LYMPHATIC VISUALISATION AND BIOPSY IN BREAST CANCER

by

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1 TABLE OF CONTENTS

2 TABLE OF FIGURES

3 ABSTRACT

"Lymphatic Visualisation and Biopsy in Breast Cancer"

3.1 Background:

Pathological examination of axillary lymph nodes is accepted as the most useful prognostic factor in patients with localised breast cancer. The therapeutic value of axillary dissection (AD) is debated, and lymphoedema is well known. A technique to adequately examine axillary nodes, avoid AD and reduce sampling error in routine pathological examination of large specimens is needed.

It was felt that the technique for targeted lymph node biopsy developed in melanoma surgery might be applicable to breast cancer.

3.2 Introduction:

A literature review was carried out looking at axillary dissection and factors leading to the concept of selective node biopsy.

3.3 Methods:

30 patients with breast cancer underwent preoperative Lymphoscintigraphy (LS) and intraoperative dye localisation to identify a sentinel node (SN) in the axilla, which was biopsied prior to AD and breast surgery. Detailed clinical and histological analysis was

carried out on the SN to assess its reliability as a marker of axillary status, and the feasibility of the procedure in various clinical and pathological circumstances.

3.4 Results:

LS located nodes in 23 patients although lymphatic channels were only seen in 5. Dye localisation identified a SN in 21 patients. Operating time increased by approximately 15 minutes. Routine histology of the AD specimen revealed one 'false negative' SN. Detailed histology of the SN revealed that 2 patients thought to be node negative had metastatic disease in the SN. Of the 14 patients who had node positive disease, 7 patients had disease only in the sentinel node.

3.5 Conclusions:

Sentinel Node Biopsy (SNB) is feasible in patients with breast cancer and is independent of tumour location, size, grade, type, or nodal status.

Various recommendations with respect to improvements in the technique are discussed.

Detailed histology of the SN alone appears to be a more accurate method of staging the axilla than routine examination of a complete AD specimen.

Sentinel node biopsy can identify the only node in the axilla containing metastatic disease.

The procedure has a false negative rate.

Consideration should be given to a trial of SNB alone reserving complete AD for patients found to have metastatic disease in the SN

4 DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university and that to the best of my knowledge and belief the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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Trevor Collinson

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6 PREFACE

An investigation into the incidence of lymphoedema at the Flinders Medical Centre Breast Clinic

The practice of axillary dissection in breast cancer is a part of surgical treatment which has changed little in the last fifty years whilst surgery to the breast itself has changed completely. Reassessment of the place of axillary dissection has occurred over the last decade and a number of techniques for limiting or replacing the procedure have been proposed. It was felt that techniques which have recently been successful at reducing the number of nodal dissections in patients with melanoma might have application in breast surgery. The particular technique referred to is known as 'Lymphoscintigraphy' and 'Dye localisation', together being known as "Sentinel Node Biopsy".

The following description of 'axillary dissection' summarises current practice throughout the world and is an important baseline from which to begin.

> " *axillary dissection is a general term that has come to describe any of several anatomic axillary procedures; therefore to precisely define the surgery it is more appropriate to identify the lymph node levels encompassed. A level I, II, and III dissection removes all nodes caudad to the axillary vein. This usually requires the transection or removal of the pectoralis minor muscle and sacrifice of the medial pectoral nerve. The long thoracic and thoraco dorsal nerves are identified and preserved, while the intercosto brachial nerves are taken or preserved at the discretion of the surgeon. On average 20 - 25 nodes are identified when using standard pathology procedures..... A level I and II dissection removes all nodes*

beneath and lateral to the pectoralis minor muscle usually without transecting the muscle. The long thoracic and thoraco dorsal nerves are identified and treated as in levels I, II and III dissections; however in addition the medial pectoral nerve can be preserved or transected at the discretion of the surgeon.... An average of 15 - 20 nodes are retrieved. Lower axillary dissection or axillary sampling consists of the removal of tissue lateral to the pectoralis minor muscle. This is not a standardised procedure and the technique varies from surgeon to surgeon. Anatomic structures usually are not identified and an average of 4 - 6 nodes are retrieved...."

