15% when IHC staining is added to routine H&E evaluation of axillary nodes[44,97].

De Mascarel et al[97] stained a single level of each tumour-free lymph node with a cocktail of monoclonal antibodies against epithelial cell antigens. With this technique they were able to detect micrometastases in 41% of previously node negative invasive lobular carcinoma patients and 10% of previously node negative ductal carcinoma patients[97].

McGuckin et al[97], detected micrometastases in 25% of specimens examined by performing both serial sectioning and IHC staining on axillary nodes that were negative on routine H&E staining.

REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)

RT-PCR is even more sensitive than immunohistochemistry in detecting metastatic tumour. It is able to detect approximately one cancer cell per 10^7 normal cells compared to one per 10^5 for IHC and one per 10^4 normal cells in routine histologically examination. Ironically, the marked sensitivity of conventional PCR has hindered its clinical application since the currently defined breast tumour associated markers are detectable in normal tissue. It is not able to quantitatively distinguish tracer amounts of gene expression in normal tissue from excessive amounts in metastatic tissue. However, real time RT-PCR technology can quantify gene expression from metastatic tissue. The assay allows for the simultaneous analysis of multiple genes and is highly automated, rapid and reproducible[96].

CLINICAL SIGNIFICANCE OF MICROMETASTASES

In the new TNM classification[98];

- A micrometastasis is defined to be between 0.2 and 2 mm in its greatest diameter.
- A tumour deposit less than 0.2 mm is referred to as an isolated tumour cell and to be recorded as pN_0.
- A tumour deposit in a lymph node drainage area with no evidence of lymph node architecture is considered a lymph node metastasis if its contour is smooth; and if irregular, it will fall in the pT category (irregular margin of the primary tumour) or it may be due to venous invasion.

Detecting a nodal micrometastasis (≤ 2 mm) is related to its size, position (subcapsular marginal sinus or parenchyma)[99]. However, its clinical relevance is controversial because not all micrometastases will progress and grow to form distant tumours[100]. A definite survival disadvantage is associated with occult nodal metastases but the size at which they become significant is undefined. In the era of lymphatic mapping and SLN biopsy, the detection of micrometastases is enhanced by focusing the pathologist's efforts on one or two dissected SLNs compared to the analysis of all nodes in a complete axillary dissection which is costly, labour intensive and time consuming. With the trend towards early mammographic diagnosis of smaller tumours, a method to optimise detection of micrometastasis with the aim of maximising tumour detection while at the same time, minimising labour and costs will become more important in the future[97].

RELIABILITY OF INTRAOPERATIVE SLN ANALYSES

INTRAOPERATIVE FROZEN SECTION

There is still some debate about the optimal intraoperative pathological evaluation of the SLN. This is a crucial issue since the primary aim of SLN biopsy is to stage the patient's axilla reliably so that axillary dissection together with the removal of a primary tumour and SLN can be performed as a one-stage operation[101]. It must be realised that during a regular frozen section 25-50% of the tissue is lost. This may lead to missing some SLN tissue with micrometastases. It is difficult to estimate the chance of this occurring, since this depends on the size and distribution of micrometastases[102,103].

The sensitivity of frozen section analysis of SLN, ranges from 56.7-100% and depends largely on the technique applied, the experience of the pathologist, the histological type and size of the tumour. This point was clearly illustrated by the Van Diest's[101] group who reported significant variation in the sensitivity between 56.7 and 87% depending on the experience of the pathologist. Metastatic diseases in patients with larger tumours metastasise more readily and are easily identified on frozen section than metastases from a smaller primary tumour. In an intensive study involving 890 patients, nodal metastases were diagnosed on frozen section in only 6 of 143 patients with stage Ia tumours as opposed to 45 of 119 patients with Stage II tumours. Metastatic lobular cells may not be much bigger than the native lymph node cells making interpretation difficult[101]. The delay of 20 minutes caused by frozen section analysis is acceptable since during this delay the primary tumour can be excised[102].

This allows the surgeon to perform immediate axillary lymph node dissection when the SLN is positive on frozen section. In case of a negative SLN frozen section, the final paraffin section result will ultimately determine

whether axillary lymph node dissection will be performed at a later stage. This occurs in less than 10% of patients as verified by Van Diest et al[102] and Giuliano et al[104].

An alternative to frozen section is intraoperative touch imprint cytology. The sensitivity ranges from 93-100%[105] with no false positives and a false-negative rate of 4.9%. The false-negative rate is associated with metastatic lobular carcinoma as it is more difficult to identify and to interpret. The false-negative rate is also associated with the difficulty in identifying micrometastases[105].

TABLE 4

The advantage of touch imprint cytology versus frozen section[103,105].

Touch imprint cytology	Frozen section
Rapid (< 10min) => less anaesthetic & operative time	Time consuming (30min)
Inexpensive	More expensive
Preserve tissue allowing further investigations if required.	25-50% of tissue loss
Highly specific	Less specific
No artefacts	Freeze-thaw artefacts
Easy to perform multiple sections	Serial sectioning – time consuming

IHC has shown to decrease false-negative rates of imprint cytology or frozen section by upstaging 10-20% SLN negative to SLN positive patients[106]. Although the most accurate way to detect micrometastases intraoperatively remains unknown, there are still concerns about the implication of false-negative results and the clinical relevance of micrometastases detected only by IHC and reverse polymerase chain reaction. With improved technology the ability to detect smaller metastases

is becoming a reality. However, our understanding at present of its biological relevance is still lacking.

