LYMPHATIC MAPPING AND THE AXILLA IN PRIMARY BREAST CANCER

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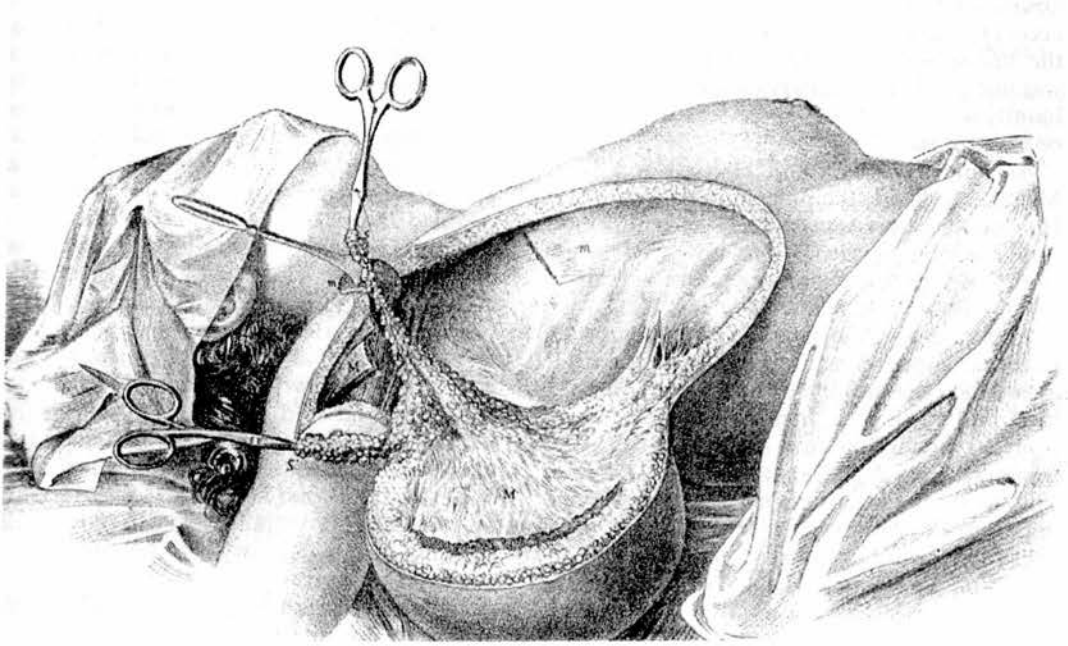
To my parents Paul and Sheila Lambah
for their unconditional love and support
throughout my life and years of study



Halsted's original drawing showing the excision of the pectoral muscles along with the breast before the axillary contents are removed during a radical mastectomy. (from Halsted. W.S., Annals of Surgery; **20;** 497; 1894.

Declaration

I declare that this thesis is entirely my own work and composition.
This thesis has not been submitted for any other degree or professional qualification.

I acknowledge that the work reported in this thesis has been carried out by myself with the exception of immunohistochemistry of imprints carried out by Mr L Brett, Chief MLSO, department of pathology, Western General Hospital, lymphoscintiscans were performed by the radiographers in the department of nuclear medicine, Western General Hospital, immunohistochemistry of lymph nodes in the micrometastases study was performed by Mrs. Frances Ray and Mr Tim Ingman and staff at the University of Edinburgh Department of Pathology, help with follow–up arm measurements and occasional recruitment of patients whilst I was on leave to enable the project to continue was provided by Sister L Renshaw, research nurse, Edinburgh Breast Unit.

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Abstract of Thesis

Assessment of axillary lymph node status in breast cancer is a collaborative exercise between surgeons and pathologists that continues to provoke debate. A positive result for lymph node metastases is the primary determinant for subsequent therapy decisions.

At present wide variation exists in the surgical approach to the axilla ranging from a complete level III clearance of all axillary nodes to no treatment in some centres. The technique of sentinel node biopsy has recently been suggested as a less invasive method of staging the axilla sparing the morbidity associated with an axillary clearance.

Despite existing guidelines for the histological processing and reporting of lymph nodes in the UK, practices continue to demonstrate considerable variation within and out with these guidelines. New ways of staining lymph nodes, such as immunohistochemistry are reported to improve on the sensitivity of conventional haematoxylin and eosin in the detection of lymph node metastases but are not considered standard practice in most pathology laboratories. The optimal surgical and histopathological management of the axilla needs clarifying to allow correct selection of patients for adjuvant treatments.

The chapters of this thesis present the results of a group of related studies examining existing and new methods of surgical and histopathological assessment of axillary lymph nodes in breast cancer patients.

The long-term results of randomised trials of 866 patients comparing a level III axillary node clearance to a non-targeted four-node axillary sample reveal no difference in long-term survival between the two procedures after a median follow up of 8.2 years. Axillary recurrence appears to be more frequent following an axillary node sample than after an axillary clearance.

A two-phase, randomised Multicentre trial (the ALMANAC trial) is currently aiming to validate the role of sentinel node biopsy in breast cancer patients in the United Kingdom. The early results of 153 patients recruited into the ALMANAC trial by Edinburgh Breast Unit suggest that sentinel node biopsy is an accurate and reliable method of staging the axilla in T1-2 node-negative breast cancer with minimal morbidity.

Accurate intraoperative assessment of sampled lymph nodes allows a surgeon to decide whether or not to proceed immediately to a full axillary clearance in node-positive patients without the need for a second operation. Imprint cytology is reported to improve upon frozen section histology in the intraoperative detection of lymph node metastases. Imprint cytology of 238 freshly examined lymph nodes from 53 patients with and without immunohistochemistry suggests the technique is at least as accurate as frozen section histology and can be useful in the intraoperative assessment of axillary lymph nodes.

The prognostic significance of occult lymph node metastases (or micrometastases) is uncertain. Lymph nodes from 26 node-negative patients who developed axillary recurrence and from 26 patients who developed no axillary recurrence found no clinical significance for axillary lymph node micrometastases after examination by immunohistochemistry.

Aims of Thesis

1. To collect and report results of conventional surgical techniques currently practised to stage and treat the axilla. A retrospective review is made of the long-term results of two randomised clinical trials comparing axillary node sample with axillary node clearance.

2. To introduce the technique of lymphatic mapping and sentinel node biopsy as a new method of staging the axilla in clinically node-negative stage T1-2 breast cancer.

3. To compare the results of sentinel node biopsy with conventional axillary staging techniques as a means of staging the axilla in a randomised clinical trial of clinically node-negative patients with stage T1-2 breast cancer.

4. To study the technique of imprint cytology of axillary lymph nodes as a rapid method of identifying lymph node metastases intraoperatively. To compare two methods of staining lymph node imprints and discover a suitable staining agent.

5. To study the significance of occult axillary lymph node micrometastases on breast cancer disease progression.

6. To provide important information for breast surgeons and pathologists on the optimal management of the axilla in clinically node-negative breast cancer based on current evidence and the results of this thesis.

1. INTRODUCTION

1.1 HISTORY

1.1.1 Pre 1950

A full understanding of modern surgical management of breast cancer requires a knowledge of its history, which dates back to the latter half of the nineteenth century. The commonest of female malignancies was certain to cause death in women suffering from it prior to 1867 when simple local excision was the only surgical procedure performed. In 1863 Sir James Paget wrote, "I am not aware of a single instance of recovery, that is that the patient should live more than ten years free of the disease.....In deciding for or against the removal of a cancerous breast we may, I think, dismiss all hope that the operation will be the final remedy for the disease.". Charles H. Moore, surgeon to the Middlesex hospital published a revolutionary paper in 1867 advocating the wider excision of the tumour along with involved skin and lymphatics if necessary so that "the various undetected prolongations of the tumour and outlying nodules be included in the operation." Mitchell Banks of Liverpool supported his ideas and published two papers in 1878 and 1882 in which he wrote "the axillary glands as well as the breast in *all* cases should be removed". Thus, the first operations of axillary dissection were performed for breast cancer over a hundred years ago.

Richard von Volkmann, the leading German surgeon of his time took things a step further and recommended excision of the pectoral fascia " because microscopical examination showed repeatedly what I had not expected that the fascia was already carcinomatous, whilst the muscle was certainly not involved." Many surgeons adopted this technique for a time and these included Billroth in Vienna. However, this technique was later found to have abysmal results including an 18.5% operative mortality, 82% 3-year local recurrence rate and only 4.7% 3-year survival when an up-and-coming American surgeon reviewed Billroth's results. It was this man who was the principal pioneer of modern surgical management of breast cancer and his name was William S. Halsted.

He was born in 1852 into a prosperous New York mercantile family and entered Yale University in 1870. It was here that he became interested in medicine and he entered the college of physicians and surgeons in 1874. He graduated in 1877 and

soon afterwards sailed for Europe where he spent more than a year in Vienna observing Billroth, the dean of European surgery. Halsted returned to New York in 1880 and became eminently successful performing the famous "radical mastectomy" for the first time in 1882 at the Roosevelt hospital. In this operation, the entire breast, skin and subcutaneous tissue over the breast are removed in continuity with the pectoralis major and minor muscles plus the axillary lymph nodes. Halsted would then cover the defect with a skin graft.

By 1894, Halsted had performed 50 radical mastectomies and reported the 3-year results: there were no operative deaths, the local recurrence rate was 6% and 50% of the 8 patients operated on more than three years previously were well. It must be noted that there were no specific selection criteria for these patients, many of whom had advanced disease and that all 50 had axillary metastases. He followed this series with a second paper in 1898, reporting the results of the first 133 radical mastectomies. In it he wrote that the local recurrence rate was 9% and 52% were alive and well [1]. In the last paper which he wrote reporting on his results of 232 patients published in 1907, the operative mortality was 1.7 % and 42.3 % were alive and well after 3 years [2]. These results clearly reflect a significant improvement on those of the earlier surgeons performing von Volkmann's technique. The radical mastectomy was the most important and successful of the pioneering surgical techniques for cancer of the breast and its practice was continued throughout the first half of the twentieth century helping thousands of women to recovery from this dreadful disease.

Halsted's operation was practised on both sides of the Atlantic almost unquestionably until David Patey published his results in 1948 suggesting that it may not be necessary to remove the Pectoralis major muscle in all cases of radical mastectomy. Patey argued that more modern studies on lymphatic anatomy had shown that firstly, the dermis was a plane rich in lymphatics and hence a rich potential plane of spread particularly in the case of more superficial cancers and secondly that the deep fascia was a plane devoid of or very poor in lymphatics and consequently less important in its potential to spread cancer cells. He theorised that this would suggest more aggressive excision of skin and less excision of muscle would be a more logical strategy in ensuring adequate excision.

His results compared 45 cases of Halsted's radical mastectomy with 46 similar patients having a "modified" radical mastectomy in which more skin was excised but only the Pectoralis minor muscle was removed with the Pectoral major muscle being preserved.

There was no significant difference in 3-year survival or local recurrence rates between the two groups. Patey therefore concluded that there was no evidence to support the view that the addition of the removal of the Pectoralis major muscle brings any increase in the survival figures[3].

At around the same time, Dr Robert McWhirter from Edinburgh published his results from the 5-year follow up of 2809 cases of breast cancer referred to the Royal Infirmary of Edinburgh between 1930 and 1945. He introduced the technique of simple mastectomy followed by radical post-operative radiotherapy to the chest wall and regional lymph nodes, and locoregional recurrence rates fell using this regime. Within 5 years, the rates of locoregional recurrence fell from 40% to 14% and 5-year survival rose from 37% to 51% (43.2% if those with distant metastases included) in those treated with adjuvant radiotherapy [4].

These key, pioneering publications provided the foundations for the modern management of breast cancer.

1.1.2 Surgery to the axilla (post 1950s)

Both Halsted and Patey practised radical axillary clearance and in the majority of Halsted's 1907 series, he also went on to include a supraclavicular neck dissection if apical axillary glands were involved [2]. McWhirter's practice differed from theirs by his use of simple mastectomy to remove the primary tumour plus axillary node irradiation to treat the lymph nodes instead of axillary clearance [5]. Radical radiotherapy was thus practised but was not without serious morbidity and in 1968 Forrest and Kunkler suggested extension of the operation of mastectomy to include the lower axillary lymph nodes lying close to the axillary tail of the breast [6]. It was suggested that were these free of tumour, treatment might safely be limited to mastectomy alone, immediate radiotherapy only being given to those patients whose nodes were histologically proven to be involved by tumour.

During the late 1960s and early 1970s randomised trials were carried out in Cardiff and St Mary's Hospital, London comparing the regime of lower axillary node sampling as a determinant for radiotherapy following simple mastectomy with radical mastectomy [7, 8]. These studies used a conservative policy of selective radiotherapy to the axillary lymph nodes if histological examination of the axillary nodes showed involvement. Patients with no disease in the axillary nodes received no further treatment of the axilla. In the conservative group treated by simple mastectomy, lymph nodes were taken from the axillary tail of the breast and described as pectoral lymph nodes. These nodes are palpated on the medial aspect of the axillary tail of the breast at the junction with the axillary fat and just behind the outer border of Pectoralis major muscle. The results revealed significant reductions in arm morbidity particularly a reduction in arm swelling and shoulder stiffness in patients spared radical radiotherapy and radical surgery. This was at the expense of more axillary recurrences in the conservative group although this was not statistically significant. These trials set the stage for the move towards a more conservative and selective approach to the management of breast cancer. This trend has continued to the present day management of breast cancer, which will be considered in the following pages.

1.2 ANATOMY

1.2.1 Breast structure

The adult female breast lies in the superficial fascia of the anterior thoracic wall. The base of the breast lies over a fairly constant area from the sternal edge medially to near the mid-axillary line laterally and from the second to sixth ribs craniocaudally. It overlies Pectoralis major and overlaps onto Serratus anterior and a small part of the rectus sheath and external oblique aponeurosis inferiorly. The axillary tail is an extension of the upper outer quadrant towards the axilla and usually lies in the subcutaneous fat but rarely penetrates the deep fascia of the axillary floor to lie adjacent to axillary lymph nodes.

Structurally, the breast is composed of three parts:

1. glandular tissue
2. fibrous tissue connecting its lobes
3. interlobar adipose tissue.

The breast has fifteen to twenty lobes containing many lobules supported by loose connective tissue, which supports blood vessels and ducts. The smallest lobules (terminal duct lobular units) are the functional component of the breast and drain into ductules which unite to form larger tributaries of the terminal lactiferous ducts which in turn drain a single lobe centrally to open onto the nipple. Each lactiferous duct has a dilated sinus at its terminal portion behind the nipple.

Behind the breast, the superficial fascia, which extends superiorly as the continuation of the superficial abdominal (Scarpa's) fascia, is condensed to form a posterior capsule. The breast is suspended by strands of fibrous tissue or stroma (the suspensory ligaments of Cooper) extending from the dermis of the skin to this fascia. This helps to lift the breast in young women but ageing causes them to atrophy leading to pendulous breasts. These ligaments become contracted when tugged on by carcinomas and this leads to pitting (or tethering) of the skin. Between the posterior capsule and the pectoralis fascia is the relatively avascular retromammary space which contains lymphatics and a few blood vessels.

Blood supply

1. **the lateral thoracic artery** (a branch of the 2nd part of the axillary artery) is the main provider to the lateral half of the breast
2. **the internal thoracic artery** sends branches through the intercostal spaces close to the sternum to the medial half of the breast
3. similar **branches** perforate **from the intercostal** vessels themselves
4. **pectoral branches of the thoracoacromial artery** (a branch of the second part of the subclavian artery) supply the upper part of the breast and also supply Pectoralis major and minor.

Venous drainage is mainly by deep veins that run with the arteries to the internal thoracic and axillary veins. Some drainage to posterior intercostal veins presents a link to the vertebral veins, which hence can allow metastatic spread to bone.

1.2.3 Lymphatic Drainage

i. Background lymphatic anatomy

The lymphatic system comprises plexuses of minute vessels (lymph capillaries) that commence blindly in the tissue spaces of the body and ultimately drain into the brachiocephalic veins via the thoracic duct and right lymphatic trunk. Lymph nodes, epitheliolymphoid tissue of the alimentary canal, respiratory tract, spleen and thymus together with circulating lymphocytes make up the rest of the lymphatic system. Lymph capillaries commence with a dilated, bulbous, blind-ended extremity in the interstitial space. The wall of a lymph capillary consists of a single layer of endothelial cells similar to a blood capillary although the basement membrane is often lacking and specialised attachments between cells are few. This allows the endothelial wall of a lymph capillary to be permeable to substances of much greater molecular size than those, which can pass through the walls of a blood capillary. This forms a pathway for absorption of colloidal material, particulate matter, cell debris and microorganisms. Thus, if the lymph vessels become obstructed, the tissues draining them become distended and oedematous with a fluid containing much protein. Experimental evidence suggests that absorption of macromolecular and particulate substances takes place through intercellular fenestrations between the endothelial cells or by micropinocytosis across the cells.

Several factors are responsible for propelling lymph along the capillaries including the filtration pressure in the interstitial fluid, contraction of surrounding muscle causing compression of lymphatics, respiratory movements creating a negative pressure in the brachiocephalic veins, and arterial pulsation compressing lymphatics, which lie close to arteries. Lymphatic vessels also contain valves similar to veins, which prevent retrograde flow of lymph ensuring its continuous flow towards the regional drainage basins. If there is an obstruction in a lymphatic such as may occur from a malignant tumour, the valves become incompetent and then lymph must travel in a retrograde fashion to find an abnormal pathway out of the tissues to the regional lymph nodes.

Lymphatic channels permeate the lymph nodes after lymph has entered through the afferent lymphatic at the cortex of the node. These channels allow lymph to percolate

and ensure maximum exposure of lymph to the macrophages and lymphocytes, which line them. The afferent lymphatics enter at different points on the periphery of the node and after branching and forming a dense plexus in the substance of the capsule, open into the subcapsular sinus, a cavity running around the periphery of the cortex except in the region of the hilum (a slight depression on one side of the node allowing blood vessels to enter and leave). From the subcapsular sinus, numerous radial cortical sinuses lead towards the medulla of the lymph node eventually coalescing to form the medullary sinuses, which are confluent at the hilum and drain into the efferent lymphatic or lymphatics. Arteries and veins serving the interior of the lymph node enter and leave at the hilum and give off straight branches, which traverse the medulla in company with connective tissue trabeculae, giving off a few minor vessels en route. On reaching the cortex, the arteries divide to give off a dense plexus of arterioles and capillaries forming numerous anastamosing loops before passing back to the highly branched venules and veins. This rich vascular network allows extensive movement of lymphocytes from the blood stream into the lymph node [9].

The lymph nodes of the axilla can be divided into five groups:

1. **Anterior (pectoral**) nodes lie medially at the lower border of pectoralis minor alongside the lateral thoracic artery. This important group receives lymph from the major part of the breast and from the anterior thoracic wall.
2. **Posterior (subscapular)** nodes also lie medially but along the subscapular artery posteriorly in the axilla. They receive lymph from the upper half of the trunk posteriorly and from the axillary tail of the breast.
3. **Lateral** nodes lie along the medial border of the axillary vein. These receive lymph from the upper limb.
4. **Central** nodes lie in the fat of the axilla centrally and receive lymph from the above groups.
5. **Apical** nodes lie in the apex of the axilla and receive lymph from all the other groups. From here lymph passes by the subclavian lymph trunk to the supraclavicular nodes which then drain into the thoracic duct or the right lymphatic trunk.

There are often lymph nodes present in the axillary tail or upper outer quadrant of the breast substance itself, the so-called **intramammary** lymph nodes and there are several nodes lying between Pectoralis major and minor, the **interpectoral** nodes [9].

ii. Lymphatics of the breast

The lymphatics of the breast were first described in detail by Cruickshank in 1786 who described two main lymphatic pathways one draining externally to the axilla and the other perforating the intercostals to drain into the internal mammary chain. Mascagani published similar findings a year later in 1787 and found additional lymphatics accompanying the epigastric and intercostals vessels. Approximately 100 years later in 1885 Sappey discovered a plexus of lymphatics in the subareolar area of the breast to which he believed the entire breast parenchyma drained before draining into the axilla. He specifically denied that any lymphatics left the posterior surface of the breast or that any lymph vessels reached the internal mammary chain. Numerous lymphatic capillaries exist in the breast substance and the overlying skin. Turner-Warwick's excellent paper "The Lymphatics of the Breast" describes in detail the many lymphatic pathways of the breast from his extensive studies of breast lymphatics in 88 patients by injection of vital blue dyes and radioactive colloidal gold into the breast parenchyma and observing the drainage patterns [10].
His observations were as follows:
The lymphatics of the breast like other areas of the body accompany the blood vessels. The blood supply of the breast is mainly derived from the axillary and subclavian arteries and the perforating branches of the internal mammary artery with a minor contribution from the lateral perforating branches of the intercostals vessels. The lymphatic drainage in these three directions is approximately proportional to the blood supply with the majority of the lymph (75%) draining to the axilla, a considerable proportion to the internal mammary nodes (or parasternals) and only occasional drainage into the posterior intercostal nodes, which lie between the necks of their corresponding ribs, close to the vertebrae.
The lymphatics of the main **axillary pathway** arise in the breast lobules and were observed by Turner-Warwick to drain directly to the lateral thoracic (pectoral) group of lymph nodes through the breast substance and not via the subareolar plexus in

contrast to Sappey's theory. These main lymphatic trunks were at no time observed to run in the deep fascial plane.

The **internal mammary** lymph nodes lie along the internal mammary vessels extending upwards from the 5th intercostal space to the supraclavicular nodes. They usually lie between the costal cartilages but also lie behind them at times. These nodes receive lymph not only from the perforating lymphatics that accompany the blood vessels but also from lymphatics that accompany the lateral perforating branches of the upper intercostal vessels thus constituting a further **intercostal pathway**. The upper three or four intercostal spaces have large internal mammary nodes with relatively smaller posterior intercostal nodes. Lymph tends to drain anteriorly into these nodes from the lateral perforating branches of these upper spaces while those entering lower spaces may drain into the posterior intercostal nodes. Perforating branches also accompany the intercostobrachial nerves and such branches in the upper intercostal spaces constitute at least a theoretical communication between axillary and internal mammary pathways.

Most of the lymphatics which leave the posterior surface of the breast perforate the pectoralis major muscle to reach the internal mammary chain and axilla creating an **interpectoral pathway**. Those vessels following the thoraco-acromial vessels drain into the interpectoral nodes on their way to the upper axilla. No lymphatics are observed to perforate the pectoralis minor muscle although lymph nodes lie in front of it (interpectoral), above it (infraclavicular) and behind it (lateral thoracic/pectoral group).

Many studies have suggested that the breast drains centripetally into the subareolar plexus (of Sappey) and from here to the axilla. The **subareolar plexus** is known to exist and communicates with the lymphatics around the lactiferous ducts. Turner-Warwick acknowledges this in his study but suggests that 10% of random injections into the breast fill the lactiferous ducts causing this central drainage pattern commonly observed and mistaken for lymphatic drainage. He concluded that the subareolar plexus may be overemphasised in some studies.

The presence of a deep fascial plexus of lymphatics in the retromammary space is not well demonstrated and it is believed that this area contains only a few lymphatics, which drain the fascia itself but do not appear to be an important drainage pathway

from the breast. As described above, several lymphatics pass across this space to perforate the pectoralis major or intercostals but do not form a plexus of lymphatics. Fascial spread of tumour is unusual in the early stages of breast cancer but is seen in later stages probably as a result of direct mechanical infiltration of tumour.

The **subcutaneous lymphatics** of the breast form a coarse mesh network over the breast similar to other areas of the body lying in the same plane as the superficial venous plexus. They extend in the same plane across the midline, over the clavicles, and down onto the anterior abdominal wall and they anastamoses with the deeper lymphatics of the breast especially in the regions of the nipple. These lymphatics drain only a small proportion of the breast's lymph but are of considerable importance in the spread of carcinoma.

The **supraclavicular lymph nodes** receive lymph from the breast indirectly after it has passed through the axillary and internal mammary drainage basins. There is little significant direct drainage from the breast to the supraclavicular nodes.

Lymphatics from the breast rarely enter the para-xiphisternal area unless the main pathways are obstructed but are not implicated as a pathway to the abdominal cavity. In late stages of the disease, deposits in the internal mammary nodes may lead to retrograde communication with the peritoneal lymphatics and similarly via the intercostals to the pleural cavity.

There is no significant drainage of lymph to the contralateral axilla or internal mammary chain under normal conditions. Contralateral deposits are occasionally seen in late stages of the disease when the ipsilateral drainage pathways have been obstructed by tumour or destroyed by surgery or radiotherapy.

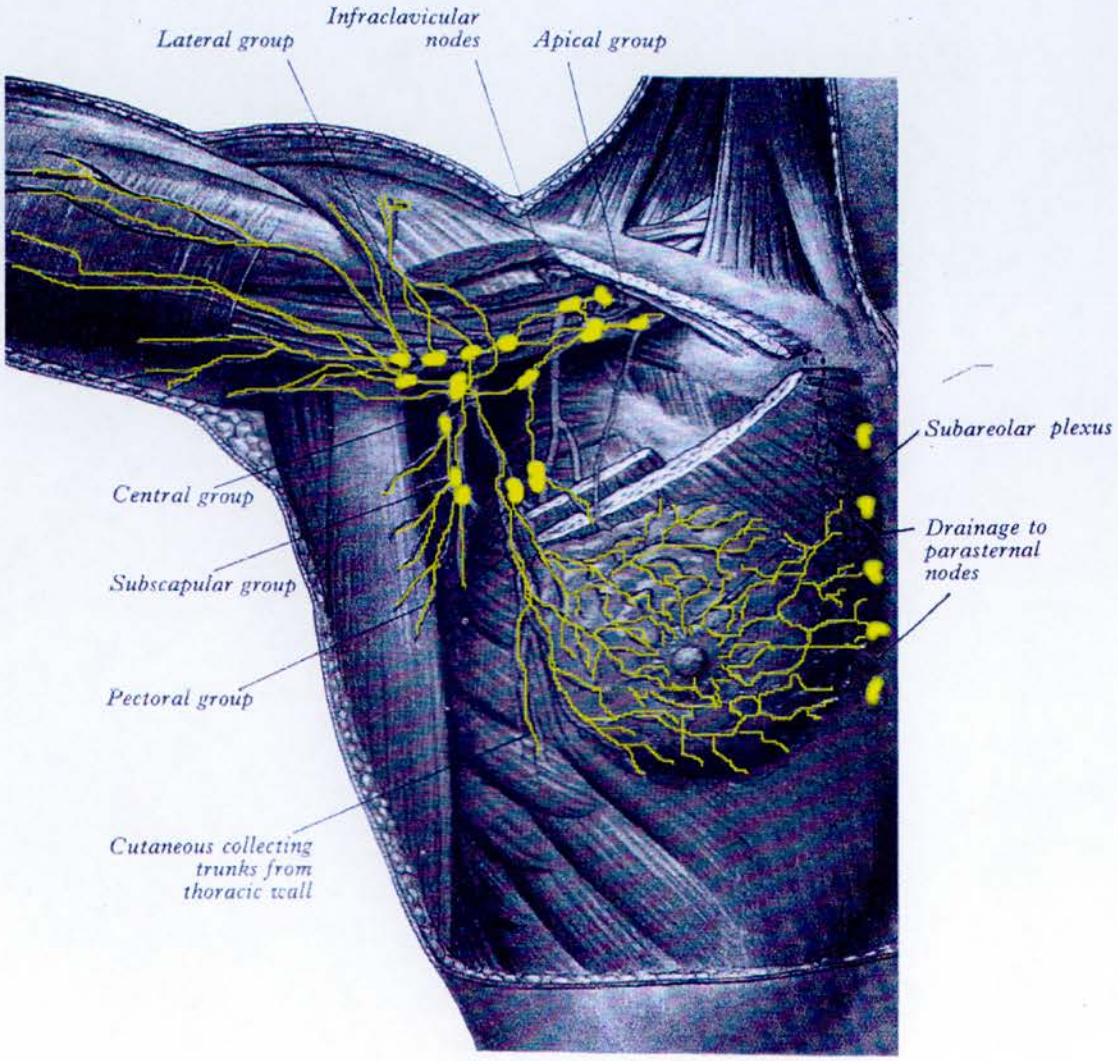
In summary, the breast drains mainly to the ipsilateral axilla, which receives 75% of the total lymph from the breast with the remainder draining into the internal mammary chain. As there are no valves in the intramammary lymphatics, lymph can pass freely across the breast from one half to another so both the internal mammary nodes and the axillary nodes can freely receive lymph from both lateral and medial halves of the breast. The posterior intercostal lymph nodes have been shown to receive lymph from the breast in a small proportion of patients but the great majority of patients have no drainage along this pathway at all unless the main pathways become blocked. A rich subareolar plexus of lymphatics does exist but it may not be

the main pathway of lymph drainage from the breast as some studies suggest and other pathways clearly exist.

There is little evidence to suggest a significant deep fascial or retromammary plexus of lymphatics so this is unlikely to be of importance in the early spread of breast cancer. Superficial lymphatics can cross the midline and this can allow lymph to pass to the opposite breast, to cervical nodes, to the peritoneal cavity and liver through the diaphragm or rectus sheath or even to the inguinal nodes via the anterior abdominal wall. This is unlikely to occur unless the usual pathways become blocked, as is sometimes the case especially in the later stages of carcinomas of the breast .

Figures 1.1 & 1.2 anatomical diagrams of breast and axilla

Figure 1.1 *Lymphatic drainage of the breast (taken from Gray's Anatomy 37th edition, p848, illustration **6.**207, Churchill-Livingston)*



iii. Surgical lymphatic anatomy

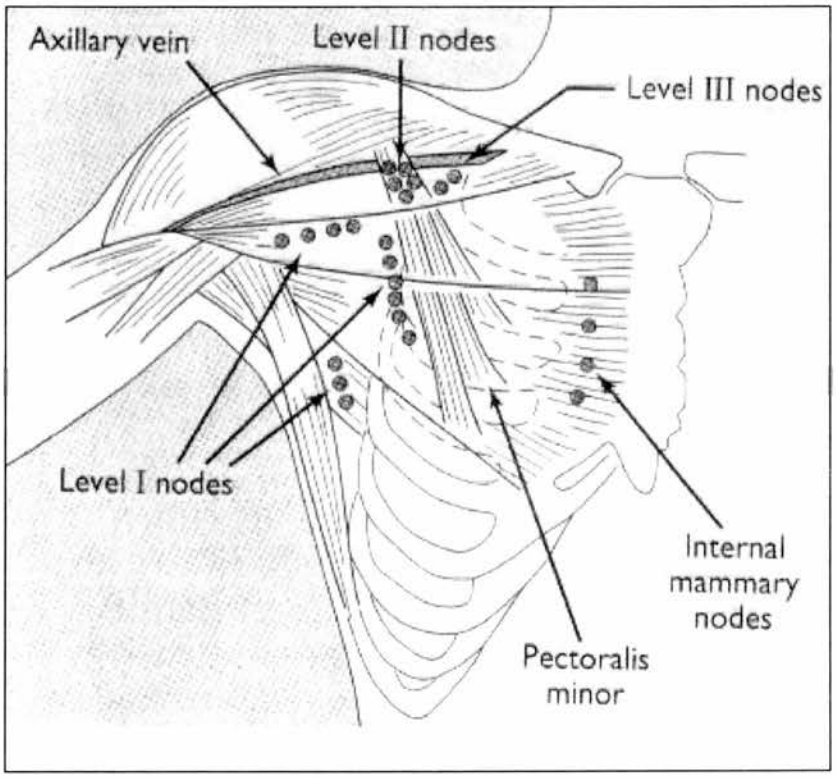
For surgical purposes, the axillary nodes are often divided into three levels according to their relation to the Pectoralis minor muscle:

Level I lymph nodes lie below and lateral to the **lateral** border of Pectoralis minor

Level II lymph nodes lie behind the Pectoralis minor muscle between its medial and lateral borders

Level III lymph nodes lie above and medial to the medial border of Pectoralis minor muscle usually up to the level of the 1st rib.

Figure 1.2 *Levels of axillary lymph nodes (taken from the ABC of Breast Diseases 2nd Edition Ch 8, Page 44 Figure 8.2, © BMJ Books 2000)*



1.3 BREAST PHYSIOLOGY

During the reproductive years, cyclical changes occur in the breast in response to oestrogen and progesterone levels.

Oestrogens cause proliferation of mammary ducts and are largely responsible for the breast enlargement seen at puberty in girls. They have been described as the growth hormones of the breast.

Progesterone stimulates the growth of breast lobules and alveoli and supports the secretory function of the breasts during lactation.

Prolactin is secreted by the anterior pituitary gland and has an important role in lactation of the breast. Its levels rise steadily throughout pregnancy until parturition when levels begin to fall again. It aids oestrogen and progesterone in the full lobulo-alveolar development of the breasts during pregnancy [11].

During the menopause, which usually occurs around 52 years of age, the breast substance undergoes involution in response to falling levels of oestrogen and progesterone. Involution leads to the replacement of breast stroma by fat causing the breast to become less dense, softer and ptotic. The glandular tissue of the breast also changes and may develop small cysts, areas of fibrosis or an increase in the number of glandular elements (adenosis) [12].

1.4 PATHOPHYSIOLOGY OF BREAST CANCER

1.4.1 Aetiology

Breast cancers arise from the epithelial cells that line the terminal duct lobular unit [12].

Three sets of influences appear to play a role in the aetiology of breast cancer. These are:

1. genetic factors
2. hormonal factors
3. environmental factors.

Genetic predisposition to breast cancer is only responsible for about 10% of breast cancers. Two breast cancer susceptibility genes have been located on chromosome 17q21 known as BRCA1 gene and chromosome 13q known as BRCA2 gene, which account for breast cancer in a substantial proportion of very high-risk families. Mutations of the p53 tumour suppressor gene and PTEN gene are implicated less frequently and are respectively associated with the Li-Fraumeni and Cowden's familial syndromes. Amplification of the c-erb-B2 oncogene is thought to be related to between 5-30% of cancers. In particular, its protein product p185erb, is associated with poor prognosis in node-positive breast cancer patients [12, 13].

A relative excess of the hormone oestrogen is clearly important in the development of some breast cancers. This may be endogenous in the case of early menarche, late menopause, nulliparity or late age at first pregnancy, which are all associated with an increased risk of breast cancer. Exogenous oestrogen, as in the case of hormone replacement therapy (HRT) and the oestrogen-containing oral contraceptive pills, is also a risk factor when prolonged exposure occurs. Normal breast epithelium possesses both oestrogen and progesterone receptors and these receptors are found on some but not all breast cancers in varying proportions. When present, they can be stimulated by oestrogen to accelerate tumour growth. Human breast cancer cells secrete a variety of growth promoters: transforming growth factor-alpha (TGF-α), epithelial growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast

growth factor (FGF). Studies suggest that they are involved in the autocrine regulation of tumour growth.

Environmental factors are suggested by the considerable variation in the incidence of breast cancer geographically. A high-fat diet and moderate to high alcohol consumption have been implicated as possible risk factors but evidence for these is neither strong nor consistent [14].

1.4.2 Histological type

Breast cancers are classified histologically under two main headings:

1. Invasive (infiltrating) ductal carcinoma of no special type (NST) or not otherwise specified (NOS) - 65-80% of breast cancers
2. Invasive carcinoma of special type – lobular, medullary, tubular, mucinous, apocrine, papillary – 20-35% of breast cancers

The invasive ductal carcinomas of no special type are graded I, II or III according to the frequency of mitoses and degrees of nuclear pleomorphism and glandular formation. These cancers tend to be of hard, 'scirrhous' consistency caused by the marked increase in dense, fibrous stromal tissue with frequent invasion of perivascular and perineural spaces as well as blood and lymphatic vessels.

Of the special types, invasive lobular carcinoma is the commonest accounting for 5-10% of breast cancers and is characteristically rubbery and poorly circumscribed. It is associated with a high percentage of bilateral breast involvement (20%) and multicentricity.

Some of the special type cancers are associated with a more favourable prognosis than other invasive cancers. Tubular carcinoma, mucinous and medullary are included in these.

1.4.3 Metastasis to lymph nodes

The preferred route of breast cancer spread is through the lymphatics to the regional lymph nodes. There are two methods by which this may occur and both are quite possible. The favoured theory is that cancer cells breaking off from the primary tumour are spread by embolism to the regional lymph nodes. The lymphatic channels, which run between the primary breast cancer and the lymph nodes, are usually free of carcinoma on histological examination giving weight to this theory[1]. The opposing belief is that the cancer spreads by permeation of the lymphatics as the tumour slowly grows along their walls. However, even some small, very early cancers can have associated lymph node metastases making the embolic theory far more likely in these patients when lymphatic permeation would not have had time to occur. It is most likely that both patterns of spread do occur although lymphatic permeation appears to be associated with advanced disease and emboli probably occur earlier as well as later in the disease process. The difficulty lies in predicting those patients who are most likely to metastasise

Several characteristics are associated with an increased risk of development of lymph node metastases. These may be conveniently divided into patient and tumour characteristics:

i. Patient characteristics and lymph node metastases

Age below 60yrs has a positive association with lymph node metastases in T1 tumours on univariate analysis[15]. Young age has also been found to be an independent predictor of lymph node metastases in patients with T1a and T1b breast cancer[16] on multivariate analysis. A Canadian study of 4,660 patients also reported pre-menopausal status and clinical palpability of lymph nodes as well as young age to be significantly associated with a higher incidence of positive lymph nodes [17]. The presence of overexpression of the oncogene c-erbB-2 has been reported to have a significant association with the number of involved axillary lymph nodes and has also been related to decreased overall patient survival [18]. Obesity is associated with an increased risk of breast cancer in post-menopausal women [19, 20] and is reportedly associated with poorer disease-free survival in breast cancer particularly

when combined with an elevated serum cholesterol [21]. Some studies have also reported an increase in the risk of lymph node metastases in obese women [22, 23].

ii. Tumour characteristics and lymph node metastases

Lymph node metastases are associated with increasing tumour size, presence of lymphovascular invasion, moderate or high nuclear grade, high S-phase fraction and multifocality or multicentricity on univariate analysis[15, 24]. Multivariate analysis states palpable axillary lymph nodes, presence of lymphovascular invasion, increasing tumour size and multifocality or multicentricity of the primary tumour as independent predictors of lymph node metastases [15, 24]. Oestrogen receptor status does not appear to be an independent predictor of lymph node metastases from reported studies [15, 17, 25-28]. The absence of oestrogen receptors (ER) does, however, appear to be associated with aggressive disease in that more lymph node metastases per patient are encountered and the rate of cell proliferation is higher within these metastases than in those with ER-positive tumours. In addition, the incidence of distant metastases is higher in ER-negative patients than in ER-positive patients [29].

Axillary lymph node metastases are more commonly associated with tumours of the upper outer quadrant (26%) than in other areas of the breast and similarly tumours of the lateral half of the breast have a 28% prevalence of lymph node metastases compared with 11.5% in the medial half of the breast [30]. The simple explanation for this is that there is less distance for the cancer to travel than from the medial half of the breast but this may also represent the different drainage patterns of the two halves of the breast. Medial tumours are more likely to drain into the internal mammary nodes (24.6% c.f 17.7% in lateral tumours) [31] making spread to the axilla relatively less likely. Medial tumours are more likely to be understaged because the internal mammary nodes are not routinely biopsied in the majority of breast units because use of the procedure does not reportedly improve disease-free or overall survival [31]. It has been suggested however, that a subgroup of patients with T1-2 tumours of the medial two quadrants and axillary node metastases may have a survival benefit from internal mammary biopsy [32].

Tumour angiogenesis has been proposed as an independent indicator of prognosis and axillary lymph node metastases [33, 34]. The presence of a high microvessel density within the primary breast tumour has also been reported to be strongly associated with relapse-free survival and overall survival [35]. Additional reports have also suggested neovascularisation and microvessel density within the lymph node metastases could also be associated with disease-free and overall survival[36]. There may be a role for tumour microvessel density in helping to select patients who would benefit from systemic adjuvant therapy[35, 37]. Evidence that tumour angiogenesis is a predictor of lymph node metastases is inconsistent however, with no firm evidence to confirm it as a useful marker of this [38-40].

There is little data reporting association of histological type of breast cancer with lymph node metastases. It has been reported however, that the risk of lymph node metastases is lower for tubular carcinoma than for other histological types [41, 42].

The presence of lymph node metastases in a breast cancer patient is internationally recognised as the most serious adverse prognostic indicator of long-term survival and is widely quoted in peer-reviewed journals [43-45]. Knowledge of lymph node involvement is critical in making decisions on whether to give regional radiotherapy and/or systemic adjuvant therapy.

1.4.4 Lymph node micrometastases

A lymph node micrometastasis (occult metastasis) is defined as a metastatic deposit of less than 2mm in diameter detected in a single lymph node after extensive review of the original histological material [46]. Such deposits are not normally appreciated during initial routine assessment of lymph nodes by light microscopy.

Lymph node micrometastases can be detected by a variety of processing methods. The simplest and most commonly used is to assess multiple levels of the same lymph node by serial sectioning of the node every 2-3mm as opposed to bisection of the node, which only examines it at two levels. Conventional haematoxylin and eosin (H&E) staining is used on each level. Detection of micrometastases in node-negative patients is reported as 7-17 per cent using this technique [47-49].

The use of immunohistochemistry to detect micrometastases in axillary lymph nodes has created much interest but currently is not used as a standard in the processing of lymph nodes histologically in the United Kingdom. The anticytokeratins AE-1, AE-3 and CAM 5.2 are reported to give a greater yield of micrometastases (11-20%) when compared with H&E staining methods [48, 50, 51]. This technique of processing lymph nodes, in particular sentinel lymph nodes, has become popular in the United States where some large cancer centres are now using immunohistochemistry routinely. Polymerase chain reaction (PCR) of extracted RNA has been used to detect micrometastases and has reported a yield of 28% in lymph node negative patients[52]. Similarly, another study using mammaglobin B gene transcript with a reverse transcription polymerase chain reaction (RT-PCR) assay revealed occult metastases in 31% of node negative patients [53]. The use of PCR and RT-PCR in detection of lymph node micrometastases is not yet validated, is still experimental and is largely unavailable in the majority of hospital laboratories, however. Specificity of these molecular biology techniques has also been questioned thus increasing the risk of false positive results. These findings give rise to the fact that detection of micrometastases is a function of probability in that the harder one looks, the more likely one is to find additional information in the form of micrometastases [54]. For example in the Ludwig International Study of micrometastases [55], the probability of detection of deposits between 100μm and 500μm was 6% if a single section was examined rising to 36% if six evenly spaced sections were examined [56].

The role of lymph node micrometastases in the natural progression of malignant breast disease is unclear. Reports of a significant decrease in disease-free and overall survival have been made from a relatively large, multicentre research group suggesting that micrometastases have a negative prognostic effect on breast cancer patients [57]. These findings are supported by a Japanese study of 129 node-negative patients using CEA-specific RT-PCR analysis of lymph nodes, which reported a significant difference in disease-free and overall survival in patients without micrometastases compared to patients with micrometastases [58]. These reports have been contested by other studies, which suggest no adverse prognostic association with lymph node micrometastases [46] and no independent association of the

presence of occult metastases with overall survival [59, 60]. There was, however a significant association between the presence and increasing size of occult metastases and poorer disease-free survival in one of the latter studies [59].

Closer examination of micrometastases suggests certain sub-groups of micrometastases may be more important. Those measuring less than 0.2mm were not associated with worse prognosis but micrometastases greater than 0.2mm were [47]. Similarly, in a study of 109 patients with micrometastases in sentinel lymph nodes who subsequently had complete axillary dissection, the frequency of non-sentinel lymph node metastases was significantly higher in patients with SLN micrometastases measuring greater than 1mm than in those with SLN micrometastases measuring 1mm or less [61]. Another study of micrometastases in sentinel lymph nodes reported the risk of lymph node metastases in non-sentinel lymph nodes following complete axillary dissection as being 15.2% [62]. This information could be important in making an accurate choice of patients for further axillary dissection after a staging sentinel node biopsy. A review paper of 8 studies with at least 5 years' follow-up concluded that occult metastases were independently predictive of survival in only one of these studies and that the size of the micro metastasis was important in the clinical outcome stating those of less than 0.5mm as small micrometastases with no proven prognostic relevance[63]. Another review of large studies concluded that most studies did show a statistically significant, negative effect of micrometastatic disease on disease-free and overall survival [64].

Histological type may also be important as two studies suggest with invasive lobular carcinoma micrometastases showing no adverse effect on prognosis whereas invasive ductal carcinoma (NOS) micrometastases was strongly associated with poorer recurrence rates and to a lesser extent survival [65, 66].

The proliferation rate and angiogenesis of micrometastases has been shown to be significantly lower than for macrometastases (those seen on routine light microscopy) [67]. Although only a small number of patients were studied, these results suggest that the two types of metastases are biologically different in their behaviour and may explain their differential growth patterns.

Logic would say that the presence of cancer cells in the lymph nodes is a bad sign and should create cause for concern. It is well known however, that breast cancer is

not a homogeneous entity but consists of a broad spectrum of vastly differing biological behavioural patterns. It would seem reasonable therefore, to accept the view that micrometastases should be managed not with the indiscriminate use of powerful adjuvant therapies and surgical procedures, but by following carefully constructed guidelines according to other tumour and patient characteristics. For example, patients with invasive ductal carcinoma (NOS) and with micrometastases measuring greater than 0.5mm could be considered as a higher risk group and treated more aggressively than those with invasive lobular carcinoma with small micrometastases of less than 0.5mm. At present there is no general consensus as to the optimum management of micrometastases but it appears that certain subgroups may have more prognostic significance than others and further studies are needed to increase our knowledge of this subject to produce surgico-pathological recommendations. Guidelines so produced need to take into consideration the labour involved and the corresponding patient benefit together with the resources available to the majority of breast cancer units.

1.5 AXILLARY SURGERY AND STAGING

Breast cancer cells spreading along the lymphatic channels from the primary breast tumour may affect axillary lymph nodes at any level in addition to the intramammary and internal mammary lymph nodes. The commonest site of spread is to the level I lymph nodes (lateral to the lateral border of Pectoralis minor muscle) which are affected alone in 58.2% of node-positive patients. Levels I and II are affected in 21.7% and levels I, II, and III are affected together in 16.3% of node-positive patients. In total this regular and progressive distribution occurs in 96.2% of node-positive patients. Skip metastases, in which the first level is not involved by disease but higher levels are involved, are found in 1.5% of cases. The first and second levels are skipped in 0.4% and the second level is skipped in 2.6% when nodes are involved in the first and third levels only [68]. Other studies report higher incidences of skip metastases [69]. The level of involved axillary nodes is not an independent predictor of overall and disease-free survival but the number of involved axillary lymph nodes appears to be of greatest importance in prediction of survival[70].

The question thus arises of which patients need axillary surgery and how much axillary dissection is necessary to provide staging information and control of axillary disease? Current practices vary extremely between centres and range from no axillary surgery to a complete level three axillary clearance [71-75]. The advantages of performing a full axillary node clearance are obvious, more accurate information is provided on axillary node status leading to better selection of adjuvant therapies, local control is excellent with failure rates of 0-2% and long-term disease-free survival is improved[76]. It is only, however, the node-positive group of patients who will benefit from such surgery and the remaining 60-70% of patients who are node-negative following axillary clearance will achieve no benefit from the surgery save the knowledge of a clear axilla. Haagensen showed that approximately 40% of all breast cancers have metastasised to the axilla at the time of diagnosis [1] leaving 60% of women node negative at presentation. The difficulty lies in the identification of node-negative and node-positive patients prior to surgical staging of the axilla. As a result, many studies have tried to identify those patients likely to benefit most from

axillary node clearance and those patients likely to be node-negative who may not require this operation. Options available to the breast cancer surgeon are many and include a level I axillary dissection[77], level I and II axillary dissection[78] and a level I-III axillary clearance[79]. Accurate axillary staging information is vital to enable the clinician to select appropriate patients for local and systemic adjuvant therapy.

1.5.1 Level I axillary dissection

The number of axillary lymph nodes excised as part of an axillary dissection has been related to disease-free survival. Removal of 10 or more lymph nodes is reported to give better disease-free survival than removal of less than 10 nodes [71]. Five-year survival increased from 75.7% to 86.2% in this study on patients with T1N0 breast cancer. This finding was also reported in a large Danish study which, found significantly better axillary recurrence-free survival, overall recurrence-free survival and survival in patients in whom 10 or more negative lymph nodes were removed [80]. Other studies have reported varying numbers of nodes as critical in conferring a survival advantage and are discussed below in the section 'axillary node sampling'. Excision of all axillary lymph nodes in level I is reported to provide reliable information as regards axillary node status. A study examining the efficacy of a level I axillary node dissection reported an average of 14 lymph nodes per patient at level I with a 3.5% incidence of 'skip' metastases to levels II and III without involvement of level I [81]. This study reports an accuracy of 94.5% in correct identification of node-negative patients and a false-negative rate of 8.7%. These figures are comparable to those reported for axillary node sampling as discussed below. Schwartz et al reported a mean of 27 nodes being excised per patient in a series of level I axillary dissections[77]. Node-negative patients were subjected to a complication rate of 13% with persistent axillary seroma formation occurring frequently in this series of 154 patients and node-positive patients were noticed to have a risk of late lymphoedema when treated by post-operative radiotherapy to treat higher echelon lymph nodes left inside the axilla.

Lloyd et al reported that a level I axillary dissection could detect more than 98% of patients with axillary metastases and the incidence of skip metastases was 3.2% [82]. This study also reported that there was a 45% probability of higher echelon lymph node metastases if metastases existed at level I. This finding is significant as it leaves 50% of node positive patients with an under treated axilla post-operatively leaving the unsatisfactory choice of a second axillary operation to clear the remaining axillary lymph nodes or axillary radiotherapy together with the risk of lymphoedema.

1.5.2 Axillary Dissection/Clearance to levels II and III

Level I and II axillary dissection is the preferred technique for the staging and treatment of the axilla in many breast cancer units. It is reported to have a low recurrence rate and limited morbidity[78, 83]. Morbidity from axillary clearance, is however, more frequent than in patients having axillary sampling or no axillary surgery[84]. Similarly, a higher frequency of late arm complications was reported in a series of over 800 patients receiving level III axillary clearance as opposed to level I and II axillary clearance and there was no significant difference in survival between the two groups of patients [85]. Arm morbidity thus appears to be more frequent (and severe), the more extensive the surgery to the axilla.