(Mackarem, 1992)

In addition to the presence of axillary metastatic disease, the level of axillary nodal involvement is often taken as another indicator of prognosis: the higher the level involved, the worse the prognosis. In many cases a small portion of tissue at the most superior part of the axillary dissection is removed and sent for separate analysis - being designated 'apical tissue'. Involvement of this tissue is seen as a grave prognostic sign.

On observation of the pathological processing of a specimen, however, it is seen that there is a significant margin for error between clinical and pathological descriptions of the levels of the axilla. Once removed, the axillary dissection is an amorphous mass of tissue, roughly spherical in shape. Careful surgeons may place a suture at the apex of the axillary dissection in order to assist the pathologist in orientation of the specimen. The pathologist then usually takes a large knife and cuts the specimen into equal thirds designating them as levels I, II and III.

Most surgeons would agree that the current Australian practice of axillary dissection does not include removal of level III lymph nodes (those medial to the medial border of pectoralis minor) therefore making the pathological descriptions mentioned above

somewhat inaccurate. Also inaccurate may be the figures quoted in the literature for lymphodema since they may not apply to a standard Australian style axillary dissection. It was felt that a documentation of the incidence of lymphodema arising as a result of level I and II axillary dissection with removal of a portion of 'apical tissue' as practiced in Australia should be carried out. Apical tissue is usually the highest part of the level II dissection, rather than tissue from level III. The following analysis of all axillary dissections carried out at Flinders Medical Centre Breast Clinic for breast carcinoma during 1994 was carried out.

All axillary dissections were of the type described immediately above and were carried out by one of the three consultant surgeons on the unit , or a surgical registrar under their direct supervision in a similar fashion. All patients were reviewed in the breast clinic post operatively and any patient complaining of or found to be suffering from lymphodema was referred to the Lymphodema Clinic where detailed measurements were made. The results are presented below.

82 patients underwent axillary dissection for breast cancer during the calendar year 1994. Two patients who had axillary sampling, one who had an axillary biopsy and one who had an excision of a melanoma mass in the axilla were excluded from the study.

51 of the 82 patients agreed to detailed preoperative examination in the lymphoedema clinic which included measurements of the limbs, tonometry, plethysmography and bioimpedance studies.

Patients who presented with lymphoedema post operatively were then remeasured and changes resulting from the surgery assessed. The definition of lymphoedema used is set out in the section on lymphoedema in the introduction, on page 36.

All patients were reviewed in the clinic and results as at June 1996 are as follows: Four patients presented with lymphoedema during 1994. One patient suffered acute onset arm swelling whilst in hospital which resolved shortly after discharge. Five further patients presented with lymphoedema during 1995. One patient lost a very significant amount of weight as well as having treatment with chemotherapy and laser in the lymphoedema clinic with resolution of the lymphoedema. Thus with a period of follow up ranging from eighteen months to two and a half years, ten patients or 12.2% suffered lymphoedema which had not resolved in eight patients or 10% of cases. Seven of the ten patients with lymphoedema had both pre and postoperative arm measurements carried out.

Analysis of patient and treatment factors in these ten patients failed to reveal any particular risk factors. With respect to type of surgery, five had breast conserving surgery and five had total mastectomy. five were node positive and five node negative. five had adjuvant chemotherapy and five did not. five had adjuvant radiotherapy and five did not. five had a postoperative wound infection and five did not. 54 of the 82 patients developed a postoperative seroma in the axilla with an average duration of 24 days (range: 0-57 days). Eight of the ten patients with lymphoedema had a post operative axillary seroma.

Analysis of surgeon related factors revealed that nine different surgeons were involved in operating upon these patients, with a registrar and consultant being jointly responsible for the operation in several cases.

Thus the incidence of lymphoedema as it relates to breast surgery carried out in South Australia is at least 10%, perhaps slightly higher than the reported incidence from around the world. Since late onset lymphoedema is well known, this is a minimum figure and long term prospective studies would need to be carried out to determine the eventual true incidence, which would probably be higher than 10%.