SIGNIFICANCE OF NON-SENTINEL LYMPH NODES

The true benefit of lymphatic mapping and SLN biopsy is that an accurate histopathological status of the axilla can be determined without the patient incurring the costs and morbidity associated with complete axillary lymph node dissection. However, for patients with a tumour positive SLN, the true extent of axillary involvement is not known until a complete axillary lymph node dissection is performed. 50% of patients with a tumour positive SLN will have no metastatic tumour detected in the remaining axillary nodes after axillary lymph node dissection. Several groups have searched for potential predictors of non-sentinel lymph node metastases in patients with a positive SLN metastasis[97]. They found that 4 factors have a possible significant association with non-sentinel lymph node metastases namely[97];

- The size of the primary tumour,
- The peritumoral lymphovascular invasion,
- The size of the SLN metastasis and;
- The extracapsular extension of the SLN metastasis.

Failure to accurately identify and evaluate the true SLN could lead to incorrect staging and inadequate treatment that may lead to regional recurrences. This may adversely impact on patient prognosis, as the identification of the wrong SLN will result in metastatic disease remaining in the axilla[9].

REASONS FOR FALSE-NEGATIVE SLN BIOPSY

- Learning curve and surgical experience.
  This is the single most important factor for determining success for SLN biopsy, which is dependant on correct patient selection and adherence to protocol and technique[107,108].
- Prior excisional biopsy[109].
- Age of 50 or more years[109].
- Obesity due to fatty replacement of breast tissue leading to poor lymphatic flow[84].
- Clinically undetected involved axillary nodes. It has been hypothesised if there are more than 5 positive nodes, the lymphatics progressively become obstructed with malignant cells and the injected radiopharmaceutical and blue dye are redirected via collaterals to false-negative SLNs[109].
  Leidenius et al[110] disputed the fact that removal of more than 5 lymph nodes does not decrease the false-negative rate.
- The method used for histopathological analysis[111,112].

PATIENTS AND METHODS

The study was devised as a prospective evaluation of sentinel lymph node mapping and biopsy for early breast carcinoma. It was initiated in April 2000 and was completed in January 2004 in patients attending our surgical outpatient breast clinic.

PATIENT CRITERIA

The selection criteria were based on the recommendations in the literature.

INCLUSIONS[3,32,45]:

- Clinical stage I (1-2cm) or stage II (> 2cm - 5cm).
- Non-palpable axillary lymph nodes.

EXCLUSIONS[109,113]:

- Tumour size more than 5cm (Stage III and above),
- Palpable axillary lymph nodes,
- Previous surgery or radiotherapy to the axilla,
- Previous excisional biopsy of the primary tumour
- Previous neoadjuvant chemotherapy,
- Pregnancy or lactating,
- Multifocal / multicentric tumour.

STUDY PROTOCOL

The technique to identify the SLN included preoperative lymphoscintigraphy after a single intratumoral injection of ^{99m}Tc nanocolloid. This was followed by the intraoperative removal of the SLN, guided by the handheld gamma probe and methylene blue dye.

The first 36 procedures included a SLN biopsy followed by complete axillary lymph node clearance. The SLN/s together with the remaining axillary lymph nodes was sent for histological analysis.
This was done in order to assess the accuracy of the intratumoral injection technique in identifying the true SLN and to predict the status of the rest of the axillary lymph nodes.

Based on the experience gained and the high identification rate of the true SLN in the first 36 procedures, the subsequent surgical management of the axilla changed as a complete axillary lymph node clearance was done only if the SLN was positive at frozen section.

ETHICAL AND LEGAL CONSIDERATIONS

The Research Ethics Committee of the University of Cape Town approved the study protocol and informed consent was obtained from all patients by the surgeon.

PROCEDURE AND TECHNIQUE

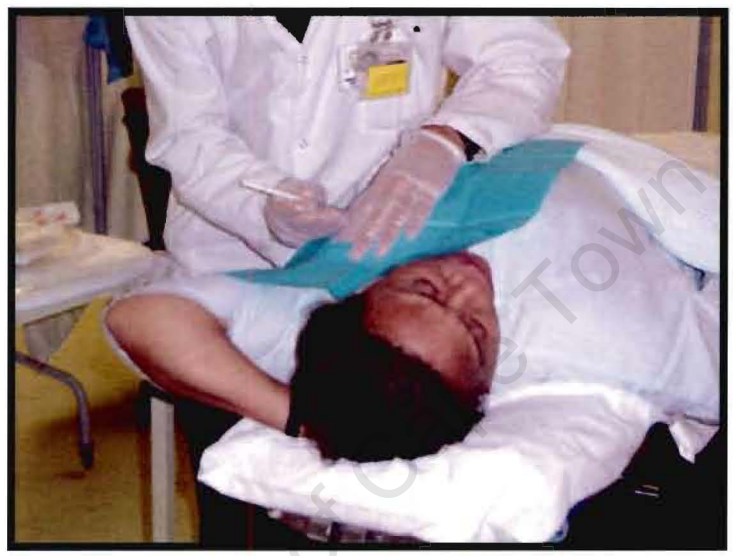
Lymphoscintigraphy was performed on all patients the day before surgery in the Nuclear Medicine Department.