Evidence from a large American cancer database has shown that fewer patients had axillary node dissection in 1993 than in 1983 [86]. This suggests a trend towards more conservative management of the axilla. An American review of axillary dissection found that whilst randomised studies have shown improved loco-regional control of breast cancer with axillary clearance procedures, there has been no impact on overall survival and the main role of axillary dissection is limited to staging and prognostication [87]. A meta-analysis of six randomised controlled trials comparing standard treatment with axillary dissection to standard treatment without axillary dissection conflicted with these results concluding that axillary node dissection did confer a mean survival advantage of 5.4% [88]. It was noted however; that few of the patients in the six trials had T1a breast cancer and no patients were treated with adjuvant therapy unlike current practices suggesting the risk reduction may be less

apparent if adjuvant therapy had been given. The large NSABP B-06 trial comparing patients showed no difference in survival between node-positive patients treated by either radical mastectomy including axillary clearance or simple mastectomy without axillary dissection but with axillary radiotherapy [89]. Such contradictions have resulted in more careful selection of patient groups in particular with regard to tumour size in an attempt to rationalise these findings. In some centres there is a selective process identifying high-risk patients for axillary clearance and low-risk patients for an axilla-conserving operation such as axillary node sampling [90]. The prevalence of axillary node metastases falls to 12.1% in stage T1a and T1b breast cancer thus further questioning the need for complete nodal dissection in early breast cancer [91]. The introduction of breast cancer screening along with the increase in public awareness has led to earlier detection and hence more patients than ever before now fall into these groups.

1.5.3 Axillary node sample

There is good evidence from randomised trials to suggest that axillary node sampling is a reliable method of providing accurate information about the status of the axillary lymph nodes[92]. In the Edinburgh series, all 135 node-positive patients undergoing axillary node clearance had a positive axillary node sample in a 4-node procedure performed immediately before a level III axillary clearance. Results from randomised studies comparing a 4-node axillary node sample with a level III axillary clearance have provisionally reported no significant difference in survival or axillary recurrence between the two groups after median follow-up of 11 years and 4.1 years in patients treated by mastectomy or breast conservation respectively [79, 84]. Another study from Sweden reported a sensitivity of 97.3% with a five-node biopsy of the axilla in clinical stage T0-3,N0-1,M0 breast cancer suggesting an alternative to full axillary clearance in operable breast cancer as a staging method[93]. Radical mastectomy plus axillary node clearance was compared with simple mastectomy plus no axillary surgery if lymph nodes were not clinically palpable or local excision of nodes if they were, in patients with T1-2, N0-1, M0 breast cancer in

Capetown, South Africa. It was noted that there was an increase in axillary recurrences in the simple mastectomy group at 40 months leading to the termination of the trial. Long-term review of patients however, revealed no difference in recurrence at other sites, time to recurrence or survival between the two groups at 10 and 25 years [94].

A Danish study of over 3,000 patients reported that false negative staging of the axilla was related to the number of nodes examined histologically. The false negative was modest at 2% when at least three axillary lymph nodes were removed and found to be negative on histological examination. The false negative rate increased to 7% if only two nodes were excised and 12% if no lymph nodes were excised [95]. A follow up study by the same group found that the estimated five-year probability of developing axillary relapse was 3% whether 5-10 lymph nodes were removed or whether greater than 10 lymph nodes were removed from the lower axilla. As progressively fewer nodes were removed, there was a progressive increase in the estimated axillary relapse rates up to 19% if no nodes had been removed. Median follow-up was 6.5 years in this study [96].

1.5.4 Omission of axillary surgery

It has been suggested that certain patients, in particular those older patients with small, screen detected, impalpable breast cancers may not benefit from axillary dissection as such patients are at low-risk of axillary disease and their nodal status would not influence choice of adjuvant treatment[97, 98]. This theory is controversial and other research suggests that treating all patients with an axillary node dissection will prevent axillary recurrences and does not adversely affect arm morbidity in the absence of axillary radiotherapy [99]. Patients with small T1 breast cancers are less likely to have axillary metastases. Lymph node metastases were found in 3% of T1a cancers, 13% of T1b cancers and 25% of T1c cancers in an American study that found no difference in survival between patients who had an axillary node clearance compared with those who had no axillary surgery[73]. It was suggested that patients with T1a tumours did not need axillary surgery but those with

T1b and T1c should still receive axillary dissection because of the higher risk of axillary disease. These findings were supported by a similar study in which it was suggested that axillary dissection was only necessary in patients with a greater than 15% probability of having lymph node metastases and supported its use in T1b and T1c tumours as a staging procedure [100].

It is clear from the above amalgam of data that there is no clear consensus on how best to manage the axilla inoperable breast cancer. It is clear however, that patients with breast cancer, rather than a group suffering from the same illness, have become a heterogeneous population inflicted with a diverse spectrum of clinical conditions and who need compartmentalising into specific treatment groups.

1.5.5 alternative methods of staging the axilla

Clinical examination is not accurate in identification of axillary lymph node involvement [101]. Ultrasound of the axillary nodes has not been helpful in detection of metastases with a reported sensitivity similar to that for clinical examination in the region of 56-72%[102-104]. Even when used in combination clinical examination with ultrasound still misses axillary metastases in one-third of patients [102]. Colour Doppler has also been studied as a possible technique to improve the sensitivity of standard ultrasound in the detection of axillary nodal metastases but is only reported to detect 70% of involved axillary nodes [105].Axillary mammography has been unsuccessful in improving these figures and only has a reported sensitivity of 39% [104]. The use of magnetic resonance imaging (MRI) is reported to have a greater accuracy than either ultrasound or clinical examination with a sensitivity of 83% [106] but still compares unfavourably with lymph node biopsy whether by axillary dissection or axillary node sample in staging the axilla[92]. The use of ultrasound-guided fine needle aspiration of clinically non-palpable lymph nodes has also been attempted but failed to detect positive nodes in 37% of cases [107].

A number of other methods of staging the axilla have been examined. CT scanning of the axilla may have a role in defining the extent of disease in local and regional recurrence when used in combination with chest CT[108]. It's role in axillary staging , however, has been assessed as inadequate with a sensitivity of only 50% (similar to

clinical examination) and a negative predictive value of only 20% [109]. More recently, however, the use of thin section CT has reported improvement on these results with a sensitivity of 93.8% for involved lymph nodes but with a specificity of 82.1% runs the risk of a false positive result [110]. Positron emission tomography (PET) scanning of axillary nodes is a relatively new technique, undergoing evaluation in detection of lymph node metastases but the results reported are not favourable when compared with the accuracy of sentinel node biopsy either in cases of breast cancer or malignant melanoma [111-114]. It has a sensitivity of about 79% in detection of axillary lymph node metastases overall, which increases to 94% in primary tumours of greater than 2cm in diameter but unfortunately drops to only 33% in T1 tumours [115].

1.6 AXILLARY RADIOTHERAPY

The use of radiotherapy as adjuvant treatment following a complete lymph node clearance of the axilla is no longer considered appropriate in view of the high incidence of lymphoedema (38.3%) in patients treated in this way [116]. Instead axillary radiotherapy is commonly used in two clinical settings nowadays. Firstly to treat node-positive patients who have had less than a complete axillary clearance and in whom further positive axillary nodes possibly persist within the axilla. Secondly as a complete treatment to the axilla in patients who do not undergo surgical staging of the axilla.

The NSABP-06 study in America found no difference in the disease-free survival, distant disease-free survival and overall survival after ten years' follow-up of a large group of clinically node-negative patients with invasive breast carcinoma treated by either radical mastectomy, simple mastectomy plus axillary radiotherapy or simple mastectomy alone plus delayed axillary node clearance only if positive nodes were subsequently found clinically [89]. Similarly, this same study observed no difference in disease-free or overall survival in clinically node-positive patients whether treated by radical mastectomy or simple mastectomy plus axillary radiotherapy. This study suggested that complete axillary dissection was no longer the only method of treating the axillary lymph nodes but that axillary radiotherapy was an acceptable alternative with equal results and thus spared the patient more extensive surgery. Since this trial was performed in the 1970s, there has been a gradual trend towards the more conservative approaches to stage and treat the axilla. The technique of axillary node sampling to avoid the morbidity associated with axillary clearance was introduced [92]. In a randomised trial, patients with no disease in four sampled axillary nodes received no further treatment to the axilla and those with positive nodes received axillary radiotherapy. Outcomes were compared with those of standard level III axillary clearance without axillary radiotherapy in patients treated by simple mastectomy and no significant difference was observed in overall survival or loco-regional recurrence [79]. A similar trial in patients treated by breast conservation

came to the same conclusions and recommended a selective policy in approaching the management of the axilla[84].

Subsequent trials have tried to identify patients who would most benefit from axillary radiotherapy as opposed to an axillary clearance. Zurrida and Veronesi recently reported results from a trial in Milan, which found no significant difference in overall survival or axillary recurrence in clinically node-negative patients with small cancers < 1.2cm randomised to receive no axillary treatment or axillary radiotherapy after breast conservation [117]. Hoebers specifically targeted postmenopausal women with node-negative breast cancer and treated them with adjuvant breast, axillary and supraclavicular fossa radiotherapy after breast-conserving surgery and reported no isolated axillary recurrences after 5 years' follow-up although two patients developed synchronous axillary recurrence and distant metastases [118]. These studies suggest the use of axillary radiotherapy in selected patient groups may negate the need for axillary surgery.

Axillary radiotherapy is itself, not without risk. The potential long-term risks of its use include lymphoedema, brachial plexopathy, radiation pneumonitis, rib fractures, cardiac toxicity, and radiation-induced second neoplasms [119]. Most of these side-effects are relatively infrequent with the exception of lymphoedema which occurs in approximately 4% of patients after radiotherapy alone [41].

A study examining extracapsular extension of lymph node metastases as a cause for axillary failure, found no benefit from giving axillary radiotherapy to patients with node-positive breast cancer after a level I-II +/-III node clearance whether or not they had extracapsular extension and concluded that extracapsular extension did not lead to an increase in axillary failure rates but was probably associated with poorer overall and disease-free survival [120]. These patients appear adequately treated by axillary clearance and may represent one group in whom radiotherapy is not necessary.

The American Society of Clinical Oncology has recently recommended not to use axillary radiotherapy in patients having a complete (level I-III) axillary clearance or a level I-II axillary dissection in view of the risk of lymphoedema and the adequacy of the surgical procedure in treatment of the axilla[119].

1.7 ARM MORBIDITY

Arm morbidity is commoner the more extensive the surgery and includes: cellulitis, seroma, lymphoedema, shoulder joint stiffness, arm pain and altered sensation [121-124] particularly of the axillary skin and the skin of the medial upper arm supplied by the intercostobrachial nerve (the lateral cutaneous branch of the second intercostal nerve[125]) which some surgeons sacrifice during a routine axillary node clearance. In addition to these physical arm side-effects, patients suffer from psychosocial maladjustment and increased psychological morbidity [126]. Consequently, if these complications can be avoided or minimised then we should endeavour to find a safe and reliable method of doing so.

1.7.1 Lymphoedema

Lymphoedema is the accumulation of protein-rich fluid in the interstitial spaces resulting in an abnormal enlargement of the affected part [127]. There is little literature defining the specific increase in arm volume necessary for lymphoedema to be present with studies using 100mls [122] and 200mls [116, 128] increase in ipsilateral arm volume as well as percentage increase in ipsilateral arm volume compared with the premorbid arm volume as cut-offs [84]. It is important to recognise that arm volume can have significant variation when compared with the contralateral arm of up to 10% difference between the two arms in normal individuals. This has been noted by the author from pre-operative arm assessments made in phase 2 of the ALMANAC study (chapter 4) but is a well-recognised entity. Assessment of arm swelling and lymphoedema must therefore allow for this by documenting premorbid arm measurements. Several methods have been used to objectively measure lymphoedema including arm circumference 15cm above and 10cm below the olecranon [84, 116, 121], arm volume by water displacement [84, 116, 128] and arm volume by circumferential measurements along the limb usually every 4cm with subsequent volume calculation using equations for the volume of a

cylinder or frustrum (truncated cone) [129]. Altered circumference of the arm provides a crude measure and limb volume is now the accepted standard. The water displacement method is highly reproducible but is messy and can be logistically impractical and arm volumes from circumferential measurements show good correlation with water displacement data in repeated series [129-132]. In Edinburgh Breast Unit the lymphoedema therapists request that any patient with a perceived increase in arm volume, i.e. any subjective increase in volume, be referred for assessment in addition to patients with a measured increase in ipsilateral arm volume of 10-20% compared with the premorbid measurement.

The incidence of lymphoedema of the arm is reported at between 2 per cent and 62.5 per cent [129]. These figures represent data collected on patients over the last century and whom varying combinations of surgery and radiotherapy to the breast and axilla has treated. Kissin et al studied 200 patients and found that the objective incidence of lymphoedema was 25.5 per cent in all groups. The incidence of subjective late lymphoedema varied according to treatment received. After radiotherapy alone the incidence was 8.3 per cent rising to 9.1 per cent following axillary sampling plus radiotherapy. Axillary clearance alone revealed an incidence of 7.4 per cent but when patients had axillary clearance followed by radiotherapy, the incidence rose to 38.3 per cent. He concluded that radiotherapy should be avoided in axillary clearance patients [116]. A more recent series of 121 patients treated by breast conservation compared the rates of lymphoedema and other arm morbidities between groups of patients according to the extent of axillary surgery [128]. Results showed that increases in arm circumference were significantly greater in patients having more extensive axillary surgery when comparing axillary dissection to levels II and III with axillary sampling. The lymphoedema rate in this study was 32 per cent. Findings also confirmed those of Kissin et al reporting substantially higher rates of arm swelling in patients having axillary dissection to level II followed by radiotherapy. Patients having an axillary node clearance to level III did not have radiotherapy to the axilla in this study. Both of these studies used a figure of 200mls increase in arm volume as their definition of lymphoedema.

In another study, there was no difference in lymphoedema rates whether or not patients had post-operative radiotherapy [133] but this conflicts with most other reports [116, 123, 129, 134, 135] .

A number of other reasons have been given as contributors to lymphoedema and these include positive axillary nodes, tumour size and grade, pre-operative arm volume and body mass index

Lymphoedema causes considerable distress to the patient and is a common fear amongst pre-operative patients who often ask "Will my arm swell up, doctor?" during their pre-surgical consultation. It can significantly impair a patient's quality of life with 37.5% complaining of pain in the affected limb whilst choice of clothing, sleep, ability to perform household activities or sports and problems with employment may also contribute [136].

The treatment of lymphoedema falls into three main groups: conservative measures, drug treatments and surgery. Conservative measures include massage, scrupulous attention to personal hygiene to avoid skin and subcutaneous infection, compression bandaging and dynamic muscle exercises. These comprise the mainstay of treatment for lymphoedema.

The use of drugs in lymphoedema focuses on attempts to encourage breakdown of proteins deposited within a brawny limb. The benzopyrone group of agents, including coumarin, is thought to activate proteolysis by macrophages and to induce an increase in total macrophage numbers. Administration may be topical or orally in tablet form. The topical form of the drug is of limited use as it penetrates to a maximum depth of 1-2cm.

There are concerns that long-term use may lead to hepatotoxicity and 2-5% of patients experience mild gastrointestinal upset. Autologous lymphocyte infusion has been used and stimulates cytokine release by lymphocytes, which subsequently activate macrophage proteinases. Such pharmacological therapy should be limited to those patients in which conservative measures have failed. There is no place for the use of diuretics in lymphoedema.

Surgery for lymphoedema can be divided into two groups, debulking procedures and those that aim to improve lymphatic flow. Debulking operations involving radical excision of large portions of tissue have met with little success although a recent

study using liposuction has given promising initial results with an average reduction in limb volume of 106% after twelve months [137]. A variety of procedures have been used in an attempt to improve the lymphatic drainage of the affected limb including rubber tubes, nylon thread, tube pedicles to carry fluid to the chest wall, buried dermis flaps to encourage lymphatic drainage via the deep channels and microsurgical lymphaticovenous anastamoses. The results have been mixed although there has been some success with the microsurgical techniques. However, as with drug treatments surgery is really only used in extreme cases resistant to conservative management [129].

Evidence suggests that lymphoedema rates are falling with less aggressive modern approaches to treatment [138] and this supports the introduction of more conservative surgical techniques in selected patients.

1.7.2 Arm pain and altered sensation (neuropathy)

Both axillary surgery and axillary radiotherapy run the risk of damage to nerves located within or crossing the axilla. Whilst all surgeons will endeavour to avoid damage to the important nerves of the brachial plexus and the long thoracic and thoracodorsal motor nerves, there is occasional difficulty in the preservation of the sensory intercostobrachial nerve and lower intercostobrachial nerves during axillary operations. A study in Edinburgh showed that a significantly higher frequency of numbness was seen in patients whose intercostobrachial nerve was divided during an axillary clearance than in patients having an axillary sample or an axillary clearance in which the intercostobrachial nerve was preserved [139]. Patients having a node sample had significantly less numbness than those having a clearance even when the nerve was preserved. In addition, significantly fewer patients experienced moderate or severe pain if their nerve was preserved rather than divided during an axillary clearance. These findings suggest that careful preservation of the nerve limits the morbidity of an axillary clearance procedure and that axillary sampling causes less damage to the nerve than axillary clearance. Ivens confirmed the presence of significant arm morbidity six months-2 years following an axillary clearance reporting subjective numbness of the ipsilateral arm as the commonest complaint in

70% of patients with pain 33%, weakness 25%, limb swelling 24% and arm stiffness 15% as the other side-effects in descending order of frequency. Objective assessment confirmed decreased sensation in 81%, weakness in 27% arm swelling in 10% and arm stiffness in 10% [140]. Similar results are reported in a longer-term study 2-5 years post axillary dissection. Warmuth et al reported a prevalence of 35% for numbness, 30% for pain, arm swelling in 15% and restricted arm movement in 8% [141]. Younger age was associated with significantly higher incidence of numbness, pain and arm swelling. Arm swelling was also commoner in patients with a greater body surface area and numbness commoner in smokers in this latter study. These studies reveal that a significant proportion of patients experience pain and numbness following axillary node dissection. These symptoms whilst mostly considered mild, may contribute to psychological distress[142]. Numbness and pain in the upper arm and axilla are most likely related to nerve damage and to the extent of axillary dissection. Performing a more conservative procedure may help to limit these side effects.

1.7.3 Restricted arm movement (shoulder joint stiffness)

Both surgery and radiotherapy are associated with a reduction in the range of movement of the shoulder and about 5% develop a frozen shoulder [143]. Radiotherapy following axillary surgery appears to increase the risk of shoulder stiffness both subjectively and objectively[84, 121]. A significant reduction in shoulder stiffness is seen when surgery is performed without post-operative radiotherapy suggesting radiotherapy is responsible for the morbidity here. Other studies have reported incidence of shoulder stiffness as 12-21% after an axillary node dissection without radiotherapy [144, 145], suggesting significant morbidity from the surgery alone. The incidence of shoulder stiffness after a trial using axillary radiotherapy alone to treat node-negative postmenopausal patients was reported as 35% subjectively and 17% when objectively measured [118]. From these studies it is clear that restricted arm movement can occur with either surgery or radiotherapy

used independently and the use of the two treatment modalities in combination appears to lead to a magnified risk compared with their independent use. Thompson et al reported reduction in upper limb mobility in all four groups of patients treated by breast conservation whether they received axillary node sample, axillary node clearance, axillary sample plus radiotherapy or level II axillary node dissection plus radiotherapy but it was most pronounced in patients treated by level II axillary dissection plus radiotherapy [128]. It was concluded that the incidence of shoulder stiffness is related to the extent of axillary surgery and radiotherapy with significant reductions in mobility seen when surgery and radiotherapy are combined. The author recommended that level II axillary dissection plus subsequent radiotherapy should no longer be performed.

1.8 SENTINEL NODE BIOPSY

1.8.1 Background

The technique of sentinel node biopsy as a means of staging the axilla in breast cancer is a highly topical and exciting concept. The 'sentinel node' concept was described as far back as 1960 when Ernest A. Gould published a series of 28 total parotidectomies during which; a specific lymph node ('sentinel node') at the junction between the anterior and posterior facial veins was excised and sent for frozen section histology. If the node was shown to be positive, a radical neck dissection was carried out. If the node was negative, no further surgery was performed. Follow-up of five patients who had malignant parotid tumours and a negative sentinel node revealed no evidence of recurrence or metastasis [146].

Ramon Cabanas described the sentinel node as a specific lymph node centre to which cancer cells from a tumour would spread to first. He used the technique of lymphatic mapping in penile carcinoma between 1965 and 1973 by injecting contrast material into the dorsal lymphatics of the penis or the dorsum of the foot to follow the drainage of contrast to the inguinal lymph nodes. It was observed that there was a fairly constantly located node to which the tumour would always drain first. It was located in the superficial epigastric group of nodes within the inguinal region close to the superficial epigastric vein. There were lymph node metastases in 33% of patients in whom sentinel node biopsy was performed and in 80 per cent of these the sentinel node was the only node to contain metastatic disease. In no patients was the sentinel node biopsy negative when other nodes were found to be positive. Cabanas concluded that anatomically, clinically and pathologically the sentinel node was the first site of metastasis and may be the only node involved. He recommended bilateral sentinel lymph node biopsy followed by inguinal lymph node dissection for positive cases. If the sentinel node is negative, he recommended no further surgery in the initial management of the primary cancer [147]. Cabanas' study was criticised for its small patient numbers and for the loss to follow-up of many of his patients. Subsequent use of the technique in penile carcinoma as recommended above led to

early regional recurrence in some cases [148] and the technique was not thought to be reliable.

The next person to use the technique was Donald Morton from the John Wayne Cancer Institute in Santa Monica, California. He presented a paper detailing the use of patent blue V or isosulfan blue to identify the sentinel lymph node in 223 patients with clinical stage I malignant melanoma. After induction of anaesthesia, 0.5-1ml of the dye was injected intradermally with a 25-gauge needle at the site of the melanoma. The injections were repeated every 20 minutes during the procedure. Repeated injections were thought to keep the blue dye flowing into the first echelon sentinel node(s) and avoid errors in the identification of sentinel nodes. It was felt that if sentinel nodes were not identified quickly then blue dye would pass into higher echelon nodes leaving the lower ones only faintly stained as the dye filtered through leaving higher nodes more deeply stained and mistaken for true sentinel nodes. The injection site was gently massaged to promote the passage of dye along the lymphatics. An incision was then made over the regional lymph node basin and the lymphatic channel from the primary site was dissected carefully away from the subcutaneous fatty tissue of the lymph node basin to the draining lymph node(s) which stained blue. In this way the sentinel lymph node was distinguished from other lymph nodes of the lymphatic basin. The surrounding area was then examined for additional blue nodes. Sentinel nodes were sent separately for frozen section and routine haematoxylin and eosin histological examination. Lymph nodes were also stained using immunohistochemical techniques using antibodies to S100 protein and the melanoma-reactive monoclonal antibody NKI/C3. Patients went on to have excision of their primary melanoma following this and completion of the regional lymph node dissection. The incidence of metastases in the sentinel nodes and other lymph nodes was measured. Morton identified a sentinel node in 82% of cases and 18% (47) of these contained metastases. He claimed a false negative rate of 1% with 2 positive cases identified from other nodes when the sentinel nodes were negative. However, his results actually showed a false negative of 4%. He concluded that the technique could accurately identify patients with nodal metastases and stage I melanoma who would benefit from radical lymph node dissection [149].

The technique was first used in breast cancer by Armando Giuliano also at the John Wayne Cancer Institute with the help of his colleague Donald Morton. He used a similar technique of injecting isosulfan blue dye in a volume of 3-5 mls directly into the breast mass and surrounding breast parenchyma. Injection was performed after induction of anaesthesia approximately five minutes before axillary incision. Sentinel nodes were identified in 65.5% of procedures. Nodal status was accurately assessed in 109 of 114 cases (95.6%) in which the sentinel node was identified. The false negative rate was claimed to be 4.3% in this series. This percentage was actually the failure rate of the technique's prediction of axillary status however, the false negative rate (i.e no of false negatives/no of true positives plus false negatives) being 11.9% (5/42).

The rate of axillary metastases detected in sentinel nodes was 32.4% (37/114) compared with 35.6% of cases in total suggesting the techniques accuracy is close to that of a complete axillary dissection. Giuliano performed an interesting analysis to determine whether the uptake of blue dye by lymph nodes containing metastases was random by examining all the nodes of 14 cases in which only one positive lymph node and at least one sentinel node were identified. Of the 285 lymph nodes examined, 18 were sentinel and 13 of these (72.2%) contained metastases. Only one of 267 non-sentinel lymph nodes contained metastases making it highly statistically unlikely that these findings were by chance alone. Giuliano demonstrated the presence of a learning curve for the clinician performing this technique with increasing identification of sentinel nodes as experience is gained. This is an important consideration for new surgeons learning the technique. These findings were supported by a similar study by Guenther et al [150].

In a follow-up study, Giuliano's identification rate of sentinel nodes increased to 93.5% with a 0% false negative rate [151]

Giuliano went on to perform another study this time looking at histological methods of increasing the accuracy of axillary staging with SNB. He used serial sectioning of lymph nodes and immunohistochemistry with antibodies to cytokeratin to examine sentinel nodes and found a highly significant difference in the number of micrometastases identified in sentinel nodes as opposed to non-sentinel nodes [152]. These results however, are significantly flawed in that Giuliano uses a completely

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different technique to examine the non-sentinel lymph nodes than he does to examine sentinel nodes using only routine H&E staining of fewer levels of the lymph node as opposed to immunohistochemistry and H&E staining in multiple sections of sentinel nodes. He also fails to report his false negative SNB cases in this study excluding them from his results.

Krag and Alex first studied the use of a radioisotope in cats to identify sentinel lymph node locations pre-operatively with a gamma-detection probe and reported results comparable to those using blue dye [153]. They went on to use the technique in pilot studies in both malignant melanoma and breast cancer confirming its potential for use as a mapping agent in humans[154, 155].

Reynolds et al studied the relationship of positive non-sentinel nodes to clinicopathological features to identify which patients were most at risk of further axillary node involvement following a positive sentinel node biopsy. Primary tumour size and the size of sentinel node metastases were found to be significant predictors of further axillary metastases in non-sentinel lymph nodes. From this information the investigators proposed that completion axillary node dissection may not be necessary in women with primary breast tumours 2cm or smaller and micrometastases rather than macrometastases in the sentinel node [156].

Table 1.1 overleaf shows results of sentinel node trials by several investigators.

Table 1.1 Sentinel node biopsy trials

Investigator	No of cases	Mapping agent	Volume	Injection technique	SN identification rate	Mean number of SNs	False negative rate	Primary cancer	Accuracy in predicting nodal status
Cabanas 1977 [147]}.	46	Radio-opaque Contrast **Single agent**	N/K	Dorsal lymphatics of penis	100%	Not known	0%	Penile	100%
Morton 1992 [149]}.	237	Patent blue V or isosulfan blue single agent	0.5-1ml repeated every 20 mins.	intradermal	82%	Not known	4%	CS-1 malignant melanoma	99%
Giuliano 1994 [157]}.	174	Isosulfan blue **Single agent**	3-5mls	Intratumoural and peritumoural	65.5%	Not known	11.9%	Tis-T3 breast cancer	95.6%
Van der Veen 1994 [158]}.	11	Technetium-labelled nanocolloid plus Patent blue V **Combined**	0.1-0.2mls nanocolloid 0.1-0.2mls patent blue	Intradermal	100%	Not known	N/A	Melanoma CS-1 1.35-32.mm depth	N/A
Pijpers 1995 [159]}.	41	Technetium-labelled albumin plus Patent blue V **Combined**	0.15mls of each tracer	Intradermal	100%	2.1	N/A	Melanoma 0.65-6.5mm depth	N/A
Albertini 1996 [160]}.	62	Isosulfan blue **combined** with filtered technetium-labelled sulphur colloid	Not known	Peritumoural	92%	2.2	0%	T1-3 Breast cancer	100%
Veronesi 1997 [161]].	163	**Single agent** Radioisotope Technetium-labelled microcolloidal albumin	0.2mls	Subdermal above breast mass followed by 0.2mls saline injection	98%	1.4	4.7%	T1-3 breast cancer	97.5%
Meijer 1996[162]].	30	**Single agent** Radioisotope Technetium-labelled albumin		peritumoural	100%		0%	T1-2 breast cancer	100%

Table 1.1 continued, Sentinel node biopsy trials

Investigator	No of cases	Mapping agent	Volume	Injection technique	SN identification rate	Mean number of SNs	False negative rate	Primary cancer	Accuracy in predicting nodal status
Giuliano 1997[151]	107	**Single agent** Isosulfan blue dye	3-5mls	peritumoural	93.5%	1.8	0%	T1-3 breast cancer	100%
Guenther 1997[150]	145	**Single agent** Isosulfan blue dye	3-5mls	peritumoural	71%	N/A	9.7%	T1-4 breast cancer	97.1%
Krag 1998 [163] Multicentre trial	443	**Single agent** Technetium-labelled sulfur colloid	4mls	peritumoural	91.4%	2.6	11.4%	T1-2 breast cancer	97%
Cox 1998[164]	466	**Combined** Isosulfan blue plus technetium-labelled sulfur colloid	5mls + 6mls	peritumoural	94.4%	1.9	1%	Tis-T3 breast cancer	N/A
Reynolds 1999 [156] Dual centre study using two techniques a) and b)	222	**a) Single agent** Isosulfan blue **b) combined** Technetium-labelled sulfur colloid or human albumin plus isosulfan blue	4-5mls 6mls 4-6mls	peritumoural peritumoural	97.1% 98.3%	2.5	Not given	T1-3 breast cancer	97.3%
Hill 1999 [165]	492	**Combined** Isosulfan blue plus technetium-labelled sulfur colloid	4mls	Peritumoural and intradermal	92%	2.1	10.6%	T1-3 breast cancer	
De Cicco 1998 [166]	250	**Single agent** Using 3 different-sized colloids for comparison. 40 cases used **combined** mapping with blue dye also	0.4mls	Peritumoural or subdermal	96%			Operable breast cancer	97.5%
Van der Ent 1999[167]	70	**Combined** Tc^{99m}-nanocolloid plus patent blue V	4mls 0.5-0.8mls	Peritumoural Intradermal	100%	2.6	4%	T1-3 breast cancer	98.6%

Another method of identifying sentinel nodes was introduced by van der Veen in 1994 using radioisotope and lymphoscintigraphy preoperatively to identify lymphatic drainage patterns in stage I melanoma patients followed by blue dye injection after induction of anaesthesia [158]. The use of isotope and patent blue V dye in combination increased identification of sentinel nodes to 100% although only a small number of patients were examined in this study. Pijpers used a similar technique and achieved a SN identification rate of 100% in a series of 41 melanoma patients when dynamic imaging was used following intracutaneous injection of technetium-99m-labelled albumin. 93% of patients were blue dye-positive when identified. The interesting feature of this paper was the use of dynamic lymphoscintigraphy. The first draining lymph node was assumed to be the true sentinel node and 95% of sentinel nodes were seen in the first 20 minutes of imaging. Dynamic imaging allowed differentiation between spill and multiple sentinel nodes and also revealed that the initial focus retained the highest fraction of radioactivity for at least 18 hours[159].

John Albertini from the Lee Moffitt cancer centre in Florida, first reported the technique of combined lymphatic mapping in breast cancer. He suggested the techniques were complementary and achieved a greater SN identification rate when used together. He defined a sentinel node as any node staining blue and/or any node with a radioactivity count greater than ten times that of a neighbouring non-sentinel node (activity ratio greater than 10). The neighbouring non-sentinel lymph node was removed as a control for background nodal radioactivity and radioactivity was measured after excision (ex-vivo). Further lymph nodes were sought if the gamma probe counted residual axillary radiation greater than 150% of the background count. An estimate of the background count was made by taking measurements from four areas in the axilla equidistant from the injection site and away from the SN [160].

1.8.2 Injection technique in breast cancer

i. Site of injection

A variety of techniques have been employed for injection of lymphatic mapping agents into the breast tissue including intra-tumoural, peritumoural and periareolar, subdermal and intradermal.

The intra-tumoural method used by Giuliano in his early studies[157] has since fallen out of favour. The puncture of tumour tissue percutaneously created concern that seeding of metastases along the needle track was a possible risk with this technique. Injection of dye into the tumour itself was also thought to lead to leakage of dye into the peritumoural breast tissue because of the high interstitial and intercellular pressures, which exist within tumours. This led to the development of the peritumoural method of injection now commonly preferred. This method involves injection of mapping substances at several points around the tumour in its immediate vicinity in order to be absorbed by the lymphatics, which drain the tumour. Good results are reported using this technique with consistent sentinel node detection rates of between 91-100% in the majority of studies [156, 160, 162-164, 168]

The presence of a rich layer of lymphatic vessels in the subdermal plexus of the skin is well described in the literature and this is the rationale for using a subdermal injection of mapping agent directly above the tumour. Veronesi et al who reported a sentinel node identification rate of 98% using a single mapping agent successfully employed this method of injection [161]. The periareolar injection follows a similar principle to the subdermal injection by using the subdermal plexus of lymphatics as a mapping pathway. This technique was compared to the peritumoural method in an experiment using combined lymphatic mapping using blue dye and a technetium 99m-labelled colloid. Results showed a higher rate of identification of sentinel nodes (100%) using the periareolar method compared with the peritumoural method [169]. Borgstein et al compared periareolar intradermal injection of blue dye with intradermal injection over the site of the primary tumour in patients having peritumoural mapping with radiocolloid to verify whether the site of injection of blue dye was related to the peritumoural localisation of sentinel nodes. They hypothesised

that the skin envelope and the underlying glandular tissue of the breast share a common lymphatic pathway to the same draining (i.e sentinel) axillary nodes. Similarly, the circumareolar lymphatic plexus, on the external surface of the gland substance, anastamoses with the subdermal lymphatic plexus of the overlying skin. The results of the study showed that a sentinel node was identified in 96% of patients and that 94% of sentinel nodes were localised simultaneously by both agents regardless of the location of blue dye injection. The sentinel nodes were consistently located in the same position in the lower axilla, almost without exception. They reported that there was no concordance between site of tumour, injection site and sentinel node targeting. They concluded that intradermal injection improves the efficacy of blue dye because transport by skin lymphatics is more rapid and reliable than in the breast parenchyma.

Borgstein argues that parenchymal lymph vessels accompany the lactiferous ducts centripetally to empty into the dense subareolar plexus of Sappey where pooling of lymph from all parts of the breast occurs. Two enormous lymph trunks (vasa lymphatica mammaria magna) leave the areolar region to course superficially towards the lower axillary lymph nodes thus the subareolar plexus conveys lymph produced by the whole breast towards the first lymph node filter of the axilla [170]. Van der Ent reported similar success using the intradermal technique following relatively poorer blue dye mapping with the peritumoural technique [167]. Although there is rationale supporting each technique, there is no conclusive evidence to support the use of one method over another in terms of success in sentinel node identification. However, the periareolar and subdermal techniques suggest a slight improvement in sentinel node identification rates possibly because of the improved transit of mapping agents along the subdermal plexus to the axillary lymph nodes. The only method that appears to be limited is the intratumoural method for the reasons described above and as such is probably best avoided.

ii. Timing of injection

There have been a number of studies, which have included data regarding the timing of injection of mapping agents and timing of lymphoscintigraphy. These figures are represented in table 1.2 below:

Table 1.2 Timing of injection relative to sentinel node identification

Investigator	Isotope timing	Blue dye timing	Scan timing	SN identification rate
Veronesi 1997 [161]	Day before surgery 0.2mls sub-dermally	Not used	15,30mins and 3h post injection	98%
Krag 1998 [163]	2.9h pre-op	Not used	Not scanned	91.4%
Hill 1999 [165]	Morning of surgery 2-4hrs pre-op	5-10 minutes before axillary incision	50-60 minutes post-injection	93%
Reynolds 1999 [156]	2hrs pre-op	In theatre	Dynamic scanning for 1-2hrs post injection	97.8%
Van der Ent[167]	Afternoon the day before surgery 4mls (370MBq)	After induction of anaesthesia 0.5-0.8mls	16h post injection	97% on scan 100% at surgery

Krag et al reported no relationship between method of injecting tracer, time interval between injection of tracer and surgery and the success in identifying a sentinel node. He did however, find an association between a prior excision biopsy, age 50yrs or more, a primary tumour in a medial location and failure to identify a hot spot before axillary incision [163]. The figures in table 2 above would tend to agree with Krag's finding that sentinel node identification is not related to timing of injection of tracer. Clearly a time interval is required to allow tracer materials to be absorbed into the lymphatics and to pass to the lymph nodes, however. In the studies shown in table 2, isotope was injected at least 2 hours and up to 24 hours pre-operatively. From these results and from the finding of Pijper's study in melanoma patients

mentioned earlier in this chapter [159], there appears to exist a substantial time interval during which successful lymphatic mapping is possible.

1.8.3 Injection volume

The role of injection volume in sentinel node identification is difficult to assess. A wide range of volumes has been used in the various studies producing varying success. For example, Krag [163]used a volume of 4mls technetium-labelled sulphur colloid injected peritumourally and identified a sentinel node in 91.4% of patients. Veronesi, on the other hand, injected only 0.2mls microcolloidal albumin subdermally to obtain a 98% identification rate [161]. These figures may suggest that the subdermal injection site rather than the injection volume has led to Veronesi's greater success. The microcolloidal albumin tracer used by Veronesi however, has a smaller particle size to the sulfur colloid used by Krag and this is another possible explanation for the different rates of identification between the two investigators. Giuliano used a volume of 3-5mls of isosulfan blue returning an identification rate of 93.5% [151]. Guenther used an identical technique with the same volume of isosulfan blue and only returned a 71% identification rate of sentinel nodes [150]. Van der Ent obtained a 100% identification of sentinel nodes using 4mls Tc^{99m}-labelled colloidal albumin peritumourally and 0.5-0.8mls patent blue V intradermally [167]. In this study he also included patients who had undergone previous excision biopsy injecting tracer around the biopsy cavity rather than into it. A higher radioactive dose of colloid (370MBq) was used in this study compared with the majority of other studies in which doses of less than 100MBq have generally been used [156, 158, 159, 163].

It is impossible to make a definitive statement on the true role of injection volume from these studies but results suggest that technique and site of injection may be more important than actual volume of injection.

1.8.4 Mapping agents

The various types of lymphatic mapping agents are considered in this section with respect to past performance, particle size and lymphodynamics.

i. Aqueous dye agents

A number of blue staining dyes are commercially available for use in lymphatic mapping. Patent blue V (Laboratoires Guerbet, France), a triphenylmethane dye, is commonly used in Europe for this purpose. It is available as a 2.5% solution distributed in 2ml glass vials and is commonly diluted with a similar volume of 0.9% sodium chloride solution or 1% lignocaine hydrochloride prior to injection although it can be injected undiluted. It costs £4.80 per vial in the United Kingdom. Patent blue V is also used as a food colour.

In America, isosulfan blue (Lymphazurin 1%, Hirsch Industries inc., Richmond, VA) [151] is more widely used for lymphatic mapping and is administered as above. Methylene blue has been used in the past but has been associated with fat necrosis of the breast so is not recommended for lymphatic mapping in these circumstances.

All of the blue dye agents pass rapidly into the lymphatics and into the regional lymph nodes so timing of injection is crucial in identification of the first echelon lymph nodes in contrast to timing of radiopharmaceutical injection, which allows a significant time interval in which to inject as described above in section **1.8.2ii**. In the case of breast cancer, it has been recommended that blue dye should be injected into the breast parenchyma around the tumour 5 minutes before the axillary skin incision [157].

ii. Radiopharmaceuticals

Numerous radiopharmaceuticles have been used for lymphoscintigraphy including technetium^{99m}-labelled dextran, ^{99m}Tc hydroxy ethyl starch, ^{99m}Tc human serum albumin and several labelled colloids including gold-198-colloid, ^{99m}Tc stannous phytate, ^{99m}Tc sulphur colloid (^{99m}Tc-SC), ^{99m}Tc antimony trisulphide colloid and ^{99m}Tc colloidal albumin (^{99m}Tc-CA). Among these agents only ^{99m}Tc-SC and ^{99m}Tc-CA are commercially available and licensed for use in Europe.

Colloid particles are mixed with the radioactive isotope technetium under aseptic conditions to allow parenteral administration into the patient.

Technetium 99m is an artificially produced element from the decay of Molybdenum ^{99} (Mo^{99}). There are no stable isotopes of technetium and it has almost no beta emission but emits gamma rays rendering it suitable for gamma camera scanning and gamma-detector probes [171].

$$^{99}Mo \longrightarrow {}^{99}Tc^{m} + \beta^{-} + \gamma \qquad \textbf{Half-life = 67 hours}$$

$$^{99}Tc^{m} \longrightarrow {}^{99}Tc + \gamma \qquad \textbf{Half-life = 6 hours}$$

Technetium 99m (^{99m}Tc) must be made on a daily basis, as its half-life is only 6 hours.

The importance of particle size in the dynamics of lymphoscintigraphy has been stressed by Ege in his early study into internal mammary lymphoscintigraphy [172]. Too large a particle size will lead to poor uptake of tracer into the lymphatics leaving most of it redundant within the breast interstitium. In contrast, particles of very small size will migrate too easily into the lymphatics, flowing rapidly through first, second and third level nodes leading to either complete passage of tracer into the circulation or 'lighting up' of multiple lymph nodes rather than one or two sentinel nodes. Ideally, intermediate-sized particles are needed to allow passage into the lymphatics and subsequent trapping in the level 1 (first echelon) lymph nodes.

Particle size of technetium-labelled colloidal albumin (Nanocoll ^{99m}Tc-CA, Nicomed-Amersham, Soren, Italy) ranges from 5-80nm. More than 95% of the particles are smaller than 80nm and only 1% are larger than 100nm [173].

Antimony trisulphide colloid (^{99m}Tc-ATC) is a small tracer measuring 3-12nm and runs the risk of sampling non-sentinel nodes [174]. During the 1970s and 1980s, antimony sulphide colloid was favoured for lymphoscintigraphy as it showed up the

whole lymphatic basin better than other agents allowing clinicians to look for filling defects to predict axillary and internal mammary metastases[175]. This feature is no longer desirable for the practice of sentinel node biopsy.

Sulphur colloid (99mTc-SC) has large particles in the 100-400nm range which carry the opposing risk of failed migration of tracer material [173]. This radiopharmaceutical is favoured in the USA over colloidal albumin, which is the favoured radiopharmaceutical for lymphatic mapping in Europe.

Technetium-labelled dextran has also been used with good success in sentinel node biopsy for operable breast cancer with a reported sentinel node detection rate of 98%, a sensitivity of 100% and no false negative cases [176].

Animal studies have suggested that the optimal lymph-node uptake of colloids should be achieved with particle sizes between 10 and 50nm [173] although accurate localisation of sentinel nodes has been demonstrated with all three of the commonly used radiopharmaceuticals: sulphur colloid (^{99m}Tc-SC), colloidal albumin (^{99m}Tc-CA) and antimony trisulphide colloid (^{99m}Tc-ATC) despite their different particle sizes [173].

Paganelli et al reported on the optimal size of radiocolloid in SNB for breast carcinoma examining three particles of different sizes. He concluded that a larger size of particle achieves the desired results of localising one or two sentinel nodes whereas the smaller particles led to detection of four or five sentinel nodes [177, 178].

Optimal particle size thus appears to be an issue of some controversy with mixed reports of the optimum radiopharmaceutical. It is logical to assume that there will be a variation in migration time to sentinel nodes dependant upon the size of colloid used. Clearly, the timing of surgery must also play a part in the optimisation of the technique to allow accurate and repeatable identification of the true sentinel node(s).

1.8.5 Lymphoscintigraphy and the lymphatic system

The lymphatic system is an extremely dynamic and reactive system. The normal 24-hour lymph flow is 2-4 litres. Lymph flow occurs by contraction in skeletal muscle,

the negative intrathoracic pressure during inspiration, the suction effect of high velocity flow in veins in which the lymphatics terminate and rhythmic contractions of the walls of the large lymph ducts. Lymph vessels have valves similar to veins, which prevent backflow of lymph and ensure flow is directed towards the heart. The rate of contractions in the walls of the lymphatic ducts increases in direct proportion to the volume of lymph contained within them. Lymphatic flow can increase with exercise by a factor of 10-30 and lymphatic channels are seen to contract and relax every 2-3 minutes. There is evidence that these contractions are the principal factor in propelling lymph. The lymphatic walls are permeable to macromolecules such as proteins. One of the functions of the lymphatic system is to return protein that accumulates in the interstitial fluid in the liver, intestines and other tissues back into the bloodstream from whence it came. This accounts for 25-50% of the circulating plasma protein each 24 hours [11]. When particulate substances of an appropriate size are delivered to the interstitial fluid they can cross the lymphatic capillary wall and enter the lymphatic lumen to pass along the lymphatic vessel towards the first draining lymph nodes [178]. It is in this way that tracer materials are absorbed from the interstitial fluid after injection into the breast parenchyma.

The role of lymphoscintigraphy in breast cancer was originally proposed in 1978 by Agwunobi and Boak [171]. In this study, pre-operative lymphoscintigraphy of the axillary lymph nodes was performed following subcutaneous, periareolar injection of 1ml of technetium-labelled antimony sulphide colloid into both breasts. Interpretation of the photoscan images led to a sensitivity of 93% in detection of axillary metastases confirmed by histological examination. In a similar study in Edinburgh, a sensitivity of 90% was achieved in detection of positive axillary lymph nodes in breast cancer patients [179] and several other studies produced similar results [180, 181]. These findings were however, contradicted by other studies [182, 183], which failed to reproduce these results. Ege reported a large series of patients who underwent staging of the internal mammary nodes by lymphoscintigraphy and preliminarily suggested it's use may help to predict those patients at risk of relapse, reporting a treatment failure rate of 19% in patients with axillary metastases and a positive internal mammary scan [172]. He failed to compare these results with those patients with negative axillary nodes and positive internal mammary scintiscans

however, making his proposals fundamentally flawed. The use of internal mammary lymphoscintigraphy has thus not become a routine part of breast cancer staging. Lymphoscintigraphy of sentinel nodes is considered an essential component of the sentinel node technique by some researchers as it potentially increases the sensitivity for detection of nodes [150, 159, 160]. In one report the addition of a static scintiscan identified 10% more sentinel nodes than by using the probe alone preoperatively[174]. Breast drainage patterns do not always follow the expected pathways with 32% of cases reported to show paradoxical drainage across the centre line of the breast (i.e. outer quadrant tumours draining to internal mammary nodes or inner quadrant tumours draining to axillary nodes) [184]. Lymphoscintigraphy can be used to demonstrate such drainage patterns more accurately.

A variety of methods have been studied to identify the optimal scanning practice. Pijpers examined timing of scintiscans at 2 or 18 hours post-injection of the radiocolloid. He reported that the site and number of sentinel node foci were unchanged in all but one patient in whom the first nodal uptake appeared only during the 18-hour scan. ^{99m}Tc-colloidal albumin was used in this study and was reported to give good nodal retention without overspill in over 95% of patients. He concluded that imaging was possible within a considerable time window post-injection of tracer allowing flexible timing of surgery [174]. Another study reported similar results claiming that images taken 2hrs and 24hrs post-injection were identical[185]. Pijpers also investigated the benefits of dynamic together with late static lymphoscintigraphy in melanoma patients. Dynamic imaging revealed at least one sentinel node in 95% of patients within the first twenty minutes post-injection and in all cases the first focal accumulation retained the highest fraction of radioactivity for at least 18 hours. Dynamic lymphoscintigraphy also differentiated between true sentinel nodes and 'spill' nodes (those drawing tracer from lymphatic vessels leading off from lower echelon lymph nodes) [159]. In the Milan study, scintiscans were taken at 15 minutes, 30 minutes and 3 hours post-injection of radiocolloid and the majority of patients revealed a sentinel node after the 30-minute scan [161]. In this study microcolloidal albumin was used as the tracer which has a smaller particle size than standard colloidal albumin and hence will probably reach the sentinel node more rapidly. It thus may be said that the exact timing of the scan is not critical but should

probably be performed at least 2 hours post injection in the case of colloidal albumin. Certain mapping agents may pass more rapidly to the sentinel node and in these cases dynamic imaging may help to illustrate more accurately the true sentinel node(s) before 'spill' nodes are picked up.

1.8.6 Gamma probe

The use of a gamma-detection probe to detect gamma rays emitted as radioisotope decays is the current method used to locate the position of sentinel nodes before incision to guide the surgeon to the most appropriate incision site. The probe is constructed using a lead and steel coating to protect the tip from surrounding interfering radiation. The radiation detector itself may consist of one of two types of detection material: a scintillation detector (usually a single crystal of thallium-doped sodium iodide or caesium iodide) or a semiconductor detector consisting of a solid crystal of cadmium telluride or cadmium-zinc telluride.

Scintillation detectors work by emission of a tiny burst of light created by gamma rays hitting the crystal. The photons of light thus emitted are amplified by a photomultiplier tube and converted into a measurable electronic pulse, which is also amplified to produce a measurable energy signal. The total light energy generated is directly proportional to the energy from the absorbed radiation.

When a gamma ray impacts with a semiconductor detector the crystal material becomes ionised producing charged particles creating a current, which is displayed as a voltage signal. These charged particles are produced in a quantity 10-20 times that of the photons produced by scintillation detectors making the potential for energy resolution much greater in these types of detector. The sensitivity of the detector requires a suitable energy window through which it can operate. This will keep detection of scatter radiation to a minimum whilst still allowing detection of higher energy gamma rays from the true radiation source, in this case the sentinel node(s). Typically unscattered radiation is detected at 140kev (kiloelectronvolts) with the limiting energy being approximately 200-300kev for acceptable sensitivity of a semiconductor detector in clinical practice [178]. In addition to the energy

resolution, the spatial resolution of the detector device is important. This allows two-point discrimination between two adjacent energy sources. As the probe is moved closer to the energy source, the spatial resolution will increase and vice-versa. Spatial resolution in detection of axillary sentinel nodes is obviously of great importance with many nodes lying close together in the axilla. Similarly, sensitivity is crucial to avoid failed detection of nodes containing small amounts of radiation, which could potentially contain metastases if they have drained directly from the tumour site.
It is possible to select a lower energy threshold and energy window on the probe monitor, which will alter the sensitivity and spatial resolution of the probe. Reducing the lower energy threshold leads to an increase in sensitivity and a corresponding reduction in spatial resolution and vice-versa within the finite limits of the machine. In clinical practice the depth of lymph nodes varies within the axilla and internal mammary chain of lymph nodes. This factor should be taken into account particularly when searching for deeper sentinel nodes containing weak radioactivity when it may be necessary to reduce the lower energy threshold of the gamma-detector in order to increase sensitivity. A reduction in the low energy threshold from 100 keV to 40 keV for example, results in an increase in the acceptance volume by a factor of 5 therefore increasing sensitivity but reducing spatial resolution [186].
A number of gamma-detector probes are commercially available for clinical use in radio-guided surgery and have been evaluated in studies simulating clinical environments.
Britten studied the performance of five intra-operative probes for sentinel lymph node localisation ranking them in terms directly related to their ability to localise lymph nodes in the presence of scatter background radiation from the injection site. Measurements were made of basic physical performance and clinical simulation with Neoprobe 1500 (Neoprobe Corporation, Ohio, USA) and Europrobe (Eurorad, Sevres, France) each with two probes and one probe with the Navigator GGS system (US Surgical Corporation, Norwalk, USA). It was concluded that the Neoprobe system with a cadmium-zinc telluride probe of 14mm outperformed the other probes with the Europrobe system with 16mm caesium-iodide scintillator probe second followed by the Navigator cadmium telluride probe, the Neoprobe system plus 19mm probe and lastly the Europrobe system with 11mm cadmium telluride probe.