One cannot implicate a particular patient group, surgeon, pathological pattern, complication or adjuvant treatment in the onset of this condition which appears to be unpredictable. It is this unpredictability which makes the complication of lymphoedema difficult to accept, and has led to a number of proposals designed to reduce the number and extent of axillary dissections carried out.

The following study examines one method by which such a goal may be achieved.

7 FOREWORD

Removal of the draining lymph nodes in malignant disease is a time honoured method of staging the disease and in some cases a means of curing the patient even after the primary tumour has metastasised to the lymph nodes.

This is a reasonably straightforward procedure in most cases when the area draining a tumour is easy to identify. Problems occur however in cases where the lesion is situated in the midline or in areas such as the trunk where it is not immediately apparent which lymph node field drains the tumour and is the appropriate one to remove, or whether more than one field should be removed. This has been particularly a problem in tumours of the genital organs.

It is not surprising therefore that in cases of melanoma of the trunk and tumours of both male and female genital organs, techniques were developed to assist in the identification and removal of appropriate lymph nodes.

Lymphangiography is a technique which has been used for many years and which can identify both lymphatic channels and lymph nodes. By itself however, this was not a satisfactory technique to assist with surgery because it was necessary to know which lymph node field needed to be removed.

In the case of breast carcinoma it is known that lymphatics drain primarily to the axilla but also to supraclavicular, internal mammary, abdominal and contra lateral axillary

nodes directly. The removal of axillary nodes has a well established place in the treatment of breast carcinoma because it is primarily to the axillary nodes that breast carcinomas metastasise. In the early part of the twentieth century it was routine to perform a Halsted type mastectomy in which the pectoral muscles were removed along with the breast. At this time it was also routine to remove internal mammary lymph nodes in patients undergoing treatment for breast carcinoma. Due to the high morbidity and low yield of this particular procedure, routine internal mammary lymph node excision has been abandoned. Debate continues however as to whether metastases to only the internal mammary nodes may account for disease recurrence in patients who have had a successful breast resection and removal of axillary nodes which are clear of tumour.

As a greater understanding of tumour biology has developed, the use of radiotherapy has been supplemented with newer modalities such as hormone manipulation and improved chemotherapy which has allowed surgery to become less and less radical. The progression from Halsted's radical mastectomy with removal of pectoral muscles to Patey's modified radical mastectomy with preservation of pectoral muscles to 'partial' or 'sector' resection of breast tissue seems a logical progression. Axillary dissection, although remaining part of the routine treatment of invasive breast cancer is now generally regarded as unnecessary in patients with purely non invasive tumours. The indications for avoiding axillary dissection have gradually been extended from non invasive tumours to small invasive tumours (less than 5 mms. in maximum dimension).

One of the main features of breast screening programmes which are now in place in many parts of the world is that breast carcinomas are detected at an earlier stage than if

screening were not carried out. It is to be expected therefore that the rate of axillary lymph note positivity will be declining steadily. Although advocates of axillary sampling or incomplete axillary dissection maintain that removing lower axillary nodes is adequate for patients with ductal carcinoma in situ and early tumours, most surgeons would undertake a complete axillary dissection in all patients with invasive breast carcinoma. This procedure has changed little in the last century except that lymph nodes and lymphatic channels superior to the axillary vein and 'level III' nodes (medial to the medial border of pectoralis minor) are now not usually removed. Axillary 'clearance' or 'dissection' has a well documented incidence of lymphodema of the arm which is one of the most feared complications of surgery. It has been recognised for some time that the development of lymphodema is significantly higher if the axilla is subjected to dissection and radiotherapy. The incidence of lymphodema as a result of surgery or radiotherapy has been shown to be approximately equal and both appear to be similar in their efficacy at controlling axillary metastatic disease. As the incidence of lymphodema is relatively stable and the incidence of axillary node positivity has been gradually declining it would seem that eventually the risks of axillary dissection will outweigh the benefits of pathological staging of the axilla. Proponents of the argument that axillary dissection is only diagnostic argue that primary tumour characteristics which can now be analysed in great detail will give at least as much information and possibly more than that obtained by removing the axillary nodes.