INJECTION TECHNIQUE

The patients were injected with ^{99m}Tc-nanocolloid (Nanocoll, Manufacturer: GIPHARMA S.r.l. Saluggia (Vercelli), Italy. Marketing Authorized Holder: Amersham Health). The ^{99m}Tc-nanocolloid was prepared according to the manufacturer's instruction in a concentration of 500MBq per ml. The activity in a volume of 0.2 ml was drawn up with a 1ml syringe attached to a 26 gauge needle. 0.1 ml of air was then drawn into the syringe. The syringe was inverted so that the air was placed behind the activity to ensure complete delivery of the radiopharmaceutical.

Prior to the single intratumoral injection, the breast tumour area was covered with a sterile drape with an opening to allow access to the injection site. The drape served to prevent possible droplet contamination from the needle tip on removal of the syringe after the injection (*Figure 5)*. The remaining activity in the syringe was measured to calculate the actual administered dose to the patient.

FIGURE 5



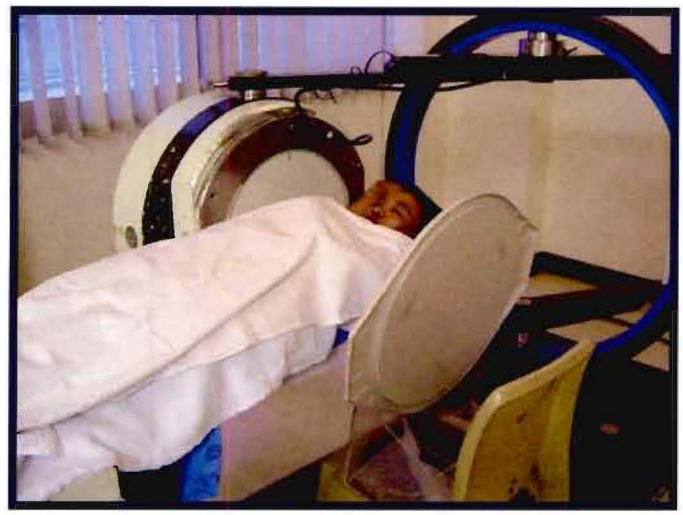
Demonstration of the injection technique.

IMAGING PROTOCOL

DYNAMIC PHASE

We used a large field of view single head gamma camera (General Electric Starcam 400 AC – Horsholm, Denmark) fitted with a low energy high-resolution collimator. The patient was in a supine position with the arm above the head. The detector was placed in a lateral projection to the patient, on the ipsilateral side to the tumour. This was done to obtain the best view of the axilla. A cobalt-57 flood source was supported in an upright position in a grooved perspex holder placed on a chair adjacent to the patient on the contralateral side. The cobalt flood source was used as a transmission source to outline the body contour. Dynamic images were then acquired at 20 seconds per frame in a 64X64 matrix for 20 minutes *(Figure 6)*.

FIGURE 6



Patient positioned between the detector head and the transmission source for dynamic and lateral static acquisitions.

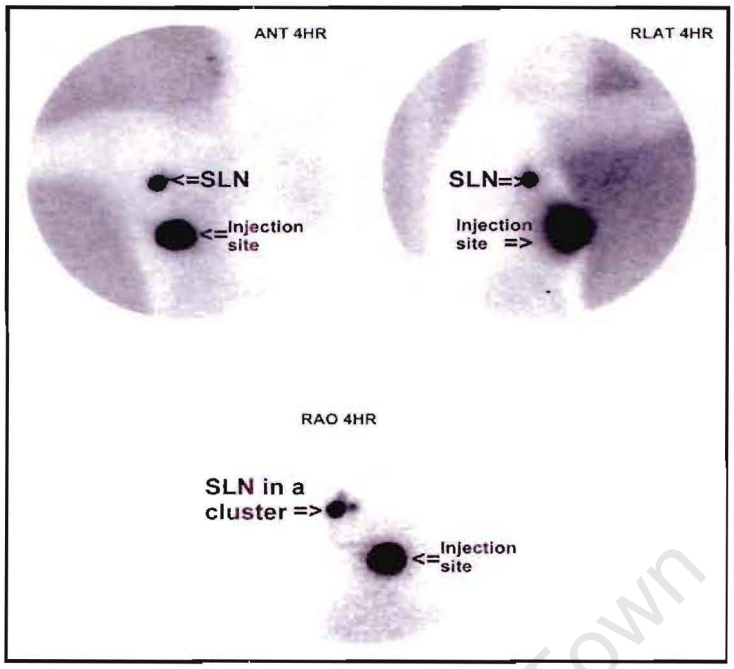
STATIC PHASE

Static images were acquired for 300 seconds in a 256X256 matrix at 30 min, 2hr and 4hrs post injection in the lateral and anterior projections, with the cobalt flood source placed in the contralateral and posterior positions respectively. For the anterior projection, the cobalt flood source was placed below the patient on a footstool.

The lateral projections were obtained with the patient in the same position as for the dynamic phase. The anterior projections were acquired with the patient supine and the arm on the affected side abducted to 90 degrees to the body in a similar position, as it would be in theatre.