One criticism of this study would be that different energy windows were set for each probe making the comparison non-uniform.

Tiourina studied the C-trak 19mm probe (Care Wise, USA), The Gammed II system (Eurorad, France), the Neoprobe 19mm probe and the CTC-4 10mm probe (RMD, USA). In her study the energy window standardised for each operating system and was also examined using two different energy windows for each probe in addition to simulation studies to more accurately measure the overall performance of each probe.

It was concluded that the Neoprobe and C-Trak systems were superior to the other two. A minimal analysis of absolute sensitivity and angular (spatial) resolution was also performed on three additional probes confirming the Neoprobe to have superior physical characteristics compared with the Europrobe 16mm and 11mm probes and the Navigator 14mm probe. This side-study also noted that there was no facility to set the upper energy threshold of the Navigator consul.

It may be concluded that a surgical gamma probe should meet the following requirements:

a. High absolute sensitivity

   Allows detection of low-uptake or deep-seated nodes

b. Side shielding

   To absorb scatter radiation from injection points

c. High spectral (energy) resolution

   Allows detection of sentinel nodes close to injection sites

d. High angular (spatial) resolution

   To discriminate between two points (nodes) closely located

e. Ergonomic characteristics

   Probe must be easy to use with optional audio signals to guide surgeon during search procedures.

   Radiation counts should be easy to measure and to read

In general when choosing a surgical gamma probe the surgeon and nuclear physicist should work closely together taking into accounts the specific operational requirements and budget of the department.

Interference from electrocautery equipment can cause the radiation detector to pick up unwanted signals so it is essential not to use diathermy whilst measuring radioactivity counts. The radiation detector device should undergo a regular sensitivity test to guard against gradual deterioration in performance over time.

1.8.7 Radiation exposure

It is important to realise correct safety procedures when handling radioisotopes. The correct method of handling and administration of the radiocolloid will be covered in the chapters on sentinel node biopsy in the results section of this thesis.
The department of medical physics at University of Wales College of Medicine, Cardiff has estimated the following measurements of radiation exposure to patients and personnel during a sentinel node biopsy procedure, see table 1.3 below.

Table1.3 Typical radiation doses per procedure when 20mbq ^{99m} Tc-labelled nanocolloid injected same day of surgery

	Area of body	**Radiation exposure**
Patient	Whole body	400microSv
Surgeon	Whole body Hands	7microSv 40micro Sv
Pathologist	Whole body Hands	<1microSv 2microSv

Using the above figures, if a surgeon performs 2 operations per week for 1 year then his/her total exposure will be 4160microSv (4.16mSv) to the hands or 728microSv (0.728mSv) to the whole body.

The legal limit for radiation exposure over 12 month period is 50milliSieverts i.e. 50,000microSv and a standard bone scan exposes the patient to 5000microSv (5mSv) dose of radiation. The figures in table 3 obviously stay well within these limits.

The radiation intensity rapidly diminishes with increasing distance from the source and follows the inverse square law. In other words, as the distance from the source is doubled, the radiation intensity is reduced by a factor of four.

A workshop conducted in Adelaide in 1998 came up with slightly different figures to those provided by The University of Wales College of Medicine depending on whether the surgeon handled the tissues manually or using forceps. The dose rates at various distances from a 40MBq point source of ^{99m}Tc using an equivalent dose rate constant for soft tissue are shown in table 1.4.

Table 1.4

Equivalent dose-rate to soft tissue versus distance for a 40MBq ^{99m}Tc point source

Distance in metres	Dose rate (μSv/hr)
0.05	250
0.10	63
0.30	6.9
0.50	2.5
1.00	0.62
2.00	0.16
3.00	0.069
4.00	0.039
5.00	0.025

*(taken from Sentinel node biopsy in breast cancer: recommendations for surgeons, pathologists, nuclear physicians and radiologists in Australia and New Zealand: Kollias, J et al; Aust NZ J Surg **70** (2); 132-6)*

It was calculated using the figures above that the dose to the surgeon's hands and torso respectively during a 15-minute procedure using forceps to handle the tumour would be 1.7μSv and 63μSv. If forceps were not used to handle the injected tumour site, the radiation dose to the surgeon's hands would be approximately 1850μSv.

This would allow a surgeon to perform up to 270 sentinel node biopsies per year before the annual occupational dose limit of 500,000μSv (averaged over any $1cm^2$) for the hands or skin is exceeded. The calculated doses to the pathologist's hands and torso during a 30-minute examination of the tumour without using forceps would be 1850μSv and 1.7μSv respectively if the examination is performed at 30cm form the pathologist's torso and 6 hours (one half-life) following the isotope injection. The dose from the sentinel nodes would be of the order of 2-5% of the dose from the tumour. Storage of specimens overnight to allow further decay of the radioisotope can further reduce these doses.

Doses to the torsos of ancillary staff in theatre would be approximately 0.16μSv and 0.04μSv for a 15-minute exposure at 1 metre and 2 metres respectively[187].

These figures should help to reassure patients and personnel that radiation exposure is minimal, conforms to legal requirements and does not place them at risk of damaging doses of radiation.

A study performed in America evaluated radiation exposure to operating room personnel, pathologist and equipment, from specimens during breast sentinel node biopsy. Twenty patients having sentinel node biopsy 1.5-3 hours following injection of 0.7-1.1mCi of ^{99m}Tc were studied. Exposure rates to the surgeon's hands and torso, scrub nurse, pathologist's hands and torso and to the operating instruments, clinical waste containers, suction canisters and pathology slides were measured using a calibrated Geiger counter. Results revealed maximum exposure was to the surgeon's hands from the breast injection site before skin incision followed by the pathologist's hands, surgeon's torso, scrub nurses torso and pathologist's torso. All operating instruments and pathology slides had radiation equal to the background rate along with 85% of clinical waste containers and 33% of suction canisters.

It was concluded that a primary surgeon could perform 2,190 hours, a scrub nurse 33,333 hours and a pathologist 14,705 hours of procedural work before surpassing Occupational Safety and Health Administration limits [188].

A review article gives similar reassurance that personnel involved in sentinel node biopsy are exposed to low levels of radiation and that these levels are not high enough to justify designated radiation workers in theatre or in the pathology laboratory [189].

Whenever exposure to radiation is occurring, it is important however, to minimise exposure by minimal time exposure to, maximum distance and if possible lead shielding from the radiation source. Protective clothing such as theatre gloves and gowns will help protect personnel from direct contact with radioactive agents. Good practice must also be followed to correctly dispose of contaminated waste and to follow local decontamination procedures in the event of spillages. The advice of the local radiation protection adviser (RPA) should be sought at the outset prior to any new plans to perform sentinel node biopsy using radiopharmaceuticles.

If administering radioactive substances, it is a legal requirement to possess or be given written permission following training by someone in possession of a certificate from the Department of Health according to MARS (Medical Administration of Radioactive Substances) regulations.

1.8.8 Pitfalls of SNB

i. Reasons for failed sentinel node identification

Guenther et al identified the presence of multiple nodal metastases as a risk factor for a failed SN identification[150]. He noted a median of nine positive lymph nodes in the axillae of a third of patients in whom a sentinel node could not be found. The reason for this is likely to be caused by the blockage of lymphatics by tumour deposits in more advanced disease thus blocking the normal pathway of tracers.
A higher percentage of failed detection (36%) in SN procedures is seen in patients if previous excision biopsy of the primary tumour has been performed for example in the case of diagnostic breast biopsy[190]. This is most likely caused by disruption to the lymphatic vessels during breast biopsy leading to poor uptake of tracer from the biopsy cavity. Hill et al found success in identifying a sentinel node was not related to tumour size, type, location or multicentricity; the presence of lymphovascular invasion; histological or nuclear grade or a previous excision biopsy [165]. This is in contradiction to the findings by Krag as described above.
Multifocality of breast tumours was associated with a 50% false negative rate in cases of sentinel node biopsy in the Milan study, most likely caused by multiple lymphatic drainage pathways leading to skip metastases. It was suggested that such patients should be excluded from sentinel node biopsy procedures [161]. This theory conflicts with the theory of Borgstein that lymphatic drainage of the breast follows a common pathway to the axilla along the two vasa lymphatica mammaria magna lymphatics [170] which would suggest that multifocal tumours should not pose a problem for sentinal node biopsy.
Reduced functional capacity of the sentinel node by tumour or fatty degeneration, as occurs in older or obese patients can lead to poor uptake of tracer materials and failure to locate a sentinel node [191]. This is a recognised entity based on surgical and histological observation. If the sentinel node is replaced by fat, its localisation is difficult due to decreased capacity of the node to retain the tracer. Increasing the dose of radiopharmaceutical injected may help overcome this problem in elderly patients.

The presence of a learning curve associated with successful performance of sentinel node biopsy is well documented in some of the earlier studies [150, 157]. The false negative rate and number of failures in identifications of a sentinel node fell with each successive 100 cases performed in a study of 500 cases in America [192]. These issues must be considered when selecting patients for a sentinel node biopsy. Whilst the presence of clinically involved nodes and multifocality are absolute contraindications to sentinel node biopsy and a previous excision biopsy a relative contraindication, none of the other problems above should prevent a patient from having a sentinel node biopsy if otherwise suitable.

ii. Side effects

A transient decrease in pulse-oximetry readings is frequently noticed with the use of blue dye as a mapping agent as it causes blue discolouration of the skin and mucous membranes [150, 193] and anaesthetists need to be warned of this as it is unlikely to be a true drop in PaO2.

Hypersensitivity to patent blue V dye is occasionally encountered and has a reported incidence of 2.2%. The commonest presentations of a hypersensitivity reaction are local oedema or generalised urticaria but there have also been reports of mucocutaneous oedema, bronchospasm and even cardiovascular collapse [194].

A study of over 600 patients undergoing sentinel node biopsy in the USA reported severe anaphylactic reactions to isosulfan blue dye in 1.1% of patients subsequently requiring vigorous resuscitation [195]. There were no deaths or permanent disabilities. It was concluded that lymphatic mapping with blue dye should be carried out in an environment where staff are trained to recognise and treat anaphylaxis.

Patent blue V is excreted through the kidneys and discolours the urine for up to 24 hours post-surgery so patients need to be warned of this before they are injected with blue dye.

1.9 INTRAOPERATIVE ASSESSMENT OF LYMPH NODES

1.9.1 Frozen Section

The performance of accurate intraoperative histopathological assessment of surgical specimens allows surgeons to make vital judgments regarding the most appropriate operation to perform on a given patient under anaesthesia. This facility renders the need for re-operation (a second procedure) redundant. Application of this principle to the dilemma experienced by breast surgeons in deciding whether to sample or clear the axillary lymph nodes could remove this uncertainty. Currently available techniques for the intraoperative assessment of surgically excised tissue have so far however, failed to match the accuracy of routine histological examination specimens. The intraoperative histological assessment of axillary lymph nodes has been performed using frozen section techniques[196]. The results of frozen section analysis are somewhat variable in accuracy. The Edinburgh study of axillary lymph nodes of breast cancer patients by frozen section reported a sensitivity of only 73% for the detection of involved nodes with a false negative rate of 27%[197]. The specificity of frozen section analysis in this study was however 100%, as was the positive predictive value but the negative predictive value was only 90%, leaving 10% of patients to face a second operation. Similar results are reported elsewhere [198, 199]. Other studies have reported slightly higher sensitivities of 83% and 87% and the same specificity of 100% [200, 201] An American study examined the benefit of frozen section in lymph nodes according to tumour size and found that its sensitivity ranged from 40% in T1a breast cancers to 76% in T2 breast cancers. It was also found that frozen section was much better at detecting macrometastatic disease (sensitivity 92%) than micrometastatic (sensitivity 17%) and it was concluded that the benefit of intraoperative frozen section increases with tumour size [202]. This finding was also found in a second American study[203]. A third American study found the opposite result however, reporting false negative rates of 5.6% and 19% for T1 tumours and T2-3 tumours respectively [204]. Serial sectioning of lymph nodes for intraoperative frozen section presents more of the

lymph node for histological assessment and this has been reported to increase the negative predictive value of the test to 95.4% with a false negative rate of 2.7%[205], an improvement on conventional frozen section, which usually only samples the node at two levels.

Despite the pitfalls of frozen section with its considerable proportion of false negative results, it has been uniformly reported as having good specificity with no false positive results. This is a reassuring characteristic if relying on the technique in deciding to proceed to full axillary clearance on the basis of a positive result. With more centres now introducing more detailed analyses such as immunohistochemistry into their routine examination of lymph nodes however, a new problem is created in that frozen section is once again made less sensitive in comparison, a finding highlighted by Turner et al[204]. The finding of Veronesi et al that the sensitivity of the technique improves with serial sectioning is certainly worth further study until more sensitive techniques become available[205]. Unfortunately, performing serial sections requires significant time and is labour intensive making it impractical in many UK laboratories where staffing levels would not allow this.

1.9.2 Imprint Cytology

The technique of imprint cytology provides an alternative to frozen section for the intraoperative examination of lymph nodes. It has been reported to have a sensitivity of 100% in some studies [206, 207]. A study from Northwick Park Hospital by Fisher et al, compared its sensitivity favourably with frozen section reporting a sensitivity of 98% for imprint cytology and only 90% for frozen section in the detection of positive lymph nodes from axillary node samples of patients with T1-2 breast cancer [208]. This finding has also been repeated by another study using the technique in patients having sentinel node biopsy for T1-2 breast cancer reporting sensitivities of 96% and 52% for imprint cytology and frozen section respectively [209]. Other studies report similar positive results with over 99% accuracy[210, 211].Other studies have reported lower sensitivities [200, 212] similar to those with frozen section.

Imprint cytology has some advantages over frozen section. Firstly, it does not distort or damage the lymph node leaving the entire specimen available for further analysis by haematoxylin and eosin histology or immunohistochemistry if necessary. Secondly, laboratory staff performing the technique have subjectively reported that good quality imprints are quicker and easier to prepare than frozen sections of lymph nodes[208]. Finally, imprint cytology does not require expensive equipment or experienced technical staff to perform it [207] although it does require the expertise of an experienced cytopathologist. One potential problem with imprint cytology lies in the examination of small lymph nodes of less than 2-3mm in diameter when slicing of the nodes to produce a cut "imprint" surface is technically difficult or impossible. In such cases nodal material may need to be examined and sliced using a fine tool under magnification to produce an imprint or simply processed for routine paraffin section thus removing the possibility of an intraoperative cytological diagnosis.

Imprint cytology has been used in the past as an adjunct to frozen section for intraoperative diagnosis of malignancy in solid tumours with a reported accuracy of 93.7%[213]. Turner et al used the combined techniques of frozen section and imprint cytology to examine sentinel lymph nodes from T1-3 breast cancer patients reporting an accuracy of 93.2% overall, detecting 98% of macrometastases but only 28% of micrometastases[204]. This finding of poor detection of micrometastases is unsurprising given that two further levels of the node were examined by routine histology in this study following the imprint cytology and frozen sections of two initial levels. As the clinical significance of lymph node micrometastases is still in doubt as described in an earlier section of this thesis it is impossible to make surgical decisions on the basis of these results. However, the finding of 98% of macrometastases using this technique may have some use in avoidance of a second axillary procedure if the decision to clear the remaining axillary nodes is made intraoperatively.

Certainly the published reports suggest imprint cytology is at least as good as frozen section with a number of studies reporting better results with imprints than with frozen section. These findings together with the possible benefits of a quicker, cheaper procedure justify more study into further improvement of this technique.

SUMMARY

The currently widespread use of axillary levels I, II and III to describe the stepwise progression of disease are no longer valid. These levels have been provided to give surgeons a landmark to use when performing axillary dissections, the Pectoralis minor muscle. This has been used as an easily located anatomical landmark but it has no biological foundation. The lymphatic vessels and lymph nodes of the axilla follow a more complex course as defined by lymphatic mapping. This can help to explain why the reported 'skip metastases' have come about [160]. The following chapters will attempt to provide useful information on the management of the axilla in breast cancer.

2. LONG-TERM RESULTS OF RANDOMISED STUDIES OF AXILLARY CLEARANCE AND NON-TARGETED AXILLARY SAMPLE

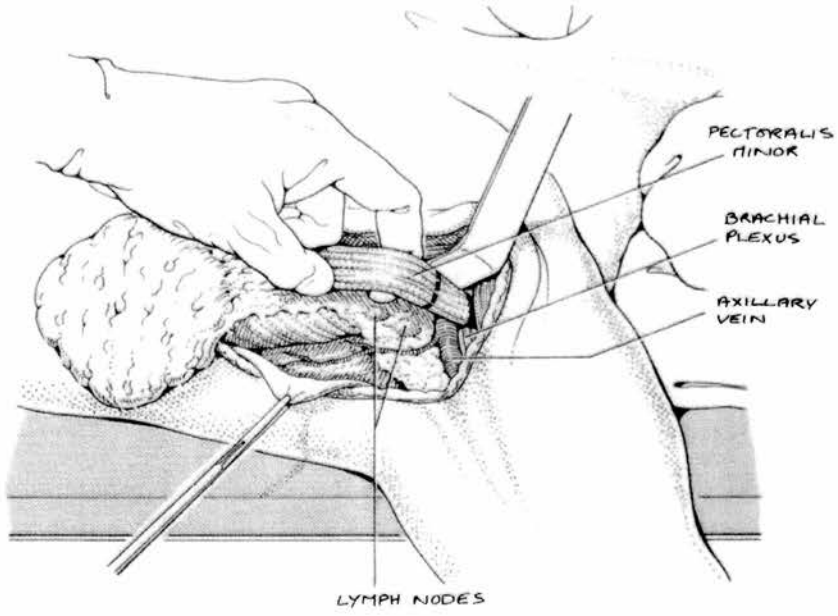


Diagram of the axilla during an axillary clearance. The dotted line marks the line of division of the pectoralis minor muscle. This gives access to the level II and III lymph nodes which are subsequently cleared away from the axillary vein.

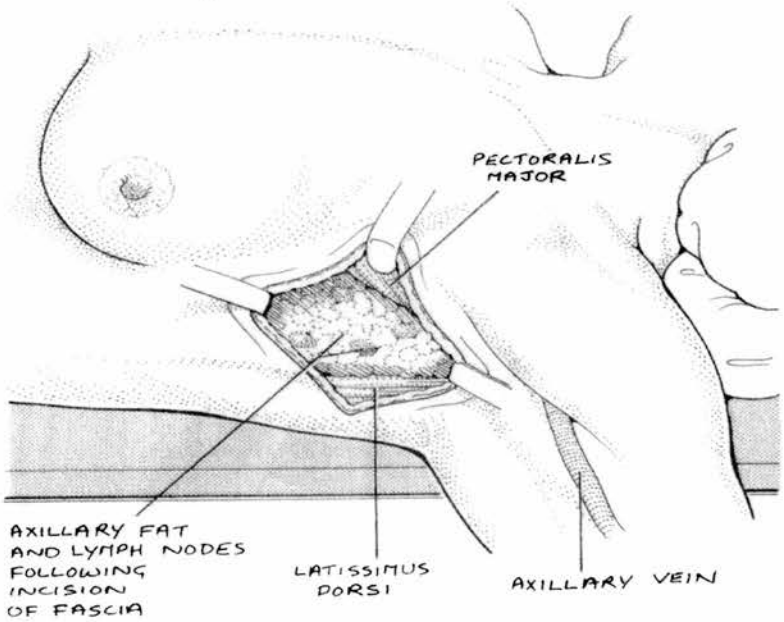


Diagram of the lower axillary lymph nodes and pectoral nodes excised during an axillary sample.

2.1 SUMMARY

Background
Axillary surgery is the only reliable method to stage the axilla in operable breast cancer. Most women having axillary surgery for breast cancer will have no disease in their axilla. Randomised trials comparing sentinel node biopsy with conventional axillary surgery will not provide information on its safety for many years. This chapter presents long-term results for axillary recurrence and mortality from two randomised trials comparing level III axillary node clearance with a four node axillary sample.

Patients and Methods
870 women with clinically staged T1-3,N0-1,M0 breast cancer were prospectively enrolled into two consecutive studies. In the mastectomy trial patients were randomised to receive a four-node axillary sample or level III axillary clearance after mastectomy (n=404) between 1980 and 1993. In the conservation trial patients were randomised to receive a four-node axillary sample or level III axillary clearance after breast conservation surgery (n=466) between 1987 and 1995. Patients who were node-positive after an axillary sample received axillary radiotherapy. Data were collected to compare axillary recurrence rates and survival between the two axillary procedures. Node negative and node positive patients were also compared separately for the two procedures.

Results
There was no difference in 5, 10 or 15 year survival between the groups treated by sample or clearance in either overall ($p=0.38$) or survival in node negative ($p=0.33$) or node positive ($p=0.99$) patients. There was a significant difference in axillary recurrence at 10 years in favour of axillary clearance in both the node negative ($p=0.023$) and the node positive ($p=0.033$) groups of patients. Patients having axillary sampling with 4 or more positive nodes were more likely to develop axillary recurrence than patients with 3 or fewer positive nodes ($p=0.016$). There was a clear association between increasing number of involved lymph nodes and decreased patient survival ($p<0.0001$).

Conclusions
Axillary sampling is a safe method of staging the axilla in operable breast cancer and does not place patients at risk of decreased long-term survival but it is associated with more axillary recurrences than complete axillary clearance. Patients with four or more positive nodes after axillary sampling are best treated by axillary clearance rather than axillary radiotherapy. Axillary recurrence developing after a node negative sample can be treated by axillary clearance combined with systemic therapy without affecting patient survival.

2.2 PATIENTS AND METHODS

870 women with clinically staged T1-3,N0-1,M0 breast cancer were prospectively enrolled into two consecutive studies on Edinburgh Breast Unit during the 1980s. The aims of this study were to amalgamate data from both of these trials (originally begun by Professor A.P. Forrest and continued by Mr U. Chetty and many other surgeons working on the breast unit at that time), obtain up to date follow-up information on patients enrolled in these trials and analyse the specific end points of survival and axillary recurrence comparing outcomes of axillary node clearance to levels I-III with a four-node axillary sample.

The mastectomy trial ran between January 1980 and October 1983; patients undergoing mastectomy (Mx) were randomised to receive a four-node axillary sample or level I-III axillary clearance. There was no age restriction as long as patients were fit for both surgery and radiotherapy and had clinically operable breast cancer (T1-2, operable T3, N0-1, M0).

All patients in the mastectomy trial underwent bilateral mammography, haematological and liver function testing, radiography of the chest and pelvis, liver ultrasonography and skeletal scintigraphy. Any abnormal results led to further investigation for metastases.

Exclusions were in-situ disease, Paget's disease of the nipple, multiple ipsilateral or contralateral breast cancer and patients unlikely to participate in continuous follow-up[214].

The conservation trial ran between 1987 and 1995; patients undergoing a breast conserving wide local excision (WLE) were randomised to receive a four-node axillary sample or level I-III axillary clearance. Patients were all under seventy years of age and had unilateral invasive breast cancer measuring 4cm or less and no evidence of metastatic disease.

All patients in the conservation trial underwent bilateral mammography, haematological and liver function testing and radiography of the chest and pelvis. Any abnormal results led to further investigation for metastases.

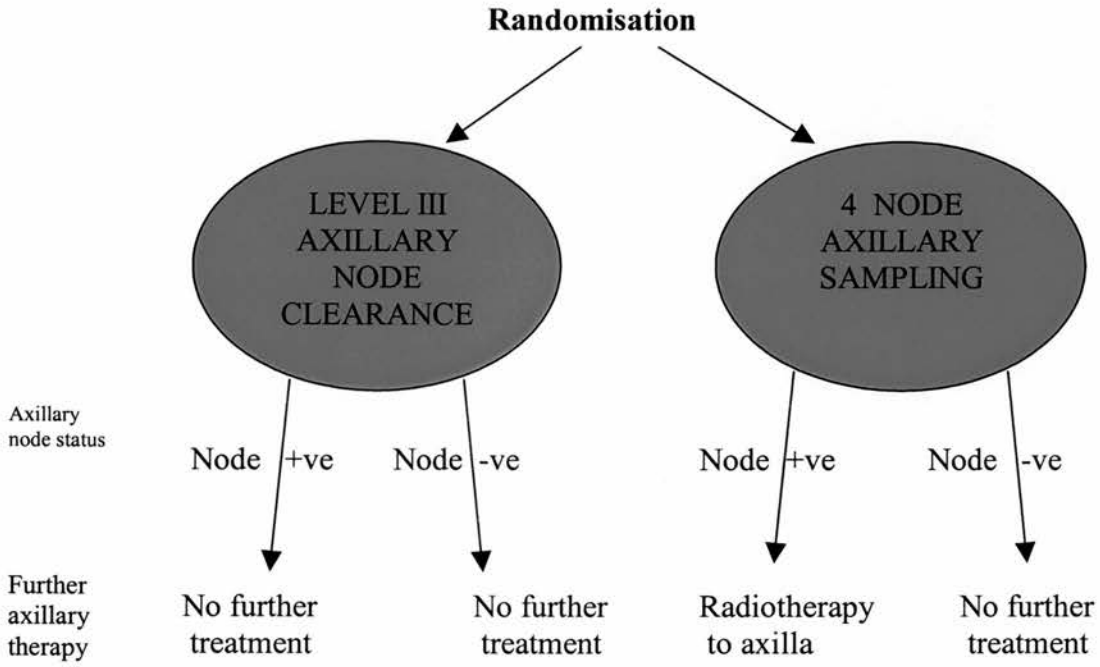
Exclusions were multicentric disease, locally advanced disease (T4), previous history of invasive cancer of any site (excluding basal cell carcinoma) and fixed axillary nodes (N2).

Records were searched by the author for data on survival and regional recurrence within the axilla. Figure 2.1 shows the study outline and axillary treatments each patient received [84].

Figure 2.1

1. Women undergoing mastectomy (Mx), n=404

2. Women treated by wide local excision (WLE), n=466



Systemic adjuvant therapy after surgery was based on node status and was identical in both randomised groups

Axillary radiotherapy was given to all node-positive sampled patients apart from 5 patients who were randomised to the 'no radiotherapy' arm of the Scottish Conservation Trial which was designed to determine whether or not radiotherapy could be avoided in patients with tumours of 4cm or less receiving systemic therapy [215].

2.2.1 Surgical technique

For mastectomy and axillary node sampling the breast was dissected from the underlying chest wall from medial to lateral and the axillary tail mobilized from serratus anterior between the pectoralis major and latissimus dorsi muscles up to the point where it merged with the lower axillary fat. Nodes were sought by inspection and palpation of the axillary tail and contiguous fat. If necessary the dissection was extended up above the intercostobrachial nerve, which was preserved. The surgeon was asked to identify and submit four separate lymph nodes for histological examination.

Mastectomy with axillary node clearance was performed by the Patey technique, with division and/or resection of pectoralis minor to facilitate access to levels II and III of the axilla. The neurovascular pedicle to latissimus dorsi was preserved. Fat and nodal tissue was removed to the level of the first rib.

With breast conservation, the axillary sampling technique was performed through a transverse incision between the latissimus dorsi and the pectoralis major muscles. The axillary tail was mobilized away from the serratus anterior muscle and nodes were identified by palpation in the lower axilla. If no lower axillary nodes were palpated, palpable nodes from level II or interpectoral regions were removed. Axillary clearance to level III was performed through a transverse axillary incision with division of the pectoralis minor muscle. The thoracodorsal and long thoracic nerves and vessels were preserved. The intercostobrachial nerve and lower intercostobrachial nerves were divided in the majority of cases.

2.2.2 Radiotherapy

Patients in the mastectomy trial were given radical postoperative radiotherapy with 6MV X-rays to the axilla if they were node-positive following a sample. During the course of the trial there were three modifications to the axillary radiotherapy regimen. These were to reduce the dose from 42.5Gy to 40Gy and to increase the

number of fractions from 10 to 20 in 4 weeks and to protect the shoulder joint with lead blocks.

Patients in the breast conservation trial with a node-positive sample were given axillary radiotherapy as a direct anterior field covering the axilla and supraclavicular fossa with a posterior axillary boost bringing the mid-axillary dose to 45Gy.

No patient in the axillary clearance group received radiotherapy.

Five patients who were node positive after axillary sampling did not receive radiotherapy as they were randomised to the 'no radiotherapy' arm of the Scottish Conservation trial.

Thirty-nine patients who were node negative after axillary sampling did receive radiotherapy to the regional lymphatics. These patients were entered into the conservation trial pre-1990 at which time all patients having axillary sampling were irradiated as a routine.

2.2.3 Follow up

Patients were followed up by clinical examination in surgical and radiotherapy clinics at 3-monthly intervals for 2 years, 6-monthly intervals from 2-5 years and then annually thereafter. Patients were also screened by annual mammography. Minimum follow-up was 4 years, apart from 4 patients lost at 26, 36, 37 and 46 months.

2.2.4 Analysis

Kaplan-Meier survival curves were plotted for survival and axillary recurrence based on 'treatment received' . Survival curves were compared using the log-rank test.

2.3 RESULTS

There were 404 patients in the mastectomy trial; there were 3 protocol violations in patients with in-situ carcinoma so 401 patients remained for analysis. There were 233 node negative and 168 node positive patients. At the time of analysis, 199 patients had died, 28 from non-breast cancer causes.

There were 466 patients in the breast conservation trial; there were 12 protocol violations, four patients had benign disease, seven patients had in-situ carcinoma and one patient had distant metastases when randomised. Thus, 454 patients remained for analysis. There were 310 node negative patients and 144 were node positive. At the time of analysis 65 patients had died, 11 of these from non-breast cancer causes.

Five node-positive sampled patients did not receive axillary radiotherapy. One of these patients developed regional recurrence in the ipsilateral axilla and supraclavicular fossa and unfortunately died 58 months after presentation. Of the other four, 3 were alive at follow up in 1999 and one was known to be alive in 1996. None of these 4 has developed any recurrence at a mean follow up of 107 months (8.9 years).

The mean follow-up was 4484 days (12.3 yrs) for women undergoing mastectomy, 2586 days (7.1 yrs) for women undergoing wide local excision and 3476 days (9.5 yrs) for the two groups combined.

The median follow-up was 5503 days (15yrs) for women undergoing mastectomy, 2650 days (7.3yrs) for women undergoing wide local excision and 3009 days (8.2yrs) for the combined groups.

Results were analysed as four specific groups according to node status and axillary procedure:

ANC -ve	negative axillary nodes after level III axillary clearance
ANS –ve	negative axillary nodes after 4-node axillary sample
ANC +ve	positive axillary nodes after level III axillary clearance
ANS +ve	positive axillary nodes after 4-node axillary sample.

The numbers of patients in the 4 groups for the mastectomy and conservation trials respectively and the patient survival, numbers of deaths and axillary recurrences with

percentages in each group are shown in tables 2.1 and 2.2. The same data combined from the two trials is shown in table 2.3.

Mastectomy trial

Table 2.1

Median follow-up 5503 days (15 yrs)

Axillary procedure plus node status	No. of patients	No. of axillary recurrences (%)	Total Deaths (% deaths excluding non-breast deaths)	non-breast deaths	Overall survival (%)
ANC -ve	121	**2 (1.7%)**	53 (**35.5**)	10	**56.2%**
ANS -ve	112	**6 (5.4%)**	44 (**35.8**)	6	**60.7%**
ANC+ve	81	**2 (2.5%)**	52 (**61.8**)	5	**35.8%**
ANS +ve	87	**7 (8%)**	50 (**55**)	7	**42.5%**

Conservation Trial

Table 2.2

Axillary procedure plus node status	No. of Patients	No of axillary recurrences(%)	Total Deaths (% deaths excuding non-breast deaths)	non breast deaths	Overall survival (%)
ANC -ve	139	**3 (2.2%)**	15 (**7.9**)	4	**89.2%**
ANS -ve	171	**10 (5.8%)**	16 (**8.2**)	2	**90.6%**
ANC +ve	83	**4 (4.8%)**	18 (**21.7**)	0	**78.3%**
ANS +ve	61	**8(13.1%)**	16 (**19.6**)	5	**73.7%**

Median follow-up 2650 days (7.3 yrs)

Combined data

Table 2.3

Axillary procedure and node status	No. of patients	No. of axillary recurrences (%)	Total Deaths (% deaths excluding non-breast deaths)	non-breast deaths	Overall Survival (%)
ANC -ve	260	**5(1.9%)**	68 (**21.1**)	14	**73.8%**
ANS-ve	283	**16(5.7%)**	60 (**19.5**)	8	**78.8%**
ANC+ve	164	**6(3.7%)**	70 (**40.9**)	5	**57.3%**
ANS+ve	148	**15(10.1%)**	66 (**39.7**)	12	**55.4%**

Median follow-up 3009 days (8.2 yrs)

There was a significantly higher rate of axillary recurrence in node-negative patients who had an axillary node sample compared with those having a level III axillary clearance (P=0.023). There was a significant difference in axillary recurrence in favour of axillary node clearance in patients who were node-positive (P=0.033). The results for axillary recurrence and survival from both trials combined were analysed statistically with 5 and 10-year estimates for axillary recurrence and 5, 10 and 15-year projections for survival and these are shown in table 2.4.

Table 2.4 Axillary recurrence

AXILLARY PROCEDURE PLUS NODE STATUS	AXILLARY RECURRENCE %				OVERALL SURVIVAL %			
	n	5y	10y	P value	5y	10y	15y	P value
NCl-ve	260	2.0	2.0	**0.023**	87.6	74.9	65.3	**0.33**
NS-ve	283	3.3	7.0		89.6	82.0	67.1	
NCl+ve	164	2.9	6.5	**0.033**	75.4	57.9	46.6	**0.99**
NS+ve	148	7.5	10.7		74.1	57.4	46.9	

The Kaplan Meier survival curves for axillary recurrence subdivided into node-negative and node-positive patients are shown in figure 2.2.

There was no significant difference in survival between the groups treated by sample or clearance either overall (P=0.38) or subdivided into node-positive (P=0.99) or node-negative patients (P=0.33) as shown in table 2.4. These figures are displayed graphically in figures 2.3 and 2.4

All patients with axillary recurrence were also analysed as a separate group. The mean time to axillary recurrence was 1627 days (4.5 years) for the combined group with a range of 131-6505 days (0.4-17.8 years). Two patients with node-positive samples recurred after nearly 18 years of follow-up. Patients having an axillary clearance recurred slightly earlier with a mean time to recurrence of 1452 days (4 years) compared with 1689 days (4.6 years) in patients having an axillary sample. Comparing sample with clearance recurrences, 45% of sample recurrences were alive compared with 36% of clearance recurrences after a mean follow-up of 1349 days (3.7 years) from time of axillary recurrence.

Synchronous distant metastases were present in 16% (5/31) of patients who had axillary recurrence after node sample and in 36% (4/11) of patients who had axillary recurrence after node clearance. The survival figures for axillary recurrence patients in both trials according to node status and axillary treatment are shown in table 2.5.

Axillary Recurrence

Table 2.5

Axillary procedure plus node status	Number of patients	Number of axillary recurrences	% Patients with axillary recurrences	Number of patients with axillary recurrences alive	% patients alive following axillary recurrence
ANC –ve	260	5	1.9%	2	40%
ANS –ve	283	16	5.7%	8	50%
ANC +ve	164	6	3.7%	2	33.33%
ANS +ve	148	15	10.1%	6	40%

Mean follow up 1349 days (3.7 years) from time of axillary recurrence

There was a significant difference in axillary recurrence in node-sampled patients with four or more positive nodes compared with those having three or fewer nodes

positive (P=0.016) suggesting axillary radiotherapy is under treating this group. There was no such difference in the axillary clearance group (P=0.258). These results are displayed in figures 2.5 and 2.6.

There was an overall negative association between survival and the number of positive axillary nodes (P<0.0001) as shown in figure 2.7.

Figure 2.2

Axillary Recurrence Rates

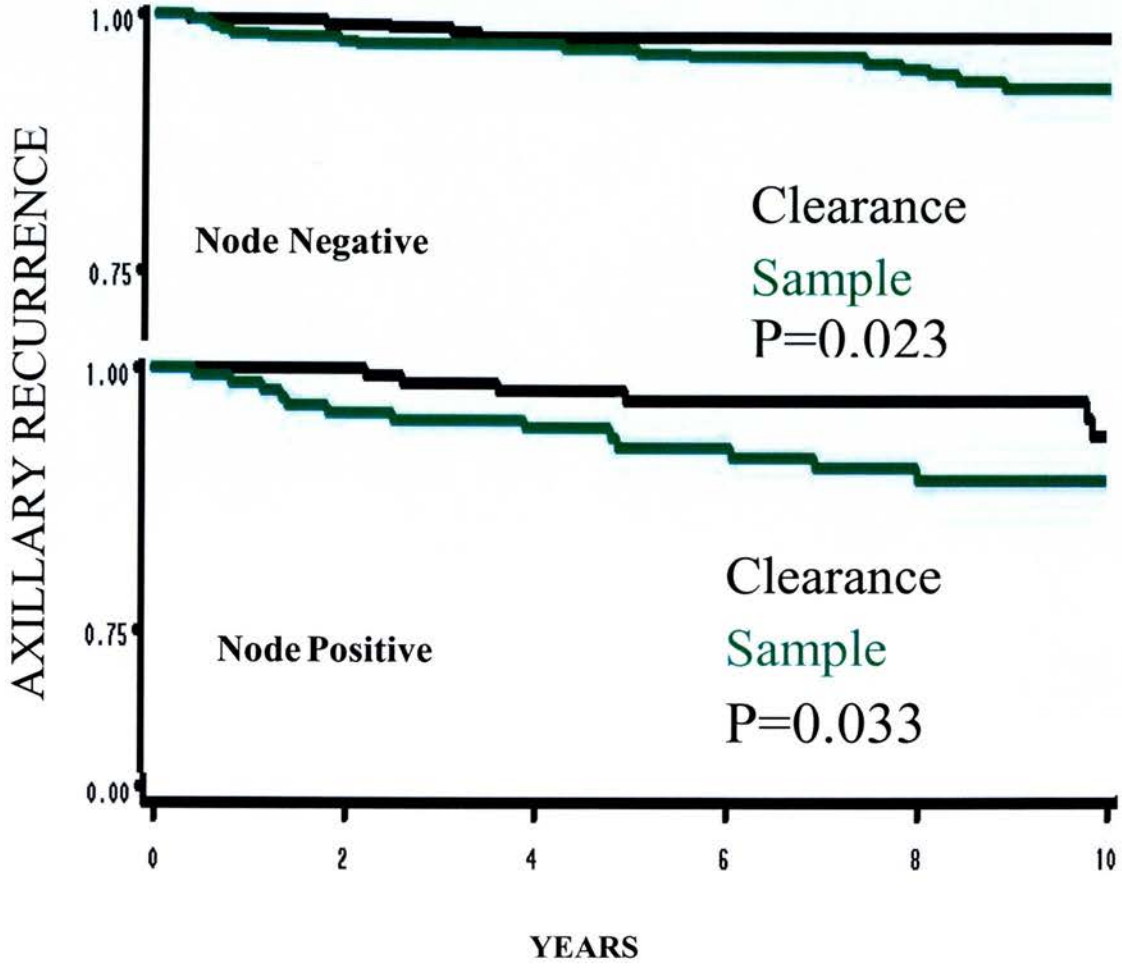


Figure 2.3

Overall Survival

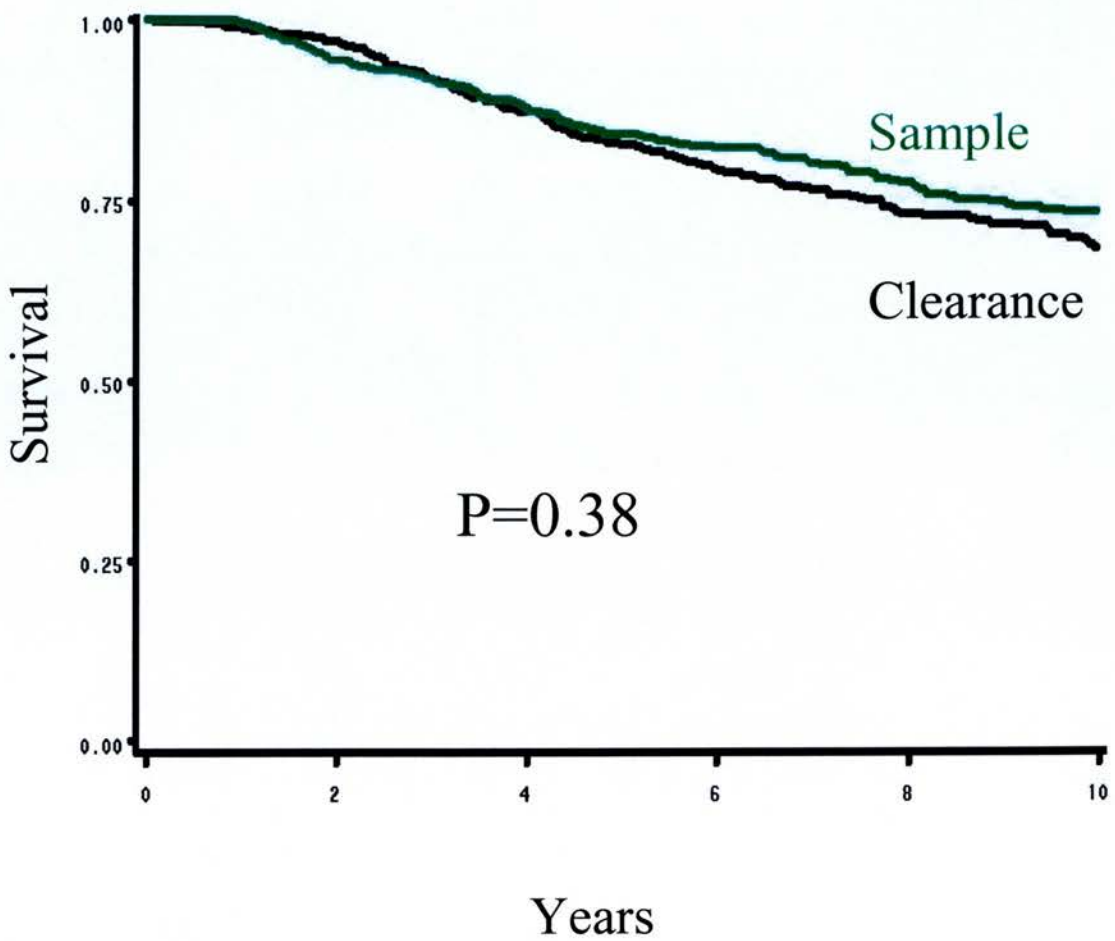


Figure 2.4

Effect of Node Status on Survival

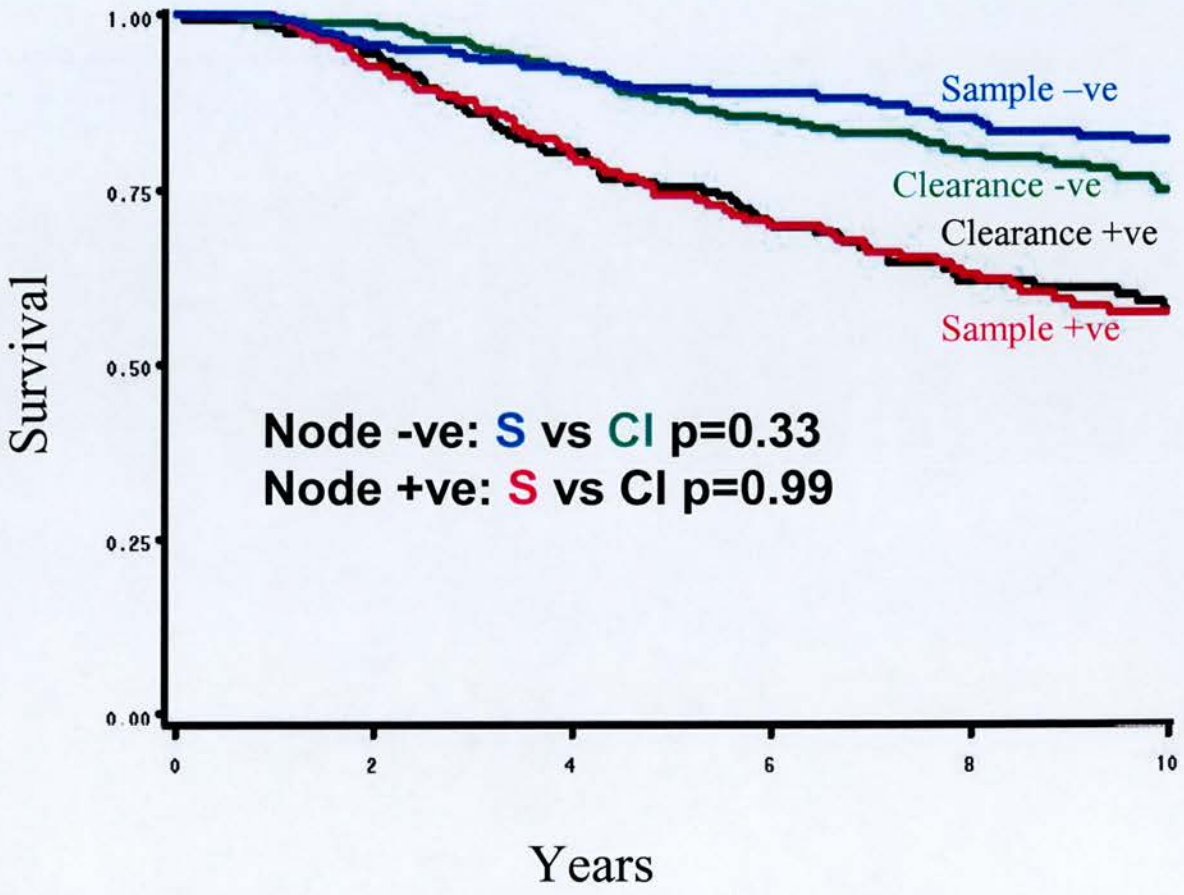


Figure 2.5

Axillary recurrence after node sample subdivided by number of positive nodes

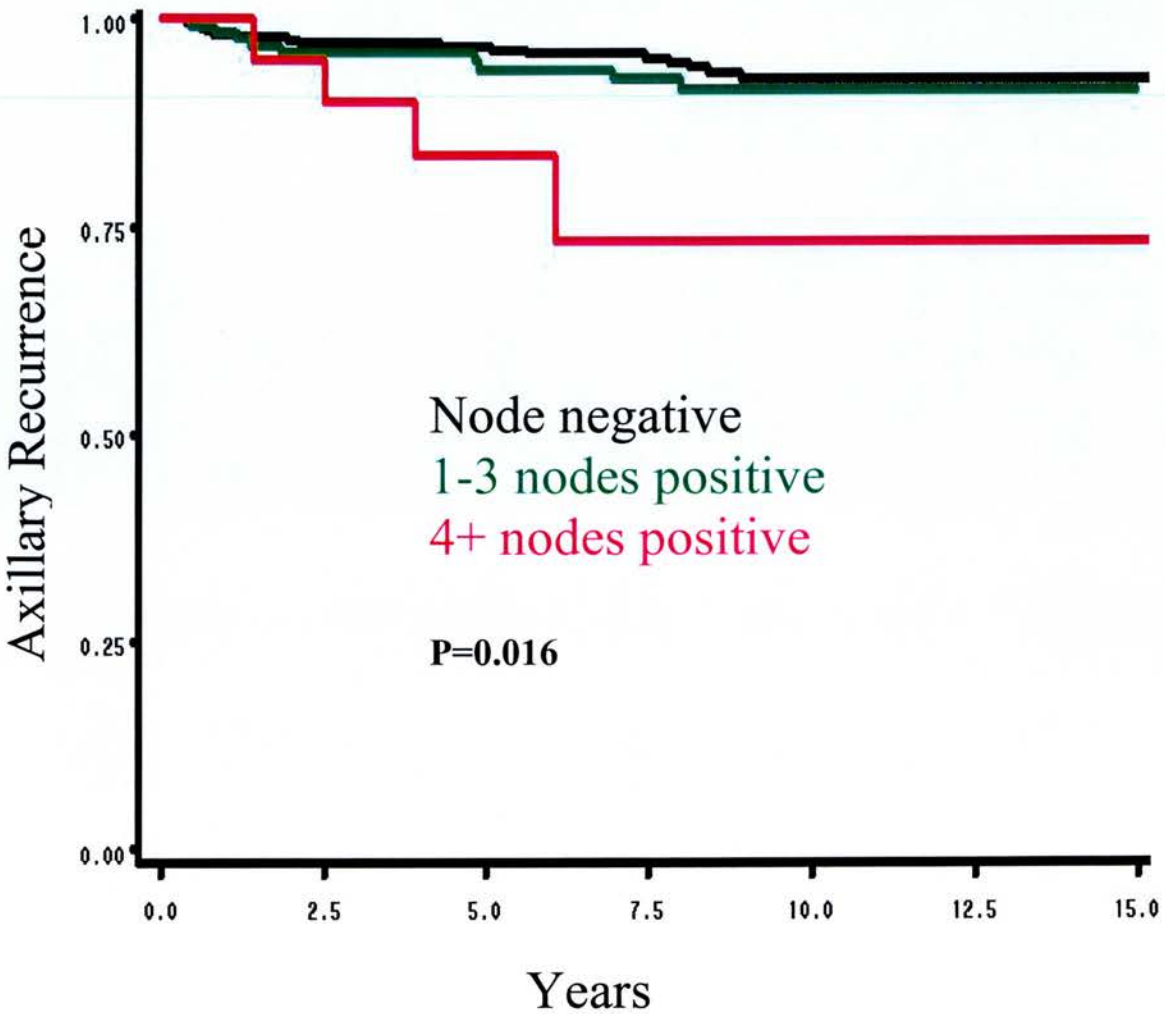


Figure 2.6

Axillary recurrence after node clearance subdivided by number of positive nodes

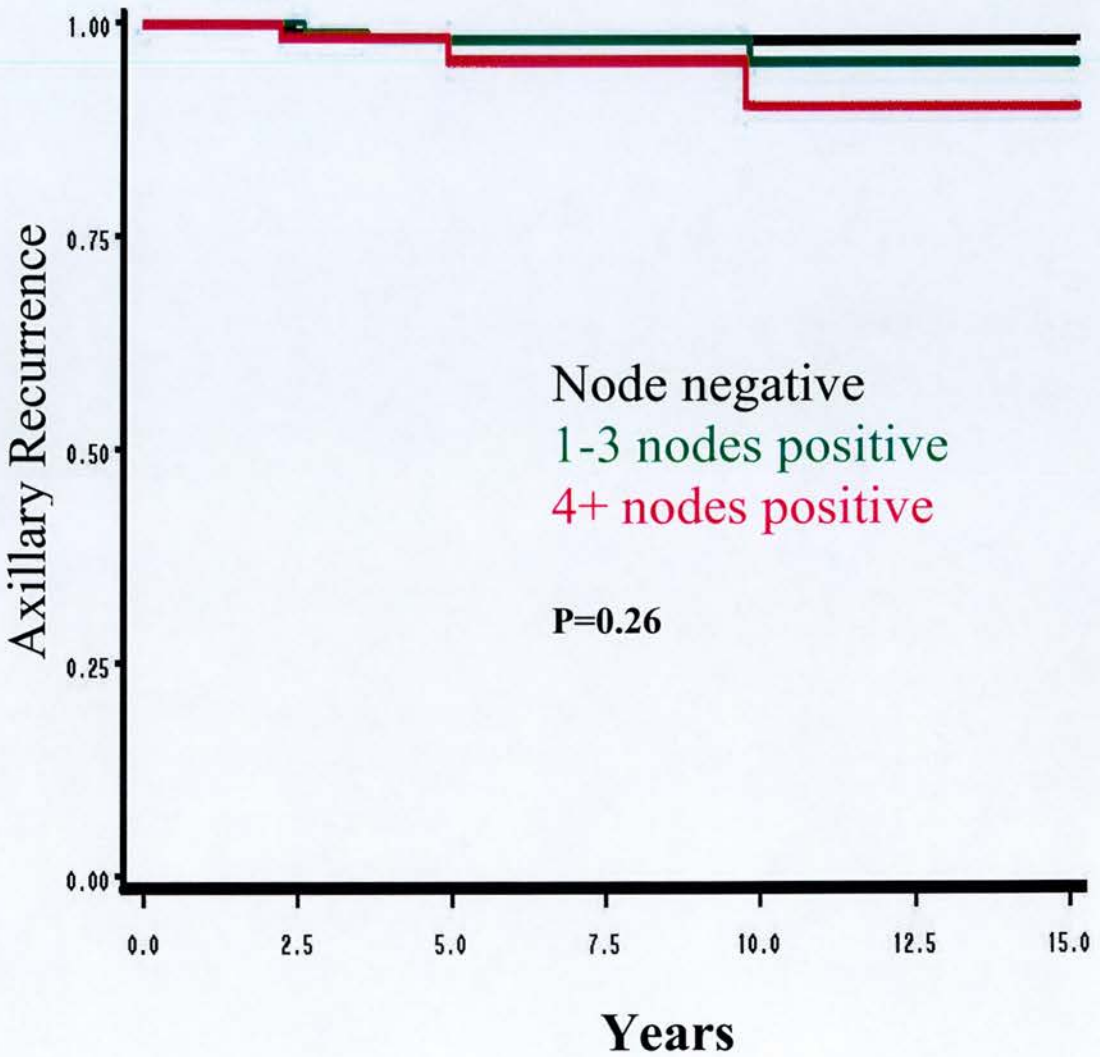
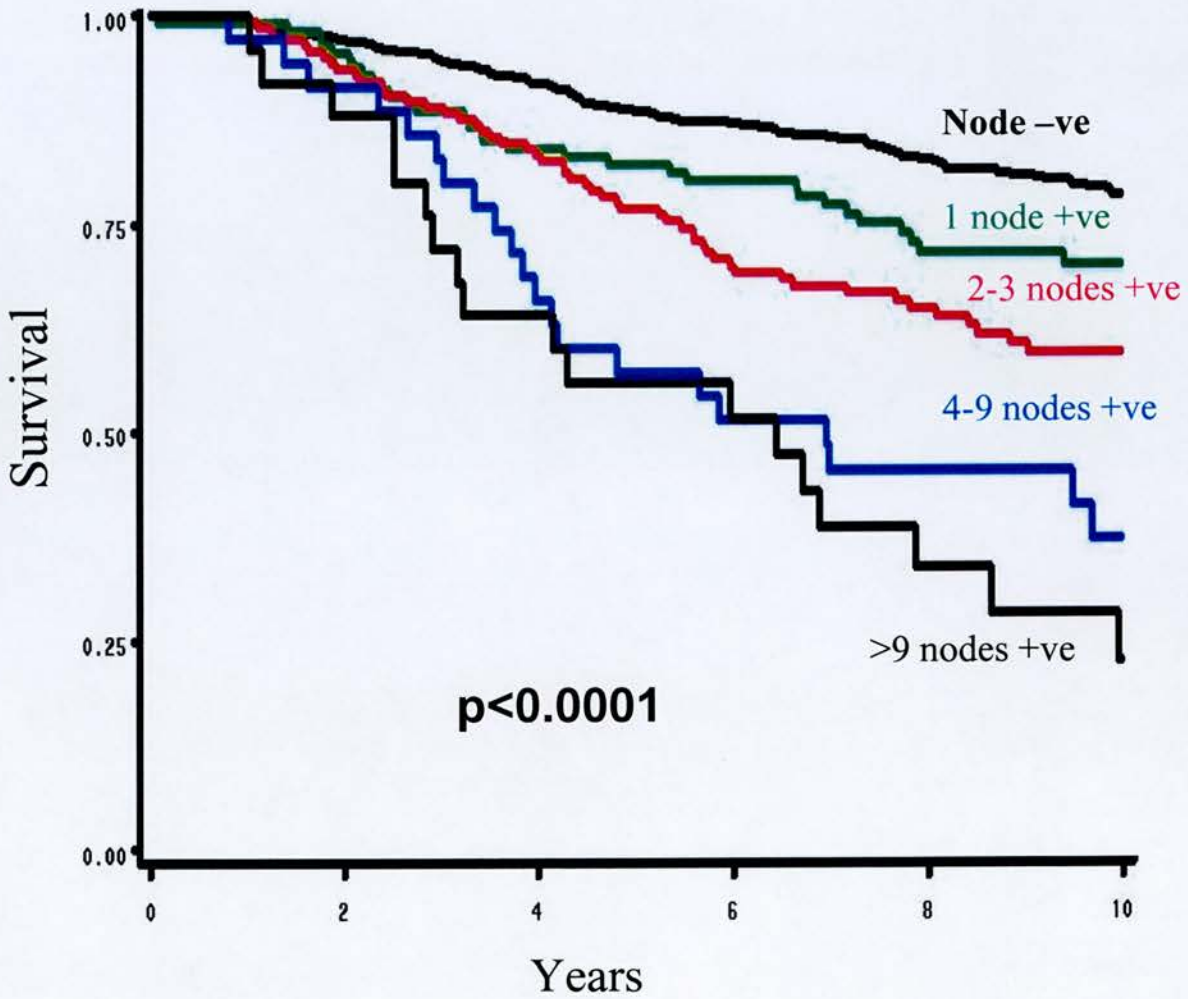


Figure 2.7

Survival vs Number of positive nodes



2.4 DISCUSSION

With no reliable non-invasive method of staging the axilla, it is still necessary to surgically excise axillary nodes to stage the axilla in operable breast cancer. Sentinel node biopsy is currently under trial as a minimally invasive technique for axillary staging. Long-term results will not be available for many years to prove the safety of this technique. Our results demonstrate that performing an axillary staging procedure such as a four-node sample rather than a full axillary dissection does not place the patient at risk of increased mortality over a 15-year period.