As it is known than the development of lymphodema is directly related to the extent of axillary surgery, the possibility of obtaining pathological material from the axilla with a limited operation using a targeted biopsy technique has generated interest.

Experience with melanoma has suggested that patients in whom an intermediate thickness melanoma has been diagnosed may benefit from dissection of draining lymph nodes. In approximately 80% of these patients, however, the lymph nodes are found to be free of tumour and therefore no benefit has been achieved. The risk of lymphodema of course applies to all patients and a group of patients will therefore develop lymphodema who need not have had a nodal dissection.

In this group of patients, and the patients with trunk melanoma referred to earlier, a technique known as sentinel node biopsy combined the procedures of lymphoscintigraphy and lymphatic mapping using blue dye to identify lymph nodes which were thought to be draining the area of the primary melanoma.

This lymphatic mapping led to two slightly surprising results.

Firstly the degree of variability between patients was seen to be much greater than had previously been imagined. Lymphatic drainage to regions where one would have not predicted this to occur was frequently seen, and nodes were identified along lymphatic channels long distances from any lymph node field. These nodes would clearly have not been removed in a standard node dissection and the logic of 'routine' node dissection was called into question.

The second finding was that in many cases the tracer (either radio nuclide or blue dye) often identified only a single node within a field (occasionally up to three nodes), passing subsequently to other nodes in the field or nodes in other lymph node fields. This led to the concept of a "sentinel node" - the single node which was the first to

receive lymphatic drainage from a particular area and thus the most likely node to contain tumour if metastasis had occurred. The possibility of removing this node alone for pathological examination arose. Correlative studies between the sentinel node and the remainder of a lymph node field carried out in melanoma patients showed that the procedure had a high degree of sensitivity and specificity for identifying metastatic disease. The finding in a subgroup of patients that the sentinel node was the only node in a lymph node field containing tumour tended to suggest that the sentinel node was likely to be the first node in a lymph node field involved with tumour. The proposal to perform sentinel node biopsy alone, reserving complete nodal dissection for patients in whom the sentinel node contained metastatic tumour, has been adopted in a number of major melanoma centres.

The possibility that this technique might be used in breast carcinoma would seem to be an attractive option because (as in intermediate thickness melanoma) most are not likely to have nodal metastasis. It may also be possible to identify a group of patients in whom nodal excision from an area other than the axilla may need to be carried out.

9 INTRODUCTION

9.1 Background

In most advanced countries around the world breast cancer is the leading cancer diagnosis in women. In some countries such as the U.S.A. other less frequent but more lethal cancers overtake breast cancer as the leading of cancer death in women, however in South Australia it is both the most common cancer *diagnosis* in women (27.7% of all cases in 1997 or 888 patients) and the leading cause of cancer *death* in women (16.2% of all deaths or 220 patients). In South Australian women breast cancer is almost twice as common as the next most frequently diagnosed cancer (Colon & Rectum: 15.6%). Additionally, the incidence of breast cancer is rising:

> *"The incidence of diagnosed breast cancer increased by 44% between 1977-80 and 1993-96, due mostly to increases since the late 1980's that followed the introduction of population-based mammographic screening. Among 50-69 year olds, the principal screening target, a 62% increase in incidence of diagnosed disease was evident. As a result, the lifetime risk of diagnosed breast cancer increased from 1:17 in 1977-80 to 1:12 in 1993-96."*
>
> *(South Australian Cancer Registry, 1998)*

9.2 Screening

The impact of mass screening for breast cancer using mammography in many centres around the world has been shown to detect cancers at an earlier stage and data is

beginning to become available to suggest that this improves the overall survival of women with breast cancer.