At 4hrs, an additional anterior oblique view (35 degrees) of the affected side was acquired for 3 minutes without the cobalt flood source. This image sought to provide more accurate localisation of the SLN and to identify possible nodal clusters *(Figure 7)*. Additional delayed static images as described above were acquired between 6 to 24 hrs after the injection in patients where the SLN was poorly visible or not seen at 4hrs.

FIGURE 7



Oblique image showing SLN in a cluster.

SKIN MARKINGS

External skin markings using a permanent marker pen were done at 4hrs with the patient's arm abducted to the same degree, as it would be in theatre. The initial markings corresponded to the nodal uptake seen on the camera images. However, the final skin markings were done using the handheld gamma probe (C-trak: Care Wise, Morgan Hill, CA, USA) *(Figure 8)* and corresponded to the area of the highest probe reading as the camera skin marking overlying a node is subject to variation depending on the angulation of the parallel hole collimator, patient size and the position of the arm[114].

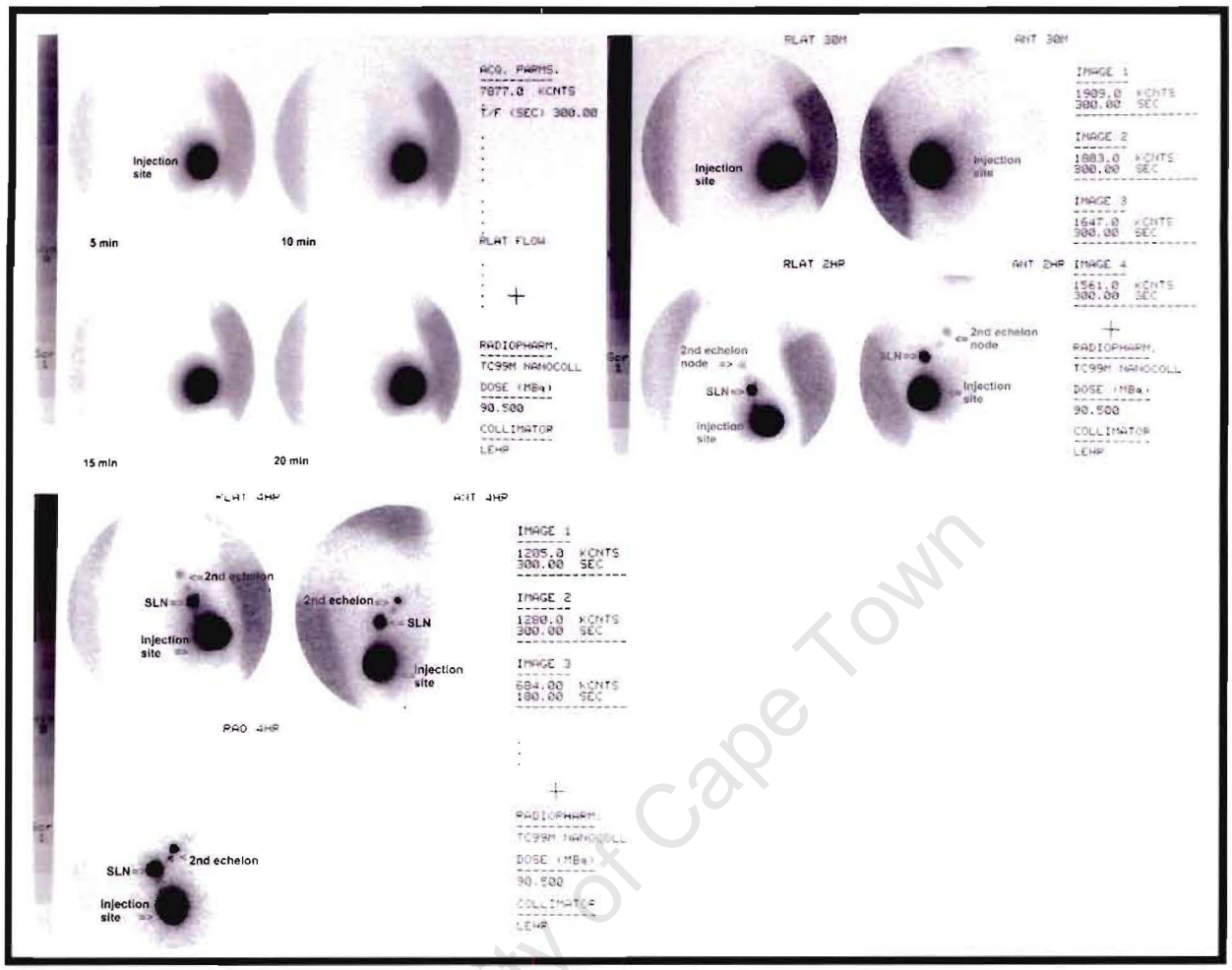
FIGURE 8

C-trak handheld gamma probe

PROCESSED DATA

The images were displayed and recorded on a single emulsion film using a GE Starimager *(Figure 9)*. The dynamic study was grouped into 4 frames at 300 sec/frame. One imaging film, which was used for a single procedure, was divided into 4 quadrants. The first quadrant of the film contained the 4 grouped images of the dynamic study while the remaining quadrants displayed the static images that were taken at the various time intervals. A copy was made for the surgeon who performed the SLN biopsy.