Axillary recurrence rates were higher in patients who were node-negative after an axillary sample. This suggests that some patients are understaged by this method. There are a number of reasons to explain this increased rate of axillary recurrence. Four of the sixteen patients who developed axillary recurrence after node-negative axillary sample had fewer than four lymph nodes removed on histology. Three of these had only three nodes removed and one had only two nodes removed. Understaging of the axilla in these four patients is probably due to poor surgical technique in the early years of the trial when axillary sampling was relatively new. This suggests there is a learning curve for this technique as has been demonstrated for sentinel node biopsy.

Two further patients with a node-negative sample were shown to have axillary metastases when their histology was reviewed by another pathologist as part of a recent study[216]. This represents failure of the first pathologist to detect metastases in these two patients.

The importance of lymph node micrometastases (those measuring less than 2mm in a single lymph node) is not clear. Studies have reported a negative effect of axillary micrometastases on overall patient survival[55, 217] and other reports have shown that closer examination of lymph nodes by the pathologist either by haematoxylin and eosin serial sectioning or immunohistochemistry leads to up to 20% more metastases being discovered[48]. Another study looked at the importance of micrometastases detected by serial sectioning and immunohistochemistry (IHC) according to tumour histology and concluded that micrometastases detected using

IHC in cases of lobular carcinoma was prognostically unimportant but that serial sectioning and examination using H&E was prognostically significant in 120 patients in whom recurrence and survival were adversely affected[66]. These findings suggest that some node negative patients will have micrometastatic deposits present in lymph nodes, which are missed by routine histological examination. As fewer nodes are examined in a node sample, the potential to miss such deposits may be greater than with a node clearance in which larger numbers of nodes are examined thus increasing the potential metastatic yield.

Another reason for axillary sampling missing involved nodes is the presence of 'skip' metastases. The incidence of skip metastases is reported at between 1% [218] and 25% [101] although most reports give an incidence between 1 and 5%[41, 219, 220]. Even with axillary clearance involved nodes may be missed [221] and the most frequently missed areas reported in this study were the interpectoral area and the area lateral to the subscapular vessels in level I. Interpectoral nodes are involved in up to 14% of patients with operable breast cancer and in a small number of patients no other disease exists in the other axillary nodes[222]. When sampling the lower axilla it is worth examining these areas paying particular attention to the axillary tail of the breast for very low intra-mammary nodes which are sometimes overlooked and may be the first location for axillary spread.

Overall mortality was not affected by axillary sampling and salvage surgery combined with systemic therapy appears to be an effective treatment for patients who relapse with axillary recurrence. It may be suggested that because more patients in the sample group had axillary recurrence than in the clearance group and hence received systemic therapy, that this may unfairly boost the sample group's survival. Looking at the data for node negative recurrences first; six more node-negative sample patients (2.1%) survive after axillary recurrence than node-negative clearance patients. If this affected survival then one would expect a rise of no more than 2.1% in overall survival for the node-negative sample group. However, table 2.3 reveals a non-significant 5% greater overall survival in the node-negative sample group compared with the node-negative clearance group suggesting that other factors are involved and that systemic therapy is not alone responsible for this. Even if this 2.1% of patients surviving recurrence were subtracted from the overall survival then the

figures would not be significantly altered. Similarly adjusted figures for node-positive patients do not significantly affect overall survival.

Survival after axillary recurrence was worse in patients who underwent axillary clearance than in those having an axillary sample. Reasons for this are unclear but over twice as many clearance patients had synchronous metastases and axillary recurrence than sample patients. The absence of any lymphatic filtering in the axilla may explain any apparent easier seeding of blood-borne metastases in the clearance group. A sampling procedure may thus preserve this route of spread for cancer cells by conserving the axillary lymphatic filtration system.

Patients who were node-positive after an axillary sample were treated by radiotherapy to the axilla and supraclavicular fossa. There was a significantly higher rate of axillary recurrence in the sample group of patients compared with those having axillary clearance. The addition of simulation of radiotherapy in the conservation trial together with the more accurate placement of the posterior axillary field and a higher overall dose of radiation (45Gy) did not reduce axillary recurrences in this group of patients. This suggests that axillary clearance gives better regional control than radiotherapy as treatment for node-positive disease although overall survival is not affected. This conflicts with results from the Institute Curie in Paris [223] who compared axillary recurrence in patients having complete axillary dissection with those having complete regional nodal radiotherapy after lumpectomy and breast radiotherapy. However there was a significant difference in distant disease-free survival in favour of the surgically treated group compared with the radiotherapy group in this study. An American trial comparing axillary dissection with axillary radiotherapy after mastectomy between 1971-1974 also showed no difference in regional recurrence rates over ten years [89] conflicting with our data although this latter trial also reported no difference in survival between the two groups in keeping with our study. Table 2.1 actually shows a small non-significant ($p\sim0.5$) survival advantage in favour of axillary node sample in node-positive patients treated by mastectomy. A more recent (as yet unpublished) analysis by Edinburgh Breast Unit with a median follow up of 17 years for patients in the mastectomy trial showed a non-significant ($p=0.66$) 6% survival advantage for node-

positive sampled patients (46% overall survival) compared with node-positive clearance patients (40% overall survival) but reasons for this are unclear.

Our survival figures show a small, non-significant difference in node negative patients in favour of axillary sampling. This is interesting given that these women in the node-sampled group had a significantly higher number of axillary recurrences. One possible explanation for this is the higher rate of metastatic recurrence in the node-negative clearance group. Patients with an axillary recurrence following clearance fared slightly worse then those following a sample suggesting that axillary recurrence is more often salvageable when occurring after an axillary sample.

Our figures support the association between increasing node positivity and decreased survival [143]. In the node-positive sample group, patients with 4 or more involved nodes had a significantly higher incidence of regional recurrence than those with 3 or fewer nodes positive. This subgroup of patients is probably best treated by axillary clearance as a secondary axillary procedure whilst those with 3 or fewer nodes positive do appear to be adequately treated with axillary radiotherapy. Radiotherapy after axillary sampling does carry an increased risk of restricted shoulder movement compared with axillary clearance which increases arm swelling and is associated with more frequent development of lymphoedema[84]. Nowadays, axillary clearance has become our standard secondary treatment in all node-positive sampled patients with 4 or more involved nodes.

In this analysis, five node-positive sampled patients did not receive adjuvant axillary radiotherapy and thirty-nine patients who were node-negative after axillary sampling did receive radiotherapy to the axilla. It may be fairly suggested that these patients do not fulfil the trial guidelines. In view of this, a subsequent analysis excluding all of these patients was performed in 2003 by the staff of Edinburgh Breast Unit to clarify this point and it was found that there were no differences in terms of the statistically significant findings reported in this chapter i.e. all of the significant findings presented here remain relevant.

Patients who are clinically node negative are unlikely to have multiple involved nodes and are now being offered sentinel node biopsy alone as primary axillary staging as part of large randomised, multicentre trials comparing sentinel node biopsy with standard axillary surgery such as the ALMANAC trial[224]. Initial

results from randomised trials of sentinel node biopsy are encouraging but until long-term figures are available, the technique is not recommended for use out with trials. Our figures demonstrate the safety of a non-targeted 4-node axillary sample in patients with stage T1-3 breast cancer. Axillary sampling correctly predicted node status in over 96% of patients and was associated with a false-negative rate of 9.75%, similar to figures reported for sentinel node biopsy[150, 163, 165]. In Edinburgh, it is our practice to perform axillary sampling in T1,N0 breast cancer with selective use in T2,N0 breast cancer according to patient age, menopausal and ER status along with patients' general health. The in-patient hospital stay for sample is shorter and post-operative arm morbidity is lower than for clearance[84]. In particular the incidence of lymphoedema is significantly reduced[84]. Returning home early with fewer side effects removes the anxiety associated with more prolonged hospitalization and delayed recovery.

This study has demonstrated that axillary sampling is a safe method of staging the axilla in operable breast cancer. Patients with 4 or more positive axillary nodes after sampling are best managed by axillary clearance rather than axillary radiotherapy. Axillary recurrence after node-negative sample is higher than after a node-negative clearance but can be treated by axillary clearance and systemic therapy without affecting patient survival.

Notes on Trial Violations and Patient Recruitment

To address the problem of the trial violations, clearly it is less than desirable for patients with benign disease and in-situ disease to have had axillary surgery. These violations may perhaps be explained by the fact that at the time of recruitment, cytological techniques were less well developed than they are nowadays and also because patients were operated on based on frozen section histology of the primary tumour on occasion. Both of these methods are associated with a false positive rate leading to the inevitable inclusion of a small number of patients with benign disease and in-situ disease. It is now the unit policy to reserve axillary surgery for patients who have a clear diagnosis of malignancy based on triple assessment of clinical, radiological and cytological or histological examination. Although only 466 patients were recruited into the conservation trial over an eight-year period, many patients were not included in the trial as they had impalpable, screen-detected cancers, which were very small and had a low risk of lymph node metastases. It was thought that these patients would be best treated by axillary sample rather than axillary clearance to limit their morbidity based on previous results [121]. Similarly, other patients were ineligible for the trial, as they required mastectomy to adequately treat their primary disease. Only two consultant breast surgeons were working in Edinburgh Breast Unit for the main period of recruitment and as such, the unit was treating far fewer cancers than are being treated today on the unit providing another explanation for seemingly low recruitment. It is hoped that these points adequately address any possible suggestions of selection bias.

Legends to Graphs and Tables

Table 2.1 *Mastectomy trial patients subdivided into axillary procedure received and node status. Numbers and percentages for axillary recurrence and patient deaths including deaths from non-breast cancer causes are shown along with a percentage for overall survival.*

Table 2.2 *Conservation trial patients subdivided into axillary procedure received and node status. Numbers and percentages for axillary recurrence and patient deaths including deaths from non-breast cancer causes are shown along with a percentage for overall survival.*

Table 2.3 *Patients from both the mastectomy and conservation trials combined subdivided into axillary procedure received and node status. Numbers and percentages for axillary recurrence and patient deaths including deaths from non-breast cancer causes are shown along with a percentage for overall survival.*

Table 2.4 *Combined trial data showing axillary recurrence and overall survival analysed statistically with 5 and 10-year projections for axillary recurrence and 5-year, 10-year and 15-year projections for overall survival.*

Table 2.5 *Patients with axillary recurrence subdivided according to axillary procedure received and node status. Numbers and percentages of axillary recurrences and for survival after axillary recurrence are shown.*

Kaplan Meier curves are shown in figures 2.2-2.7 as follows:

Figure 2.2 Axillary recurrence rates comparing axillary clearance with sample subdivided into node-negative and node-positive cases.

Figure 2.3 Overall survival comparing axillary clearance with sample.

Figure 2.4 The effect of node status on survival comparing axillary clearance with sample

Figure 2.5 Axillary recurrence in node-sample patients subdivided by number of positive nodes.

Figure 2.6 Axillary recurrence in node-clearance patients subdivided by number of positive nodes

Figure 2.7 Survival according to the number of positive axillary nodes

3. ALMANAC STUDY PHASE 1 – AUDIT PHASE

INTRODUCTION

The **ALMANAC** study (**A**xillary **L**ymphatic **M**apping **A**gainst **N**odal **A**xillary **C**learance) is a two-phase multicentre trial to evaluate sentinel node biopsy in breast cancer in the United Kingdom. The trial's principal investigator is Professor R E Mansel of the University Department of Surgery, University College of Wales College of Medicine, Heath Park, Cardiff CF14 4XN. Edinburgh Breast Unit is one of the main centre's taking part in the trial.

Phase 1 will be an audit phase and Phase 2 a randomised trial comparing conventional treatment of the axilla with sentinel node guided treatment of the axilla. Mr U Chetty and Mr J M Dixon were the two consultant breast surgeons chosen to perform sentinel node biopsy in Edinburgh during the two phases of the trial.

i. Audit phase – Phase 1

The primary objective of the audit phase is to validate each surgeon's ability to localise the sentinel node in breast cancer. All surgeons who take part will perform sentinel node biopsy in a minimum of 40 patients with invasive breast cancer using the combined mapping technique of radiocolloid and blue dye followed by axillary sampling or clearance according to the unit's routine policy. A localisation rate of 90% and a false negative rate of 5% or less in the last 40 consecutive cases is required before a surgeon can proceed to the randomised phase (phase 2) of the trial. A secondary objective of the audit phase is to follow-up patients for arm and axillary morbidity. Health service resource use and quality of life after treatment of the axilla will also form part of the national study and will not be covered in this thesis.

ii. Randomised Phase – Phase 2

The primary objective of phase 2 is to compare sentinel node biopsy with conventional axillary surgery. Three main outcome measures will be studied:

a) arm and axillary morbidity
b) resource costs
c) quality of life

Resource costs and quality of life are part of the national study and will not be covered in this thesis.

The secondary objective is to record the incidence of local recurrence in the axilla for the different axillary procedures.

In phase 2 patients will be randomised to either the **control group** who will have a wide local excision or mastectomy plus axillary sampling or clearance according to routine policy or the **sentinel node biopsy group** (study group) who will have a wide local excision or mastectomy plus a sentinel node biopsy. Sentinel node positive patients on routine histology will go on to have delayed treatment of the axilla by surgery (axillary clearance) or radiotherapy. Sentinel node negative patients will have no further axillary treatment. Patients who are node-positive after conventional axillary surgery will go on to have adjuvant treatment according to unit policies.

3.1 PATIENTS AND METHODS

3.1.1 Patients

83 patients with stage T1-2,N0,M0 invasive breast cancer were enrolled into phase one of the trial.

Two patients had DCIS alone with no invasive cancer found in the operative specimens histologically and were excluded from the results. Both of these patients had clinical, radiological and cytological features of malignancy and it is the policy of Edinburgh Breast Unit to perform surgery on the basis of such findings. These cases represent the small group of patients in whom falsely positive triple assessment can occur. Even if histological diagnosis is obtained using core biopsy, false positive results occasionally still occur. Both of these patients had axillary samples and wide local excisions of their disease and suffered no complication as a result of their surgery.

Of the remaining patients 70 were T1N0M0 and 11 were T2 N0M0.

75 patients had axillary sampling following SNB

6 patients had level III axillary clearance following SNB

Patient age ranged from 31-80 years.

Tumour size ranged from 5-40mm radiologically and from 7-40mm histologically.

3.1.2 Methods

Inclusion criteria were: age 18-80 years, presence of invasive breast cancer proven by cytology or histology, having an axillary procedure as part of the routine treatment of the patient's disease, patient's informed consent in writing.

Exclusion criteria were in-situ breast cancer, clinically involved lymph nodes, previous breast cancer in the same breast, multifocal disease, locally advanced disease, pregnancy, previous axillary surgery, previous or existing limb swelling or lymphoedema of the ipsilateral arm and history of allergy to human serum albumin or patent blue V dye.

i. Patient recruitment

Patients were chosen by consultants at the multidisciplinary meeting. Patients were listed for a standard axillary procedure of either axillary node sample or axillary node clearance according to the existing unit protocols plus sentinel node biopsy according to the entry and exclusion criteria above.

Suitable patients were counselled during a 20-30 minute consultation, which included a thorough explanation of the standard surgical procedure in addition to the technique of sentinel node biopsy. Patients were given ample opportunity to ask questions during and at the end of the consultation. A patient information sheet was given to any patients who were interested in taking part following the consultation. Patients were asked to return the signed consent form by mail if they agreed to take part in the trial. Once consent was obtained isotope was ordered from the nuclear medicine department for preoperative injection.

ii. Preoperative assessment

A preoperative assessment of all patients was made and the following features were documented:

Patient's height and weight

Side and site of the primary tumour

Clinical and radiological size of the tumour

Palpable or impalpable lesion

Screen detected or self-referred breast cancer

Mammographic abnormality if present

3.2 ARM MORBIDITY

All patients had preoperative arm measurements documented as a reference point for post-operative arm morbidity. The following measurements were made:

3.2.1 Arm volume

Right and left arm volume in mls.

Measurements for arm circumference were taken using a tape measure at 4cm intervals along the length of the arm starting at the wrist 20cm from the nail fold of the middle finger and ending at the junction of the arm and the axilla or as close to this point as possible. All values were entered into the programmable calculator ®Lymcalc 1.0 supplied by Colibri Software Systems UK which uses a mathematical equation to calculate the limb volume from your circumferential measurements. This calculator is designed specifically for use by healthcare personnel in calculation of lymphoedema.

Arm volume measurements are illustrated in figs 3.1 and 3.2.

Figure 3.1 Assessment of arm volume. Serial arm circumferences are recorded starting 20cm proximal to the nailfold of the middle finger and then every 4cm up to the axilla

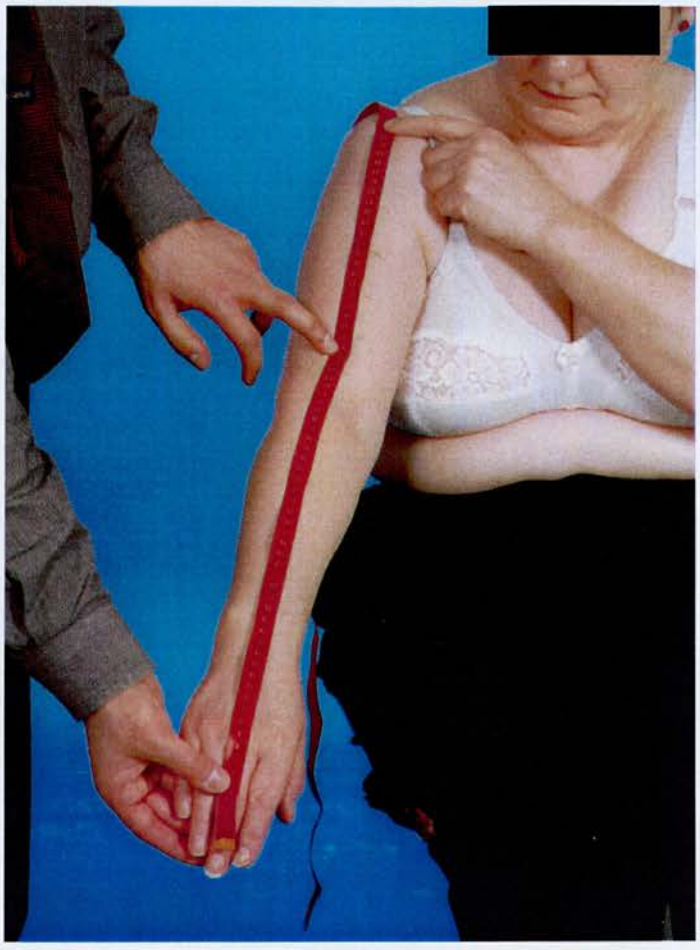


Figure 3.2 Measurement of arm circumference

3.2.2 *Shoulder joint mobility*

Shoulder movements of flexion, abduction, and internal and external rotation. Measurements were taken using a standard large-joint orthopaedic goniometer. The table below and figures 3.3-3.6 describe and illustrate the methods used.

Movement	Plane	Axis	Range
Flexion	Sagittal	½ way acromion-axilla	160-180
Abduction	Coronal	Axilla	160-180
External rotation	90°shoulder abduction/90°elbow flexion forearm horizontal move in sagittal plane	Olecranon	90-100
Internal rotation	90°shoulder abduction/90°elbow flexion forearm horizontal move in sagittal plane	Olecranon	40-60

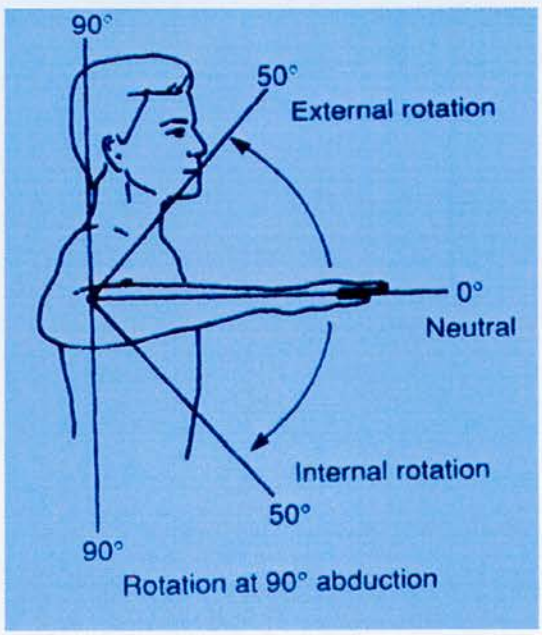


Figure 3.3

Measurement of arm rotation

Figure 3.4 Measurement of shoulder flexion

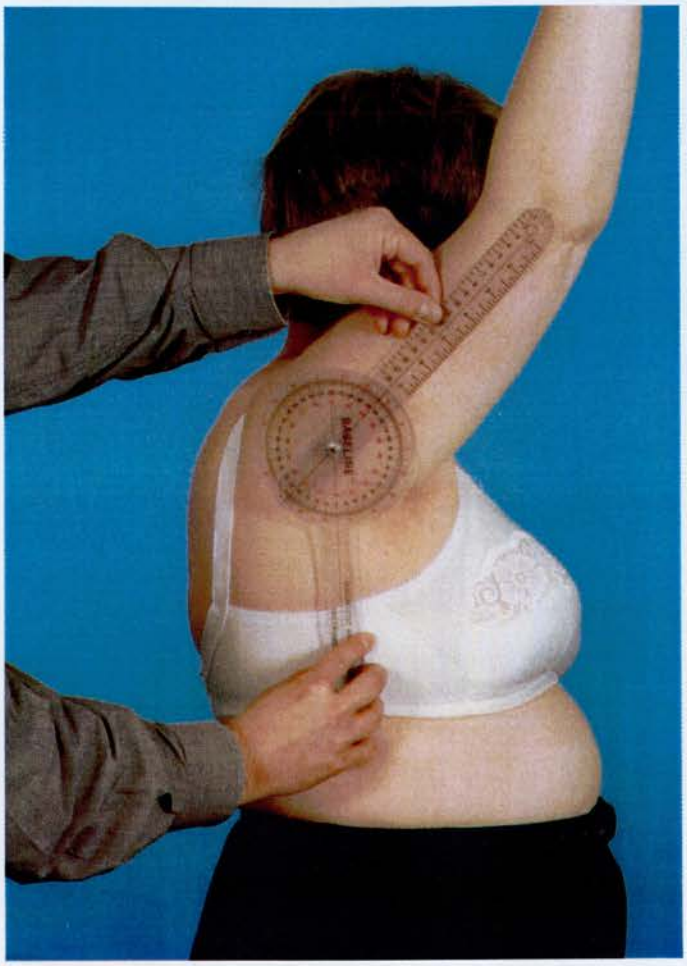


Figure 3.5 Measurement of shoulder abduction

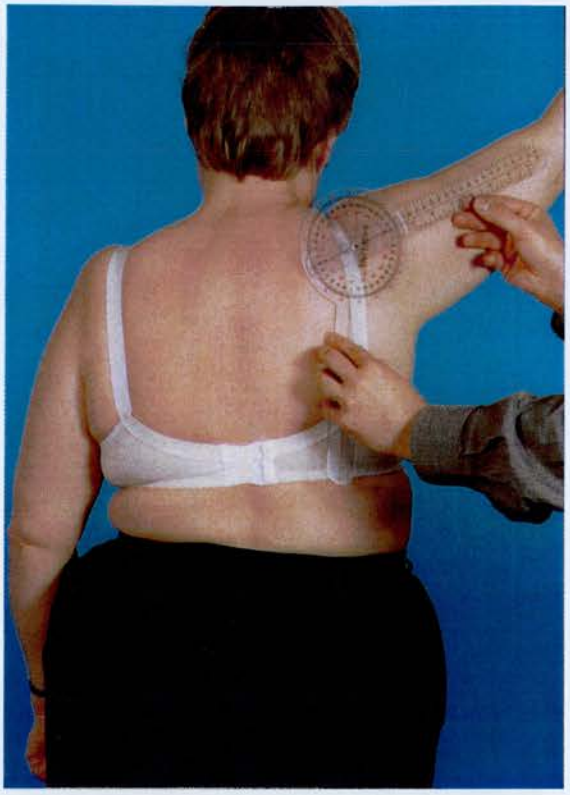
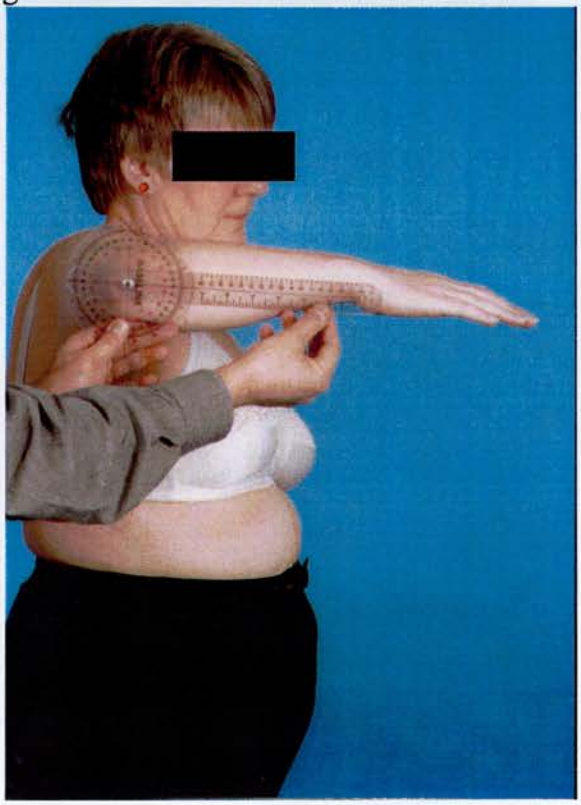


Figure 3.6 Measurement of shoulder rotation

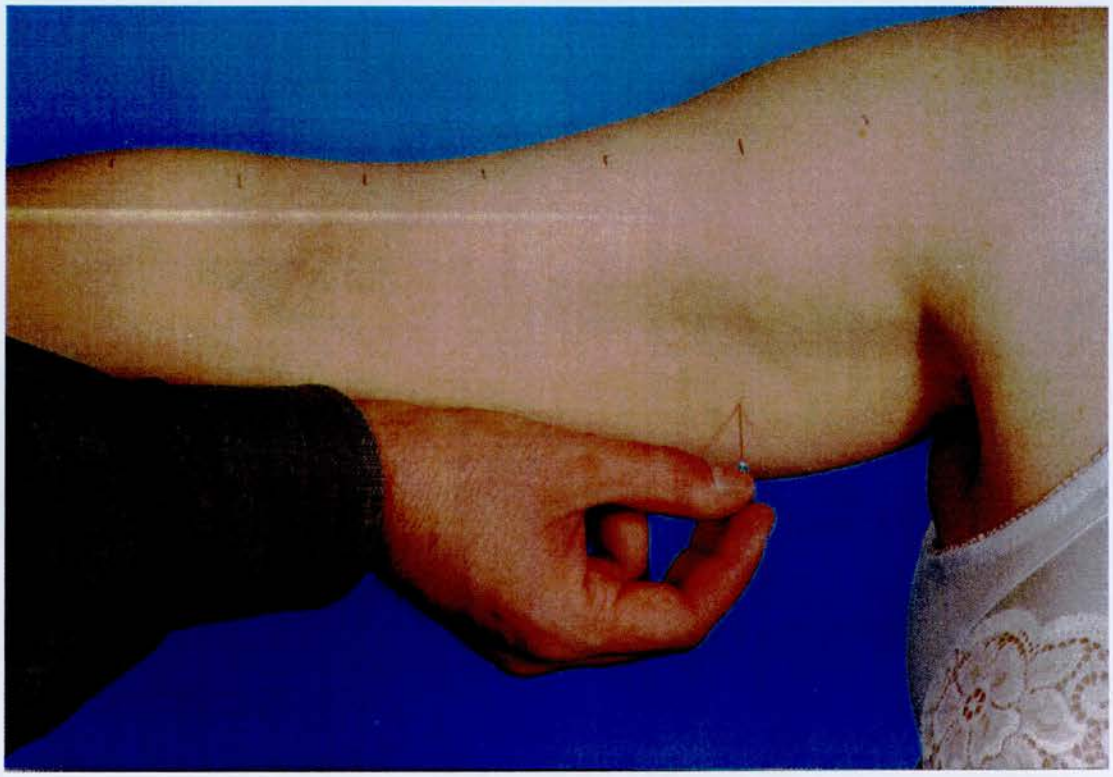


3.2.3 Arm sensation

Arm and axilla sensation to pin-prick (fig 3.7) and light touch.

A 23 gauge needle and a piece of cotton wool were used to test pin-prick and light touch respectively. All dermatomes of the upper limb were tested and any defect carefully mapped and recorded as area in square centimetres.

Figure 3.7 Assessment of arm sensation to pin-prick



3.3 QUALITY OF LIFE ASSESSMENT

Patients were asked to fill in a baseline quality of life questionnaire as part of a side study carried out by the CRC Psychosocial Oncology Group, Department of Oncology, University College London Medical School. Further questionnaires were mailed to patients at one and three months post-operatively and returned to University College London.
This study is not part of this thesis and its results will not be be discussed here.

3.4 SENTINEL NODE BIOPSY TECHNIQUE

A combined lymphatic mapping technique is performed using human colloidal albumin radiolabelled with the isotope Technetium 99M (® Nanocoll) and Patent Blue V dye (Laboratoire Guerbet, 16-24 rue Jean-Chaptal, 93600 Aulnay-sous-Bois, France).
Both agents are injected peritumorally at the 3, 6, 9 and 12 o'clock positions around the primary breast cancer in equal proportions.

3.4.1 Injection of Isotope

The nanocoll (£35 per dose) is injected in the nuclear medicine department usually on the morning of surgery for afternoon operations or in the afternoon the day before surgery for morning operations. A dose of 20MBq is used for same day injections or 40MBq for previous day injections. A volume of 2mls is used in each case, 0.5mls of the isotope is injected in each area as described above. The time of injection is recorded and the patient returned to the ward.

3.4.2 Scintiscan

Two scintiscans of 10 minutes duration each are performed approximately 3 hours after injection of isotope (fig 3.8). A straight anteroposterior (AP) scan is performed and an oblique lateral at 30-60° from vertical. Two images are thus obtained showing the injection site and in the majority of cases the sentinel node or nodes (fig 3.9). The location of the sentinel node is marked on the axillary skin in the nuclear medicine department to help guide the surgeon to the best site for the axillary skin incision (fig 3.10). Following scintiscan the patient is sent to the operating theatre.

Figure 3.8 Scintiscanner in the nuclear medicine department

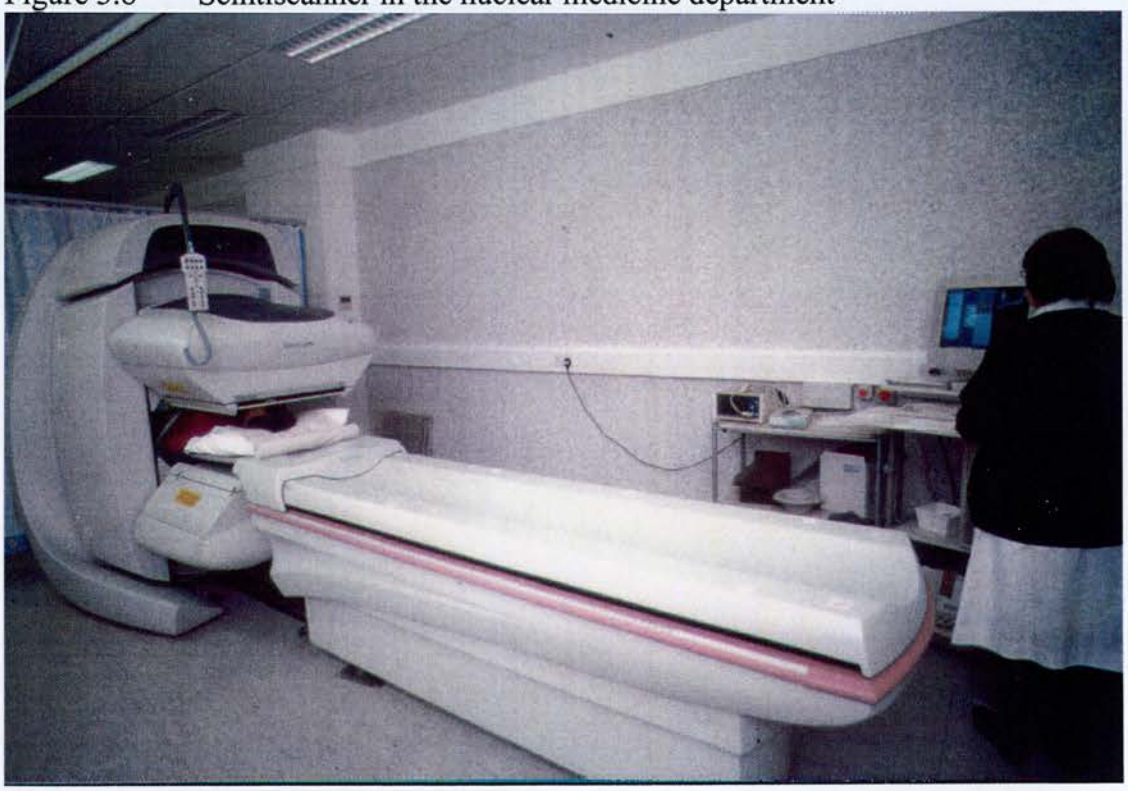


Figure 3.9 Scintiscan showing a single sentinel node in the axilla of a breast cancer patient

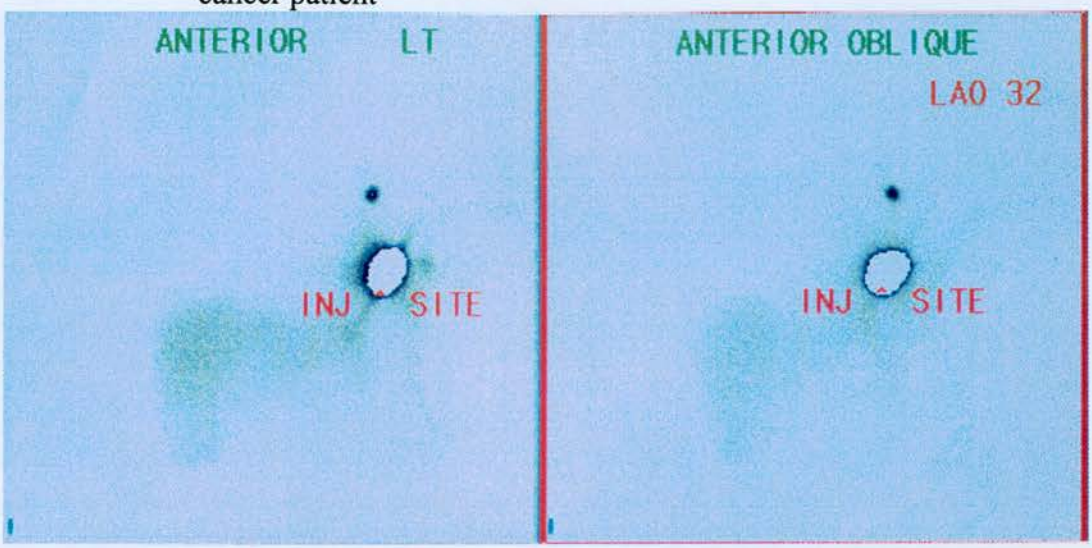
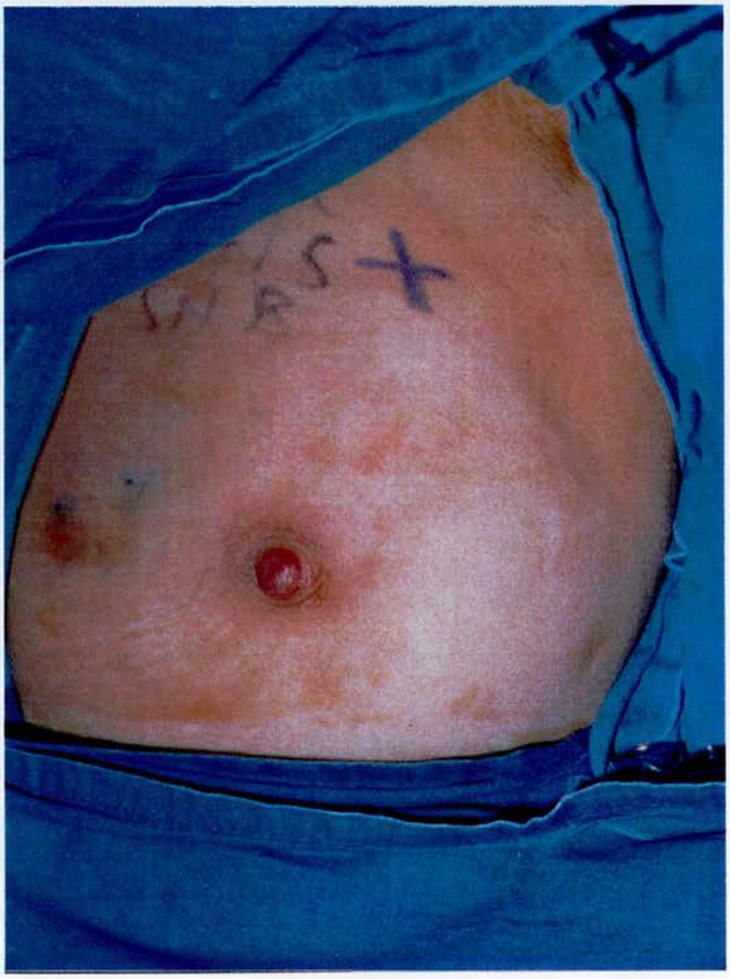


Figure 3.10 X-marking a sentinel node identified at lymphoscintigraphy



3.4.3 Injection of Blue dye

A single vial containing 2mls Patent blue V dye (fig 3.11) is drawn up into a 5ml syringe using a green (21 gauge) needle. A further 3mls of sterile normal saline solution is also drawn up into the same syringe to dilute the dye to 5mls (fig 3.12). A quarter volume (1.25mls) of this fluid is injected using a blue (23 gauge) needle into each of the four locations peritumorally as described above approximately five minutes before the skin incision is made (fig 3.13). The area of injection is gently massaged concentrically towards the ipsilateral axilla to disperse the dye into the lymphatic vessels (fig 3.14).

Figure 3.11 Vials of Patent Blue V (Laboratoires Guerbet, Aulnay-sous-Bois, France)

Figure 3.12 Patent blue V diluted from 2mls to 5mls using 0.9% sodium chloride Solution

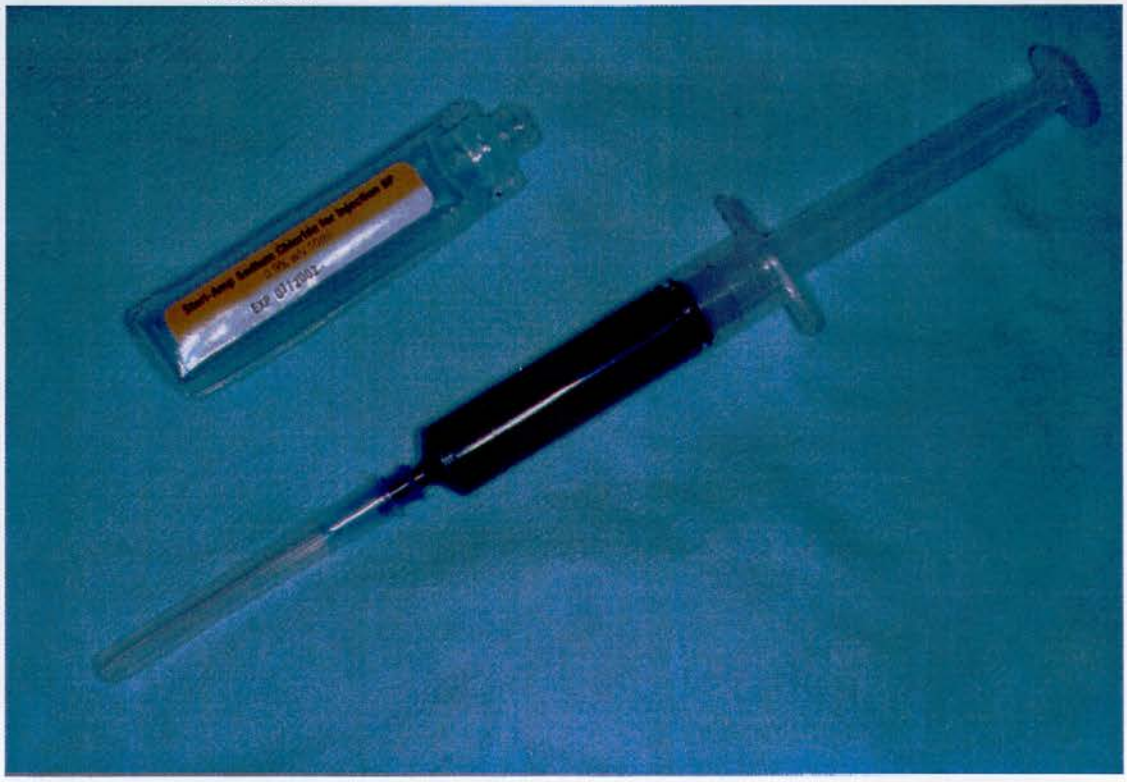


Figure 3.13 Peritumoural injection of Patent Blue V dye

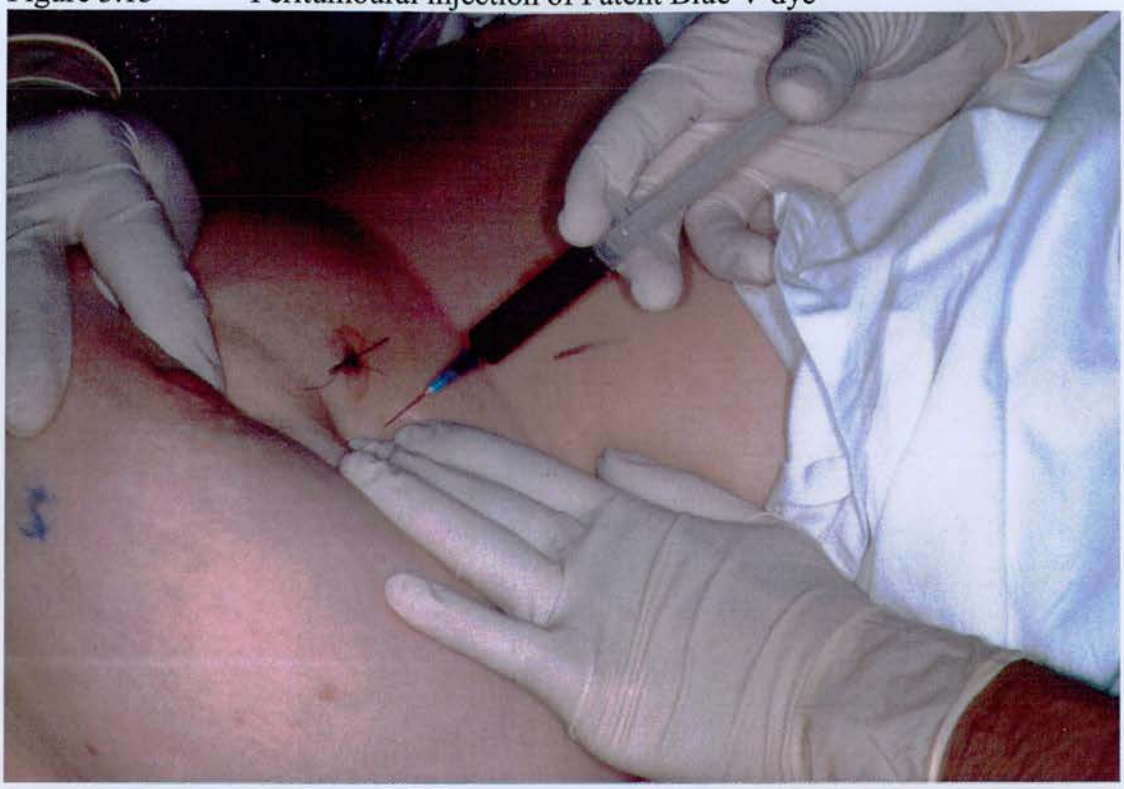
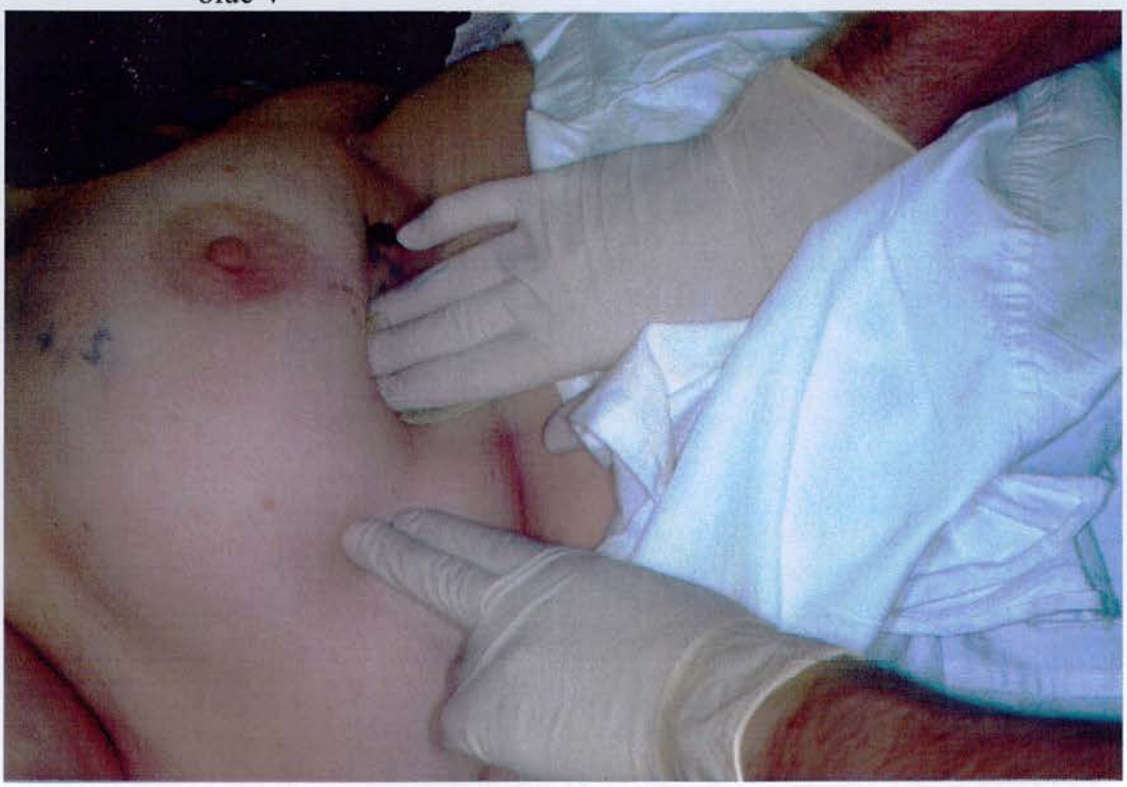


Figure 3.14 Massaging peritumourally immediately following injection of patent blue V



3.4.4 Surgical Technique

Prior to incision a 10-second background radiation count is taken from the ipsilateral upper arm using the gamma (®Navigator) probe (Navigator Gamma Guidance System, by RMD for the United States Surgical Corporation, 44 Hunt Street, Watertown, MA, USA.); figs 3.15-3.16. The gamma probe is also used to locate the maximal radioactivity in the axilla representing the sentinel node location (fig 3.17). The surgeon can then decide on the best incision site for retrieval of the sentinel node. The time of the axillary skin incision is noted.