> *"Tumours detected in screened women in South Australia have been much smaller and with less nodal involvement than observed among the unscreened population"*
> *(South Australian Cancer Registry, 1998)*

From BreastScreen South Australia, among the initial series of screening mammograms performed (the prevalent round) 75% of patients with cancers were stage 0-I of whom 79% had a tumour of less than 2 cm. in diameter and 19% were node positive. Two years later on the second round of screening (the incident round) the number of patients with stage 0 or I disease had increased to 81%, the number of patients having tumours less than 2 cms. had increased to 81% and the number of patients with node positive disease had decreased to 8.5% (Robinson, 1996). Similar data has been shown by the New South Wales Cancer Registry after two incident rounds of screening where the number of patients with stage 0 or I disease had increased from 49% to 53% and the number of patients having tumours less than 2 cms. in diameter has increased steadily from 37 to 47% (Kricker, 1995). It should be noted that the South Australian figures include all patients who are screened whereas the New South Wales figures refer only to patients aged between 50 and 69. Data for patients younger than 50 in the New South Wales experience has not shown statistically significant changes and no significant decrease in mortality has yet been observed.

Data from the United States suggests that women with smaller tumours are living longer and in one paper 88.3% of women with a cancer less than 1.1cms. in diameter were alive

these patients could be observed on the premise that observing a clinically negative axilla and treating it when a relapse occurred has not been shown to have a detrimental effect on overall survival.

Such sentiments have set the stage for the less aggressive assessment of axillary nodes, though the importance of axillary nodal status in the management of breast cancer remains paramount.

9.4 Surgery for Breast Cancer.

9.4.1 The Breast

There has been little controversy regarding the extent of surgery to the breast itself over the years and a gradual trend from the radical mastectomy of Halsted to the modified radical mastectomy named after Patey to segmental, sector or partial mastectomy has occurred so that most people would now agree that in appropriate cases segmental resection is the treatment of choice. Many centres now treat small tumours with 'lumpectomy' alone. In a major study it was shown that variations of local and regional treatment were not important in determining overall survival, irradiation of the internal mammary nodes in patients with inner quadrant lesions did not improve survival and that the location did not influence prognosis. (Fisher, 1985)

9.4.2 The Axilla

The treatment of the axilla has elicited much more controversy which still continues. The debate concerns whether or not axillary dissection is purely prognostic and valuable for staging or whether it also has a therapeutic role. It has been shown though, (Kjaergaard, 1985) that the rate of axillary recurrence is inversely proportional to the number of excised lymph nodes in the axillary dissection specimen. Figures quoted in this article range from 12% if no axillary nodes are removed to 2% if three or more nodes are removed. It has been recommended on the basis of this that any operation on the axilla should remove at least four lymph nodes. In a similar study of 960 cases (Mathiesen, 1990) it was found that in node negative patients who received no adjuvant treatment there was a significant association between recurrence free survival and the number of nodes removed at operation up to 8 - 10 nodes. There was no additional benefit in patients who had more than 10 nodes removed and many authors would regard removal of 10 lymph nodes as adequate axillary treatment. Inclusion into clinical trials where axillary nodal status is required usually specifies that at least 9 nodes be identified.

Proponents of a less radical approach to the axilla as well as the breast have suggested that axillary sampling or 'partial' axillary dissection is adequate for assessment of the axilla. These arguments have been compounded by the fact that the precise meaning of partial axillary dissection or 'axillary sampling' as well as the descriptions of 'levels' within the axilla are imprecise and unreproducible.

9.4.3 Levels Within the Axilla

The generally accepted description for 'levels' within the axilla relates to the borders of pectoralis minor muscle. Nodes lateral to the lateral border of pectoralis minor are regarded as being level I, nodes immediately posterior to (behind) pectoralis minor are regarded as level II nodes, and nodes medial to the medial border of pectoralis minor are said to be level III nodes. Since the axilla is a three dimensional structure, this rather two dimensional description has shortcomings and there is significant discrepancy between what various surgeons would regard as dissections of a certain level. In a large series of patients studied retrospectively it was calculated that the average number of nodes removed in a complete axillary dissection is 20.5, the average number in level I being 13.8, level II 4.5 and level III 2.2. (Veronesi, 1987) In this study it was found that similar numbers of nodes were removed for Halsted, modified radical and quadrantectomy type mastectomy.