FIGURE 9



Complete study: Processed images.

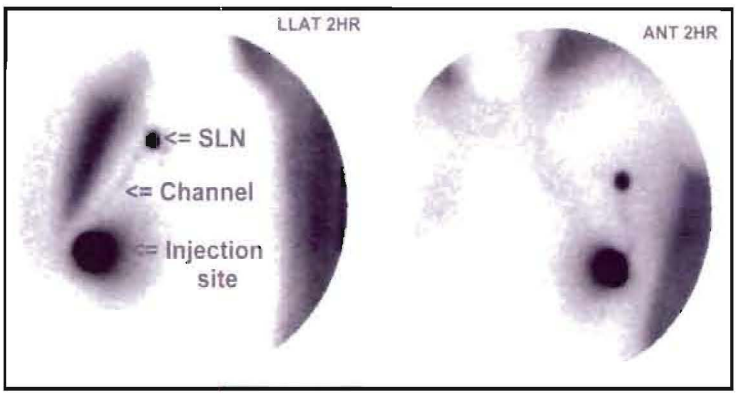
VISUAL ASSESSMENT

REPORTING

The SLN was identified as being the first lymph node seen at the end of the afferent lymphatic channel that emerged from the injection site or, if no afferent channels were seen, the first lymph node/s appearing in each regional draining basin[74].

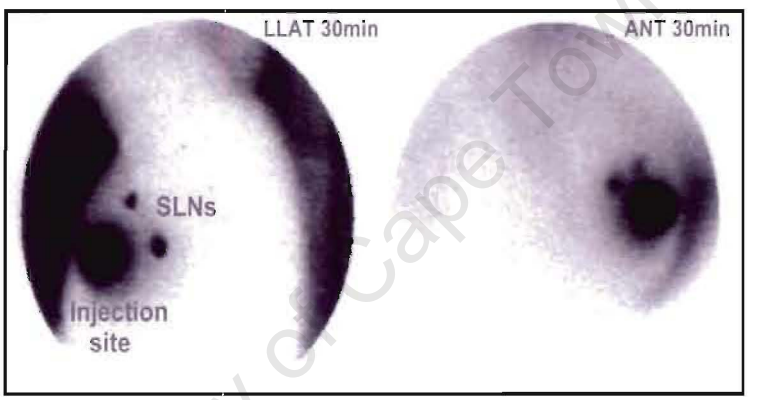
FIGURE 10

Example of scintiscans



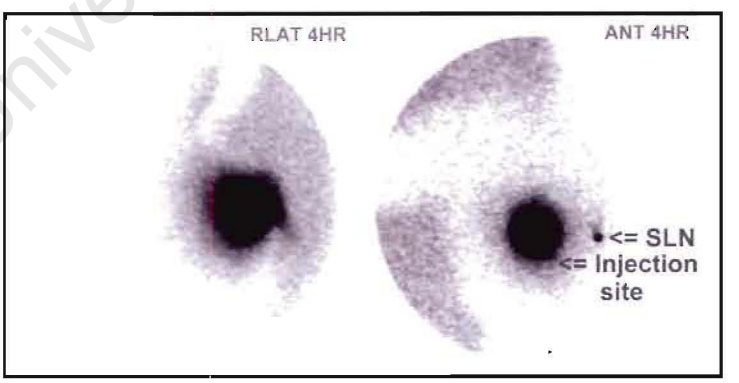
A.

A. Single channel with a single SLN in left axilla.



B.

B. Two SLNs seen in left axilla simultaneously without channels.



C.

C. Single SLN in the IMN without a channel.

A senior nuclear medicine consultant and I viewed and reported all scintiscans at the same time.

SURGERY

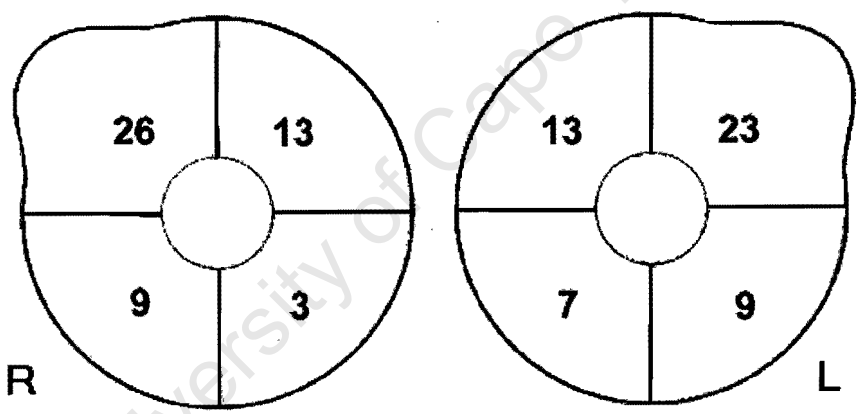
Surgery was performed within 36 hrs of the radiopharmaceutical injection. Under general anaesthesia, 0.5-1 ml of methylene blue dye was injected intratumorally immediately prior to draping the patient for surgery. The identification and excision of the SLN was the first part of the procedure followed by the planned breast surgery. Before an incision was made, the surgeon used the handheld gamma probe to confirm the location of the SLN as seen on lymphoscintigraphy. Sound-directed probing and digital display readings were then used to locate the radioactive SLN node/s, which may or may not have stained blue. According to the surgical protocol, the node was defined as sentinel if the nodal counts were 10X background or visually stained blue. An ex vivo or bench count in counts per second (cps) was taken from the excised SLN/s followed by an in vivo background count from the site of the excised node/s to confirm removal of all radioactive node/s. The SLN/s were sent for histological analysis.