The preferred technique is to make the axillary incision first. A standard curvilinear skin incision is made in an oblique/transverse orientation just below the hair-bearing skin of the axilla. The wound is carefully opened through the fat layer and clavipectoral fascia to expose the axillary fat and axillary lymph nodes. As soon as a blue lymphatic is seen, it is followed using careful sharp and blunt dissection to its draining lymph node, which is stained blue by the dye. The lymph node is mobilised either digitally or using tissue forceps to retract it forwards on its pedicle taking care not to crush the node itself. A 10 second count is taken using the gamma (®navigator) probe and this is recorded. The node is excised by careful diathermy of its blood and lymphatic supply or by using artery forceps and hand tied vicryl ligatures to secure its blood supply prior to excision. A further 10-second count is made outside the patient at a distance from the injection site to prevent contamination of the recording.

The lymph node is then labelled separately, fixed in formalin and sent for histopathology.

If there is no visible blue lymphatic, then radioactivity is sought using the gamma probe. When an area of increased activity is found, the surgeon explores the area to identify separate lymph nodes, which are then counted individually for 10 seconds as above to confirm the presence of a sentinel node or nodes. Any nodes with a ten second count greater than ten times the previously measured background count qualify as a sentinel node whether or not they contain blue dye and they are described as being 'hot'. They are removed, counted again for ten seconds outside the patient and sent separately for histopathology. A sentinel node must contain blue

dye alone, radioactivity of greater than ten times background count alone or both blue dye and radioactivity to qualify as a sentinel node. The process is repeated until all 'hot' and/or blue sentinel nodes are removed.
If the primary breast cancer is sited very close to the axilla in the upper outer quadrant of the breast or in its axillary tail, an alternative method is used excising the primary tumour first followed by sentinel node biopsy. This removes the radioactivity contained around the primary tumour and allows accurate readings to be taken of sentinel node counts that would otherwise be contaminated by the close proximity of the tumour.

Following sentinel node biopsy, the remaining axillary procedure is completed i.e axillary node sample or axillary node clearance and the primary tumour is then excised. Wound closure is performed according to the standard practice of the surgeon but usually involves a two-layer closure with an absorbable monofilament suture such as Dexon®, Monocryl® or PDS®. Two consultant breast surgeons performed all operations in phase one of the trial. A 90% sentinel node identification rate and a false negative rate of less than 10% were required to progress to phase two.

Figure 3.15 Navigator Gamma Guidance System, by RMD for the United States Surgical Corporation

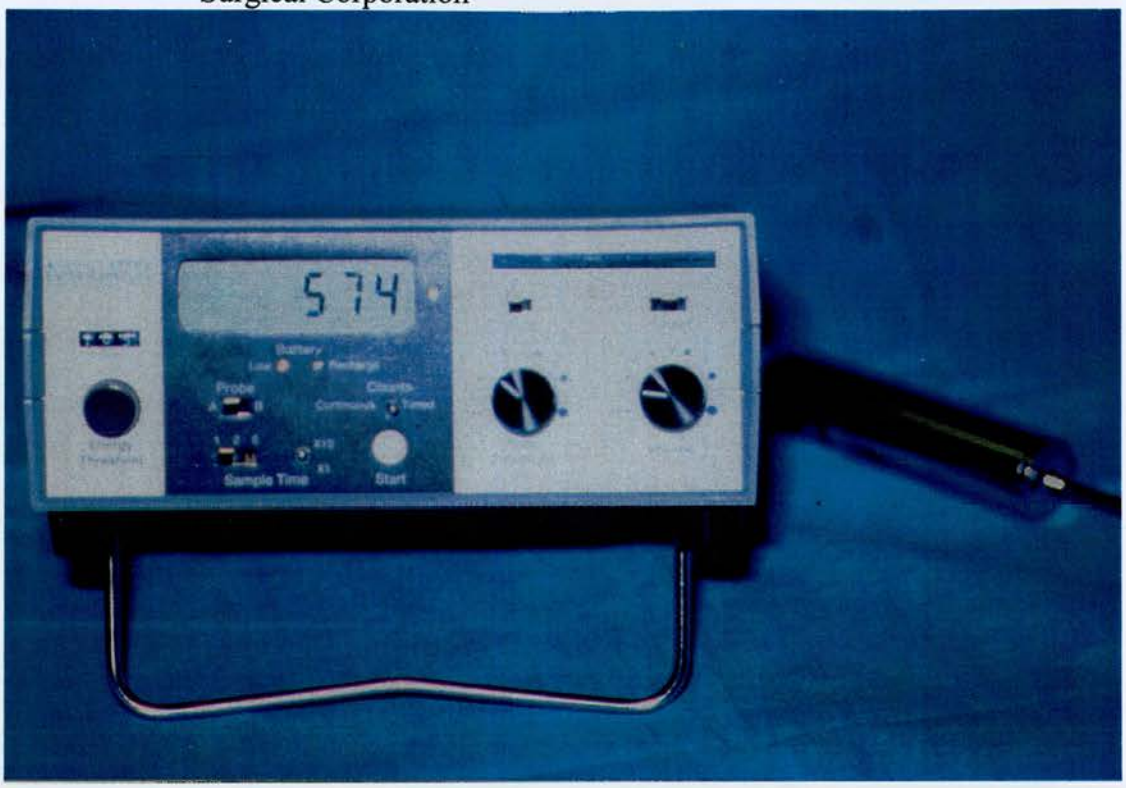


Figure 3.16 Gamma probe (®Navigator)

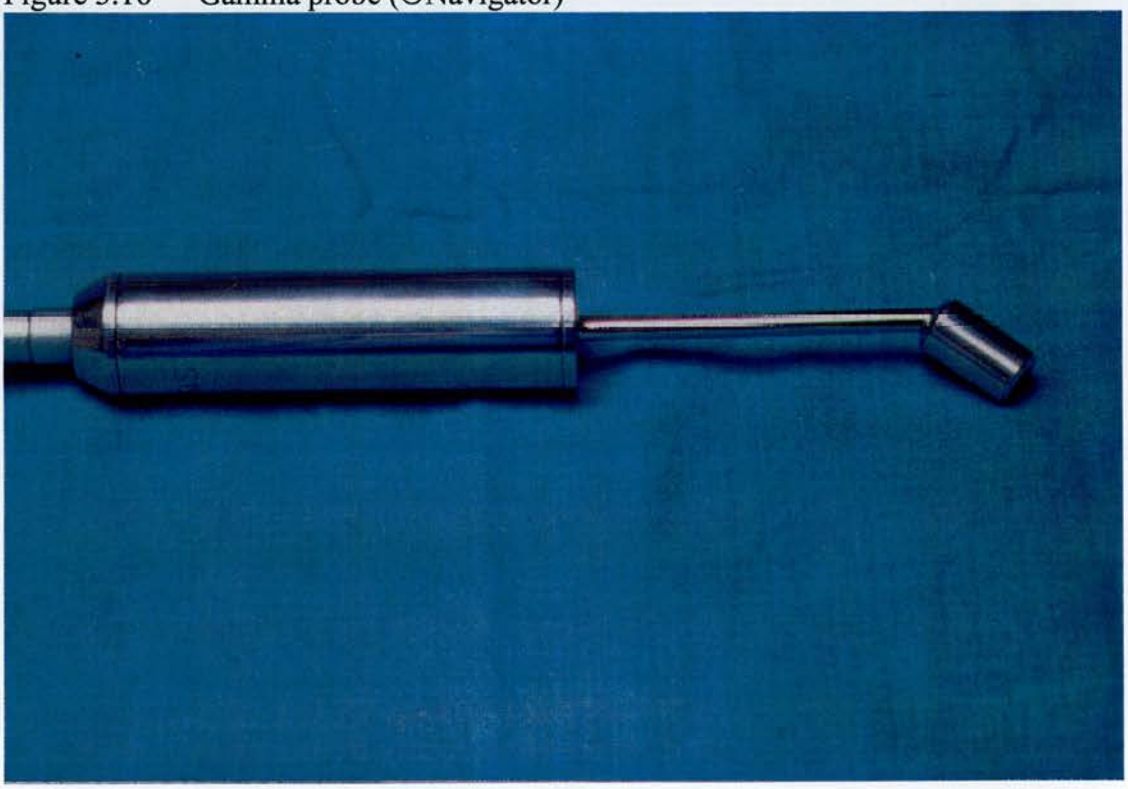
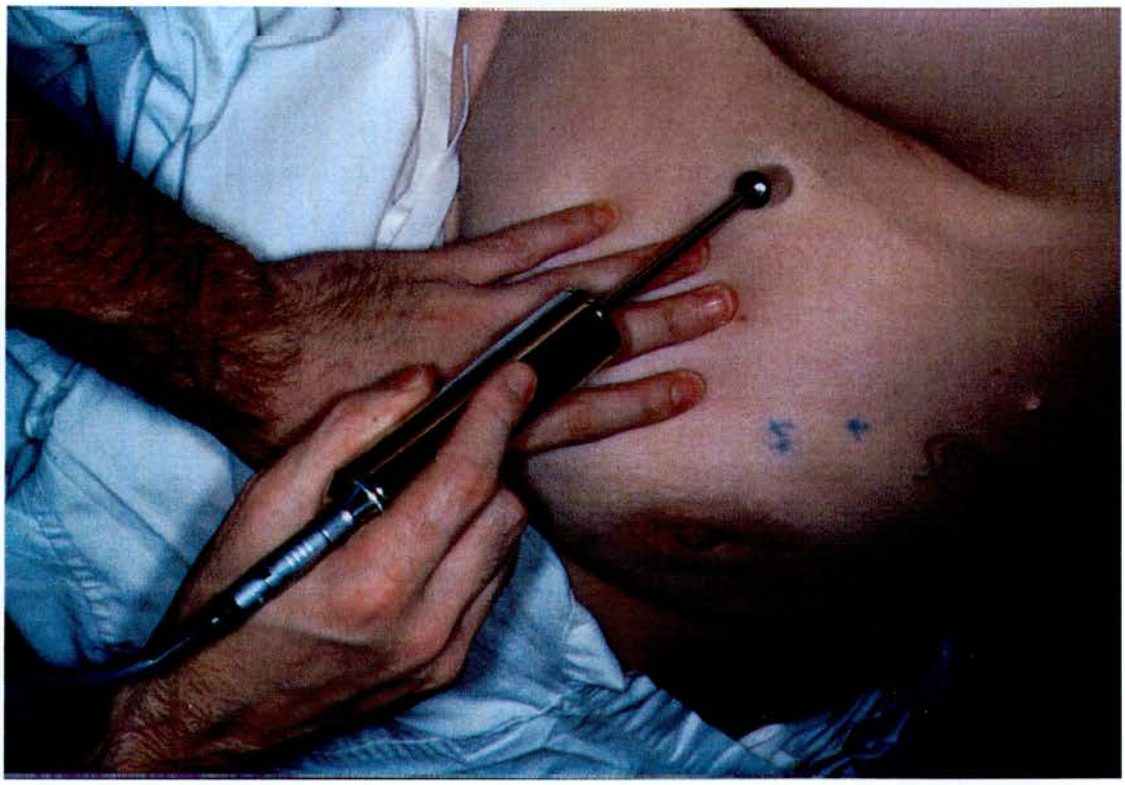


Figure 3.17 Search for sentinel node(s) using the gamma probe prior to axillary Incision



3.4.5 Post operative care

Patients were informed of the risk that their urine may be discoloured by the blue dye for 24 hours post-operatively.
Patients were seen on the ward and educated on arm exercises to reduce shoulder stiffness following discharge.
Any immediate post-operative complications were documented recording in particular haematoma, seroma or infection of the breast or axilla, pneumothorax or dye sensitivity.

3.4.6 Pathology

Pathology results from the surgical specimens were followed up and recorded for each patient including histological type, presence or absence of associated DCIS, special features, grade, lymphovascular invasion, oestrogen receptor status and presence or absence of metastatic disease in each lymph node specimen.

3.4.7 Follow up

Patients were seen at 1month and 3 months when their breast and axilla was examined and further measurements were taken to assess arm morbidity.
Any further complications, surgical procedures or extra visits to their GP, practice nurse or to the hospital were documented at this time.

3.5 RESULTS

3.5.1 Identification Rate

At least one sentinel node was identified in 79 (97.5%) of 81 patients (fig 3.18).
An average of 2.76 sentinel nodes were identified for each patient in whom a sentinel node was identified
In 2 patients (2.5%), a sentinel node could not be found.
Of these, one patient had macroscopic tumour in the axillary lymph nodes visible at surgery. The other patient was node negative but a sentinel node could not be found.

i. Blue nodes

178 blue nodes were found in 74 patients.
A mean of 2.2 blue nodes were found in each patient.
A blue staining lymph node was found in 91.4 % of patients.

ii. Hot nodes

202 hot nodes were found in 72 patients.
A mean of 2.5 hot nodes were found in each patient.
A 'hot' lymph node was found in 88.9% of patients.

The detection rate was 97.5% with both blue dye and nanocoll in combination.

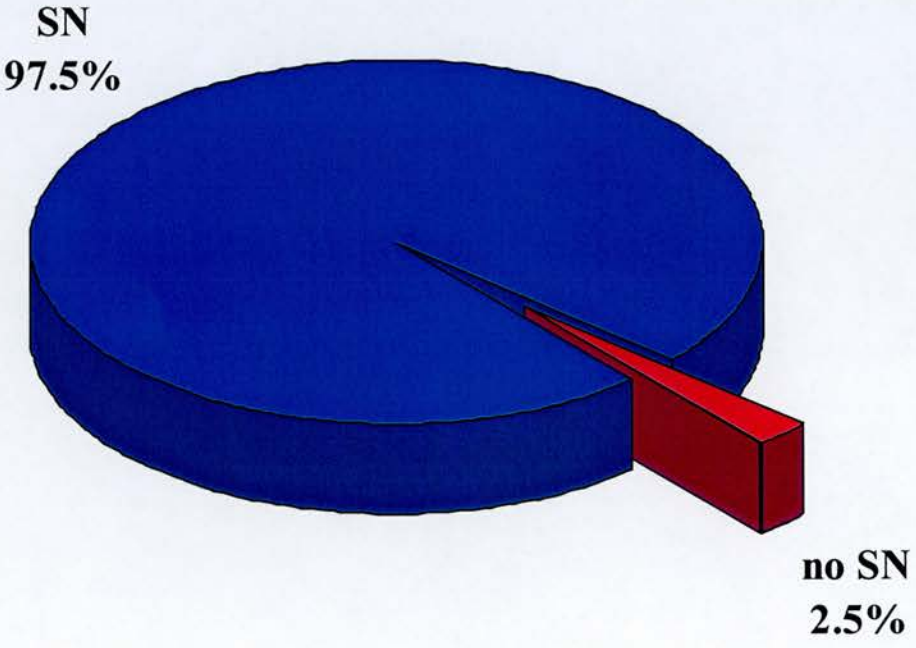
iii. Node positivity

Positive lymph nodes were found in 26.6% (21 of 79) of patients and in 76% (16) of these the sentinel lymph node(s) was/were the only positive node(s).

iv. False negative sentinel nodes

There were no falsely negative sentinel nodes found, i.e. false negative rate 0%.
A sentinel node(s) is defined as falsely negative when routine histology finds it to be negative in the presence of one or more positive non-sentinel lymph nodes from the same patient.

Figure 3.18 Sentinel node identification rate



3.5.2 Scintigraphy

Scintiscans showed 'hot' lymph nodes in 77.8% (63 of 81) of patients with a mean of 1.47 'hot' nodes per scan. A range of 0 to 5 nodes was seen on scintiscans. More 'hot nodes were found at surgery, which suggests either greater sensitivity of the Navigator probe or obstruction of some 'hot' nodes by tumour radioactivity on scan imaging. Figs 3.19-3.23 illustrate the different types of scan pattern, which can be expected.

Figure 3.19 Scintiscan showing simultaneous hot internal mammary lymph node and hot axillary node

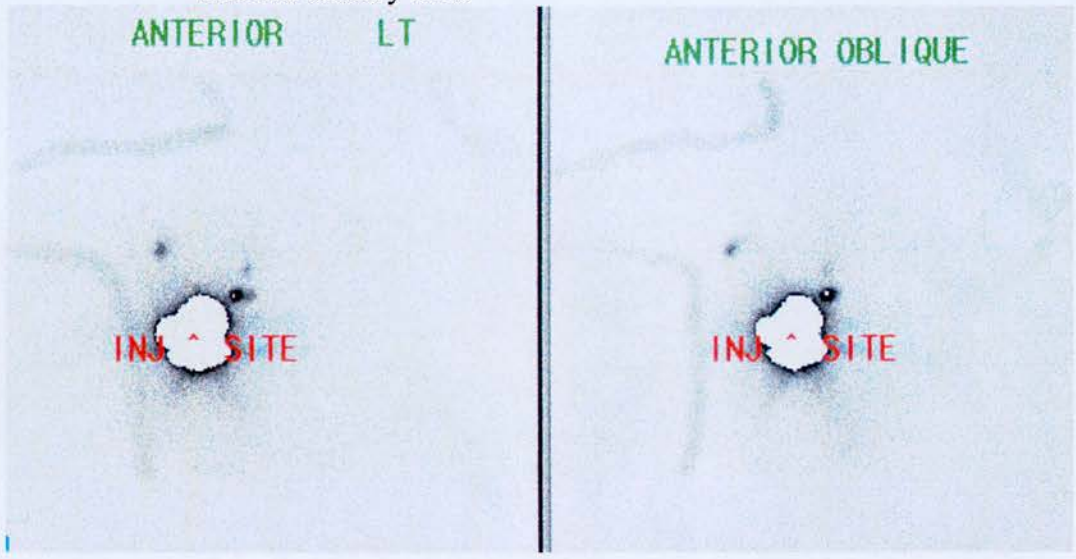


Figure 3.20 Scintiscan showing multiple hot nodes demonstrating 'spill' of tracer

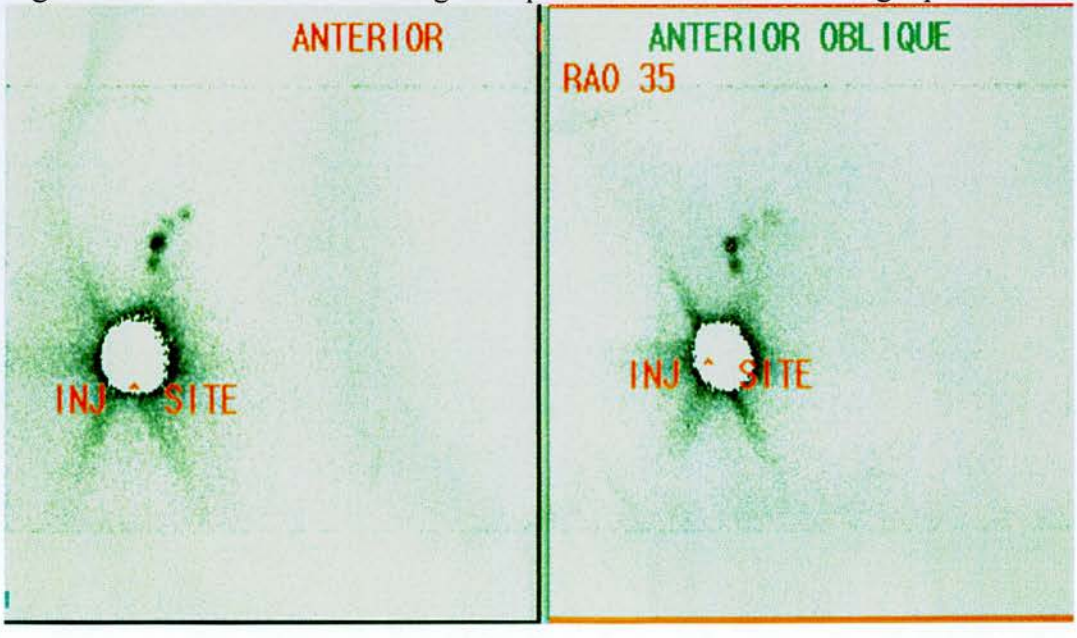


Figure 3.21 Negative Scintiscan

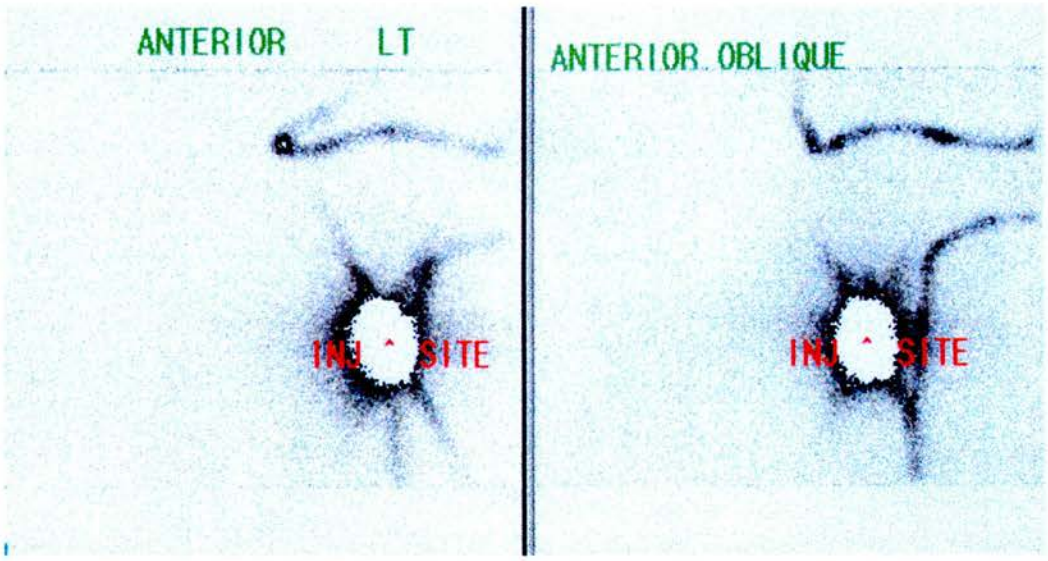


Figure 3.22 Scintiscan of axillary lymph node with laterally sited tumour

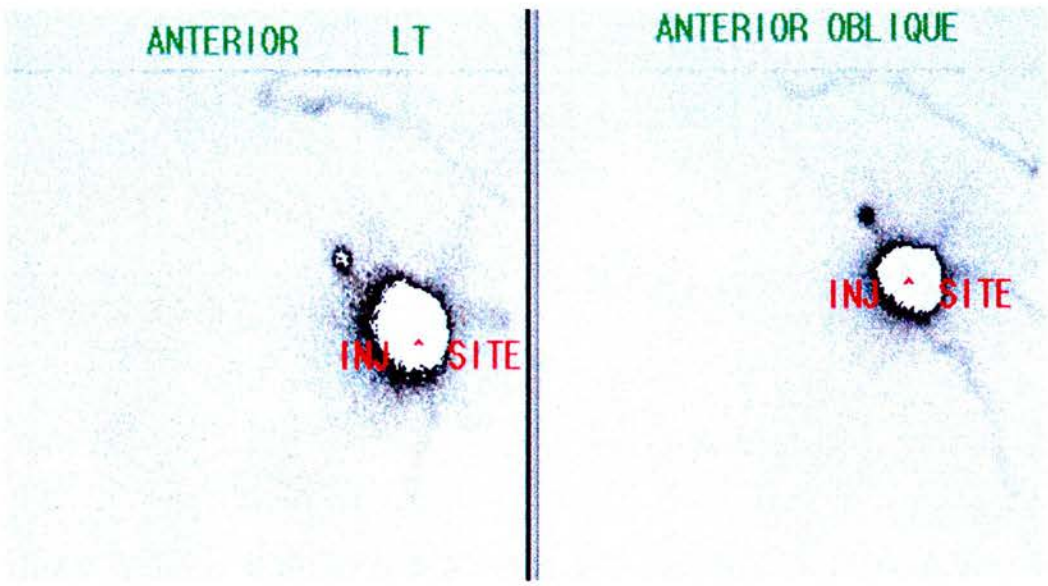
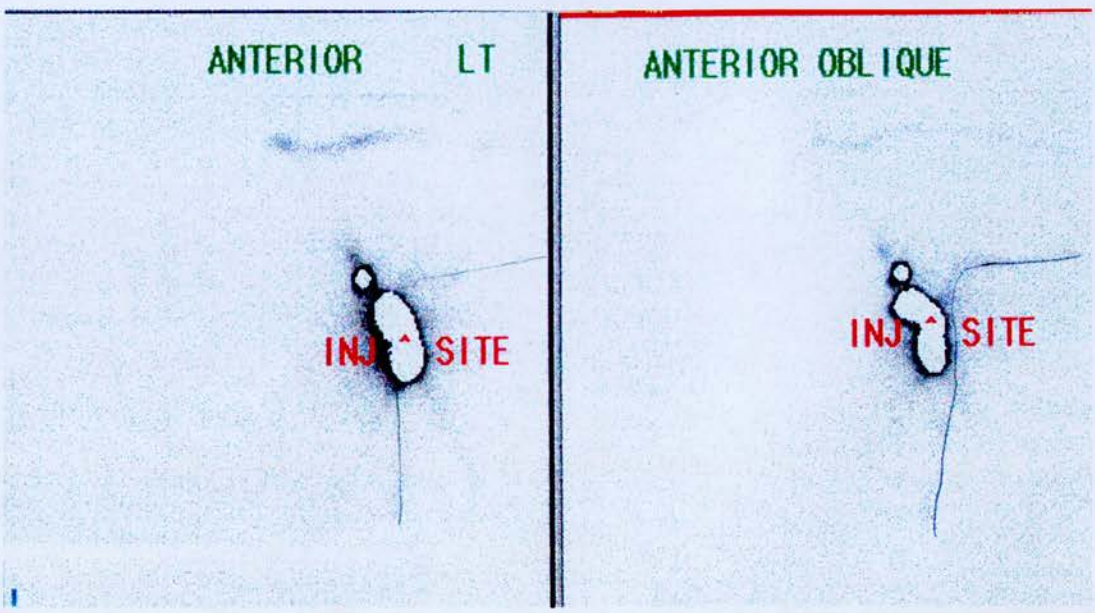


Figure 3.23 Scintiscan of a patient in whom nine sentinel nodes were identified at surgery showing lateral position of primary tumour with axillary lymph node and 'spill' of tracer to a higher echelon axillary node



3.5.3 *Internal mammary nodes*

Seven patients (8.6%) had internal mammary nodes as sentinel nodes on scintiscan. In two of these the internal mammary node was the only sentinel node. None of the internal mammary nodes was positive on histology.

Table 3.1 summarises these figures:

Table 3.1

Number of SNs	2.76 nodes (mean)
Positive nodes	26.6% (SN alone in 76%)
Blue nodes	91.4% of patients
Hot nodes	88.9% of patients
Scintiscans	77.8% (mean 1.47 nodes per scan)
Internal Mammary	8.6% (8 nodes in 7 patients)

3.5.4 Complications

A post-operative complication was encountered in 19 patients (24%). Of these, six patients had more than one complication.

Seroma of the breast was the commonest complication affecting 11 women (13.6%). Breast wound infection was the second commonest complication in 4 (5%) patients, followed by axillary wound infection and breast haematoma requiring incision and drainage in 3 (3.7%) patients each. Table 3.2 summarises these and the less common complications with actual patient numbers in parentheses.

Table 3.2

Breast seroma	13.6%(11)
Breast wound infection	5%(4)
Axillary wound infection	3.7%(3)
Breast haematoma requiring incision and drainage	3.7%(3)
Axillary seroma	2.5%(2)
Pneumothorax	1.2%(1)
Breast infection requiring incision and drainage	1.2%(1)
Blue dye sensitivity	1.2%(1)
Breast haematoma treated conservatively	1.2%(1)
Axilla haematoma treated conservatively	1.2%(1)

i. Blue dye tattooing

A number of patients were left with blue discolouration on the skin of the breast from the patent blue injections.

Twenty-five patients (30.9%) showed tattooing at 1 month post-op.

Nineteen patients (24.4%) still showed tattooing at 3 months post-op. See Table 3.3 below. Figs 3.24-3.27 illustrate some problems with blue dye tattooing.

Table 3.3

	Number of patients with tattooing/ total number of patients	% of patients with tattooing
1 month	25/81	30.9%
3 months	19/78	24.4%

Figure 3.24 Blue tattooing of breast skin adjacent to lumpectomy scar

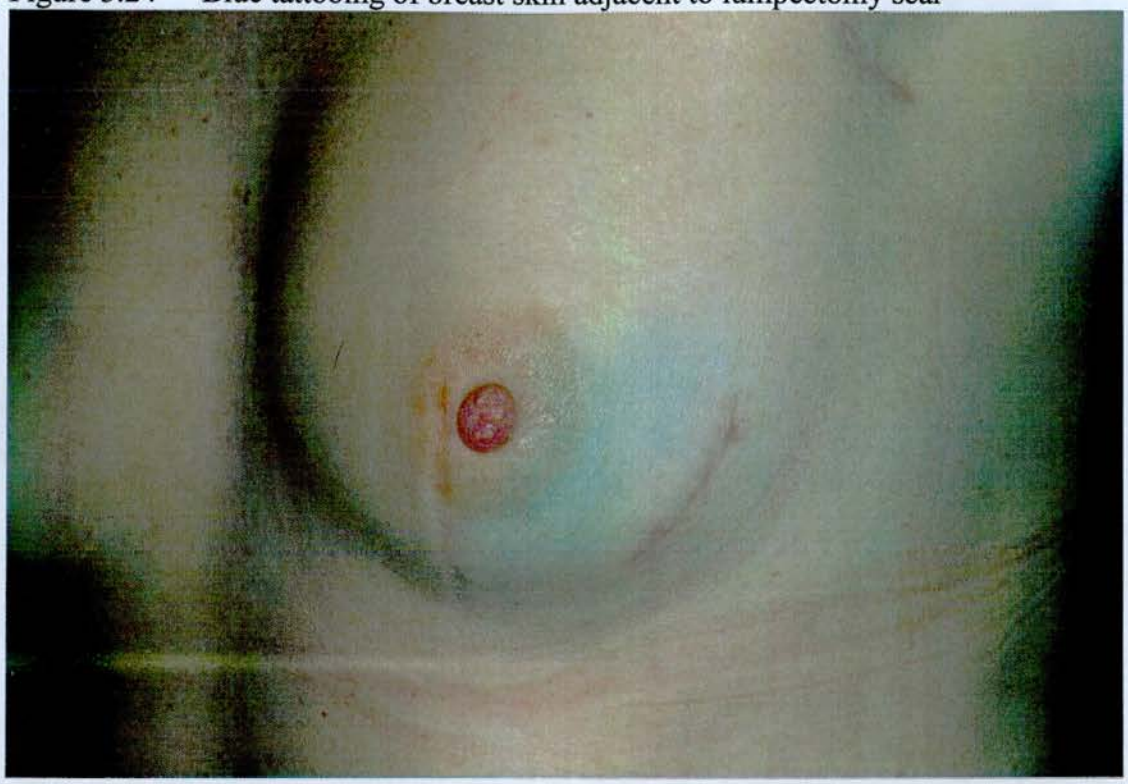
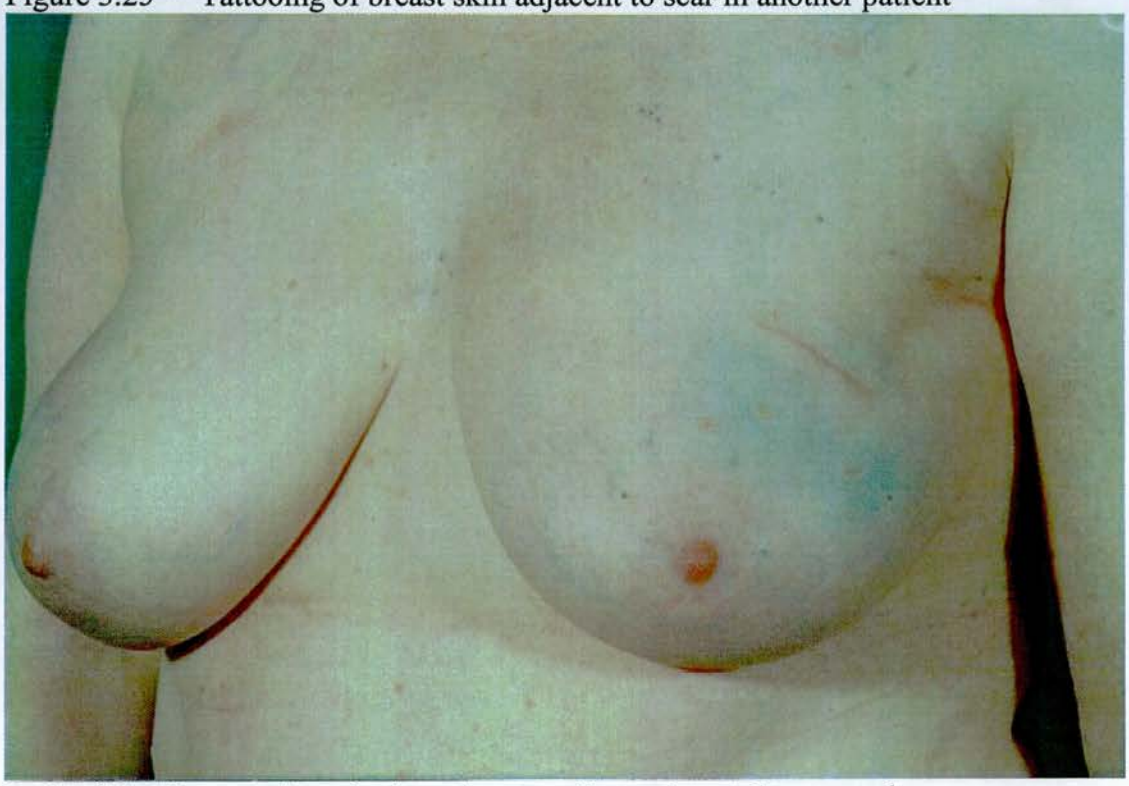


Figure 3.25 Tattooing of breast skin adjacent to scar in another patient



Both of the above patients had persistence of tattooing at three months.

Figure 3.26 Gross blue staining of the breast immediately post-operatively in a patient with a breast haematoma, which was drained. Staining had completely disappeared at one-month.

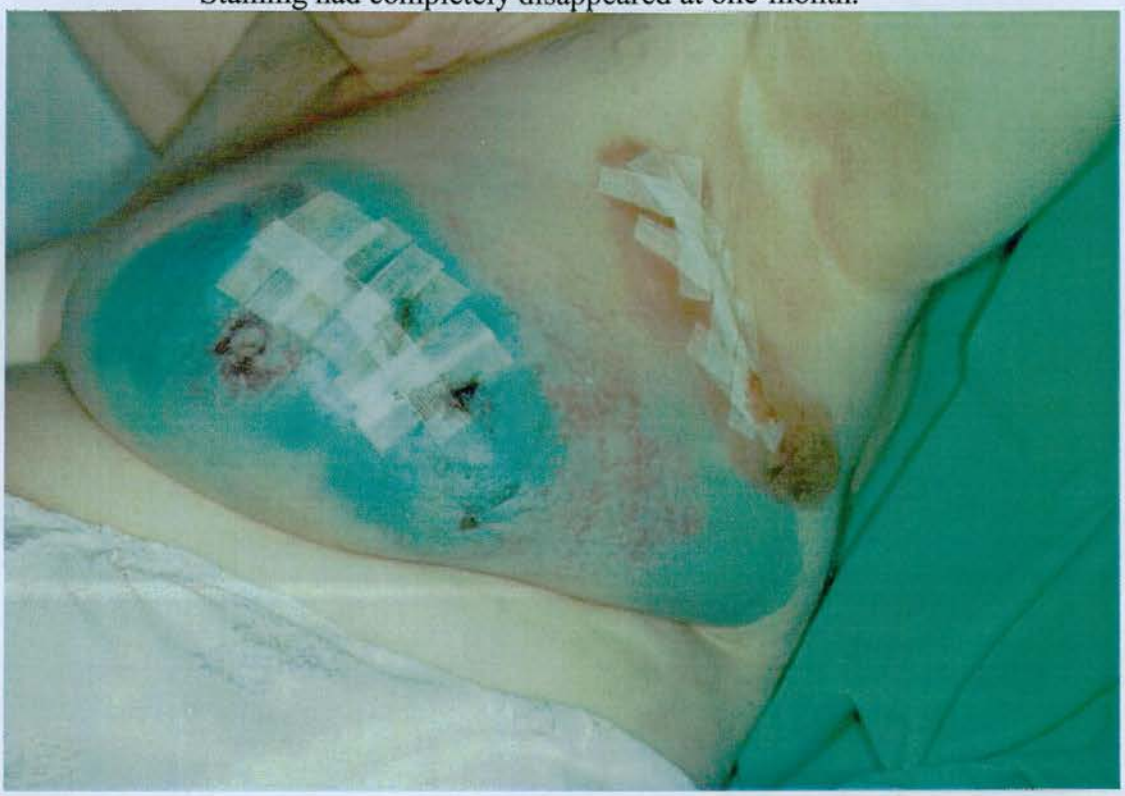
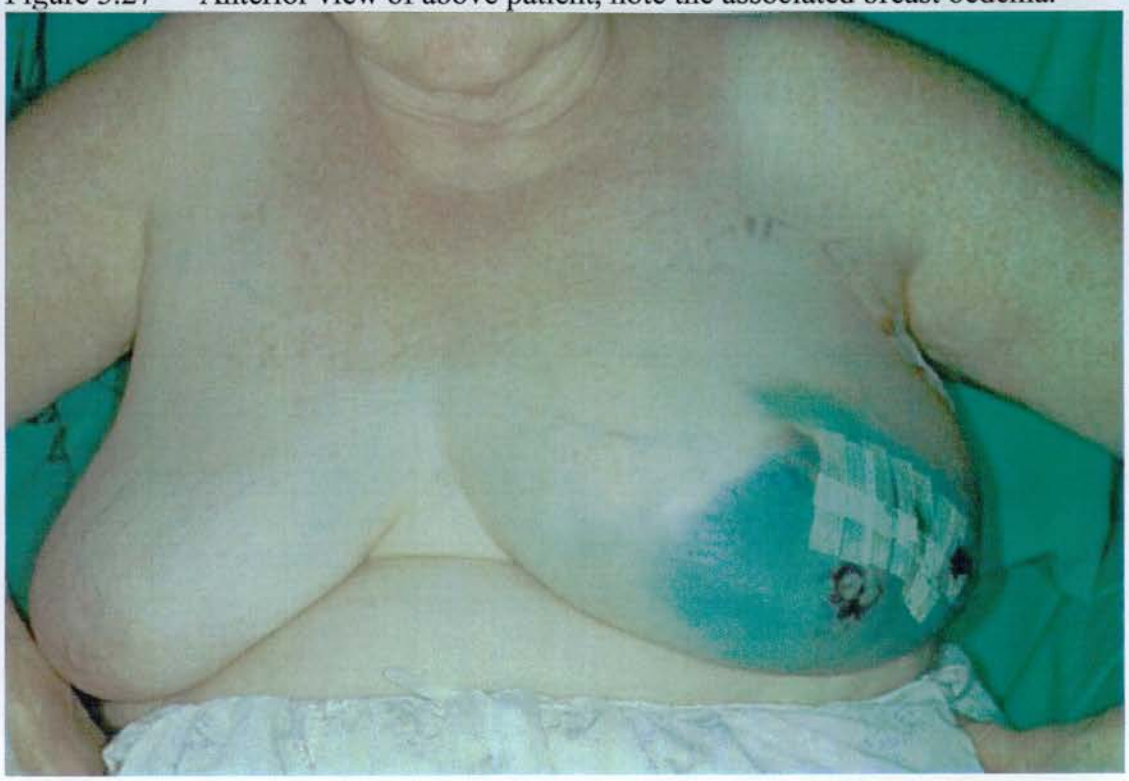


Figure 3.27 Anterior view of above patient, note the associated breast oedema.



3.5.5 *Arm morbidity*

Arm morbidity was documented according to the following criteria:

1. Any degree of sensory loss or altered sensation
2. Increase in arm volume of 100mls or more independent of the contralateral arm. This figure has been used before[122] and was chosen as opposed to the 200ml cut-off used in some studies of lymphoedema [116, 128] as it was felt necessary to detect more subtle changes in arm volume than these previous studies which have generally included more invasive procedures such as axillary clearance rather than the theoretically less invasive procedure of sentinel node biopsy.
3. Loss of 10° or more of any measured shoulder movement independent of the contralateral arm

i. Altered Sensation

At 1 month 13.6% of patients demonstrated arm sensory loss and 17.3% axillary sensory alteration. Two of these patients displayed both axillary and arm sensory alteration. In total, 28.4% of patients displayed some degree of altered sensation of the arm, axilla or both at one month post-operatively. The area of altered sensation ranged from 1x3cm $(3cm)^2$ to 12x40cm $(480cm)^2$.

At 3 months these figures dropped to 9% and 11.5% for altered arm or axillary sensation respectively. One patient displayed both axillary and arm sensory alteration. In total, 19.2% of patients displayed some degree of altered sensation of the arm, axilla or both three months post-operatively. The area of altered sensation ranged from 2x7cm ($14cm^2$) to 32x12cm ($384cm^2$).

Of the twenty-three patients with sensory alteration at one month, eleven had complete resolution of their symptoms at three months. Of the fifteen patients with sensory alteration at three months, three had normal sensation at one month and three had a smaller area of altered sensation at one month than at three months.

Sensory loss	@ 1 month	13.6% (11 of 81 patients)
(arm)	@ 3 months	9% (7 of 78 patients)
Sensory loss	@ 1 month	17.3% (14 of 81 patients)
(axilla)	@ 3 months	11.5% (9 of 78 patients)

ii. Arm swelling

Arm swelling was encountered in 9 patients (11.1%) at 1 month post-operatively and in 18 patients (23%) at 3 months post-op. These figures with ranges and percentages are shown in table 3.4 below:

Table 3.4

	1 month	3 months
Number of patients with swelling (% of total)	9(11.1%)	18(23%)
Mean % increase in arm volume	5.2%	6.4%
Range of increase in arm volume (mls)	103-196	101-388

Only one patient had an increase in arm volume of greater than 10%, with a 14.4% increase.

iii. Shoulder Stiffness

One month post-operatively, 63% (51 of 81 patients) had shoulder stiffness.
Three months post-operatively, 35.9% (28 of 78 patients) had shoulder stiffness.
Table 3.5 below shows the movements affected and the percentage of patients affected by each movement at one month and three months. The range of shoulder restriction in degrees is shown in the far-right column of the table. Figs 3.28 and 3.29 illustrate the arm morbidity from phase one of the trial.

Table 3.5

	Follow-up month	**Pt. Number**	**% of total**	**range**
flexion	1 month	27/81	33.3	10-65
	3 months	**9/78**	**11.5**	**12-33**
abduction	1 month	31/81	38.3	10-66
	3 months	**16/78**	**20.5**	**10-52**
internal rotation	1 month	17/81	21	11-48
	3 months	**5/78**	**6.4**	**14-39**
external rotation	1 month	14/81	17.3	10-25
	3 months	**10/78**	**12.8**	**10-28**

3.5.6 Tumour Characteristics

Histology

Ductal carcinoma no special type	66
Lobular carcinoma	5
Mixed ductal/lobular	2
Tubular	4
Mucinous	2
Apocrine	1
Other mixed	1

Associated features

DCIS	44
LCIS	3
LCIS and DCIS	2
Medullary features	2
Multicentric	1
Cribriform in-situ	1
No associated features	28

Lymphovascular invasion

Present	13
Absent	68

Tumour Grade

Grade 1	18
Grade 2	36
Grade 3	25
Non-gradeable	2

Pathological Tumour Size

Average tumour size	16mm
Range	7-40mm

3.5.7 Sentinel node pathology

Sentinel nodes were positive in 21 patients:

Hottest sentinel node alone was positive	9 (43%)
Hottest sentinel node plus other sentinel nodes positive	4 (19%)
Sentinel node other than the hottest was positive	5 (24%)
Unclear from pathology which sentinel node was positive	3 (14%)

3.5.8 Admission Time

Average admission duration was 2 days for patients having a node sample plus SNB and 4.7 days for patients having a node clearance plus SNB.

3.5.9 Recurrences

One patient developed a 1cm subcutaneous deposit beneath the axillary wound site and simultaneously developed pulmonary metastases.
This patient had a 20mm grade 3 ductal carcinoma of no special type with lymphovascular invasion and had a single sentinel node positive. She received adjuvant radiotherapy to the breast and axilla and was systemically treated with tamoxifen and chemotherapy.