9.4.4 Skip Metastasis

The above paper also addressed the problem of what has been described as 'skip' metastasis showing that one or other level of the axilla was not involved in cases where a higher level *was* involved in 3.7% of patients. In only 0.4% of cases were there metastases in level III nodes when levels I and II were clear. In other words metastatic disease was accurately identified by a level I and II dissection in 99.6% of patients. (Veronesi, 1987)

This has led to the 'standard' axillary dissection involving removal of nodes from levels I and II only, which has reinforced the assumption that metastatic invasion of the axillary nodes in patients with breast cancer follows a regular and progressive pattern. In another large study (Rosen, 1983) the distribution of metastatic disease in the axilla was studied. The incidence of 'discontinuous' or 'skip' metastases not following this regular pattern was found to occur in 1.6% of all cases and 3% of those with lymph nodes metastases. It was found that the presence of skip metastases was not related to size, location in the breast, or histological type of the primary tumour. The highest level as well as the number of affected lymph nodes was closely correlated with the size of the primary tumour and prognosis. It was commented however that

> *"in most modified radical mastectomies, it is virtually impossible to locate levels reliably by gross inspection alone. For consistently accurate separation of the lymph nodes according to level it is essential that they be tagged at the time of surgery."*
>
> *(Rosen, 1983)*

It was suggested that differences between descriptions of levels gave rise to differences in the incidence of skip metastases. The other explanation for skip metastases is the possible existence of micro metastatic disease or sampling error in which tumour is present in lower level lymph nodes but is not identified by the examining pathologist.

Two small studies suggested that the incidence of skip metastases may vary according to the size of the tumour and the number of positive nodes. In one, (Gaglia, 1987) patients with 1-3 positive nodes had a rate of skip metastasis of 26% compared to those with four

identified in 18% of axillary sampling procedures. (Forrest, 1976) Similarly, others have found a 42% false negative rate when sampling was followed by complete axillary dissection. (Davies, 1980)

9.4.6 The Need for Axillary Dissection

In addition to arguments about the accuracy of axillary dissection, there is also debate about whether small tumours have a lower incidence of axillary node positivity than larger tumours. In one study, 137 cases of very small cancers (1 mm. or less in diameter) were examined with the finding that 16% were node positive. It was asserted that non palpable tumours are equally as aggressive as larger tumours and that axillary dissection should not be omitted simply on the basis of the small size of the tumour. (Margolis, 1992)

On the other hand an often quoted study (Silverstein, 1991) examined 175 cases of ductal carcinoma-in-situ where axillary dissection had been performed and only one case was found to have axillary node involvement. It was noted that there is an increase in node positivity in larger insitu tumours even if the tumour was felt to comprise entirely in-situ disease because of the risk of sampling error in a larger tumour. This amounts to the increased risk of missing an invasive carcinoma. In a later paper (Silverstein, 1994) it was further proposed that axillary dissection be eliminated for patients with T1a lesion (where the largest dimension of the invasive component is less than 5mm) because of the low yield of axillary nodes which were positive for tumour. In this study in-situ tumours have no incidence of nodal metastases and T1a lesions had a 3% incidence. By contrast T1b lesions (where the largest dimension of the invasive component is between 6 and

10mm) had a 17% incidence of metastatic axillary disease. The authors propose that axillary dissection be reserved for T1b lesions or larger, or T1a lesions where other poor prognostic features were present.

> *"if 100 node dissections are preformed for T1a lesions, 3% are likely to be positive.....how can we justify 100 node dissections in an attempt to find three patients with positive nodes to treat with chemotherapy, one patient of which, at most will be helped?"*
>
> *(Silverstein, 1994)*

The inadequacy of clinical nodal staging was addressed in one paper (Moffat, 1992) noting that 39% of patients with clinically negative nodes had positive nodes pathologically. In clinical terms 18% of patients who were clinically node negative eventually developed clinical axillary disease if the axilla was left untreated. In the NSABP B-04 trial (Fisher, 1985) it was pointed out that untreated sub clinical axillary metastatic disease does not always lead to regional recurrence. This again has fuelled the debate about the wisdom of performing axillary dissection in all patients with invasive breast cancer.