RESULTS

PATIENT AND TUMOUR CHARACTERISTICS

A total of 102 patients were analysed and 103 SLN procedures were performed as one patient had bilateral synchronous breast carcinoma. The median age of the patients was 52 yrs (range 30-77 yr).
57/103 (55%) breast tumours were clinically Stage I while 46/103 (45%) were clinically Stage II. 51/103 (49.5%) of the breast tumours were located in the right breast and 52/103 (50.5%) were located in the left breast with the upper outer quadrants predominating *(Figure 11)*.

FIGURE 11



Primary tumour distribution in various quadrants.

At surgery, 36 breast tumours had a wide local excision (WLE) and 67 had a mastectomy.

The histological tumour types in the 103 breasts were:

- Ductal 94/103 (91%);
- Lobular 7/103 (7%);
- Papillary 1/103 (1%);
- Medullary 1/103 (1%).

TABLE 5

Patient and tumour characteristics

CHARACTERISTICS	n = 103
Age (yrs)	
Median	52
Range	30 – 77
Clinical Tumour Stage	
TI	57 (55%)
TII	46 (45%)
Tumour Type	
Ductal	94 (91%)
Lobular	7 (7%)
Papillary	1 (1%)
Medullary	1 (1%)
Surgery	
WLE	36 (35%)
Mastectomy	67 (65%)

LYMPHOSCINTIGRAPHY

All breast tumours received a single intratumoral injection of ^{99m}Tc-Nanocolloid with a median dose of 89 MBq (range: 65 –104MBq) in a volume of 0.2 ml.

Standard imaging up to 4 hours was performed in 101/103 (98%) procedures; of these, 8/101 (8%) procedures had additional delayed images varying from 6 to 24 hrs due to poor or non-visualisation of the SLN at the end of 4hrs. Due to technical reasons, 2/103 (2%) procedures were not imaged according to standard protocol and were terminated at two hours. However, a SLN was visualised at that time.

LYMPHATIC CHANNELS

These were seen in 68/103 (66%) procedures.

SENTINEL LYMPH NODES

NUMBER OF SLN SEEN

The first 36 procedures had a SLN identification rate of 89% as a SLN was identified in only 32 procedures. Four procedures showed no SLN on lymphoscintigraphy. The overall preoperative lymphoscintigraphy identification rate was 96% as the SLN was seen in 99/103 procedures. In the remaining 4, despite delayed images up to 24hrs, no SLN was seen. A single SLN was seen in 68/99 (69%) procedures, 2 SLN's were seen in 22/99 (22%) procedures, 3 SLNs were seen in 7/99 (7%) and 4 SLN's were seen in 2/99 (2%) procedures.

TABLE 6

Number of nodes seen at preoperative lymphoscintigraphy

No of Procedures	No of Nodes
4	0
68	1
22	2
7	3
2	4

TIMES SLN FIRST SEEN

In the 99 successful procedures a total of 141 SLNs were seen.

In 28/141 (20%) the SLNs were first seen on the dynamic phase, 19/141 (13%) at 30 minutes, 80/141 (57%) at 2 hours, 13/141 (9%) at 4 hours and 1/141 (1%) were seen at 6 hours.

127/141 (90%) SLNs were seen by two hours.

SITES OF SLN

Of the 141 SLNs, 114 (81%) were located in the axilla, 11 (8%) intramammary and 16 (11%) were in the IMN chain.

In 96/99 (97%) procedures irrespective of the location of the tumour the axilla was the commonest site, while in 3/99 (3%) the SLN was found only in the IMN. The axilla was the only site in 73/96 (76%) procedures, while in 23 the SLN was seen in the axilla as well as other sites. Of these 12/23 were in the IMN chain, 10/23 were in the intramammary area and in 1/23 the SLNs were seen in the IMN and intramammary sites.

SECOND ECHELON NODES

Second echelon nodes were seen in 53/99 (54%) procedures. The axilla was the commonest site in 49/53 (92%). In 11/49 (22%) in addition to the axilla, they were also seen in the IMN and intramammary areas. In 4/53 (8%) of the procedures, no second echelons were seen in the axilla but were seen in the IMN and intramammary areas.

SURGICAL AND HISTOLOGICAL FINDINGS

Of the first 36 procedures that were used to validate the study, only 29 (81%) had a SLN biopsy and a complete axillary lymph node dissection. A total of 64 SLNs were removed compared to 42 SLNs seen on lymphoscintigraphy due to some SLNs occurring in clusters.

In the remaining 7(19%) who had complete axillary lymph node clearances, a SLN was not removed, as 4 were not seen on preoperative lymphoscintigraphy, 1 was in the internal mammary chain whilst 2 were very poorly seen on lymphoscintigraphy in the axilla and the surgeon was unable to detect and remove the SLN node.