Figure 3.28 Arm morbidity

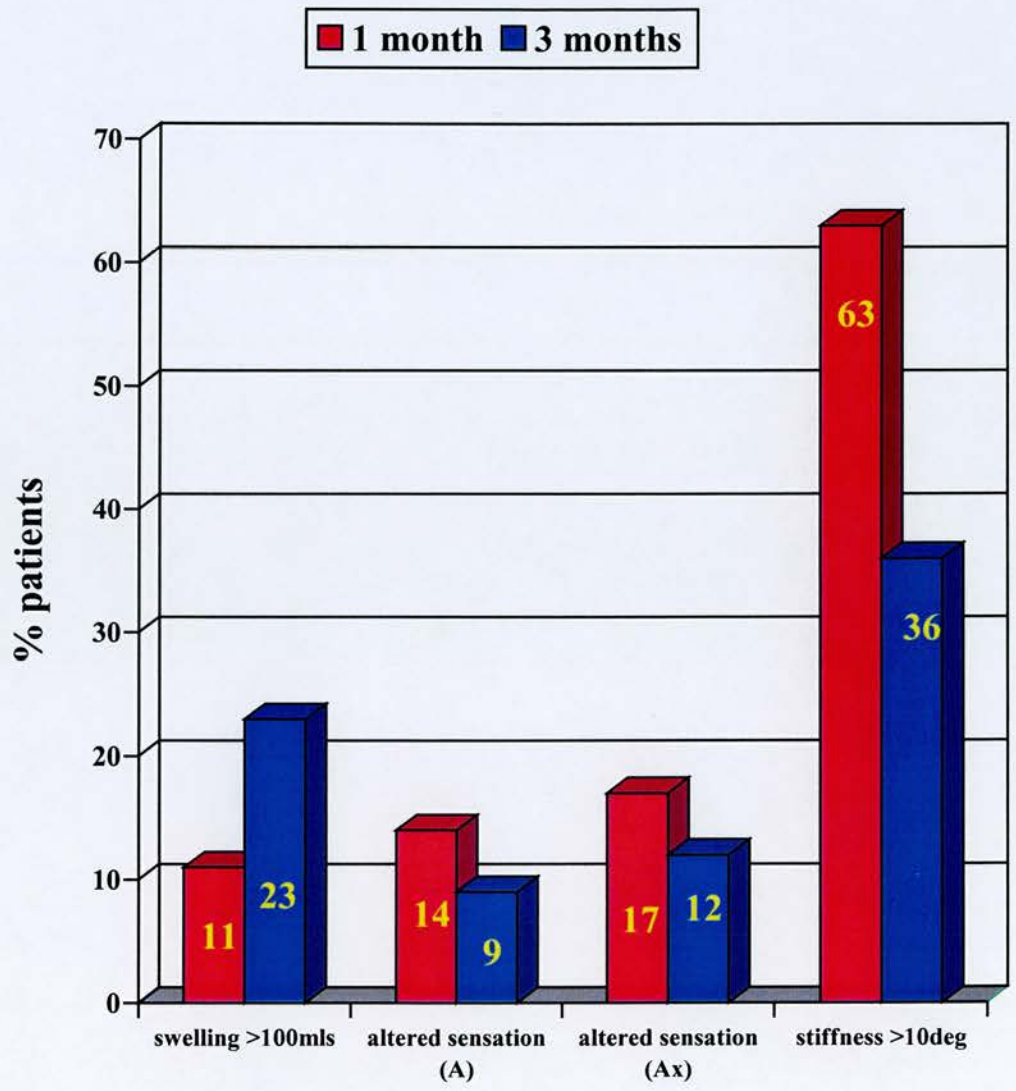
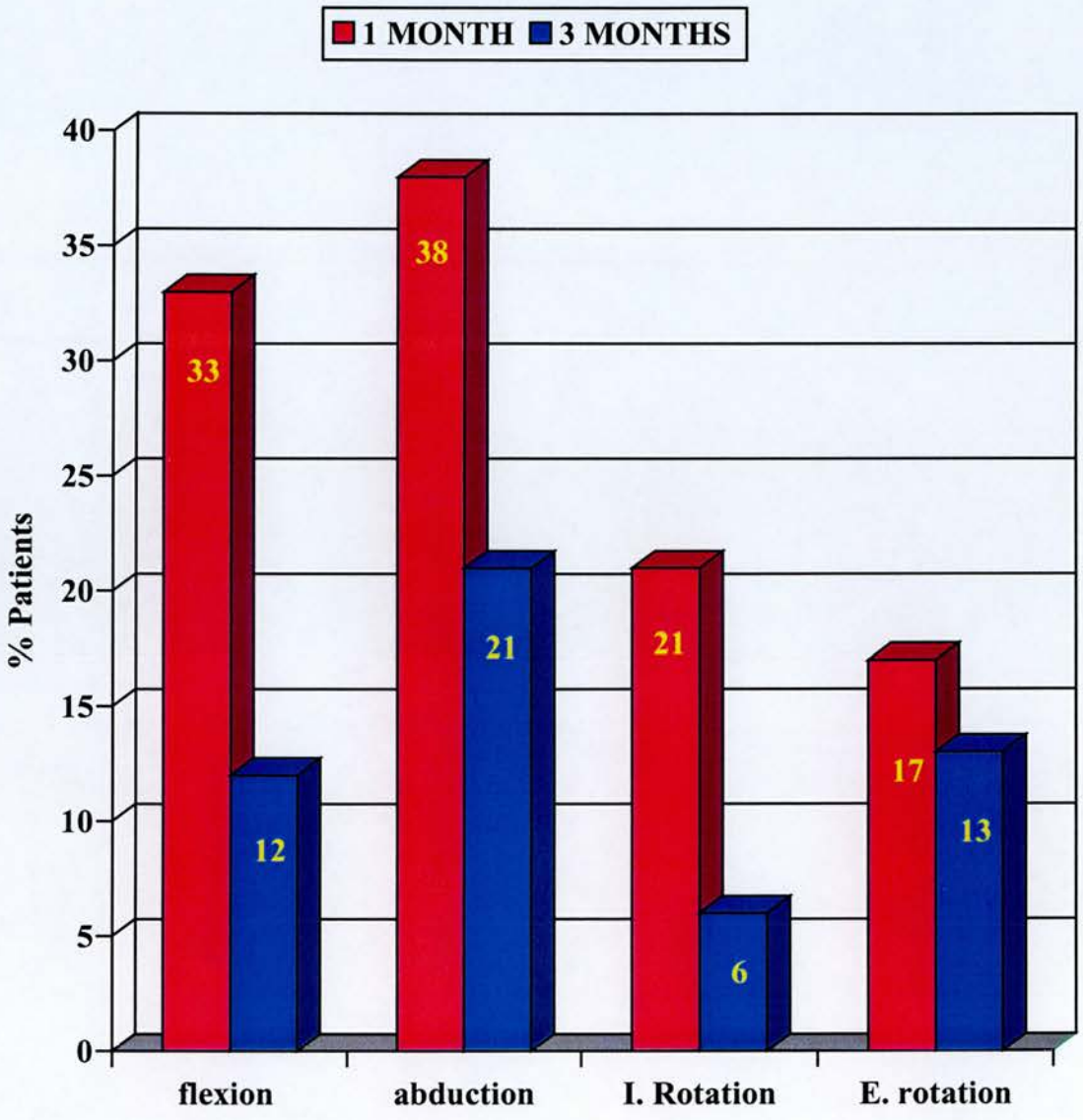


Figure 3.29 Shoulder stiffness



3.6 DISCUSSION

Sentinel node biopsy using the combined mapping technique was associated with a 97.5% sentinel node identification rate. These figures support the use of the combined agents as opposed to the use of single mapping agents as the sentinel node identification rates with either blue dye or nanocolloid alone were 91.4% and 88.9% respectively.

Of the two patients in whom a sentinel node was not found, one had macroscopic tumour visible in multiple axillary lymph nodes at operation suggesting that she had been clinically understaged and would not have satisfied the entry criteria for the trial had her node status been clinically detected pre-operatively. This highlights the importance of a careful clinical assessment preoperatively in order to select patients correctly for sentinel node biopsies. However, as clinical examination is notoriously unreliable at detecting axillary metastases it will not always be possible to do this until a more accurate non invasive method of examining lymph nodes is developed for identifying metastases pre-operatively. Lymphatic obstruction by tumour renders lymphatic drainage patterns abnormal. Mapping of sentinel nodes is thus inaccurate in patients whose lymph nodes are completely replaced by tumour as the nodes will not absorb mapping agents normally and blocked lymphatic vessels will divert mapping agents away from true sentinel nodes to other lymph nodes increasing the likelihood of a false negative result. The technique is thus more suitable only for those who are clinically node negative. If grossly positive nodes are unexpectedly encountered at surgery during a sentinel node procedure then the procedure is best abandoned and converted to an axillary clearance. The other patient in whom a sentinel node was not identified was clinically and histologically node-negative and was therefore a suitable candidate for sentinel node biopsy. This particular patient was overweight and this is a potential cause for the difficulty encountered when trying to identify a sentinel node. In the next chapter, the association of sentinel node identification with body mass index is shown in figure 4.2 'Body Mass Index'. It is made apparent that fewer sentinel nodes are identified in patients with a higher body mass index. This is possibly caused by fatty degeneration of lymph nodes leading to a high fat component to the lymph node, a phenomenon that has been described in

the literature and is occasionally observed at surgery [163, 191]. Should difficulty be encountered in identification of a sentinel node during surgery in a case such as this where the axilla is not obviously involved then the conventional operation should be reverted back to according to the unit policy. It may be wise to impose a time limit on the sentinel node procedure to avoid excessive dissection when it is clear that a sentinel node cannot be found. These cases illustrate two potential pitfalls of the technique.

The use of lymphoscintigraphy helped to detect internal mammary lymph nodes in 7 patients (8.6%). Internal mammary node biopsy resulted in one pneumothorax and one severe breast infection at the internal mammary node biopsy site requiring incision and drainage of pus in the follow-up clinic. Internal mammary node dissection as an addition to axillary dissection is not reported to improve disease-free recurrence or survival[31]. This questions the need to perform internal mammary node biopsy as part of a sentinel node biopsy procedure. Results are now available from the ALMANAC study phase 1 in the United Kingdom and revealed internal mammary sentinel nodes in 8% of cases, in keeping with the results from Edinburgh. Of these 14% were involved by tumour, which comprised 0.5% of patients overall. Only 0.2% of patients had involved internal mammary nodes and negative axillary nodes. Certainly, internal mammary node biopsy is not routinely practised in the majority of breast cancer units. It is believed by some that adjuvant breast irradiation will adequately treat internal mammary nodes involved by tumour in the majority of patients. If only 2 patients per thousand operated upon will have positive internal mammary nodes and negative axillary nodes it is difficult to justify using internal mammary node biopsy as a routine on all patients. However, with the availability of lymphoscintigraphy and sentinel node biopsy this may adequately select the small group of patients in whom the technique may be of some benefit.

Lymphoscintigraphy detected sentinel nodes in only 77.8% of patients whereas the gamma probe detected hot nodes in 88.9%. This may be explained by the fact that tracer material has had a longer time-period to travel to lymph nodes but could also be due to masking of some sentinel nodes from the view of the gamma camera by radioactivity from the tumour site. The failure of scintigraphy to detect some axillary nodes found at surgery and the questionable patient benefit in removal of internal

mammary nodes suggest that lymphoscintigraphy may be an unnecessary component of the mapping technique. Omission of these procedures would lead to a considerable saving in time and labour and would also prevent the delay to surgery, which was frequently encountered when patients were having their scintiscans. The logistics involved in performing lymphoscintigraphy and the cost:benefit ratio in terms of additional relevant patient information gleaned from scintigraphy needs to be addressed.

Tumour histological type has not been shown to influence the ability to identify a sentinel node in previous large studies [165] of sentinel node biopsy and as numbers were small in this study a further analysis of this particular aspect of sentinel node identification was not performed although tumour characteristics are presented in **section 3.5.6** showing the vast majority of tumours (81%) to be invasive ductal carcinomas with the remaining 19% being the special types or mixed tumour histology.

The procedure was associated with a relatively high complication rate although the majority of complications were minor. Breast seroma was the commonest complication affecting 13.9% of patients with breast infection as the second commonest complication. The repeated puncturing of breast skin for peritumoural injections, the massage of the breast to disperse mapping agents and the local effects of injection to the breast tissue such as oedema and mild inflammation are almost certainly the likely cause of these adverse effects. One way to minimise these side-effects would be to perform fewer needle punctures to the skin injecting the skin in two places instead of four and directing the needle around the tumour but underneath the skin. Similarly, a gentle massage technique for a shorter time would further reduce local trauma to the breast tissue. To minimise breast infection, a single intravenous dose of a prophylactic antibiotic such as co-amoxiclav given on induction of anaesthesia should also be considered.

In this study we have used a 5ml volume of diluted patent blue V. Other studies have used smaller volumes with equal success by using the intradermal or subdermal injection route [161, 166, 167]. Injection of a smaller volume of dye into the breast or subdermal area would reduce local oedema from injection and possibly reduce seroma formation. It has also been reported, however, that an increased volume of

radioisotope leads to better detection of sentinel nodes [225]. Studies of tissue adhesives in rat mastectomy models have reported a decrease in seroma formation with the use of polyethylene oxide hydrogel[226] and this may have a future role in seroma prevention should human studies mirror these results. Other measures to reduce seroma formation have been tried in mastectomy and plastic surgery patients such as pressure dressings and suction drainage but these are not ideal. A pressure dressing in a patient undergoing breast conservation may produce a concave deformity of the normal breast contour and suction drainage itself can further contribute to inflammation or infection.

A significant number of patients (30.9%) showed blue tattooing of the skin adjacent to their breast scar at one month post-operatively. Of these, approximately 20% fewer were affected at 3 months than at 1 month post-op although a quarter of the total number of patients remained affected. In the majority of cases the tattooing was only a faint blue but in a handful of cases staining was deeper. The discolouration appeared to become fainter over time and none of the patients affected was unduly concerned or distressed about this. Once this side effect became apparent, patients were warned about the risk of blue tattooing of the skin during their pre-operative trial counselling session at the staging clinic.

Nearly one in three patients experienced altered sensation to the arm, axilla or both at one month post-operatively. This fell to just under one in five after three months. This reduction in incidence of altered sensation suggests that at least some of these patients are suffering from a transient neuropraxia of the sensory nerves rather than a permanent loss of function. Serious nerve damage in these patients is thus unlikely and recovery of function suggests bruising or stretching of the nerves during surgery may be to blame for the transient loss of function here.

Six (40%) of the patients with altered sensation at three months had progression of sensory alteration from their one-month measurements and of these, half (three patients) of them were measured as having normal sensation at one month. It is possible for neuronal damage to progress over a period of time following an injury, which would explain the cases of the three patients developing further sensory alteration. It is not apparent, however, why three patients should develop new symptoms of sensory alteration at three months after an absence of symptoms at one

month. If nerve damage were present one would expect to see some signs of this clinically during the first month rather than signs to develop de novo at three months. Perhaps the best explanation would be one of operator error in that the initial one-month measurement may have been performed less than accurately and failed to detect small areas of sensory alteration, which later manifested themselves as larger areas at three months. This phase of the trial did not follow patients beyond three months so it is not possible to comment on the later progress of these patients' signs of sensory nerve injury although it would be reasonable to expect continued improvement in the majority of cases.

Arm swelling was recorded in twice as many patients (eighteen) at 3 months as at one month (nine). The extent of swelling was not large with a mean increase in arm volume of 5.2% and 6.4% at one and three months respectively. Without a suitable control group in this phase of the trial, it is impossible to determine the exact reason for the increase in incidence of arm swelling at three months. It is possible that the increased incidence of objective arm swelling may be related to patients' breast and axillary radiotherapy which was usually given at six weeks post operatively rather than resulting directly in relation to axillary surgery. It is known that the development of lymphoedema is related to the extent of axillary dissection, axillary radiotherapy and to the number of involved axillary lymph nodes[116] so it is likely that the administration of radiotherapy at 5-6 weeks post-surgery leads to a synergistic effect with axillary surgery to increase incidence of arm swelling at three months. The incidence of lymphoedema seen here lies within published figures of lymphoedema incidence of between 9-28% [116, 227] despite the use of the lower threshold of 100mls increase in arm volume as a reference point for arm swelling in this study. Only one patient had an increase in arm volume of greater than 10% of the pre-operative arm volume, at 14.4%. Patients with swelling of less than 10% are rarely referred for physiotherapy as is not usually troublesome. These figures are encouraging and whilst only preliminary, suggest that sentinel node biopsy does not have an adverse effect on the development of lymphoedema of the arm. If the figure of 200mls had been used as a threshold figure for arm swelling, no patients would have had swelling at 1month and only four patients had swelling of greater than 200mls at 3 months of follow-up thus demonstrating lower rates of arm swelling than

reported in other series which have included patients having axillary clearance. In the next chapter this will be examined again comparing axillary sample with sentinel node biopsy. As the majority of patients found to have arm swelling of greater than 100mls did not complain of problems it may be that the figure of 200mls is more clinically useful in detection of problematic swelling.

Shoulder stiffness occurred in almost two-thirds of patients at one month and this fell to just over a third at three months. Whilst these figures appear quite high, they may be explained by the fact that a very subtle definition of ten degrees loss of movement was recorded as shoulder stiffness. This inevitably results in the detection of more patients with shoulder stiffness than had a less subtle definition been used as a reference point. It is reassuring to see the incidence falling over time and it would be expected that this would continue to fall over time although follow-up beyond three months was not part of this initial phase of the trial. Abduction was the most commonly affected movement at both one and three months with flexion and external rotation being affected similarly and internal rotation least affected. These findings have some similarities with other reports, which suggest external rotation and abduction is affected by radiotherapy [121] although in others flexion was most affected by radiotherapy and a node clearance with external rotation being affected by radiotherapy [84].

In order to minimise shoulder stiffness patients were encouraged to mobilise their shoulder joints early and were given instructions on simple arm exercises before they were discharged.

In summary, it appears that sentinel node biopsy of axillary lymph nodes in breast cancer is a relatively simple and reproducible method of sampling axillary nodes. It is associated with a low false negative rate and a high degree of accuracy. Both consultant surgeons performing the technique reported good results, which were more than adequate to progress to the randomised phase of the trial. Potential pitfalls of the technique include patients with gross tumour of the axillary lymph nodes highlighting the importance of good patient selection and obese patients in whom fatty degeneration of lymph nodes may occur making sentinel node identification less straightforward. Complication rates were a little higher than expected and were

more severe in patients undergoing internal mammary node biopsy. These issues will be evaluated further in the next chapter within the randomised phase of the trial.

4. ALMANAC STUDY PHASE 2 – RANDOMISED PHASE

Phase 2 - Randomised Phase

The primary objective of phase 2 is to compare sentinel node biopsy with conventional axillary surgery. Three main outcome measures will be studied:

d) arm and axillary morbidity
e) resource costs
f) quality of life

Resource costs and quality of life are part of the national study and will not be covered in this thesis.

The secondary objective is to record the incidence of local recurrence in the axilla for the different axillary procedures.

In phase 2 patients will be randomised to either the **control group** who will have a wide local excision or mastectomy plus axillary sampling or clearance according to routine policy or the **sentinel node biopsy group** (study group) who will have a wide local excision or mastectomy plus a sentinel node biopsy. Sentinel node positive patients on routine histology will go on to have delayed treatment of the axilla by surgery (axillary clearance) or radiotherapy. Sentinel node negative patients will have no further axillary treatment. Patients who are node-positive after conventional axillary surgery will go on to have adjuvant treatment according to unit policies.

In Edinburgh we have selected patients being treated by axillary node sample as their conventional axillary procedure as the control group in phase 2 to be compared with sentinel node biopsy. It was felt that patients selected for axillary node clearance generally had larger tumours, stage T2 and above and hence the risk of axillary metastases and false negative procedures was greater than in those selected for axillary sample, generally stage T1 or small T2 tumours.

4.1 AIMS OF PHASE 2

The aims of this part of the trial were to:

1. Compare the success of axillary node sample with the new technique of sentinel node biopsy as a method of staging the axilla in stage T1,N0,M0 invasive breast cancer.
2. Compare the outcomes of the two groups for post-operative complications.
3. Compare the outcomes of the two groups for post-operative arm morbidity.
4. Identify specific patient and tumour characteristics associated with improved sentinel node detection.

4.2 PATIENTS

At the time of analysis, 70 patients with clinical stage T1-2, N0, M0 invasive breast cancer had been enrolled into phase 2 of the trial.

Three patients had in-situ disease alone with no invasive cancer found in the operative specimens histologically and these patients were excluded from the results. As stated for similar cases in the previous chapter, all of these patients had been diagnosed with malignancy based on the triple assessment of clinical, radiological and cytological examination, as is the policy of Edinburgh Breast Unit.

Of the remaining patients, 58 patients were stage T1, N0, M0 and 8 patients were T2, N0, M0. One patient was stage T3, N0, M0 after being found to have a tumour measuring 55mm on pathological examination. This patient was randomised to receive an axillary node sample.

Tumour size ranged from 0-40mm clinically, 5-37mm radiologically and 5-55mm histologically.

Patients' age ranged from 32-79 years.

35 cancers were screen detected

48 palpable tumours, 19 impalpable

19 patients had radiologically guided breast wide local excisions.

33 patients had sentinel node biopsy and 34 patients had an axillary node sample as their axillary staging operation.

4.3 METHODS

Patients were selected according to the inclusion criteria for Phase 1 of the trial and were randomised to receive a sentinel node biopsy alone or an axillary node sample. Inclusion criteria were: age 18-80 years, presence of invasive breast cancer proven by cytology or histology, having an axillary procedure as part of the routine treatment of the patient's disease, patient's informed consent in writing.
Exclusion criteria were in-situ breast cancer, clinically involved lymph nodes, previous breast cancer in the same breast, multifocal disease, locally advanced disease, pregnancy, previous axillary surgery, previous or existing limb swelling or lymphoedema of the ipsilateral arm and history of allergy to human serum albumin or patent blue V dye.
Patients details were sent to the central trials office in Cardiff and patients were randomised to receive sentinel node biopsy or standard axillary surgery.

4.3.1 Patient recruitment

Patients were chosen by consultants at the multidisciplinary meeting. Patients who were listed for axillary node sample according to the existing unit protocols were considered for entry into the randomised trial. It was decided not to include patients having an axillary clearance as their routine surgery until sentinel node biopsy has been proven to be safe. It was felt that these patients were at higher risk of nodal disease and that they were at risk of being understaged by a sentinel node biopsy should the technique prove to be inadequate at staging the axilla in the future.
Suitable patients were counselled during a 20-30 minute consultation which included a thorough explanation of an axillary node sample in addition to the technique of sentinel node biopsy. Patients were given ample opportunity to ask questions during and at the end of the consultation. A patient information sheet was given to any patients who were interested in taking part following the consultation. Patients were asked to return the signed consent form by mail if they agreed to take part in the trial. Once consent was obtained, patients were randomised to receive sentinel node

biopsy or axillary node sampling. Isotope was subsequently ordered from the nuclear medicine department for preoperative injection of patients randomised to receive sentinel node biopsy.

4.3.2 Preoperative assessment

The pre-operative assessment was identical to that in phase 1 of the study and pre-operative arm measurements were taken as before to assess post-operative arm morbidity for the two surgical techniques. Quality of life questionnaires were completed as before as part of a side study carried out by the CRC Psychosocial Oncology Group, Department of Oncology, University College London Medical School. Further questionnaires were mailed to patients at one, three, six, twelve and eighteen months post-operatively and returned to University College London.
The quality of life study is not part of this thesis and its results will not be be discussed here

4.3.3 Sentinel node biopsy technique

An identical surgical technique to that described in phase one of the trial was used. Injection of radioactive isotope, Patent blue V dye and scintiscans were all performed as described in the previous chapter for phase one of the trial.

4.3.3 Axillary node sampling surgical technique

The axillary sampling technique was performed through a transverse incision between the latissimus dorsi and the pectoralis major muscles. The axillary tail was mobilized away from the serratus anterior muscle and nodes were identified by palpation in the lower axilla. If no lower axillary nodes were palpated, palpable nodes from level II or interpectoral regions were removed. At least 4 lymph nodes were removed for histology.

4.3.4 Post operative care

Patients were informed of the risk that their urine may be discoloured by the blue dye for 24 hours post-operatively.
Patients were seen on the ward and educated on arm exercises to reduce shoulder stiffness following discharge.
Any immediate post-operative complications were documented recording in particular haematoma, seroma or infection of the breast or axilla, pneumothorax or dye sensitivity.

4.3.5 Pathology

Pathology results from the surgical specimens were followed up and recorded for each patient including histological type, presence or absence of associated DCIS, special features, grade, lymphovascular invasion, oestrogen receptor status and presence or absence of metastatic disease in each lymph node specimen.

4.3.6 Follow up

Patients were seen at 1month, 3 months, 6 months, 12 months and 18 months when their breast and axilla was examined for recurrence and repeat measurements were taken to assess arm morbidity as compared with pre-operative measurements.
Any further complications, surgical procedures or extra visits to their GP, practice nurse or to the hospital were documented at this time.
A measured increase of greater than 100mls from the pre-op measurement in the ipsilateral arm volume compared with the contralateral arm was recorded as **objective** arm swelling. If both arms had swollen equally then a general increase in patient's weight was presumed and the increase in volume was not recorded.
Patients were asked to describe any perceived arm swelling as mild, moderate or severe and this was recorded as a **subjective** increase in arm volume.

Failure to attend follow-up appointments for arm measurements

One patient did not attend (DNA) at 1 month, 2 patients DNA at 3 months, 6 DNA at 6 months, 11 DNA at 12 months and 16 DNA at 18 months (13 of these had yet to attend their 18 month appointment).
One patient declined follow-up before her 1 month appointment, one patient declined follow up after the 1-month appointment but before her 3-month appointment.
These patients were excluded from the figures when calculating percentages. These patients were often required to attend extra appointments for follow-up arm measurements to assess arm morbidity and these non-attendance statistics refer to these appointments and not to their routine breast cancer follow-up.

4.4 RESULTS

4.4.1 Tumour Characteristics

Histology

	Total	ANS	SNB
Ductal carcinoma no special type	44	24	20
Lobular carcinoma	9	5	4
Mixed ductal/lobular	2	2	0
Tubular	5	1	4
Mucinous	2	1	1
Medullary	1	0	1
Cribriform	1	0	1
Other mixed	3	1	2
Associated features			
DCIS	33	15	18
LCIS	5	2	3
LCIS and DCIS	1	1	0
Cribriform in-situ	2	1	1
No associated features	26	15	11
Lymphovascular invasion			
Present	9	5	4
Absent	58	29	29

Tumour Grade

	Total	ANS	SNB
Grade 1	17	5	12
Grade 2	33	22	11
Grade 3	17	7	10

Pathological Tumour Size

	Total	ANS	SNB
Average tumour size	15.4mm	16.1mm	14.7mm
Range	5-55mm	5-55mm	7-30mm

4.4.2 Lymph node characteristics

i. Node status

There were 55 node-negative and 12 node-positive patients (17.9%).

Eight patients (24.2%) had positive sentinel nodes.

Four patients (11.7%) had positive axillary node samples.

Chi-squared analysis $\chi 2=0.5$, $p \approx 0.5$.

ii. Node identification

119 sentinel nodes were found in 33 patients having a sentinel node biopsy.

The mean number of sentinel nodes identified was 3.6 per sentinel node biopsy.

163 nodes were found in 34 patients having an axillary sample.

The mean number of sampled nodes was 4.79 per axillary node sample.

iii. Hot nodes

A total of 101 hot nodes were found in 33 patients.

100% of patients had at least one hot sentinel node identified.

Of the 101 nodes, 63 nodes had a radioactivity count greater than 100 counts per 10 seconds and 38 nodes had a radioactivity count of less than 100 counts per 10 seconds.

A mean of 3.1 hot nodes were found in each patient.

iv. Blue nodes

85 blue nodes were found in 33 patients. A mean of 2.6 blue nodes were found in each patient.

100% of patients had at least one blue node.

52 nodes were deeply stained with blue dye and 33 nodes were faintly stained with blue dye.

v. Hot and Blue nodes

67 nodes were both hot and blue.

A mean of 2 hot and blue nodes were found in each patient.

vi. Internal mammary nodes

Two patients (6.1%) had internal mammary nodes biopsied when their scintiscans revealed drainage to the internal mammary nodes. In neither case was the internal mammary node the only sentinel node. None of the internal mammary nodes was positive.

vii Sentinel node pathology

Sentinel nodes were positive in 8 patients:

Hottest sentinel node alone was positive	1 (12.5%)
Hottest sentinel node plus other sentinel nodes positive	4 (50%)
Sentinel node other than the hottest was positive	3 (37.5%)

Of these patients, two (25%) had lymphovascular invasion on histology. Three patients had grade 1 tumours, three were grade 2 and two were grade 3. Tumour histology was ductal carcinoma no special type in five of these patients, lobular carcinoma in one patient and mixed types in one patient.

viii Axillary node sample pathology

Four patients had a positive axillary sample. Of these, three had lymphovascular invasion on histology. Two patients had grade 2 tumours and two had grade 3 tumours.

Three of these patients' histological tumour type was ductal carcinoma no special type and one patient had a lobular carcinoma

ix Recurrences

At the time of writing there had been no axillary recurrences recorded with a median follow-up period of 18 months.

4.4.3 Scintigraphy

Scintigraphy was positive in 75.8% of patients scanned.

48 nodes showed on scintigraphy in 25 patients.

8 patients had negative scintigraphy with no hot nodes showing.

Two patients (25%) with positive sentinel nodes had a negative scintiscan.

A mean of 1.45 nodes were found per scintiscan performed.

A mean of 1.92 nodes were found on each positive scintiscan.

4.4.4 In-patient duration and operating time

i. In patient duration

Mean length of in-patient stay was 2.1 days for SNB and 2 days for ANS.

ii. Operating Time

Mean operating time was 21.3 minutes for SNB.

Mean operating time was 13.1 minutes for ANS.

4.4.5 Factors affecting sentinel node identification

The number of sentinel nodes identified was not related to tumour size as shown in figure 4.1. Pearson correlation coefficient P=0.141

As body mass index increased, the number of sentinel nodes identified decreased as shown in figure 4.2. Pearson correlation coefficient P=0.009

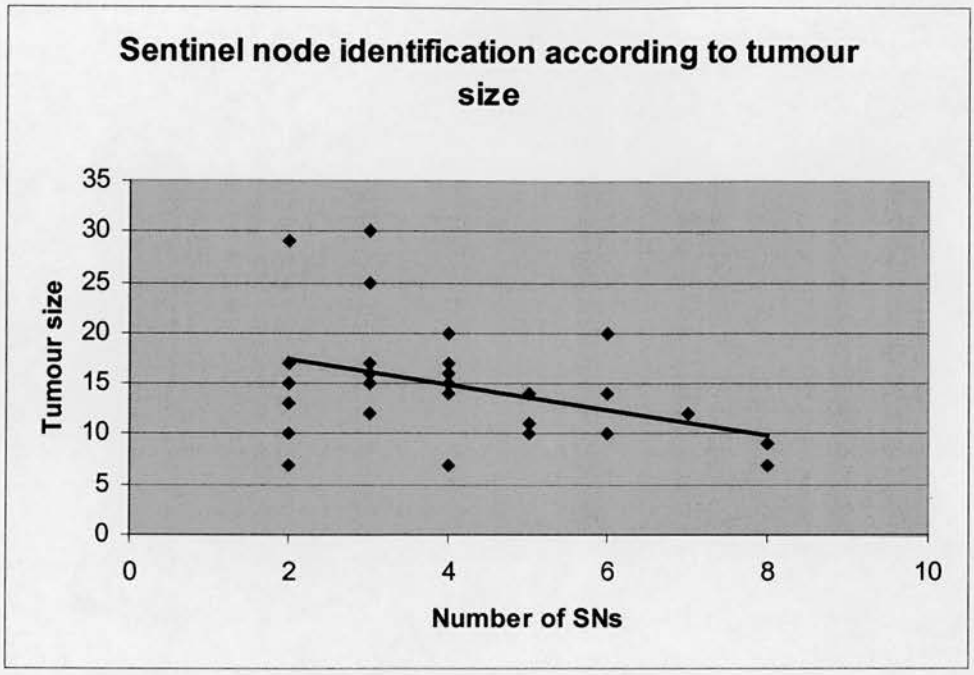
There was no association between tumour grade and the number of sentinel nodes identified, see figure 4.3. Pearson correlation coefficient P=0.903

The dose of isotope and the timing of injection of the isotope in relation to the time of scintiscan did not affect the number of nodes identified by lymphoscintigraphy as shown in figures 4.4 and 4.5 respectively. Pearson correlation coefficients P=0.761 (dose of isotope) and P=0.17 (timing of injection).*

A greater number of sentinel nodes was identified when tumours were sited in the lateral half compared with the medial half of the breast. This is displayed in Table 4.1 Pearson correlation coefficient P=0.007

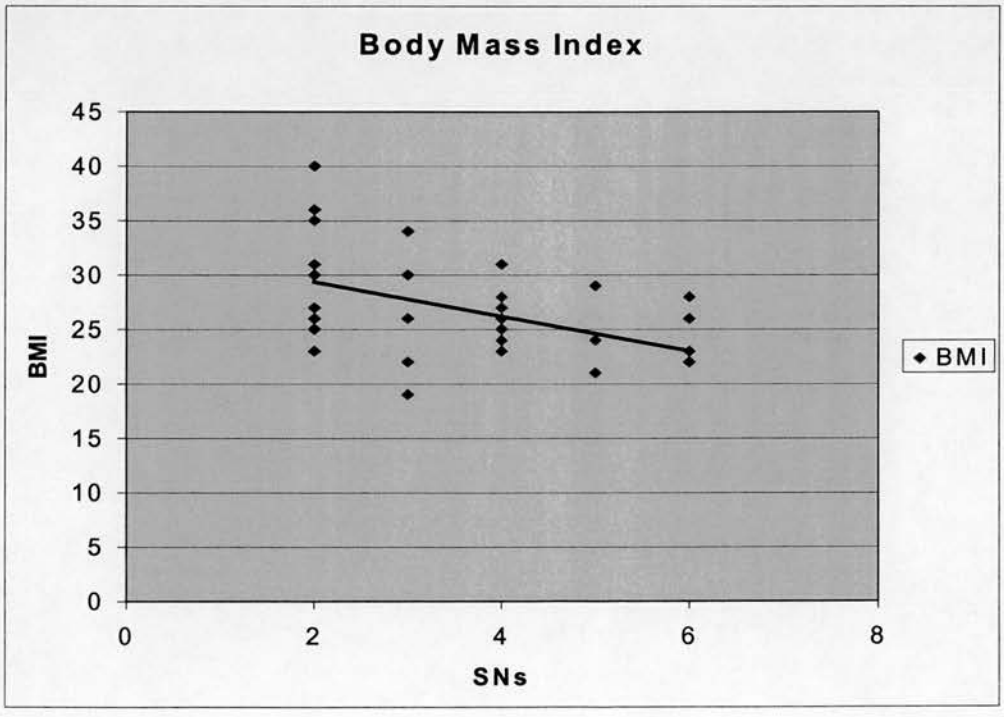
*It is important to note, when examining the scatter plots shown in figures 4.1-4.5 that the number of dots viewed does not always equal the number of patients and this is because some dots are superimposed on one another when they have equal values.

Figure 4.1 Tumour size



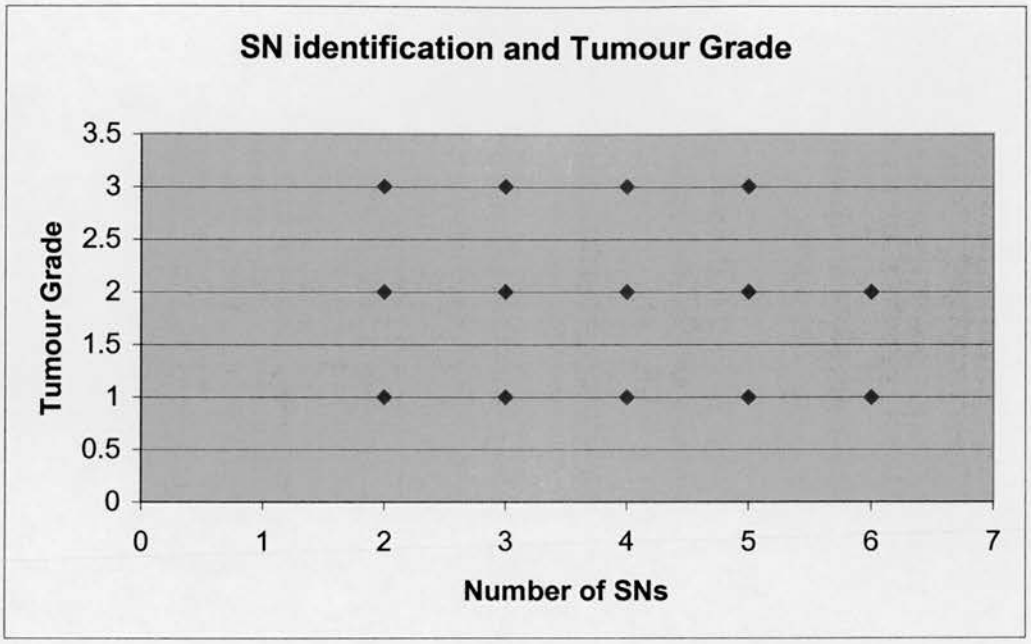
Pearson correlation coefficient P=0.141

Figure 4.2 Body mass index (BMI)



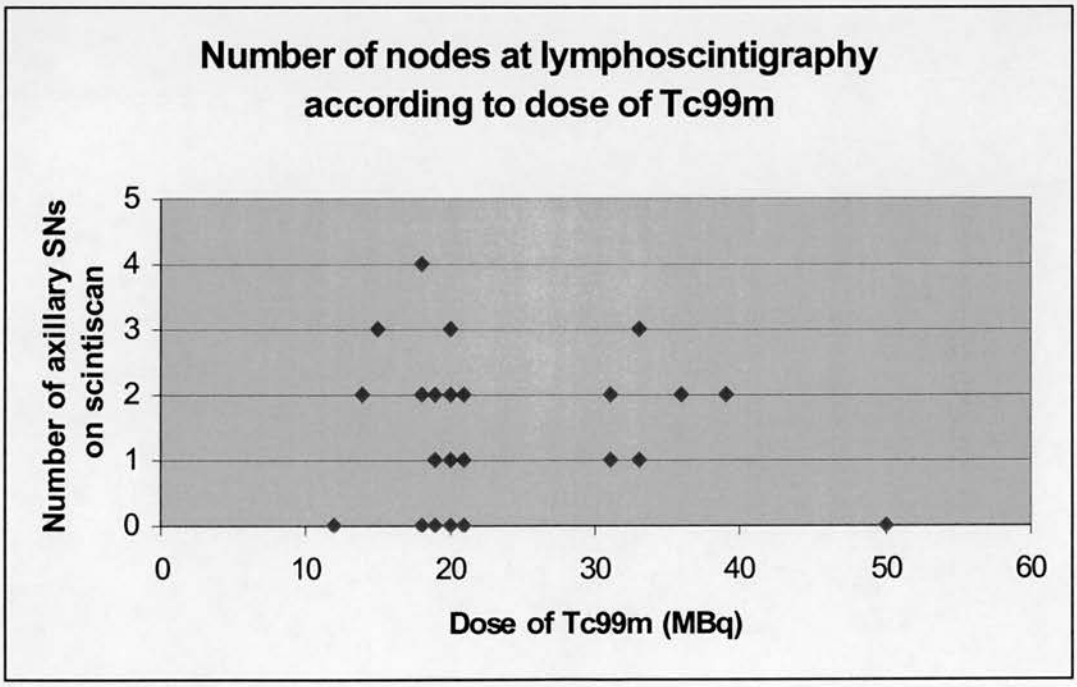
Pearson correlation coefficient P=0.009

Figure 4.3 Tumour grade



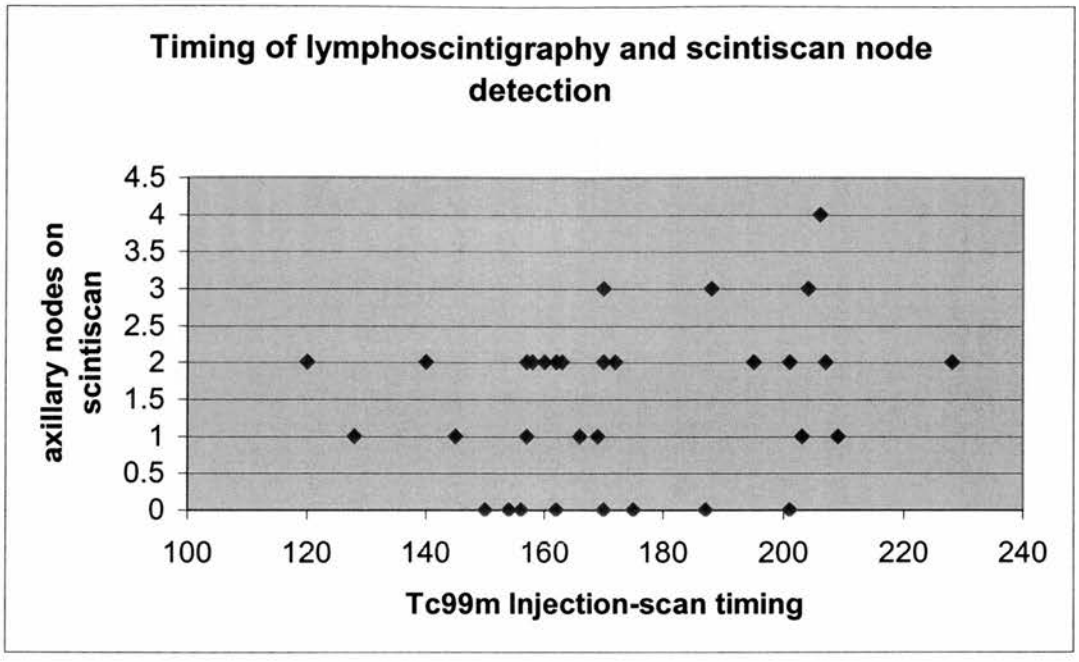
No correlation. Pearson correlation coefficient P=0.903

Figure 4.4 Dose of radioisotope (Tc^{99m})



No correlation. Pearson correlation coefficient P=0.761

Figure 4.5 Timing of lymphoscintigraphy



No correlation. Pearson correlation coefficient P=0.17

Table 4.1 Tumour site

Tumour site	Number of patients	Mean number of SNs
Lateral half	20	4.1
Medial half	13	2.8

Pearson correlation coefficient P=0.007

4.4.6 Post-operative Complications

Complications were encountered more frequently in patients having a sentinel node biopsy than in those having an axillary sample. Complications were encountered in fifteen patients (45.5%) following a sentinel node biopsy and in six patients (17.6%) following an axillary sample. In particular, more seromas of the axilla and breast and more breast infections were seen following SNB.

Statistical analysis using the chi-squared test was performed to compare the risk of breast seroma for the two procedures and was not significant $\chi 2=2.19$, $p>0.1$. Fisher's exact test was performed comparing the risk of axillary seroma between the two procedures and was approaching significance ($p=0.092$) but was not significant. The higher incidence of breast infection in the sentinel node patients was not statistically significant ($p>0.1$).

Potentially more serious complications were encountered in patients having a sentinel node biopsy with one patient each having a pneumothorax, a pulmonary embolism, and widespread cutaneous sensitivity to Patent Blue V dye.

The numbers of early post-operative complications found for each procedure are shown in Table 4.2 overleaf with percentages in parentheses. Figure 4.6 displays the main complications as a bar chart comparing the two procedures.

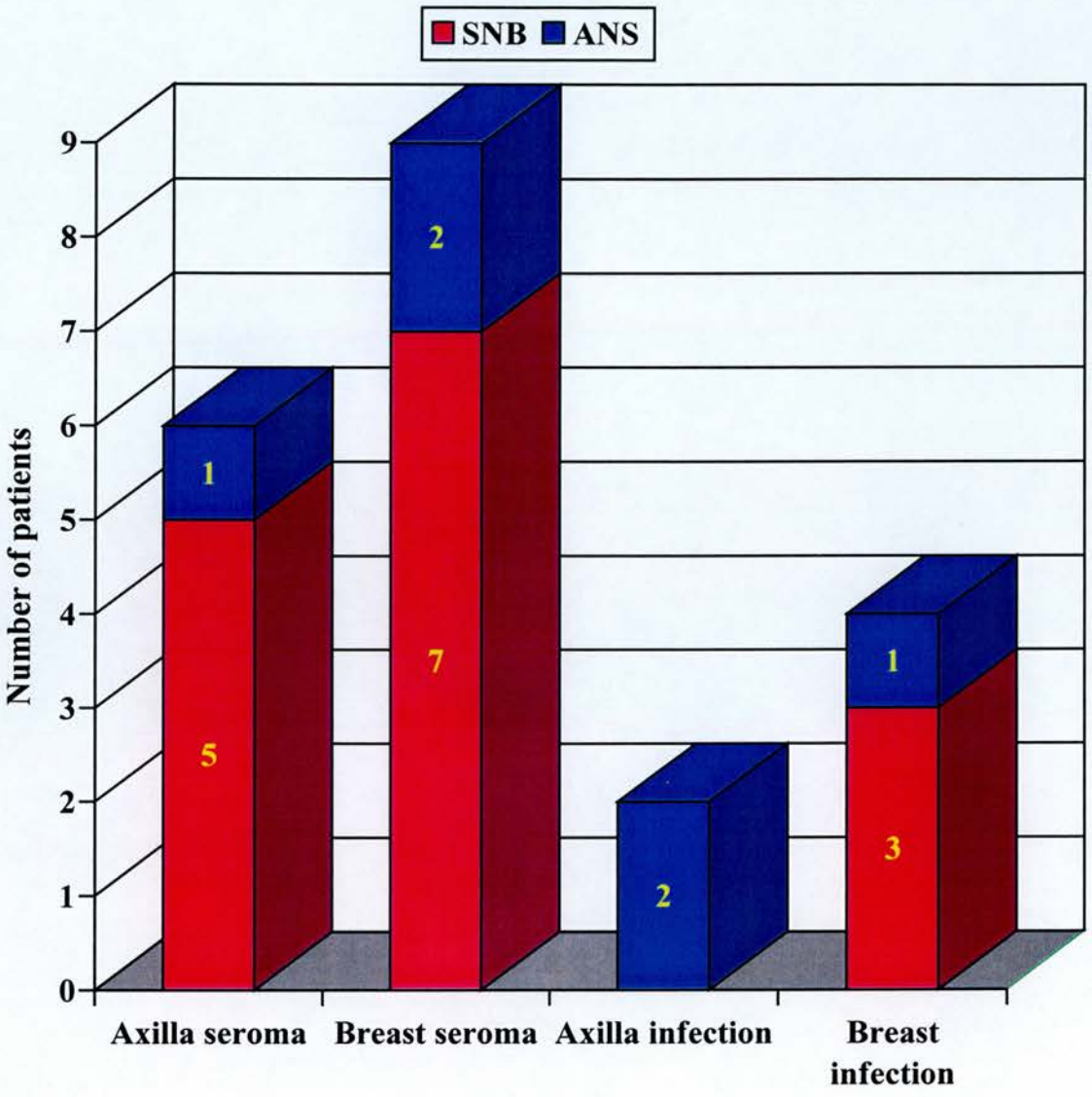
Table 4.2 Early post-operative complications

COMPLICATION	SNB	ANS
Breast seroma	7(21.2%)	2(5.9%)
Axillary seroma	5(15.1%)	1(2.9%)
Breast wound infection	3(9.1%)	1(2.9%)
Axillary wound infection	0	2(5.9%)
Axillary wound dehiscence	1(3%)	0
Pneumothorax	1(3%)	0
PE	1(3%)	0
Blue dye sensitivity	1(3%)	0
Breast haematoma treated conservatively	1(3%)	0
Axilla haematoma treated conservatively	0	1(2.9%)

4.4.7 Blue Dye Tattooing

Six patients (18.1%) had tattooing of the breast at one month, with three patients (9.1%), two patients (6.5%) and 1 patient (3.7%) showing blue tattooing at 3, 6 and 12 months respectively. The only patient with persistence of blue tattooing at twelve months had not been followed up at 18 months.

Figure 4.6 Immediate Complications



4.4.8 Arm morbidity

i. Arm Swelling

Objective Arm Swelling

There appeared to be little difference in arm swelling whether patients had a sentinel node biopsy or an axillary sample as shown in the bar chart, figure 4.7 'objective arm swelling'. The two procedures were compared using Fisher's exact test and there were no significant differences between them, $p>0.5$.

There appeared to be an increase in arm swelling in both groups regardless of their axillary procedure during the first 6 months post-operatively. The percentage of patients with objective arm swelling fell slightly at 12 months and then increased again at the 18-month follow-up appointment. Reasons for this are unclear.

The results for objective arm swelling are displayed separately for the two procedures in table 4.3 overleaf.

The overall numbers of patients with objective arm swelling combined for both procedures are described below:

7 patients (10.4%) had arm swelling at 1 month 3 had SNB and 4 had ANS.

10 patients (14.9%) had arm swelling at 3 months, 5 SNB and 5 ANS.

11 patients (18%) had arm swelling at 6 months, 4 SNB and 7 ANS.

6 patients (10.7%) had arm swelling at 12 months, 3 SNB and 3ANS.

9 patients (17.6%) had arm swelling at 18 months, 4 SNB and 5 ANS.

Table 4.3		OBJECTIVE ARM SWELLING	
		SNB	ANS
1 MONTH	Number of patients/total number of patients	**3/33**	**4/33**
	% Patients	**9.1**	**12.1**
	Range of arm swelling (mls)	**116-122mls Mean 120mls**	**102-203mls Mean 150mls**
	% increase in arm volume	**4-5.3% (4.7%)**	**3.8-6.8% Mean 5.3%**
3 MONTHS	# PTS/total # pts	**5/33**	**5/32**
	% PTS	**15.2%**	**15.6**
	Range of arm swelling (mls)	**102-254mls Mean 154mls**	**100-233mls Mean 135 mls**
	% increase in arm volume	**3.9-10.2% Mean 6.7%**	**5.1-8.9% Mean 6.6%**
6 MONTHS	# PTS	**4/31**	**7/30**
	% PTS	**12.9%**	**23.3%**
	Range of arm swelling (mls)	**107-201mls Mean 152 mls**	**107-280mls Mean 179mls**
	% increase in arm volume	**4.4-8.1% Mean 5.6%**	**3.8-10.9% Mean 7.6%**
12 MONTHS	# PTS	**3/27**	**3/29**
	% PTS	11.1%	**10.3%**
	Range of arm swelling (mls)	**105-223mls Mean 113mls**	**111-124mls Mean 116mls**
	% increase in arm volume	**3.4-9.2%** Mean 5.7%	**4.2-7.7% Mean 5.6%**
18 MONTHS	# PTS	**4/26**	**5/25**
	% PTS	**15.4%**	**20%**
	Range of arm swelling (mls)	**104-189mls Mean 151mls**	**112-378mls Mean 199mls**
	% increase in arm volume	**4.1-6.7% Mean 5.4%**	**4.2-20.3% Mean 8.6%**

Subjective arm swelling

Of the patients who reported a perception of arm swelling, all reported the swelling as 'mild' apart from two patients who reported swelling as 'moderate'. Reports of 'moderate' swelling were both in the first 3 months of follow-up and did not persist beyond that time. Only one patient reported swelling of the arm at 18 months of follow-up. These findings are shown in Table 4.4 overleaf.
There appeared to be a large difference in subjectively perceived arm swelling at 3 months and again at 6 months in favour of sentinel node biopsy as shown in the bar chart figure 4.8 'subjective arm swelling'. Analysis of subjective arm swelling at 3 months almost reached statistical significance in favour of sentinel node biopsy, $p=0.053$ using Fisher's exact test. Fisher's exact test revealed no other significant differences in arm swelling between the two groups of patients whether they were treated by axillary node sample or sentinel node biopsy, $p>0.5$.

Of all the patients who reported a subjective increase in arm volume, only four had a simultaneously measured (objective) increase in arm volume.
Reports of subjective arm swelling declined over time with 7 patients reporting swelling at 1 month post-operatively, 4 patients reporting swelling at both 3 and 6 months post-operatively and 2 patients and 1 patient respectively reporting swelling at 12 and 18 months post-operatively.

Table 4.4 Subjective arm swelling

SUBJECTIVE ARM SWELLING						
		SNB	Degree of swelling	ANS	Degree of swelling	Correlation of subjective and objective patients
1 month	**# pts**	**3/33**	**2 mild, 1 moderate**	**4/33**	**4 mild**	**0**
	% pts	**9.1**		**12.1**		
3 months	**# pts**	**0/32**		**4/32**	**3mild, 1 moderate**	**2 (40%)**
	% pts	**0%**		**12.5**		
6 months	**# pts**	**1/31**	**1mild**	**3/30**	**3 mild**	**2**
	% pts	**3.2%**		**10%**		
12 months	**# pts**	**1/27**	**1 mild**	**1**	**1mild**	**0**
	% pts	**3.7%**		**3.4%**		
18 months	**# pts**	**0/26**		**1**	**1 mild**	**0**
	% pts	**0%**		**4%**		

Figure 4.7 **Objective Arm Swelling**

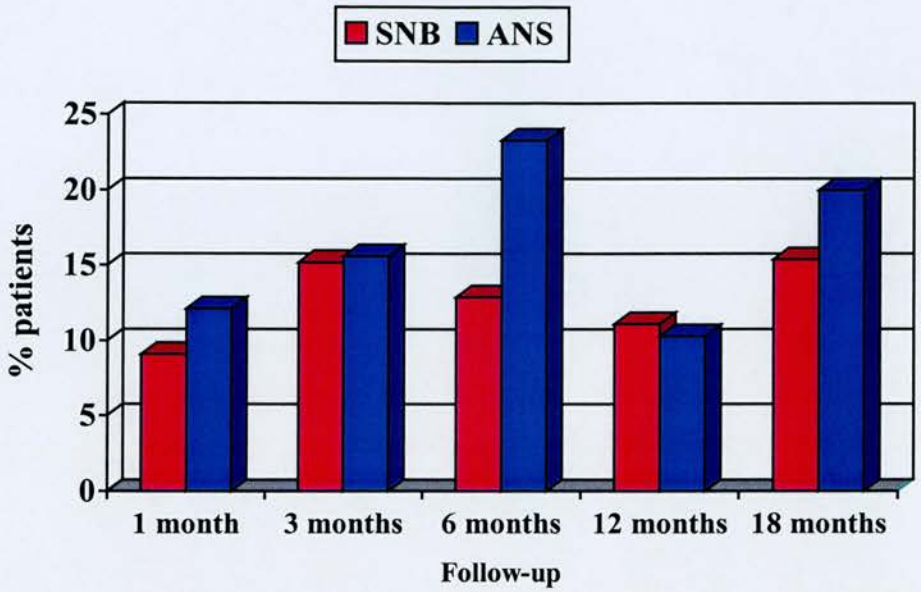
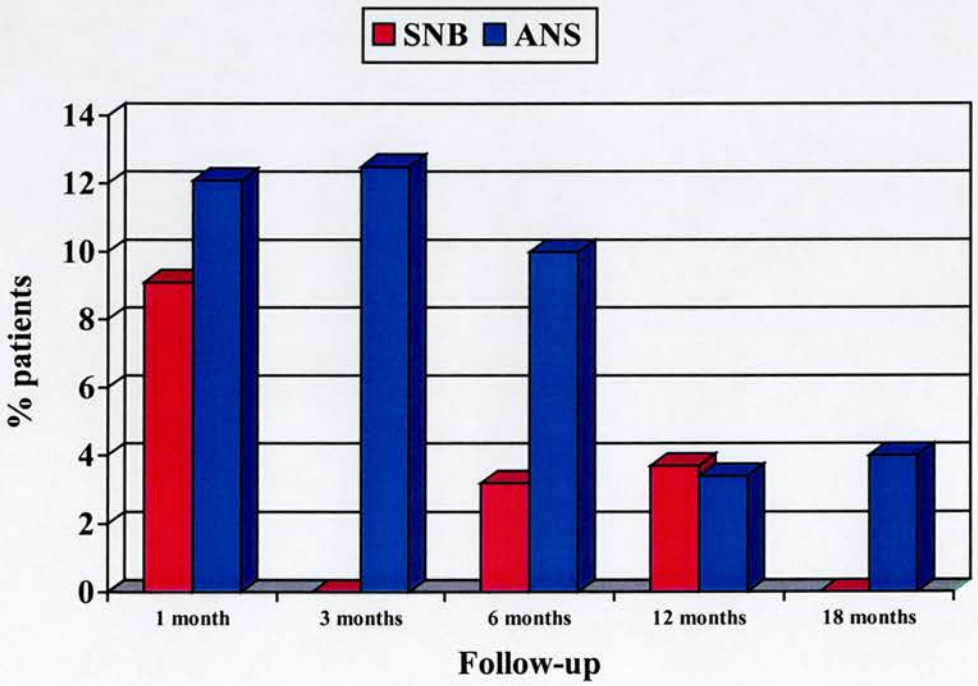


Figure 4.8 **Subjective Arm Swelling**



ii Altered Sensation

Altered sensation to pin-prick

A larger proportion of patients had altered sensation to pin-prick of the skin of the upper arm and the axillary skin following an axillary node sample than following a sentinel node biopsy. The difference was most marked at one month post-operatively to the axillary skin and three months post-operatively to the skin of the upper arm. More than three times the number of patients affected by altered sensation following a sentinel node biopsy were affected following an axillary sample at one month but this difference did not reach statistical significance using Fisher's exact test.

There was no significant difference in sensation to pin-prick between the two groups at 3 months $p>0.1$ using Fisher's exact test.

Figures for patients affected by altered sensation to pin-prick of the upper arm and axillary skin are displayed in table 4.5.

Altered pin-prick sensation appeared to decrease over time with fewer patients reporting this problem as time progressed. This was more marked in the axillary sample group of patients, a third of whom had altered axillary sensation at one month falling to just 8% at 18 months. This factor is displayed graphically in figure 4.9 for altered sensation to axillary skin and in figure 4.10 for altered sensation to the skin of the upper arm.

One patient having a sentinel node biopsy had a large area of sensory loss of the upper arm, which failed to reduce over 18 months of follow-up. This patient's results distort the mean area of sensory loss to the arm in the sentinel node group to a higher than expected value.

The majority of patients did not complain of sensory loss unless the area of loss was large.

Table 4.5 Patients reporting altered sensation to pin-prick

		SNB		ANS	
		AXILLA	ARM	AXILLA	ARM
1 MONTH	# patients	3/33	4/33	11/33	10/33
	% of patients	9.1	12.2	33.3	30.3
	Mean area(cm2)	15	65.5	36.3	48
	Range of area(cm2)	9-24	14-119	6-88	1-108
3 months	# patients	7/33	2/33	10/32	7/32
	% of patients	21.2	6.1	31.3	21.9
	Mean arca (cm2)	19	96.5	32.9	47.6
	Range of area(cm2)	4-42	25-168	1-77	16.5-91
6 months	# patients	3/31	2/31	7/30	5/30
	% of patients	9.7	6.5	23.3	16.7
	Mean area (cm2)	17	27	28.3	59.4
	Range of area(cm2)	6-36	4-50	4-50	36-98
12 months	# patients	3/27	1/27	3/29	3/29
	% of patients	11.1	3.7	10.3	10.3
	Mean area (cm2)	22.8	147	12.3	34
	Range of area(cm2)	2.25-48	147	9-16	24-42
18 months	# patients	2/26	1/26	2/25	2/25
	% of patients	7.7	3.8	8	8
	Mean area (cm2)	42	240	18.5	41
	Range of area(cm2)	36-48	240	2-35	30-52

Figure 4.9 **Altered pin-prick sensation axillary skin**

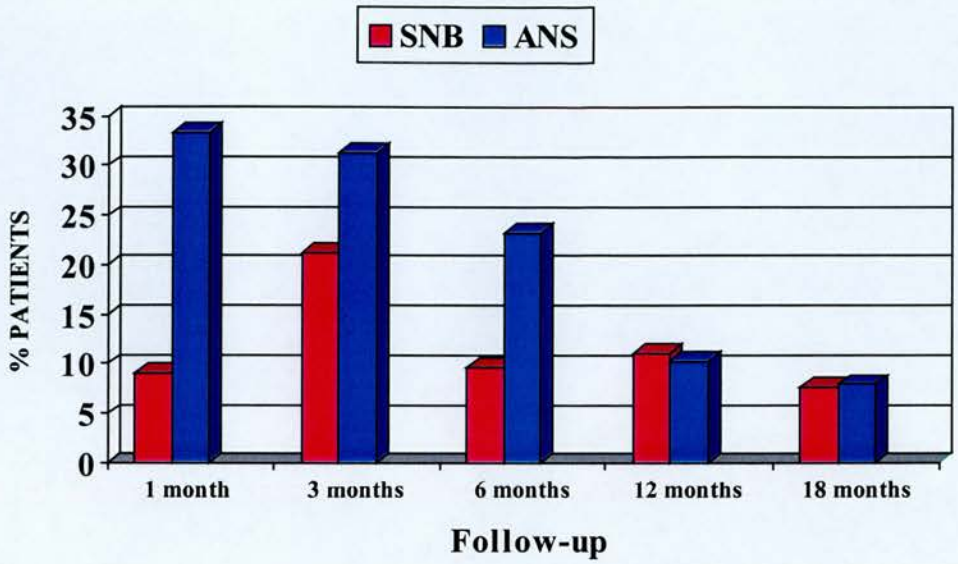
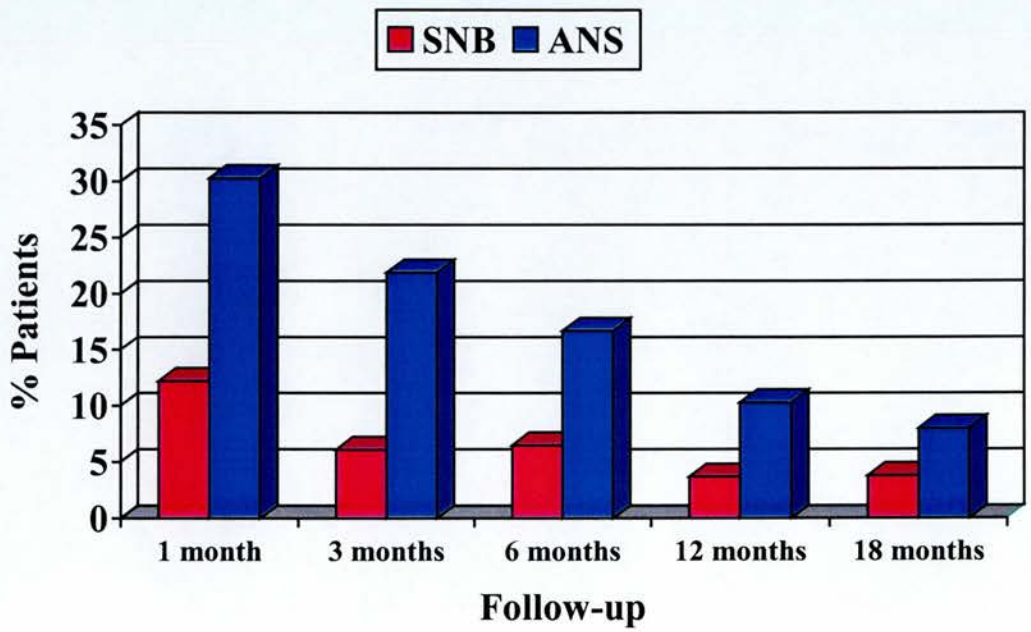


Figure 4.10 **Altered pin-prick sensation arm skin**



Altered sensation to light touch

Reduction in sensation to light-touch of the upper arm was encountered more frequently in the ANS group of patients than in the SNB group p=0.011 as measured at 3 months using Fisher's exact test.

Loss of light touch sensation appeared to reduce in frequency over the 18-month follow-up period for both groups of patients. These findings are displayed graphically in figure 4.11 for axillary sensation and figure 4.12 for arm sensation.

The frequency of alteration to light-touch sensation was lower than that for alteration to pin-prick sensation with a maximum of 18.8% of axillary sampled patients reporting an alteration to arm light-touch sensation at 3 months falling to only 4% at 18 months. No patients from the sentinel node biopsy group were shown to have altered light-touch sensation of the arm after the 1-month follow-up visit or to the axilla after the 6-month follow-up visit.

One axillary sample patient developed altered sensation to light-touch of the arm at 18 months after the 12-month follow-up visit had been normal. Reasons for this are unclear but are unlikely to be related to her surgery.

Table 4.6 displays the numbers and percentages of patients affected by altered sensation of the axillary skin and the skin of the upper arm to light-touch for both groups of patients. The mean areas of altered sensation and the ranges of area loss for both groups of patients are also displayed in the same table.

Table 4.6 **Light touch**

		SNB		ANS	
		AXILLA	ARM	AXILLA	ARM
1 MONTH	# patients	2/33	1/33	6/33	6/33
	% of patients	6.1	3	18.2	18.2
	Mean area	18	119	40.5	56.2
	Range of area	12-24	119	6-88	1-108
3 months	# patients	5/33	0/33	4/32	6/32
	% of patients	15.2	0	12.5	18.8
	Mean area	23.2	0	52.6	54
	Range of area	5-42	0	7-77	28-91
6 months	# patients	1/31	0/31	2/30	4/30
	% of patients	3.2	0	6.7	13.3
	Mean area	36	0	47	63
	Range of area	36	0	4-90	50-98
12 months	# patients	0/27	0/27	0/29	2/29
	% of patients	0	0	0	6.9
	Mean area	0	0	0	78
	Range of area				56-100
18 months	# patients	0/26	0/26	1/25	1/25
	% of patients	0	0	4	4
	Mean area	0	0	1	30
	Range of area	0	0	1	30

Figure 4.11 **Altered light-touch sensation axillary skin**

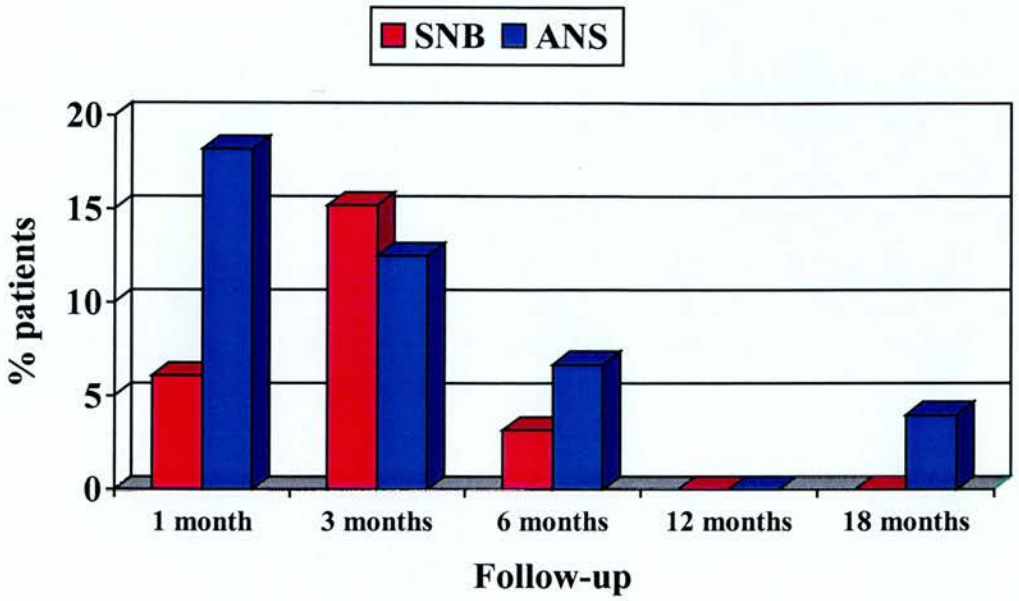
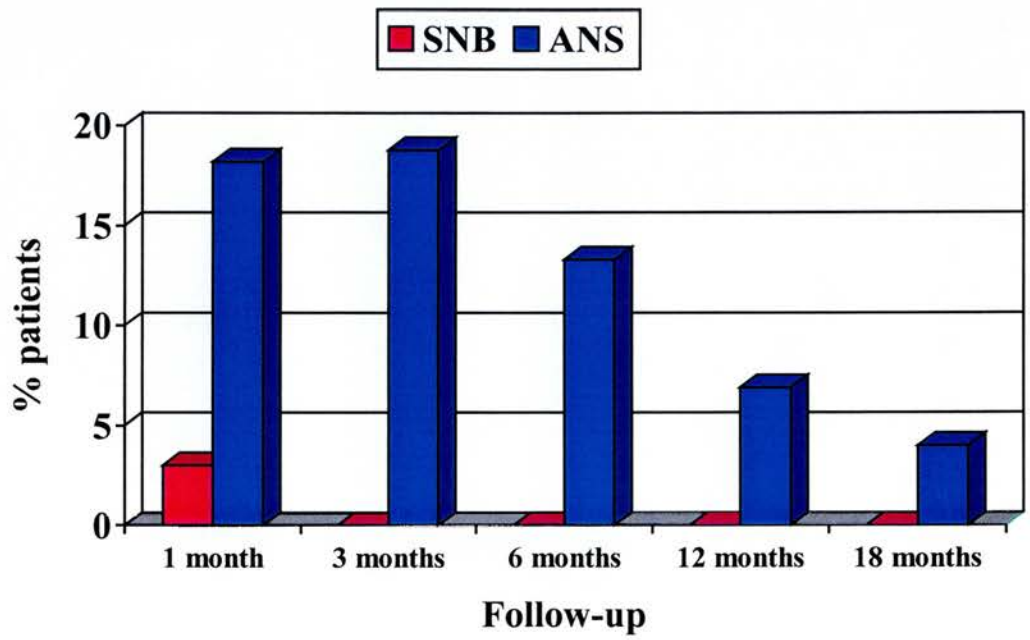


Figure 4.12 **Altered light-touch sensation upper arm skin**



iii. Shoulder stiffness

There was no significant difference in reduction in shoulder mobility between the two groups, $p>0.5$.

More patients had a measured reduction in abduction, internal and external rotation than in flexion, which was the least affected of the shoulder movements.

There was no clear trend towards a decrease in shoulder stiffness over the 18-month follow-up period.

The numbers and percentages of patients with measured reduction in shoulder joint mobility are presented in table 4.7 along with the ranges of reduction in shoulder mobility.

Figures 4.13-4.16 display graphically the trends in shoulder stiffness for all four measured movements separately and then in figure 4.17 for all four movements combined.

Table 4.7 Shoulder stiffness

		SNB			ANS		
		# PTS	% PTS	RANGE	# PTS	% PTS	RANGE
FLEXION	1 MONTH	2/33	6.1	18-41	3/33	9.1	15-18
	3 MONTHS	2/33	6.1	15-19	4/32	12.5	12-24
	6 MONTHS	3/31	9.7	10-19	1/30	3.3	17
	12 MONTHS	1/27	3.7	29	2/29	6.9	23-33
	18 MONTHS	2/26	7.7	10-12	3/25	12	15-34
ABDUCTION	1 MONTH	5/33	15.2	12-68	9/33	27.3	10-37
	3 MONTHS	5/33	15.2	10-34	9/32	28.1	12-35
	6 MONTHS	3/31	9.7	10-14	7/30	23.3	11-20
	12 MONTHS	6/27	22.2	11-49	12/29	41.4	10-46
	18 MONTHS	7/26	26.9	10-48	7/25	28	10-32
INTERNAL ROTATION	1 MONTH	1/33	3	16	4/33	12.1	11-20
	3 MONTHS	2/33	6.1	12-15	5/32	15.6	10-15
	6 MONTHS	7/31	22.6	12-18	6/30	20	10-35
	12 MONTHS	6/27	22.2	10-34	6/29	20.7	12-61
	18 MONTHS	3/26	11.5	21-68	3/25	12	13-45
EXTERNAL ROTATION	1 MONTH	3/33	9.1	15-25	5/33	15.2	10-26
	3 MONTHS	5/33	15.2	10-14	2/32	6.3	13-18
	6 MONTHS	4/31	12.9	12-19	2/30	6.7	10-20
	12 MONTHS	5/27	18.5	11-59	6/29	20.7	14-82
	18 MONTHS	5/26	19.2	10-94	1/25	4	20

Figure 4.13 **Shoulder stiffness-flexion**

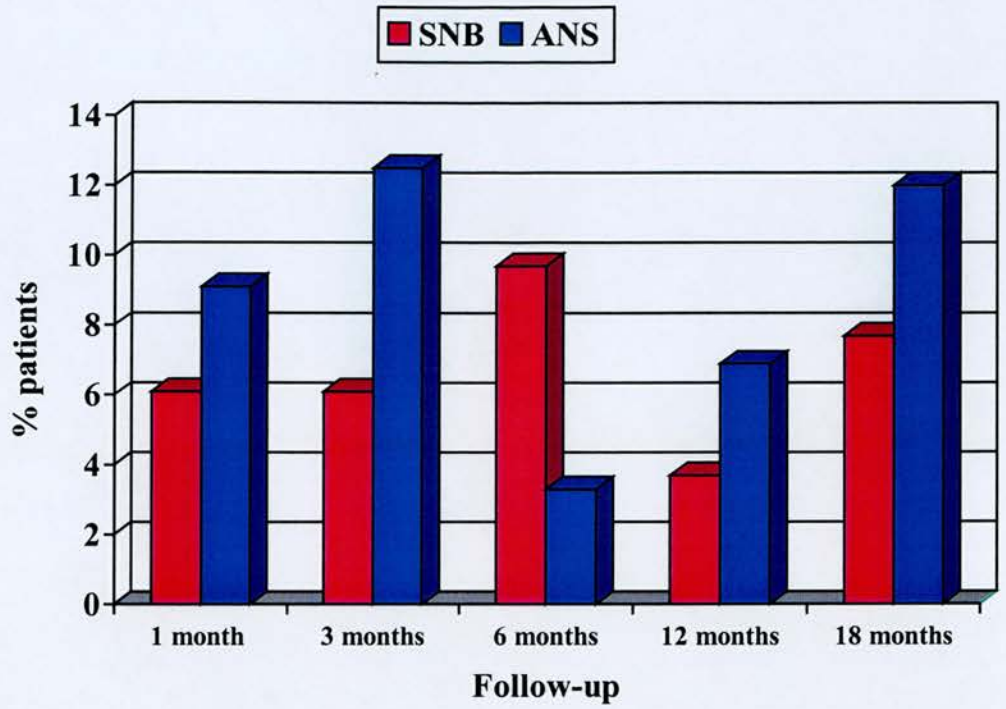


Figure 4.14 **Shoulder stiffness-abduction**

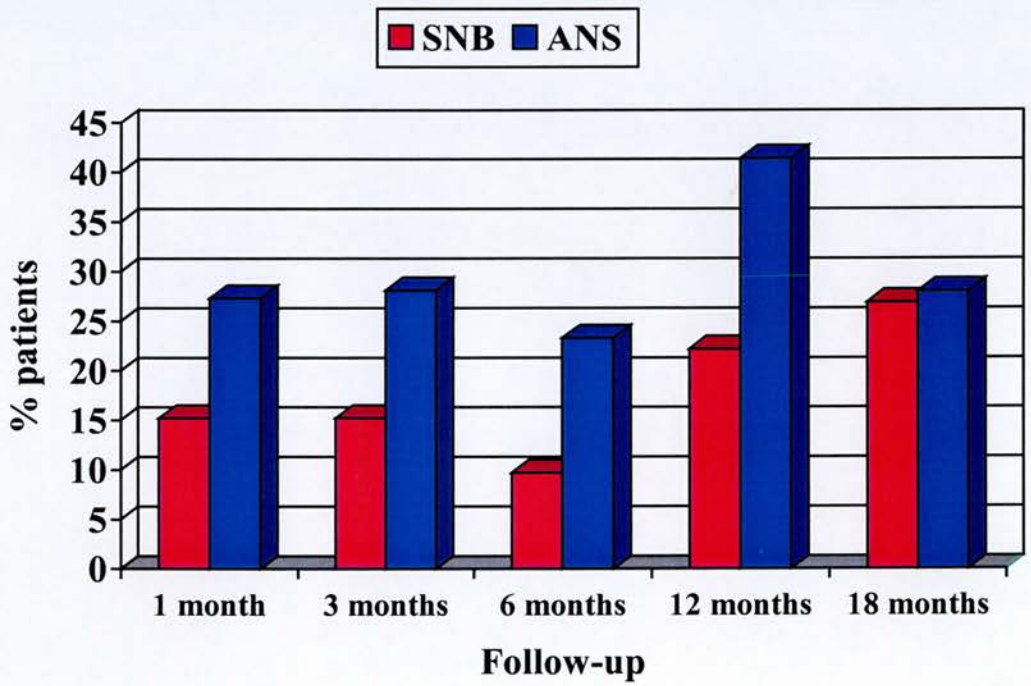


Figure 4.15 **Shoulder stiffness-internal rotation**

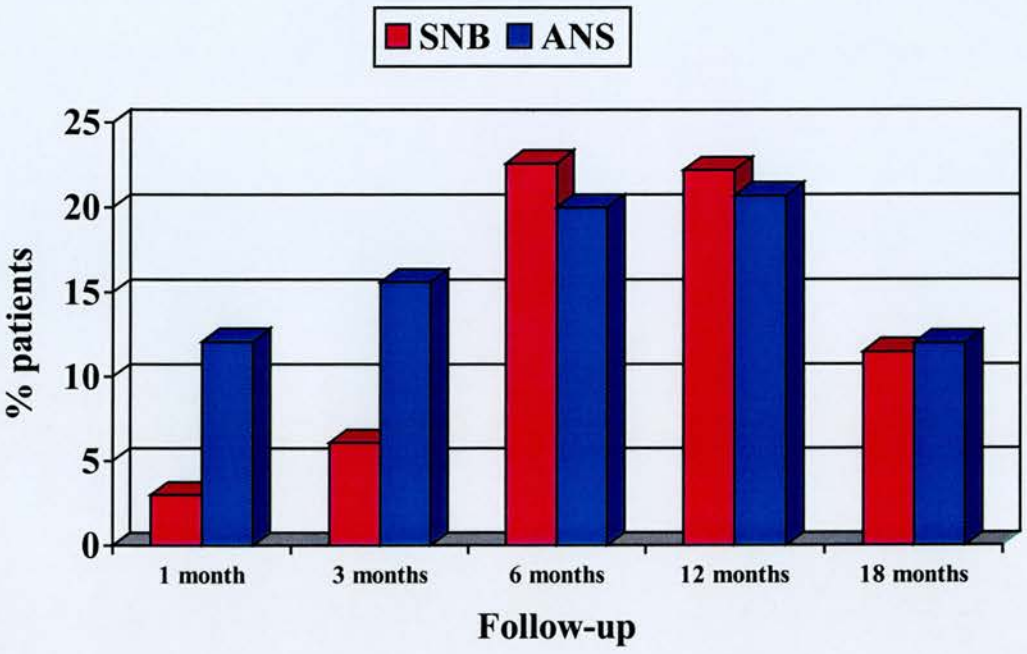


Figure 4.16 **Shoulder stiffness-external rotation**

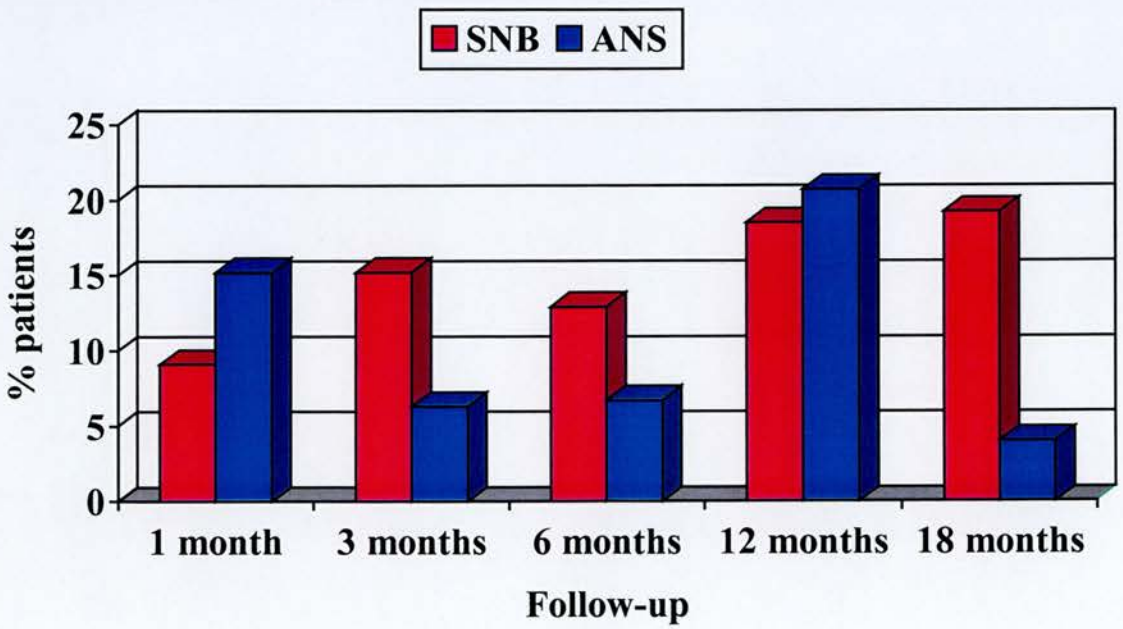
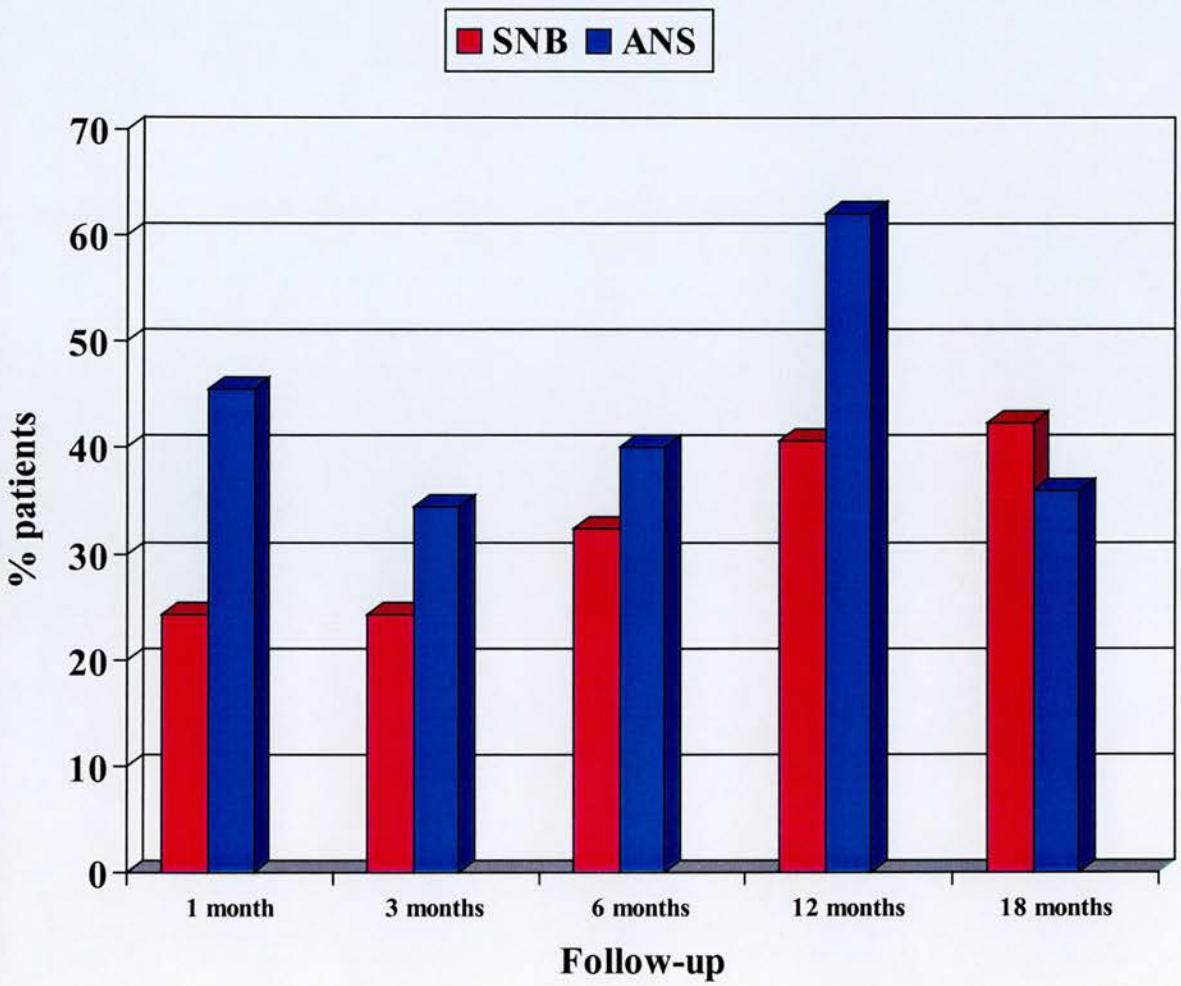


Figure 4.17 **Shoulder stiffness all movements**



4.5 DISCUSSION

Axillary node sampling and sentinel node biopsy both involve the surgical removal of a small number of axillary lymph nodes to stage the axilla. The mean number of nodes removed in an axillary sample was 4.79 per patient with a mean of 3.6 nodes removed during a sentinel node biopsy. Interestingly, only 11.7% of patients (4 of 34) having an axillary node sample had lymph node metastases in contrast to 24.2% of patients (8 of 33) having lymph node metastases following a sentinel node biopsy. These figures suggest that sentinel node biopsy may be a more exact method of identifying lymph node metastases within the axillae of patients with T1-2 breast cancer. Although the sentinel node group had slightly more special types and grade 1 cancers than the axillary node sample group, tumours from the two groups were otherwise well matched for histological type and presence or absence of lymphovascular invasion. Lymphovascular invasion (LVI) was present in 25% of tumours of positive sentinel node biopsies (2 of 8) compared with 8% (2 of 25) of node-negative sentinel node biopsies. In contrast, 75% of node-positive axillary samples (3 of 4) had tumours showing lymphovascular invasion compared with 6.7% (2 of 30) of the node-negative primary tumours. These figures are in keeping with other studies which correlate the presence of lymphovascular invasion with the finding of lymph node metastases [24, 228, 229]. However, numbers are very small and cannot be readily interpreted. The presence of lymphovascular invasion did not affect the ability to identify a sentinel node and this is in agreement with Hill's large study of 500 sentinel node biopsies [165]. The fact that sentinel node biopsy involved removal of one fewer node per patient than an axillary node sample adds strength to the theory that sentinel node biopsy is targeting those areas to which disease spreads within the axilla more accurately. Although only small numbers have been used here, the results are very promising and support further research using the technique in larger numbers of patients. All patients who had positive axillary node metastases received adjuvant axillary radiotherapy apart from one patient who was treated by axillary clearance based on unit policy using prognostic criteria.

All patients (100%) having a sentinel node biopsy had at least one lymph node, which was stained with blue dye and at least one node with radioactivity. The identification rate of 100% with each mapping agent is in contrast to phase one of the trial in which the combination of agents led to a higher identification rate than with the use of a single mapping agent alone. The mean number of sentinel nodes identified per patient was higher in phase two of the trial suggesting that the surgeons were perfecting the technique and were more successful at locating sentinel lymph nodes than in phase one. Not all sentinel nodes were labelled with both mapping agents simultaneously being frequently labelled by one or other agent alone. It is important to emphasise here that both mapping agents must be used therefore, in order to identify **all** sentinel nodes and that the use of a single mapping agent whilst having the potential to identify at least one sentinel node in each patient, will not identify **all** of the sentinel nodes in **all** of the patients.

The radioactivity count contained within a sentinel node does not appear to be a proportionate indicator of metastatic disease. In 37.5% of patients with at least one positive sentinel node, the node that contained the tumour was not the node with the highest radioactivity count. This stresses the importance of continuing to search the axilla for further radioactivity to identify such nodes after very hot nodes have been removed. The original definition of the sentinel node described it as the first draining lymph node of the primary tumour. Clearly there may be more than one node to which the primary tumour can first drain as parallel lymphatics from a similar area of the breast can cross directly to different lymph nodes within the regional lymph node basin. That said, it is still possible for a single node alone to be the only sentinel node.

A sentinel node biopsy should thus not be considered complete until all hot or blue staining nodes have been removed for histology.

A similar incidence of internal mammary node drainage was seen in this phase of the study as in phase one of the trial. Two patients (6.1%) had internal mammary node biopsies and neither of them revealed a positive node. The incidence of internal mammary drainage is lower than reported in some studies [230] although this particular study used a much higher dosage of radioisotope than in our study. Clearly, the drainage of radioisotope to the internal mammary basin is not related to

node positivity in this area so the routine biopsy of internal mammary nodes purely on the basis of a positive scintiscan does not guarantee additional staging information. The procedure was associated with a high complication rate within this study and as there is no currently agreed consensus recommending the routine removal of internal mammary nodes for staging purposes, it cannot be justified for use out with controlled randomised trials.

Lymphoscintigraphy results were almost identical to those found in phase one with a 75.8% identification rate. In keeping with the phase one findings, preoperative lymphoscintigraphy of the axillary and internal mammary lymph nodes did not offer much additional information for the operating surgeon other than to demonstrate the small number of patients in whom drainage to the internal mammary nodes was observed. The presence of a positive scan before surgery, however did reassure the surgeon that sentinel nodes would be found at surgery whereas a negative scan was associated with the feeling that sentinel nodes might be more difficult to find at surgery. This would be in keeping with the finding of Hill et al [165], that a positive result on lymphoscintigraphy was significantly associated with the successful location of a sentinel node($P=0.0001$).

The in-patient stay was the same for both groups of patients staying a mean of 2 nights in hospital. Sentinel node biopsy was, however, associated with a longer operation time taking on average 8 minutes longer than an axillary node sample to perform. When a sentinel node biopsy was performed, extra time was taken up recording 10-second radioactivity counts for each sentinel node excised. Counts were recorded both in-vivo and ex-vivo. The two consultant surgeons performing the sentinel node biopsies were both very experienced in the technique of axillary node sampling before this study began, whereas they had performed significantly fewer sentinel node biopsies. This provides another reason for the longer operating time in the case of sentinel node biopsy. It is reasonable to expect the operating time for sentinel node biopsy to approach that of an axillary node sample as experience is gained. Searching for lymph nodes under direct vision by following the course of blue lymphatics is also likely to be a slightly slower process than reaching directly into the axillary tissue and plucking out nodes by palpation, as it requires more exact dissection than the latter. The small amount of extra time required to perform a

sentinel node biopsy may be justified if larger trials prove the greater accuracy of this technique over axillary node sampling.

Analysis of the relationship of patient and tumour characteristics with the number of sentinel nodes identified revealed that high body mass index was significantly associated with identification of fewer sentinel nodes. The reasons for this are unclear and may either be related to differences in lymphatic dynamics between overweight and slimmer patients or possibly to a greater degree of technical difficulty in locating sentinel nodes surgically in overweight patients. It is perhaps more likely that overweight patients have a less dynamic lymphatic system than slimmer patients causing the migration of tracer agents to be slower and thus reaching only one or two nodes as opposed to four or five. This finding may have some relevance to the success of sentinel node biopsy in certain very obese patients in whom the procedure may have to be abandoned in favour of a conventional axillary node sample to stage the axilla should sentinel node identification fail.

Increasing tumour size was not significantly associated with identification of fewer sentinel nodes although there was a weak correlation as shown by the trend line on the scatter plot figure 4.1. Again reasons for this are unclear but possibly relate to the relatively smaller volume of mapping agent injected per unit of breast volume in patients with larger tumours as the volume of tracer is spread over a greater volume of breast tissue. As has been mentioned in the previous chapter, one study reported greater success in sentinel node identification when injecting a greater volume of radiopharmaceutical [225] and this may be worth considering in patients with larger tumours. Another explanation is that increasing tumour size is associated with an increased incidence of lymphatic spread and this may lead to obstruction of some lymphatics by tumour deposits, which may in turn reduce the number of sentinel nodes to which the mapping agents will travel.

The location of the primary tumour in the lateral half of the breast was associated with the identification of significantly more sentinel nodes than with tumours located in the medial half of the breast. The relatively shorter distance for mapping agents to travel to the axillary lymph nodes perhaps explains this finding. This is likely to result in the spread of mapping agents to a greater number of lymph nodes by the time of surgery. This finding is in contrast to the findings of Krag and Hill who

reported no significant association between tumour location and sentinel node identification although Krag does note a non-significant relatively higher rate of failure to identify a sentinel node in medial tumours [163, 165, 231]. Another explanation given by Krag for this finding was the possibility that medial tumours may mask internal mammary 'hot spots' by the interference of radioactivity from the overlying tumour following injection of radioisotope. Certainly in our group of patients we did not consider any particular tumour location to be a significant hindrance to sentinel node identification although it was felt that upper outer quadrant tumours occasionally caused interference from local radioactivity or diffusion of blue dye from the primary injection site.

The dose of radioisotope injected was unrelated to the success at locating a 'hot spot' on scintiscan but timing of injection did appear to have some bearing on this although this was not a strong link and was not statistically significant. It is probably wise to allow at least 3 hours after injection of isotope before performing a scintiscan, as this is likely to maximise the chance of a positive scan. Krag et al reported that the type of radiopharmaceutical, amount of technetium, volume injected, tumour size and the time interval between injection and surgery were significantly associated with success at finding a hot sentinel node [231], which is in agreement with some of our findings mentioned above. In contrast to our results, however, Krag did not find an association between tumour location and success at identifying a hot sentinel node. Whilst these findings are relatively subtle and are unlikely to significantly change the outcome of a sentinel node biopsy, they do provide us with a little more information in helping us to understand the exact dynamics of lymphatic mapping and sentinel node identification.

The rate of immediate post-operative complications was higher in the sentinel node biopsy group of patients than in the group having an axillary sample. In particular, breast seroma, axillary seroma and breast infection rates were all at least three times more frequently encountered in patients having sentinel node biopsy than in those having an axillary node sample. The numbers of patients in this phase of the study are small and statistical analysis did not reach significance for these results. These findings are closely associated to those found in phase one of the trial, however, and are surely related to the repeated needle punctures of the breast and the localised

oedema caused by injection of mapping agents. It was suggested in the previous chapter that fewer needle punctures should be used when injecting mapping agents, for example two punctures should suffice for each agent, as the clinician can direct the injection subcutaneously around the full 360° circumference of the tumour using this method without the need for multiple skin punctures. In addition, as mentioned in the last chapter it may be sensible to administer a prophylactic dose of antibiotic, such as co-amoxiclav 1.2g intravenously, at induction of anaesthesia in an attempt to reduce the post-operative infection rate.

One of the two patients having an internal mammary node biopsy developed a moderate post-operative pneumothorax requiring a single aspiration on the ward. This patient's discharge was delayed by one day but she made a complete recovery. Another patient in phase one of the study also developed a pneumothorax following an internal mammary node biopsy, which resulted in a significant delay to her discharge. As mentioned earlier in this discussion and in the previous chapter, internal mammary node biopsy did not add any staging information to any of the patients in this study and was associated with a very high complication rate with some potentially serious complications. It is not routinely practised out with trials in Edinburgh and it is likely to be proved to be of little value when adequate numbers of patients have been investigated.

The single patient who developed a widespread cutaneous hypersensitivity reaction to Patent blue V dye recovered within 12 hours requiring supportive management and observation only. A single patient in phase one also developed a cutaneous hypersensitivity reaction to blue Patent blue V dye and followed a similar course of recovery. The incidence of such a hypersensitivity to blue dye in this study is similar to that reported in other studies of 2.2% [194]. Any patient developing such a reaction should be informed of the product to which they are sensitive, advised to avoid further contact with that particular product, a report should be sent to the Committee for Safety of Medicines (CSM) detailing the adverse effect(s) witnessed and a further report should also be sent to the patient's general practitioner. Clear documentation of the incident must be made in the patient's medical notes with an allergy-warning message written preferably on the front of these notes. It is also wise

to inform any patient who is considering undergoing lymphatic mapping of the small risk of a hypersensitivity reaction.

Blue dye tattooing of breast skin was less frequently encountered than in phase one and appeared to decrease over time with only one patient having persistent tattooing at 12 months. None of the patients with tattooing considered this to be a problem as the discolouration faded with time in the majority. It is not clear why so many patients were left with blue tattooing after phase one but from the results in phase two it could be expected that the persistence of blue tattooing in phase one patients at 3 months would fade and disappear in the majority of cases.

Arm swelling was not a major problem in the majority of patients. Although a record has been made of any patient who was observed as having an increase of over 100mls in arm volume when compared to their pre-operative measurement, independently of the contralateral arm, few patients had an increase of greater than 10% of their pre-operative arm volume. A 10% increase in arm volume is the level at which lymphoedema therapists would usually begin to commence treatment regimes for patients starting with simple massage and skin care programmes. One patient in the axillary sample group was measured as having a 20% increase in arm volume at 18 months but interestingly she did not report any subjective awareness of an increase. It is possible that this particular measurement may be spurious and a result of a measurement error. The technique used to measure arm volume in this study is particularly prone to error particularly if not performed by the same operator on each occasion. The author of this thesis and one research nurse who had been instructed on the measuring technique by the author made the majority of arm measurements although on one or two occasions towards the end of the follow-up period, a research fellow in breast surgery made the measurements due to unavailability of the other two staff members. It is worth considering a more foolhardy technique for measuring subtle arm volume changes such as a water displacement tank in which the arm is submerged up to a constant mark and the volume of water displaced is recorded. This method is likely to be more readily reproducible than the tape measure method employed here. An alternative option would be only to record cases of arm swelling which the patient perceives to be troublesome as this is a more realistic indicator of morbidity.

Patients having axillary node sample were more frequently likely to have altered sensation of the skin of the axilla and upper medial arm than those having a sentinel node biopsy. The difference between the two groups was statistically significant for light touch sensation of the upper arm at 3 months post-operatively in favour of sentinel node biopsy. More patients had altered pin-prick sensation to the skin of the axilla and upper arm at one and three months after an axillary sample than after a sentinel node biopsy although figures did not reach statistical significance because of small numbers. It would appear from these results that sentinel node biopsy is less invasive than axillary sample in terms of damage to the intercostobrachial nerve. This is possibly explained by the more exact dissection under direct vision required for this procedure as opposed to the blind and blunt dissection used to extract lymph nodes during axillary sampling. Having stated this, it is clear from the results that the majority of patients will only have a transient loss of sensation, which will most likely return completely within 6 months of surgery. Fewer patients suffered loss of light touch sensation than pin-prick sensation regardless of the procedure performed. This suggests that the Aδ (or 'fast pain') nerve fibres of the intercostobrachial nerve are more susceptible to trauma than those which conduct light-touch sensation. Recovery of pin-prick sensation was similar to that for light touch over the 18 months with less than 10% of patients in either group being affected at 18 months. Abduction was the most commonly affected movement at the shoulder joint followed by rotational movement. Other studies of arm morbidity have reported shoulder flexion and rotation as the most commonly affected [84, 128] but related the shoulder stiffness to axillary radiotherapy or an axillary node clearance. Rotational shoulder stiffness and abduction appeared to be slightly more frequently measured from the 6-month follow-up visit onwards. The effects of radiotherapy on the shoulder joint rather than the axillary surgery may possibly explain this. There was no significant difference between the two groups in terms of shoulder joint stiffness suggesting neither procedure is more damaging than the other to the shoulder's mobility. The fact that shoulder mobility did not improve considerably over the 18-month follow-up period seems disappointing. The rather minimal surgical invasion of the axilla might suggest that the surgery is an unlikely cause of this persistent stiffness in some patients and that radiotherapy might be a more likely cause. Certainly, a greater

proportion of patients having axillary radiotherapy were found to have persisting shoulder stiffness than those who had no radiotherapy to the axilla.

There were no axillary recurrences in either group of patients during the median 18-month follow-up period. These are encouraging preliminary results although a longer period of follow-up is required before sentinel node biopsy can be confirmed as being safe.

5. IMPRINT CYTOLOGY OF AXILLARY LYMPH NODES AS AN INTRAOPERATIVE DIAGNOSTIC TOOL

5.1 BACKGROUND

Imprint cytology is a special variation of applied cytology. It can be used as an adjunct to frozen and paraffin section as a diagnostic tool. Imprints can reveal very subtle diagnostic changes and also make it possible to make a diagnosis in a shorter time than with paraffin section [232].

Sentinel node biopsy is being validated as an alternative to axillary node clearance in staging the axilla in breast cancer. This will inevitably lead to the development of a group of patients with positive sentinel nodes either requiring a second operation to clear the axilla of its remaining lymph nodes or being treated by axillary radiotherapy. Currently, some centres use an axillary sampling technique to stage the axilla by removing a small number of selected lymph nodes, those with positive nodes likewise requiring further axillary treatment. A reliable and accurate intraoperative technique of examining lymph nodes would mean that a second operation could be avoided because patients with positive nodes could have an axillary clearance during the same sitting. This would avoid the considerable anxiety and emotional upset associated with a second operative procedure.

It is also possible that a significant number of sampled patients with stage T1-2 breast cancer could avoid an axillary clearance if nodes were negative following intraoperative examination [206]. This would lead to a shorter time in hospital than is usual following complete axillary dissection and would reduce the rates of arm morbidity, particularly lymphoedema, which is more commonly associated with lymph node clearance.

Frozen section histological examination of lymph nodes is known to have a high false negative rate of up to 27% in some studies[197]. Imprint cytology has been reported to have a lower false negative rate with the sensitivity reaching one hundred per cent in some studies[207].

It is important to realise that when performing a frozen section procedure, 25-50% of the material may be lost[200]. A potentially serious consequence of this could be the failed detection of a small area of micrometastasis within part of a lymph node.

Imprint cytology does not lead to any loss of material and the entire specimen is available for histological and immunohistochemical examination if necessary. The aim of the current study was to assess the reliability of imprint cytology as an intraoperative diagnostic tool in patients having an axillary sampling procedure as part of breast cancer surgery.

5.2 PATIENTS AND METHODS

We examined 238 lymph nodes in 53 patients having an axillary node sample of at least four lymph nodes for T1–2 breast cancer. There was no age restriction and patient age ranged from 32 to 82 years. All but three patients had a breast conserving wide local excision to remove their primary breast cancer with the remaining three patients having a mastectomy.

Axillary node samples were performed through a transverse curvilinear incision just inferior to the hair-bearing area in the axillary skin crease. At least four lymph nodes were removed from the lower axilla using a standard technique of digital palpation. Each fresh lymph node was processed immediately. Lymph nodes were sliced every 3-4mm and imprints were taken from each cut surface. Two identical sets of imprints were prepared on two separate slides for each lymph node. All slides were immediately fixed in a methanol and acetone 50:50 mix. One set of slides was stained with toluidine blue before drying and mounting cover slips. The second set was stained with anti-pancytokeratin (Pan-CK) immunoglobulin as an immunohistochemical marker to determine whether this improved the sensitivity of the test. Immunohistochemistry was performed using a standard technique of antigen retrieval, buffer washes and incubation with primary and secondary antibodies using a streptavidin-biotin complex and immunoperoxidase with the labelling antigen diaminobenzidine. Cells were then counterstained with haematoxylin to show up the nuclei before undergoing fixation and mounting.

A single consultant histopathologist (Dr. M. A. McIntyre) examined all of the slides. A non-pathologist (the author, PAL) also examined all of the slides to determine whether using a technician to screen slides could decrease a pathologist's workload. The non-pathologist received regular feedback on imprint examination from the consultant histopathologist during the study. Both examiners were blinded to the routine histology results prior to examining the nodes.

Lymph node metastases were defined as those measuring greater than 2mm in size (i.e macrometastases not micrometastases) and were compared against the currently accepted standard of haematoxylin and eosin (H & E) histology.

5.3 RESULTS

Of the 53 patients in the study, six patients were found to have in-situ disease only on H & E histology. These patients were excluded from the analysis. This left 217 lymph nodes from 47 patients with invasive T1-2 cancer. Imprints were not possible in five of these lymph nodes as they were too small leaving 212 nodes available for analysis. None of the five lymph nodes excluded were subsequently shown to have metastases on H & E histology.