A total of 450 axillary nodes were harvested in only 35 procedures, as the axillary specimen of one patient was lost in transit to the pathologist.

Thus in the final histological analysis only 28 SLN biopsies were compared to 28 complete axillary lymph node clearances.

There was metastatic involvement in 10/28 (36%) SLN biopsies. Complete axillary lymph node clearance in this group revealed that in 4 the SLN was the only positive node while in the remaining 6 other axillary nodes had metastatic involvement.

There was no metastatic involvement in 18/28 (64%) SLN biopsies. Complete axillary lymph node clearance in this group revealed 17 who had no metastatic involvement, while 1 had metastatic involvement in the remaining axillary lymph nodes.

TABLE 7

Histological findings of SLN versus the remaining axillary nodes.

SLN		REMAINING AXILLARY NODES	
		POS	NEG
POS	10	6	4
NEG	18	1	17

The false-negative rate was 1/18 (5%) and the sensitivity of the SLN biopsy was 10/11 (91%), specificity 17/17 (100%) with a negative predictive value of 17/18 (94.4%) and a positive predictive value 10/10 (100%). There was concordance between the SLN and remaining axillary nodes in 23/28 (82%).

In the remaining 67 procedures 19 had complete axillary lymph node clearances. Of these, 14 had a positive SLN at frozen section, in 2 the SLN was in the IMN while in 3, the SLN biopsies were cancelled. In 48

procedures the SLN was negative and no axillary lymph node clearances were done.

Of the 14 who had a positive SLN intraoperatively, the SLN was the only positive node in 3 whereas the remaining 11 had other axillary nodes involved.

DISCUSSION

Lymphatic mapping and SLN biopsy is rapidly replacing complete axillary lymph node dissection in the staging of the axilla in early breast carcinoma due to its lower morbidity, but it is not yet universally accepted[46]. Currently, there is no consensus on the optimal method for defining and identifying the SLN[115,116].

The main issues pertaining to SLN biopsy are the following:

- What is the optimal technique?
- Does it accurately predict axillary lymph node status?
- Does the SLN technique actually decrease axillary morbidity?
- Does a false-negative SLN have an adverse effect on patient management and outcome?

A number of techniques have been proposed as the optimal method for identifying SLN/s. The main debate centres on whether to use a blue dye or radiopharmaceutical method either singly or in combination.

Some prominent authors support using only the blue dye method (Guiliano)[46] while others recommend only the radiopharmaceutical method (Veronesi)[46].
Literature reviews by Hoefnagel et al[64], Nieweg et al[77] and studies by Kraft et al[94], Cody et al[117] and d'Eredita et al[118] reported that the best results for localising the SLN are obtained with the combination of lymphoscintigraphy, a handheld gamma probe and blue dye.

In this study we evaluated whether the single intratumoral injection technique is a reliable and valid method to identify SLN/s. In the first 36

procedures we assessed the accuracy and false-negative rates of lymphatic mapping using this technique.

Currently, there is no consensus on the radiopharmaceutical of choice for lymphoscintigraphy and SLN identification, its injection volume, activity, site and imaging protocol after injection[46,114,119]. In this study, we used ^{99m}Tc-nanocolloid with a median dose of 89 MBq in a volume of 0.2 mls.

The analysis of the first 36 procedures showed a SLN identification rate of 89% and a false-negative rate of 5%. The results of which influenced the subsequent management of patients with early breast carcinoma as only those at surgery with a positive SLN at frozen section, continued to have a complete axillary lymph node clearance.

The overall SLN identification rate was found to be 96% (99/103) with 90% visualised by two hours. The present study therefore supports other authors who reported increasing success in SLN identification as they gained experience[4].

At the Netherlands Cancer Institute[13] using the single intratumoral injection technique the sentinel lymph node identification rate was 97% as the SLN was identified in 516/531 patients.

In Giuliano's original study[115,120], he used a peritumoral injection of blue dye, with a SLN biopsy success rate of 66%, with 96% accuracy. Subsequent studies by the same group and others using the blue dye alone have reported success rates from 71% to 94% and accuracy between 97% and 100%. Krag et al[115,120] used the peritumoral injection of radiopharmaceutical to achieve a SLN biopsy success rate of 82% with

100% accuracy. Albertini et al[115,120] achieved a success rate of 92% with 100% accuracy using a combination of blue dye and radiopharmaceutical in a series of 62 patients. Veronesi and colleagues[115,120] used the subdermal injection of radiopharmaceutical in 163 patients and achieved a SLN biopsy success rate of 98% with 98% accuracy.

Kern[121,122], using subareolar injection of blue dye alone, achieved a success rate of 98% with an accuracy of 100%.