Ten positive lymph nodes in six patients were found using H & E histology of the 217 lymph nodes examined. Imprint cytology and toluidine blue staining detected metastases in eight of these. One further positive node was identified by imprint cytology, which was reported as negative on routine histology. The H&E histology was reviewed by the consultant histopathologist and thought to be equivocal rather than definitely negative or positive. Immunohistochemical marking using Pan-CK (antipancytokeratin immunoglobulin) was performed on this lymph node and this confirmed the presence of metastatic deposits measuring greater than 2mm in diameter (i.e macrometastasis not micrometastasis) within this lymph node. Consequently there were 11 positive lymph nodes identified in six patients. Imprint cytology detected 9/11of these giving a sensitivity of 82% and a false negative rate of 18%. There were no false positive results amongst the cases analysed giving a specificity of 100% and a positive predictive value of 100% (9 of 9). The negative predictive value was 99% (201 of 203). Routine H&E detected 10/11 of the positive nodes giving a sensitivity of 91% and a false negative rate of 9%.

Six of 47 patients had positive lymph nodes. All six patients had lymph nodes identified as positive by the consultant histopathologist on imprint cytology preparation. Analysis of data according to the lymph node sample as a whole rather than for single lymph nodes eliminates the false negatives as all patients with positive samples were detected by imprint cytology and would thus have gone on to have a lymph node clearance or axillary radiotherapy in clinical practice. Using this analysis the sensitivity is 100% with a false negative rate of 0%.

Using anti-pancytokeratin antibody (Pan-CK), seven positive lymph nodes were detected by the consultant histopathologist. Four of these were positive on routine H & E histology. The sensitivity was thus only 36.4% with a false negative rate of 63.6% using immunohistochemistry. Three lymph nodes were found to be positive with Pan-CK but were negative on routine H & E histology. The original H & E histology for these three lymph nodes was re-examined but malignant cells were not seen. The positive predictive value was thus 57% (4 of 7). The false positive rate was 1.5% with a specificity of 98.5% for immunohistochemistry. The negative predictive value was 98% (201 of 205).

A non-pathologist (surgical research fellow, PAL) also examined all lymph nodes to determine whether an untrained technician could help in screening for positive cases. Only five of the eleven positive lymph nodes were detected on examination of the toluidine blue imprints giving a sensitivity of 45% and a false negative rate of 55%. There were also 12 false positive cases giving a false positive rate of 6% and a specificity of 94%. The positive predictive value was thus 29.4% (5 of 17), negative predictive value was 97% (189 of 195). A diagnosis could not be made from ten of the slides and these were checked by the histopathologist and found to be negative. Examination of the Pan-CK slides revealed 6 of the 11 to be positive giving a sensitivity of 54.5% and a false negative rate of 45.5%. There were 2 false positives giving a specificity of 99% and a false positive rate of 1%. Positive predictive value was 75% (6 of 8). Negative predictive value was 98.5% (201 of 204). There were two slides in which a diagnosis was unclear and these were checked by the histopathologist and found to be negative.

All metastatic deposits identified and used in the results were defined as those measuring greater than 2mm in size.

Table 5.1 shows the figures for each staining method and for each examiner for sensitivity, specificity, positive and negative predictive values.

Table 5.1 Values of each staining method as examined by a consultant histopathologist and a non-pathologist

.

	Consultant Histopathologist		Non-Pathologist	
	Toluidine Blue	Pan-CK	Toluidine Blue	Pan-CK
Sensitivity	82%	36.4%	45%	54.5%
Specificity	100%	98.5%	94%	99%
Positive Predictive Value	100%	57%	29.4%	75%
Negative predictive Value	99%	98%	97%	98.5%

Legends to Figures 5.1-5.6 (Imprint photomicrographs)

Figure 5.1 Negative Toluidine Blue Imprint with a typical mixed picture of lymphoid cells predominantly lymphocytes.

Figure 5.2 Positive Toluidine Blue Imprint showing a cluster of cells with large nuclei and nuclear pleomorphism against a background of lymphocytes.

Figure 5.3 Positive Toluidine Blue Imprint. Abnormal cells appear in loose clusters with poor cell-cell adhesion, high nuclear:cytoplasmic ratio and nuclear pleomorphism.

Figure 5.4 Strongly positive brown staining of malignant cells with Pan CK against a background of non-malignant, unstained lymphocytes.

Figure 5.5 Weakly positive Pan CK staining of a group of malignant cells.

Figure 5.6 Strong positive Pan CK staining of malignant cells detected initially on Toluidine blue imprint of a lymph node, which was reported as being negative on routine H&E histology

Figure 5.1- Negative Toluidine Blue Imprint x40

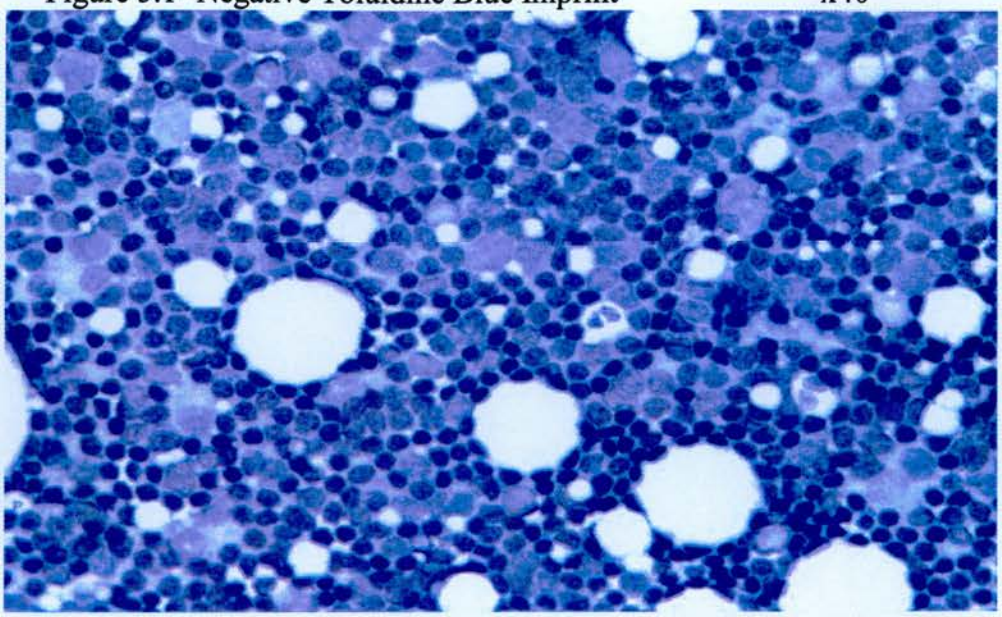


Figure 5.2-Positive Toluidine Blue Imprint x40

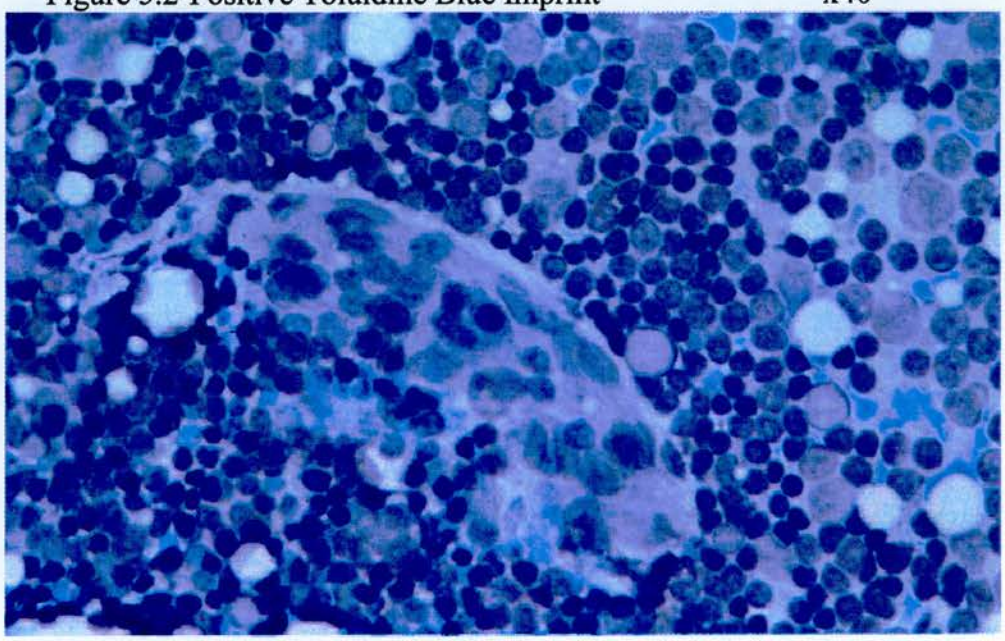


Figure 5.3-Positive Toluidine Blue Imprint x40

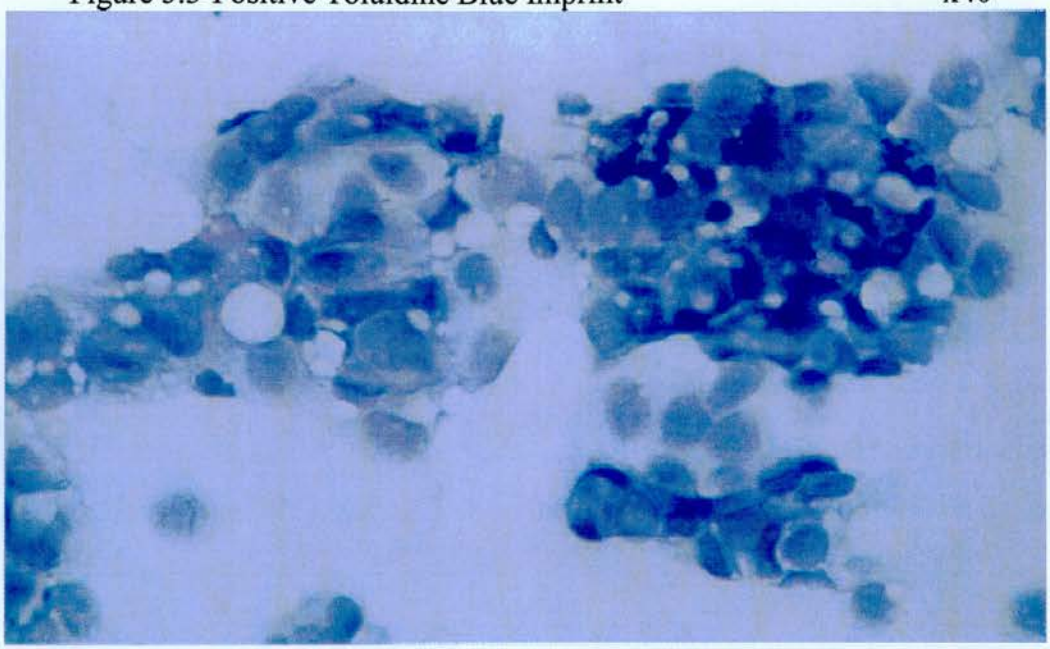


Figure 5.4- Pan CK positively staining malignant cells x40

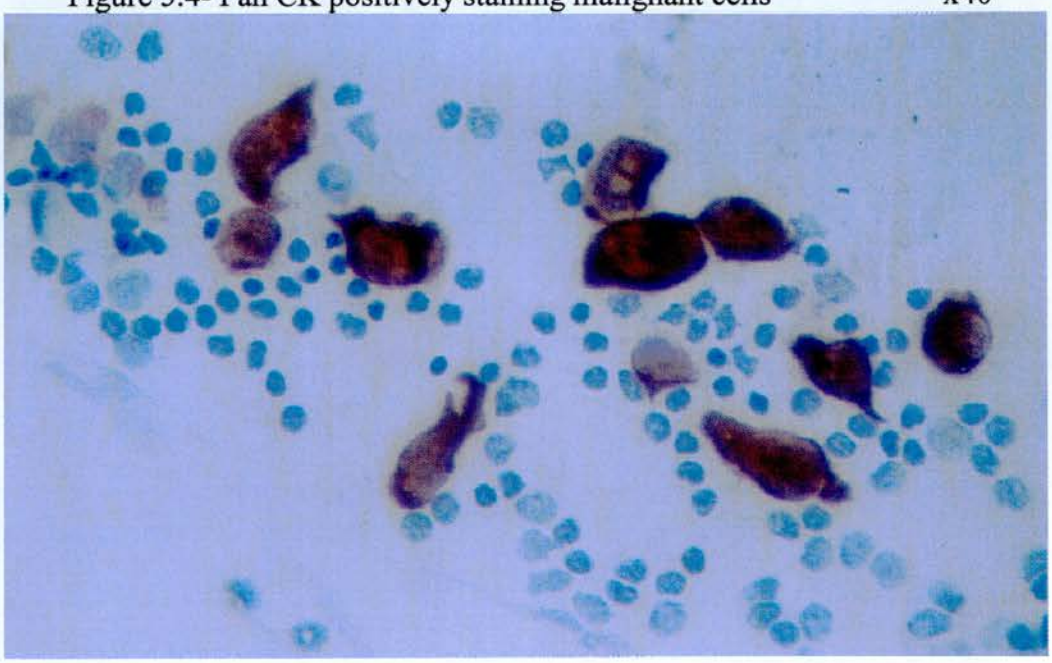


Figure 5.5-weakly positive Pan CK staining x25

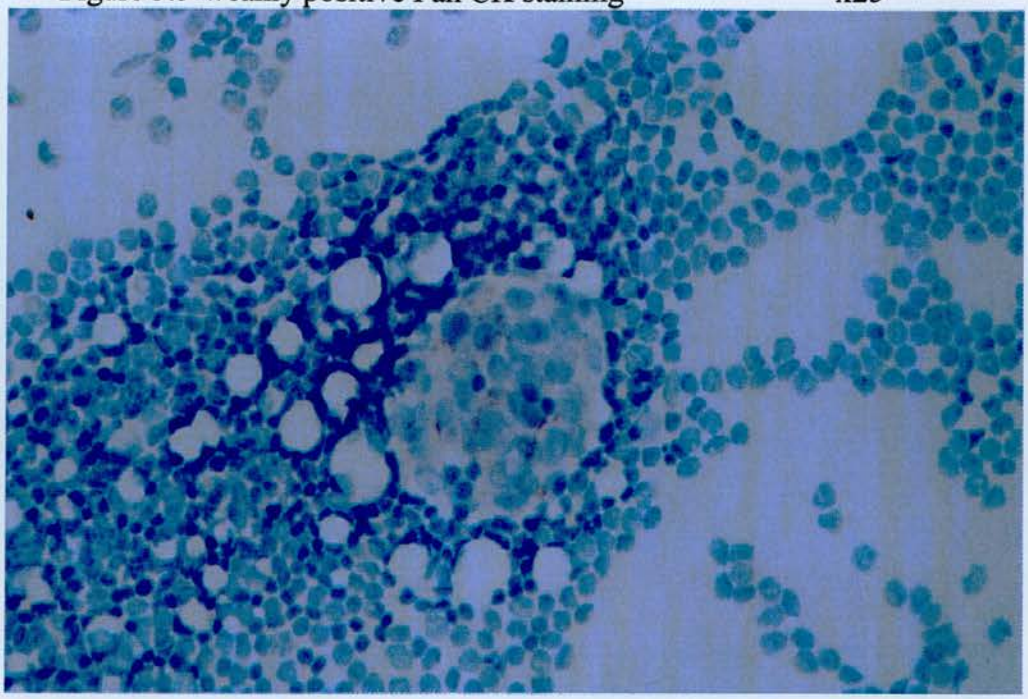
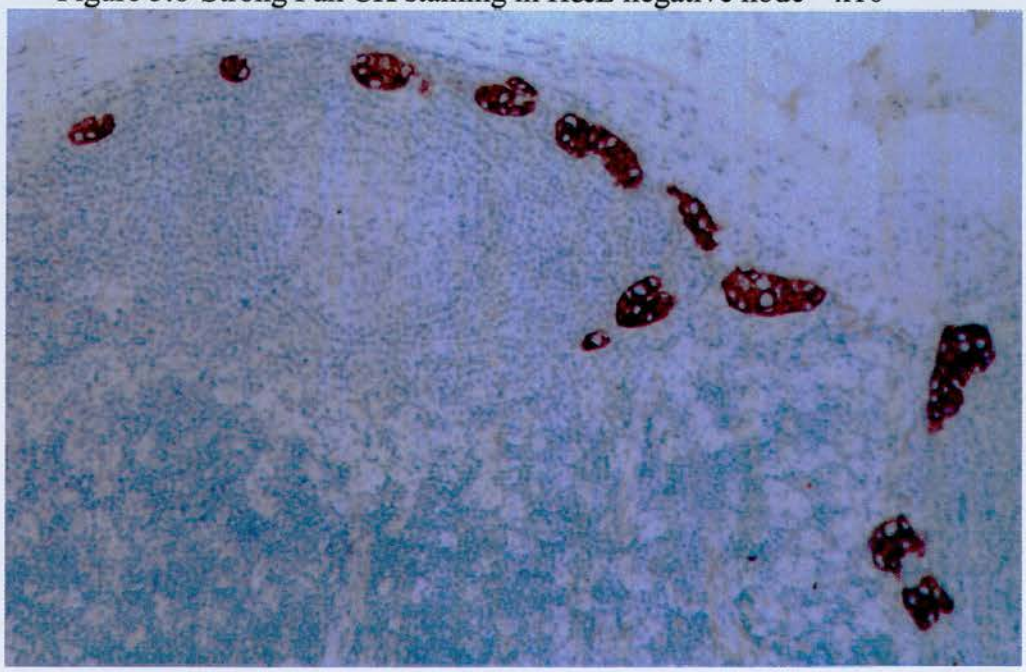


Figure 5.6-Strong Pan CK staining in H&E negative node x10



5.4 DISCUSSION

Our results show a similar false negative rate to frozen section histology when imprints are stained with toluidine blue dye and examined by a consultant histopathologist. This is in contrast to several studies which report a higher sensitivity of imprint cytology than frozen section histology[207, 209, 233]. The addition of an immunohistochemical staining with Pan-CK did not increase yield in our series and was actually associated with a higher false negative rate than that with toluidine blue. In some of the positive lymph nodes, malignant cells could be clearly seen but were not staining positively with Pan-CK. This suggests a failure of the antibody marker and it is possible that the use of a different antibody may improve these disappointing results.

Examination of lymph node cytology by an untrained non-pathologist does not appear to be a reliable option for reducing pathologists' workload. Cytology is a specialised subspecialty of pathology and requires considerable expertise to make an accurate diagnosis. However, when immunohistochemistry is used the yield of a non-pathologist is improved. This is of potential value should a more reliable immunohistochemical marker become available, as detection rates were much closer to those of the histopathologist when using this technique. Some centres have used the monoclonal antibodies AE-1 and CAM 5.2 with reasonable success in lymph nodes and these may prove useful in imprint cytology[48].

The positive and negative predictive values of the test using toluidine blue and a consultant histopathologist were very high. This suggests that the technique could be applied as an intra-operative diagnostic tool with the reassurance that a positive result can be wholly relied upon. This is obviously of great importance when performing axillary node clearance on the basis of such results. Patients would need to be warned of the risk of a false negative result in a minority of cases along with the possible need for further surgery later. Despite this failure rate, a second operation is potentially avoidable in at least 4 out of 5 cases with positive lymph nodes. In our series we would have avoided a second operation in six of 53 patients (11.3%) and there were no patients who would have required re-operation to the

axilla. The additional cost to the pathology department in materials by using toluidine blue imprint cytology is certainly lower than that for frozen section histology as it uses a single inexpensive staining agent only as opposed to the use of complex equipment such as that required for frozen section. Imprint cytology is likely to be faster to perform than frozen section thus requiring less technician time and further reducing costs. The cost of re-admission and a second axillary operation in six patients is a potentially greater expense than the imprint cytology of lymph nodes in 53 patients although a detailed cost-analysis has not been carried out in this study. Should sentinel node biopsy become a standard method of axillary staging in early breast cancer in the future, fewer lymph nodes would require examination than with an axillary sample. This would further reduce the pathology costs and workload involved with imprint cytology.

The two positive lymph nodes which were not picked up on imprint cytology by the consultant histopathologist were reviewed and a positive diagnosis could not be made as malignant cells were not present even in retrospect on these slides. Both these nodes were from patients in whom multiple nodes were involved and other involved nodes were identified on imprint cytology with Toluidine blue. The most likely explanation for this discrepancy between histology and cytology is that different levels of lymph nodes were sectioned for histology compared to those levels taken for imprints and unless the node is entirely replaced by tumour it is quite logical for some levels to be involved whilst others are not. Similar discrepancies have been reported in rates of detection of micro-metastases when serial sections of a lymph node are sampled as opposed to a single section. The presence of such micrometastases has been reported to adversely affect five year and overall survival[55] although the importance of micrometastases is not clear and remains a subject of considerable debate.

In conclusion, intra-operative imprint cytology has a similar sensitivity to frozen section when used as a diagnostic aid for axillary lymph node metastases. Imprint cytology does have one technical advantage over frozen section histology resulting in little interference with nodal material leaving the whole specimen available for routine and/or special analysis. Staining of imprints with antibody to pancytokeratin does not appear to be of added value. Further studies are required to assess whether

alternative immunohistochemical markers will improve the sensitivity of plain (non-immunohistochemical) imprint cytology. A non-pathologist (e.g MLSO/surgical trainee) without specific training is not acceptable to screen slides but whether or not such an individual would be more accurate at detecting involved nodes stained by a better immunohistochemical marker, should be assessed.

Imprint cytology may have a role in reducing the re-operation rate on the axilla in patients undergoing axillary sampling or sentinel node biopsy. The cost implications of performing imprint cytology on all patients having such axillary surgery as opposed to the cost of a second axillary operation in a small number of patients requires further study.

5.5 IMMUNOHISTOCHEMICAL STAINING OF IMPRINTS

After fixing slides in methanol and acetone, they were stained by the immunohistochemical technique. This involves a complex series of events as follows:

5.5.1 Preparation

1. Wash in xylene to dissolve any wax (not necessary in the case of imprint cytology).
2. Two changes of 100% ethanol to eliminate excess xylene.
3. One change of industrial methylated spirits (IMS)
4. Into water
5. Blocking of endogenous peroxidase from red blood cells with 0.5% hydrogen peroxide plus 0.1% sodium azide (10 minutes).
6. Antigen retrieval (histology only) – any cross-links in the protein chains need breaking to expose any hidden antigen lying within a loop of the protein chain. This may be done by two methods:
    1. Using an enzyme e.g. trypsin
    2. Heat specimen to boiling point or higher (specimens must be kept moist whilst heating).

Incubation – slides then irrigated in buffer bath of phosphate buffered saline (PBS) at pH 7.6. This keeps specimens damp.

5.5.2 Blocking stage

Before addition of antibody, the slides are treated with egg-white protein. This neutralises any positively charged proteins, which would otherwise be attracted indiscriminately by the antibodies' negative charges.
Wash in PBS for five minutes to remove excess egg-white from the specimens.

5.5.3 *Antibody staining*

Slides are processed using the "Shandon Sequenza" which is a sequenced set of containers for holding slides whilst they are treated with antibody.

1. Antibody diluted in PBS with biotin and bovine serum albumin (same function as egg-white) is added to the specimens
2. Incubation for thirty minutes with primary antibody
3. Rinse in PBS to wash off any unbound primary antibody
4. Incubate with secondary antibody (goat anti-mouse antibody) for thirty minutes
5. Incubate with Streptavidin ABC complex (streptavidin-biotin complex with peroxidase attached) for thirty minutes
6. Add peroxidase-labelling antigen

$$H2O2 + DAB\ (diaminobenzidine) \xrightarrow{peroxidase} H2O + O + DAB$$

$\downarrow$

Brown dye (stains cytoplasm)

Cells were then counter-stained with haematoxylin to show up the nuclei.

5.5.3 *Fixation*

Slides then undergo reverse sequence through alcohols:
IMS x1, two changes in ethanol 100% and xylene x1 before adding coverslip and mountant.

6. OCCULT LYMPH NODE METASTASES

Occult lymph node metastases in patients with node negative breast carcinoma following axillary node sample

6.1 AIMS

1. To compare the incidence of occult metastatic disease in the axillary lymph nodes of node-negative patients who subsequently developed axillary recurrence with those without axillary recurrence when treated by an axillary node sample and breast conserving wide local excision.
2. To examine the clinical significance of occult metastatic disease in the axillary lymph nodes of node-negative patients following a lymph node sample.
3. To assess the reliability of a non-pathologist after a limited training period, in the detection of lymph node micrometastases in slides stained immunohistochemically.

6.2 PATIENTS AND METHODS

6.2.1 Patients

Lymph nodes from 26 patients with node-negative T1-3 breast cancer who subsequently developed axillary recurrence after treatment by breast-conserving wide local excision and axillary node sample between 1987 and 1995 were re-examined for metastases. Lymph nodes from a further 26 patients with node-negative T1-3 breast cancer who did not develop any axillary recurrence after a breast conserving wide local excision and axillary node sample were examined similarly and used as control cases. Patients were matched for tumour size, grade, and type, menopausal status and number of lymph nodes sampled.

6.2.2 Methods

Each paraffin-embedded lymph node from the study and control groups was re-examined at two additional levels 100 microns apart. Three sections were taken from each level and one was stained with haematoxylin and eosin (H & E). The remaining two sections from each level were stained with antibodies to pancytokeratin (MNF116, Dako, UK) and MUC1 protein (HMFG2, Prof J Taylor-Papadimitrou, ICRF, London). Each immunocytochemically-stained slide was examined for metastases by a senior histopathology registrar (KLM) and by a non-pathologist (PAL) with no post-graduate experience of histopathology. The senior histopathology registrar also examined each H & E-stained slide in addition to the immunocytochemically-stained slides. Lymph node sections were examined using standard light microscopy. Both the histopathologist and the non-pathologist were blinded to the patients' clinical outcome whilst examining the nodes. Positive cases were noted and the incidence of micrometastases compared between the two groups. Statistical analysis using the chi-squared test compared the results from the axillary recurrence group of patients with the control cases with regard to the number of micrometastases identified.

6.2.3 Staining methods

Sections were dewaxed and rehydrated. Antigen retrieval was necessary for cytokeratin staining and was carried out by microwaving sections in Vector Retrieval Solution 3 for five minutes and then washing in water. The HMFG2 antibody did not require antigen retrieval. All sections were then immersed in a solution of 1.5% hydrogen peroxide in methanol for ten minutes and subsequently loaded into a Biogenax Optimax staining machine and incubated with 1:5 normal rabbit serum (NRS) diluted in Optimax buffer for thirty minutes. The MNF116 and HMFG2 primary antibodies were applied for sixty minutes at dilutions of 1:500 and 1:4 respectively. Sections were then further incubated following a buffer wash for thirty minutes in a 1:400 dilution of biotinylated rabbit anti-mouse antiserum (DAKO). After a further buffer wash, sections were incubated in ready-to-use ABC (Vector

Labs) for thirty minutes and after a final rinse in buffer solution sites of peroxidase activity were developed using 3,3-diaminobenzidine (DAB) for five minutes.

6.3 RESULTS

133 lymph nodes were examined from each of the axillary recurrence and the control groups with a mean of 5.1 lymph nodes per patient. A total of 596 slides were examined, two for each of two levels sectioned from each lymph node. There were micrometastases present in four patients with known axillary recurrence and in three patients with no axillary recurrence as detected by the histopathologist. The non-pathologist detected micrometastases in six (85.7%) of these patients but in one patient who had a subcapsular metastatic deposit present in one of the nodes, the metastasis was missed. This gave a false negative rate of 14.3%. The sensitivity of the non-pathologist in detecting metastases in lymph nodes was thus 85.7%.

The results analysed as number of slides examined gives a different picture. Eighteen slides demonstrated micrometastatic deposits and 10 (55.6%) of these were identified by the non-pathologist. Eight slides (44.4%) were consequently falsely diagnosed as negative. Sensitivity in detection of positive slides was thus 55.6%.

Of the pancytokeratin slides showing metastases, six of nine slides (66.7%) were detected but only four of nine slides (44.4%) showing metastases with HMFG2 were detected. Sensitivity is thus greater with pancytokeratin at 66.7% than with HMFG2 at 44.4%. In terms of levels identified, metastatic disease was seen in nine levels of the sectioned nodes of which the non-pathologist identified seven levels (77.8%).

False positive cases were found by the non-pathologist in 16 slides. This translates to a false positive rate of 2.8% and a specificity of 97.2%. Nine false positives (3.1%) were from CK stained slides and seven (2.4%) from HMFG2 slides. Positive predictive value of the non pathologist was thus only 38.5% (10 of 26). The negative predictive value was 97.3% (570 of 586). Positive predictive values for Pan CK and HMFG2 were 40% and 36.4% respectively. Negative predictive values were 96.9% and 97.6% for Pan CK and HMFG2 respectively.

Nine slides were referred to the pathologist for checking but these slides were not thought to be positive by the non-pathologist.

The results as defined by the histopathologist revealed that in two patients from the study group (those who developed axillary recurrence) macrometastases had been

overlooked on routine H & E histology. This suggested that the pathologist had originally understaged these two patients. Of the remaining patients, two patients from the study group (7.7%) and three patients from the control group (11.5%) were found to have micrometastases on immunohistochemistry. All of these micrometastases measured less than 1mm in diameter and were either intraparenchymal, subcapsular or both. Statistical analysis using the chi-squared test revealed no significant difference in the rate of micrometastases between the two groups (P=1) whether or not they developed axillary recurrence. Both the antibodies to pancytokeratin and to MUC1 protein showed similar intensity of staining.
Table 6.1 overleaf displays the numbers of metastases identified by the pathologist. Table 6.2 overleaf displays the sensitivity, specificity and predictive values of each antibody separately and in total as examined by the non-pathologist.

Table 6.1 Metastases and micrometastases identified by pathologist

	No of patients	No. of lymph nodes	Patients in whom metastases found	Patients in whom metastases overlooked	Patients with micro-metastases
Axillary recurrence group	26	133	4 (15.3%)	2(7.7%)	2(7.7%)
Control group (no axillary recurrence)	26	133	3(11.5%)	0	3(11.5%)

Table 6.2 Results of slide examination by a non-pathologist

	No. of slides examined	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Pan CK	298	66.7%	96.9%	40%	96.9%
HMFG2 (MUC1)	298	44.4%	97.6%	36.4%	97.6%
Total	596	55.6%	97.2%	38.5%	97.3%

6.2 DISCUSSION

The results of this study have shown that in this relatively small group of patients, micrometastases do not have clinical relevance in relation to the development of axillary recurrence. These findings are in contrast to those of the Ludwig international study group who reported a significantly poorer disease-free and overall survival in postmenopausal patients with occult lymph node metastases (micrometastases) [48].

The same study also reported that the presence of lymph node micrometastases was an independent and highly significant predictor of recurrence in post-menopausal women. The study performed in Edinburgh, however did not stratify patients to separate groups of pre and postmenopausal women, as numbers were small. It is possible that a study of larger numbers of patients could produce different findings particularly if specific patient groups are studied. Other studies have reported no clinical significance to the presence of lymph node micrometastases[46, 60] in terms of overall survival. It should be mentioned here that these two studies were carried out during the 1970s and were studies of serial sectioning using standard H & E histology rather than modern immunohistochemistry techniques. In addition, both studies were of smaller numbers of patients (227 patients and 78 patients) than the Ludwig study (736 patients). It becomes apparent from the findings of these different studies that lymph node micrometastases may have more significance in certain patient groups than in others. Subsequent studies which examine their clinical significance would perhaps be better set up prospectively to study separate patient groups in large numbers. For example, patients with a particular histological type of tumour or patient groups according to menopausal status or age. Until this issue has been clarified, it will continue to be a subject of some debate.

The introduction of sentinel node biopsy allows a small number of lymph nodes to be subjected to a more detailed histological analysis and one study reports a high incidence of lymph node micrometastases in sentinel nodes examined by immunohistochemistry, which were negative for metastases on conventional histology [234]. If such detailed analysis is to be carried out on sentinel lymph nodes,

then it is essential that the true significance of occult metastases be understood before patients are subjected to highly toxic and potentially harmful chemotherapy.
Pressures on pathologists to perform a greater number of sections on each lymph node examined and to examine further slides stained by immunohistochemistry are likely to impose a considerable workload on an already stretched specialty with staff recruitment problems. The ability of a laboratory technician such as an MLSO (medical laboratory scientific officer) to examine and screen slides for pathologists could greatly reduce this burden. In this study the non-pathologist was able to identify over half of the positive slides stained using antibody to pan-CK after only a very limited period of training. The use of the microscope to screen slides is a potential area for error as by scanning too fast or too far at each sweep of the lens, it is easily possible to miss a small area of micrometastasis. In addition, the detection of sixteen false positive slides suggests that extra training in the distinction between true positive staining and background staining would be useful. Although some positive slides were missed, it is possible that with a longer period of training and with closer supervision early on in the training, these figures could be improved.
The figures achieved with the HMFG2 antibody were significantly lower than with the antibody to pan-CK. Reasons for this are unclear as the pathologist stated that the intensity of staining was similar with each antibody. The non-pathologist disagreed with this and felt that the pan-CK staining was easier to see than the staining with HMFG2 and this is worth consideration if the technique is to be tried with a non-trained member of staff in the future. Other antibodies may prove to be more specific in the detection of occult metastases without leading to background staining of normal tissue and this would allow an untrained person to identify abnormalities more easily. The antibodies AE-1, AE-2, AE-3, CAM 5.2, MCA (mucous-like carcinoma antigen) and EMA (epithelial membrane antigen) amongst others have all been used to detect lymph node micrometastases of the axilla in a number of different studies [235].
On the basis of this study however, an untrained technician should not attempt to screen histology slides outside the confines of a controlled clinical trial.
In summary, this study did not associate the presence of axillary lymph node micrometastases with axillary recurrence. Further studies on larger numbers of

specific patient subgroups need to be performed to clarify the debate on the significance of lymph node micrometastases in breast cancer. All micrometastases identified in this study measured less than 1mm in diameter and this may be an important threshold point in the clinical significance of micrometastases as found a previous report [61]. It may be possible to train a pathology technician to screen large numbers of slides stained immunohistochemically if a longer period of training is provided with close supervision from a trained pathologist. Some antibodies may allow easier detection of lymph node micrometastases than others. It is vital that an international consensus is reached giving clear guidelines to pathologists, surgeons and oncologists on how best to process and manage lymph node micrometastases before clinical decisions are made on their presence. Until a decision has been reached, studies into this must be confined to clinical trials.

7. GENERAL DISCUSSION AND CONCLUSIONS

7.1 GENERAL DISCUSSION

7.1.1 Purpose of these studies

These studies were set up in an attempt to discover the most suitable approach to the management of the axillary lymph nodes in operable breast cancer both surgically and histologically. The following pages will bring together all the information provided by the previous chapters and analyse it critically against today's up to date research, which has been published since this thesis started. The result of this it is hoped, will offer some guidance as to the current preferred management of the axilla in this complicated disease. The fight against breast cancer is an ongoing process and the suggestions drawn from this thesis only offer a further drop in the ocean of knowledge, which is being diligently gathered by medical researchers around the globe. Each piece of medical research goes some way however, in directing new research towards the next stepping-stone in the journey towards a cure for this terrible disease as the pioneering work of Halsted, McWhirter, Patey and Forrest and many others has done in bringing us to the point where we lie today.

7.1.2 Current research and views for the future

Early results of randomised studies comparing sentinel node biopsy with standard axillary dissection are now being published. A Multicentre trial of 965 patients based at the Moffitt Cancer Centre, Tampa, Florida, reports that there have been no recurrences to date in node-negative patients treated by sentinel node biopsy to stage the axilla after a median follow-up of 16 months [236]. These results are in keeping with ours found in Edinburgh. No recurrences have yet been reported in our small group of 33 patients whose axillae were staged with sentinel node biopsy for node-negative disease after a median follow-up of 18 months. These reports are very encouraging. It is too early however, to draw final conclusions from these findings as the mean time to axillary recurrence in patients treated by axillary clearance or axillary node sample is 4.5 years (see chapter 2) suggesting that a follow-up period

of at least 5 years is required to confirm the safety of this technique. Our figures are for patients with stage T1-2 breast cancer although it has now been reported that sentinel node biopsy is no less accurate when T3 tumours are staged using this technique following a large Multicentre study of over 2000 patients with stage T1-3 breast cancer based at the University of Louisville, USA [237]. Patients enrolled into this trial were initially staged as having T1-2 cancers clinically but some were subsequently found to have T3 tumours on pathological examination. It is likely that these were small T3 tumours and other reports have found that sentinel node biopsy is less accurate in T2-3 tumours than in T1 tumours [238]. It is probably wise to reserve the technique of sentinel node biopsy for use in patients with clinical stage T1-2 N0 M0 until further information on the subject of T3 tumours becomes available. The increasing risk of nodal metastases with increasing tumour size makes sentinel node biopsy less suitable for patients with T3 tumours as their risk of requiring further surgery to clear the axilla is higher.

The original aim of the sentinel node biopsy was to reduce the extent of axillary surgery required in breast cancer patients with the hope of reducing post-operative morbidity without sacrificing staging information. Reports now suggest that morbidity is found to be lower following a sentinel node biopsy than following a standard axillary dissection or clearance [239, 240]. In particular the number of symptoms, tenderness, soreness, tightness, pain, limited range of motion of the operated upper extremity, numbness, paraesthesias, and arm swelling as well as perceived disability in activities of daily living were significantly less common in patients having sentinel node biopsy than in those having an axillary dissection to level II. These results are in agreement in part with our preliminary results, which suggest that less sensory alteration of the axilla and upper medial arm is encountered following a sentinel node biopsy than following an axillary node sample.

A recent report on the arm morbidity of patients receiving a level I-III axillary dissection reported that shoulder stiffness, pain and loss of arm strength were frequent complications with pain and loss of arm strength occurring in half the patients and a reduction of 20 degrees of abduction, dorsal or ventral elevation of the arm occurring in 12%. The arm morbidity appeared to persist rather than to improve with time with no difference in morbidity between patients treated 6-12 months ago

and those treated 5 years ago [145]. These findings would agree with the findings of phase 2 of the ALMANAC trial, which showed persistence of shoulder stiffness rather than improvement over the 18-month follow-up period (see figures 4.14 to 4.18).

Another report suggests that there is less arm swelling and that patients returned to their normal activities sooner after surgery following a sentinel node biopsy than following axillary node dissection [241]. These findings add further support to the validation of sentinel node biopsy as a non-radical method of staging the axilla.

Certain patient and tumour characteristics have been associated with a higher risk of failed lymphatic mapping such as patient age, body mass index and lower inner quadrant tumours [242, 243]. These findings are in agreement with the findings reported in this thesis (see chapter 4 figs 4.1 – 4.3).

Axillary node sample of at least four lymph nodes has been shown to be safe in terms of long-term overall patient survival when compared with a level III axillary node clearance as part of randomised clinical trials (see chapter 2). It is however, associated with a false negative rate of 9.75% that does adversely affect a small group of patients who will develop axillary recurrence. It is because of this that some breast surgeons have been sceptical of the technique, as they have found it difficult to perform and have felt that there is a risk of leaving positive disease behind in the axilla. Sentinel node biopsy may offer some benefit over axillary node sample in terms of its accuracy and reproducibility. Sentinel node biopsy has been shown to be easy to perform and reproduce by two consultant surgeons who achieved an identification rate of close to 100% after performing 40 cases each (see chapter 3). In phase 2 of the ALMANAC trial (chapter 4) the sentinel node identification rate was 100%. Surgeons sceptical of axillary node sample may find sentinel node biopsy easier to perform and reproduce as it offers a more definite and precise way of targeting specific lymph nodes as opposed to the less defined method of 'cherry-picking' of lymph nodes as in a sample.

It is important to remember that should the sentinel node biopsy procedure fail to identify a lymph node then conventional axillary surgery should be reverted back to as an alternative. Similarly, it is essential not to forget that sentinel node biopsy is not suitable for all groups of patients and it should not be seen as a substitute for patients

with large or multifocal tumours or with palpable axillary nodes who are better treated with an axillary node clearance when they have their primary tumour excised because of the high risk of nodal metastases in these patients.
Two recent papers have reported that intraoperative imprint cytology of sentinel lymph nodes is helpful in determining which patients should go on to have a completion axillary node clearance [244, 245]. Both papers reported a specificity of 100% with no false positive results and an accuracy of over 80% in keeping with the findings of chapter 5 of this thesis. A false positive lymph node imprint thus does not appear to be a likely problem with this technique and this should reassure the surgeon that a positive imprint is likely to be a true positive.

7.1.3 Cost implications of sentinel node biopsy

After the initial expensive outlay of purchasing a gamma probe for between £10,000 and £15,000 the cost of sentinel node biopsy is relatively inexpensive. A single dose of Nanocoll costs £35 and a single vial of Patent blue V costs £4.80 in the United Kingdom. If scintiscan is avoided, the extra cost over that of an axillary sample is likely to be less than £50 per patient. If sentinel node biopsy proves to be more accurate at staging the axilla, it has the potential to recoup these costs in prevention of axillary recurrence and the additional healthcare costs associated with it.
An American study profiling the cumulative treatment costs of 555 patients treated by sentinel node biopsy reported inconclusive results regarding the cost-effectiveness of sentinel node biopsy in the average breast cancer patient and recommended measurement of longer term costs and outcomes before conclusions could be reached on this subject [246].

7.1.4 Problems of sentinel node biopsy

The various pitfalls and side effects of sentinel node biopsy have been discussed in the introduction so do not need to be repeated here. The problem of a false negative

sentinel node biopsy however, needs addressing. A meta-analysis of eleven studies of sentinel node biopsy followed by axillary node dissection concluded that the false negative rate for the technique was 5% and that sentinel node reflected the status of the axilla in 97% of patients [247]. This figure of a 5% false negative rate is likely to be realistic with the majority of studies reporting false negative rates at between 0% and 11.9% [150, 157, 160, 161, 163, 164, 168]. As demonstrated in chapter 2 of this thesis, axillary node sample was associated with a false negative rate of 9.75%, which assumes that all axillary recurrences were a result of missed disease present at the time of surgery. Level III axillary clearance was also associated with a false negative rate of 2.9% in that five patients who were node-negative subsequently developed axillary recurrence. Perhaps the axillary relapse rate is not a true reflection of false negatives but this representation may help to bring into context the small number of false negatives associated with an axillary node sample and with sentinel node biopsy.

It is clear that some patients will always develop axillary recurrence despite apparently adequate surgery and this may not necessarily be fairly blamed on the surgical technique but rather blamed on the nature of aggressive disease. A figure of a 5% false negative rate may thus be considered an acceptable compromise against the unnecessary indiscriminate clearance of all patients' axillae. This figure may be minimised by the careful selection of patients for sentinel node biopsy, by the use of a combined mapping procedure, by the careful attention to detail during surgery which should result in removal of **all** lymph nodes taking up mapping agents not only the hottest or most deeply stained and by the intraoperative palpation of the surgically opened axilla to check for and remove clinically suspicious lymph nodes in addition to sentinel nodes. This coupled with careful follow-up should help to reduce the false negatives to a minimum whilst detecting any recurrent axillary disease early on when salvage axillary clearance still remains an option.

The other problem of sentinel node biopsy highlighted by this research thesis is that of post-operative breast and axillary seroma formation and of breast infection. These complications whilst minor, can be upsetting for the patient but are easier to deal with if patients are warned of the risk before surgery. Prophylactic antibiotics and wound drainage have been discussed earlier and most seromas resolved rapidly with

one or two aspirations in the outpatient clinic. If clinicians are aware of the slightly higher risk of these complications they can be easily detected and managed with minimal disturbance to the patient.

7.1.5 Findings of thesis and conclusions

A retrospective review of 855 patients with T1-3 N0-1 M0 breast cancer has revealed the survival for node-negative breast cancer to be over 70% after a median follow-up of 8.2 years. The treatment received to the axilla by these patients was either a surgical clearance of all axillary lymph nodes up to and including level III nodes or an axillary node sample of at least four lower axillary nodes. Neither group of patients had axillary radiotherapy. The survival was similar regardless of the axillary procedure carried out. The suggestion here is that a complete clearance of axillary lymph nodes to level III is an unnecessarily invasive operation in node-negative patients, as it does not improve long-term survival. Conversely, axillary recurrences were significantly more common in patients who received a four-node axillary sample rather than an axillary clearance. The logical assumption was to conclude that some of these patients were understaged by a four-node axillary sample and that positive axillary disease was missed by this operation.

The survival for node-positive breast cancer patients was approximately 20% lower than for node-negative patients at around 55% after a median follow-up of 8.2 years. Treatment received to the axilla was either an axillary clearance of all axillary lymph nodes up to and including level III nodes or a four-node axillary sample of lower axillary nodes followed by post-operative axillary radiotherapy. Survival figures were similar regardless of the axillary treatment received. The suggestion here is that axillary radiotherapy following an axillary sample is equivalent treatment to a level III axillary clearance of the involved axilla. As in the case of node-negative patients however, axillary recurrences were significantly more common in patients who received a four-node axillary sample plus axillary radiotherapy rather than an axillary clearance. The conclusion here was that axillary clearance provides better local control of the involved axilla than axillary radiotherapy. Further analysis revealed

that patients with four or more involved nodes following an axillary sample were significantly more likely to develop axillary recurrence than those with three or fewer nodes involved. It was concluded that patients with four or more involved axillary lymph nodes after an axillary sample should have a second operation to clear the remaining lymph nodes but that patients with three or fewer involved nodes are adequately treated by axillary radiotherapy.

The clear difference in survival between node-positive and node-negative patients demonstrates the importance of accurate axillary staging. Axillary recurrence is infrequent occurring in less than 10% of patients overall during a 10-year period of follow-up but the effects of it are devastating to the patient both physically and psychologically. Although axillary recurrence does not affect overall patient survival, it does affect the individual survival of patients who develop it. Survival following axillary recurrence is less than 50%. The mean time to axillary recurrence is 4.5 years in this study. Close observation of the axilla is thus mandatory for all patients post-operatively. Node-positive patients treated by axillary node sample followed by axillary radiotherapy had the highest rate of axillary recurrence with a rate of 10.7% over a 10-year follow-up period. It is hoped that this rate of recurrence will fall if all patients return for an axillary clearance if found to have four or more positive lymph nodes after an axillary sample. Complete control of the axilla in all patients is not possible using present management as a few patients continue to return with axillary recurrence after an axillary clearance even if they were node-negative. In these patients aggressive primary disease may be responsible and this is another subject outside the scope of this thesis.

Axillary sampling thus potentially misses axillary disease in a small number of patients. The technique of sentinel node biopsy and lymphatic mapping aims to improve on this problem associated with axillary sampling without the need for radical surgery and the logic behind this has already been explained in the introduction. Phase one of the ALMANAC trial in Edinburgh has proved that sentinel node biopsy is not difficult to perform and is easily reproducible. Using a combined mapping technique with the use of blue dye and a radiopharmaceutical it can identify a sentinel node in close to 100% of patients. Patients with obvious nodal disease should not have sentinel node biopsy and should be treated by axillary

clearance and systemic therapy if they have operable disease and are clinically fit. Patients in whom a sentinel node is not easily found (i.e. a failed mapping procedure) should be treated by a conventional axillary procedure according to their clinical disease stage (i.e axillary sample or clearance).

Sentinel node biopsy in clinically node-negative patients with T1-2 breast cancer detects twice as many axillary metastases than axillary node sample as demonstrated in chapter 4. Although numbers are too small to reach statistical significance the figures are encouraging. It may prove necessary to remove fewer lymph nodes during a sentinel node biopsy than during an axillary node sample in order to acquire this information but the longer-term results must be awaited before we can assume this. If this proves true, sentinel node biopsy has the potential to provide the breast surgeon and breast oncologist with more accurate information regarding the status of the axillary lymph nodes with a non-radical operation and in turn allow better decisions to be made on subsequent regional and systemic therapy. Sentinel node biopsy has not been associated with any axillary recurrences after a median follow-up of 18 months. Sentinel node biopsy was associated with more immediate post-operative complications such as breast seroma and infection but caused less post-operative sensory alteration than an axillary sample.

Imprint cytology of axillary lymph nodes can rapidly provide accurate intraoperative information on the axillary status of over 99% of lymph nodes when using toluidine blue to stain lymph nodes. It has the advantage over frozen section of allowing all lymph node tissue to be retained for further histological analysis. It is easy to perform and nodes can be processed very rapidly without the need for expensive equipment such as that required for frozen section histology. Immunohistochemistry of imprints using antibody to pancytokeratin does not improve on the results with toluidine blue.

Intraoperative imprint cytology is worth considering as an option to avoid two operations in a small group of node-positive patients (up to 15-20% in T1 breast cancer). It is not associated with false positive results when using toluidine blue so a positive result allows the surgeon to proceed to immediate axillary clearance whilst the patient is on the operating table.

The clinical significance of occult lymph node micrometastases remains a subject of considerable debate. The findings of the study detailed in chapter 6 are not in agreement with the majority of larger studies published during the last 10 years, which have reported negative effects on disease-free and overall survival from lymph node micrometastases [48, 58, 64, 235]. Other studies have however, reported similar to results to those reported in chapter 6 of no clinical correlation between lymph node micrometastases and axillary recurrence or overall survival [248, 249]. Clearly, as breast cancer is a heterogeneous entity with multiple possible confounding factors, it is vital that all factors are taken into consideration including patient characteristics, tumour characteristics, the number of levels of each node examined by the pathologist, whether or not to perform immunohistochemistry, and so on, to allow a structured and critical analysis of the many different clinical and histological possibilities that face us in our every day work. It is essential that a clear definition of what constitutes a micrometastasis is agreed upon based on its clinical relevance otherwise continued poorly structured study merely becomes an academic exercise. Until a consensus has been reached on the best way to manage lymph node micrometastases, it cannot be advised to base therapy decisions on their presence. Patients with lymph node micrometastases who are otherwise lymph node negative should have their progress followed carefully.

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Publications and Abstracts

Lambah PA, Dixon JM, Prescott R, Jack W, Forrest P, Chetty U.
Long term Results of Randomised Studies of Axillary clearance vs Non-targeted Axillary Sampling.
Abstract published in Breast Cancer Research And Treatment Vol 64, 1, Page 38, Nov 2000.
Abstract published in the British Journal of Surgery Vol 88, Suppl. 1:40, May 2001.
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Lambah PA, Dixon JM, Jack W, Forrest APM, Rodger A, Chetty U.
Randomised study of axillary clearance versus 4 node sampling.
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Abstract published in The European Journal of Cancer 2001; 37 (suppl 5): 2.

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Imprint Cytology As An Intraoperative Diagnostic Tool In Breast Cancer.
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Murray KM, Lambah PA, Dixon JM, Anderson TJ.
Patients who are node negative on axillary node sampling: do they recur because of occult lymph node metastases missed by the pathologist? The Breast Vol 11: 3, 249-251 June 2002.
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