Embryologically, the breast develops from the ectoderm. Its network of lymphatics communicates with the subdermal plexus and drains predominantly into the ipsilateral axillary nodes[7,115]. Borgstein et al[7,115] demonstrated 100% concordance in localising the sentinel lymph node using an intradermal injection of blue dye and a peritumoral injection of radiopharmaceutical. This high rate of concordance not only supported the theory that the intraparenchymal lymphatics drain into the same nodal basin as the lymphatics of the overlying skin; it showed that the two regions drain to the same sentinel lymph node. Linehan et al[123] who reported a 95% concordance between intradermal radiopharmaceutical injection and peritumoral injection of blue dye provided additional evidence for this theory. Klimberg et al[124] found a 95% concordance with the subareolar injection of radiopharmaceutical and peritumoral blue dye. Pelosi et al[72] found a 92% concordance with a subdermal injection of radiopharmaceutical and the periareolar injection of blue dye. These studies all suggest that, regardless of tumour location within the breast, all lymphatics drain to the same sentinel lymph node[7,115].

The different injection techniques described in the literature all seem to reliably identify the "true" SLN/s in the axilla. It can thus be hypothesised

that predefined SLNs representative of the entire breast, can be found irrespective of the injection site of the radiopharmaceutical or blue dye[7,8].

Lymphoscintigraphy is used as a "road map" and increases one's level of confidence in identifying the SLN at the end of a lymphatic channel. This facilitates differentiation of a SLN from a 2nd echelon node. Using the intratumoral injection technique, lymphatic channels were observed in 68/103 (66%) of our procedures.

Lymphoscintigraphy also allows visualisation of the number of SLNs in the regional drainage basin and may facilitate the skin incision needed for their removal. Without the benefit of imaging, the surgeon has to remove all radioactive nodes, as it may be impossible to distinguish between the SLN and echelon nodes. In this study preoperative lymphoscintigraphy showed SLNs in 99/103 (96%) procedures of which 31/99 (31%) had more than one SLN. Second echelon nodes were seen in 53/59 (54%) of the procedures.

Drainage to the axilla predominated irrespective of the site of the primary tumour[125,126,127]. This pattern was observed in this study as 96/99 (97%) drained to the axilla.

The final skin markings of the SLN were based on the handheld gamma probe as the patient's size and arm positioning influenced the gamma camera image.

The pattern of lymphatic drainage in any one patient to non-axillary nodes is unpredictable and difficult to determine from the location of the primary tumour[4]. According to Nieweg et al[13] and Uren et al[128], non-axillary nodes

are present in up to 27% and 35% of patients respectively. They are usually seen with the intratumoral and peritumoral injections and not with the subdermal / intradermal and periareolar / subareolar injection techniques[129,130]. Our study demonstrated lymph nodes in the breast tissue (intramammary) in 11/99 (11%) and in the IMN in 16/99 (16%) of the procedures.

Poor visualisation of the SLN at the end of 4hrs necessitated additional delayed imaging between 6 and 24 hrs in 4/103 (4%) procedures, 1 had extensive tumour infiltration of the lymph nodes and 3 had no direct relationship to tumour presence in the nodes as all the axillary nodes removed were free of tumour.

Non-visualisation of SLNs occurred in 4/103 (4%) procedures;

- 2 had extensive metastatic lymph node infiltration.
- 2 had no obvious reasons.

Failure to accurately identify and evaluate the true SLN could lead to incorrect staging and inadequate treatment, and lead to late regional recurrences. This may adversely impact on patient prognosis[9].

There was a single false-negative SLN in the first 36 procedures. There was no obvious explanation for this finding.

Limitations of the study:
The first 36 procedures included a SLN biopsy followed by a complete axillary lymph node clearance to assess the accuracy of the intratumoral injection technique in identifying the true SLN and to predict the status of the rest of the axillary lymph nodes.

In the subsequent 67 procedures 14 had a SLN biopsy followed by a complete axillary lymph node clearance, as the SLN was positive at frozen section. In the remaining 53 procedures we were unable to determine whether the SLN identified at lymphoscintigraphy truly reflected the status of the remaining axillary nodes i.e. the false-negative rate could not be determined. In 48 of these the SLN was negative and no axillary lymph node clearances were done. In the remaining 5, complete axillary lymph node clearances were done but there was no SLN biopsy in 2 as the SLN was in the IMN, and in 3 the SLN biopsies were cancelled due to theatre unavailability.

In this study we were able to identify the SLN in 99/103 procedures most of which were in the axilla 96/99. However, in 3/99 procedures the SLNs were located only in the IMN. A SLN biopsy was not done in these 3 who went on to have a complete axillary lymph node clearance.

Currently, there is no reliable data on local axillary recurrence and survival rates in patients who have had a node negative SLN biopsy.
The American College of Surgeons Oncology group, Z0011[35,97], the National Surgical Adjuvant Breast Project, B-32[35,97,127,131] and the Almanac study[132] are currently collating data on regional recurrence and survival of patients with breast carcinoma who have undergone SLN biopsy.

CONCLUSION

The SLN concept is established and will play an increasingly important role in the staging and management of the axilla in early breast carcinoma.

An initial study consisting of both SLN biopsy and complete axillary dissection should be performed. Once the nuclear medicine physician and surgeon are sufficiently experienced and the identification and false-negative rates of the SLN are acceptable, the technique can be adopted and complete axillary dissection can be abandoned in SLN negative patients. This technique also results in less radical surgery with a decrease in morbidity.

Close communication and co-operation between nuclear medicine physician, surgeon and pathologist is essential for the successful implementation of the SLN technique.

The single intratumoral injection technique with preoperative lymphoscintigraphy has been shown in our study to be a valid and reliable method in identifying SLNs.

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