9.4.7 Axillary Dissection: Complications

Axillary dissection is a procedure with definite complications. In the immediate term these include nerve injury, resulting in parasthesia, anaesthesia and muscular weakness. In the medium term seroma formation, cellulitis and skin necrosis may occur. In the longer term the development of lymphodema is the most significant complication. In

severe cases longstanding lymphoedema may lead to malignant change in the form of lymphangiosarcoma. (The 'Stewart-Treves syndrome'.) Other more frequent complications of lymphodema include recurrent cellulitis or lymphangitis, shoulder dysfunction and nerve entrapment syndromes such as thoracic outlet syndrome and carpal tunnel syndrome. Of the immediate and medium term complications of axillary dissection, most are not serious and/or are treatable. The long term complication of lymphodema however is one of the most feared complications of breast surgery and one for which there is little effective treatment. The subject of lymphoedema will be addressed in the next section.

9.5 Lymphoedema

9.5.1 Definition

Chronic, non pitting oedema of the arm following mastectomy is an unsightly, obvious complication and may range from very mild which is hardly noticed by the patient to extremely severe with gross swelling of the entire limb. Most papers investigating it define lymphodema as a condition in which there is an increase in volume of the affected arm distal to a point 15 centimetres above the olecranon of greater than 200 mls. over the opposite side after allowing for hand dominance. Severe lymphodema is classified as a volume difference greater than 500 mls. (Hoe, 1992). Arm circumference measurements correlate poorly with volume differences measured at plethysmography. (See figure Figure 24, page 80)

9.5.2 Incidence

The mean time to development of lymphodema post operatively is 36 months (Mackarem, 1992) but it may occur up to 15 years later (Aitken, 1983). The incidence of lymphodema is lowest with the least extensive procedures on the axilla and most common in those patients undergoing surgery and radiotherapy combined. This is illustrated in the following table:

Procedure	**Incidence of Lymphoedema**
Axillary sampling	0 - 2.8%
Partial axillary dissection	2.7 - 7.4%
Complete axillary dissection	6 - 8.0%
Radiotherapy alone	2.1 - 8.3%
Surgery plus radiotherapy	0 - 38%

Table 1 Incidence of Lymphoedema

(Moffat, 1992)

9.5.3 Complications

With respect to the complications of mastectomy and axillary dissection, symptoms are surprisingly common even though in general only 10% of patients have objective limb swelling. One paper reported that 24% had subjective limb swelling, 70% experienced numbness, 33% pain, 25% weakness, 24% limb swelling and 15% stiffness. (Ivens, 1992) Similarly, in a group of 200 post axillary dissection patients, objective evidence of some

degree of limb swelling (not all classifiable as lymphodema) was found in a quarter of the patients. (Kissin, 1986)

9.6 Limited surgery on nodal regions: the beginnings of 'Sentinel Node Biopsy'

In tandem with radiological efforts to identify lymph nodes in patients with melanoma and breast cancer were surgical efforts aimed at limited procedures on a lymph node region which would still give useful prognostic pathological data. This became particularly important in young patients with intermediate thickness melanomas. Such patients routinely underwent prophylactic lymph node dissection which in around 80% of cases showed no evidence of metastatic disease and as a result the development of lymphoedema became harder and harder to accept. This paved the way for pioneering work in targeted node biopsy by Donald Morton (see below). Radiological studies aimed at identifying lymph nodes are known as 'Lymphoscintigraphy'. These studies were combined with the use of blue dye marking at operation to target a single lymph node in a region.

The technique has subsequently become known as 'Sentinel Node Biopsy', and after early acceptance in Melanoma surgery it is a technique now under investigation in various other types of cancer.

9.7 Lymphoscintigraphy

9.7.1 Development

An early application of lymphoscintigraphy (Ege, 1983) reported 6,500 studies of internal mammary lymphoscintigraphy by injecting radio colloid into the rectus sheath at the costal margin. This study noted that there was considerable inherent variability in the scintigraphic images even under normal circumstances. It was noted that lymphatics may or may not be symmetrical bilaterally, cross drainage often occuring between parasternal lymphatics. It had been hoped that "breast" lymphoscintigraphy might allow the visualisation of pathological nodes in the same way that bone scanning visualised pathological lesions in the skeleton. It was not clear however whether suppression of uptake of radio colloid due to impaired macrophage phagocytic function or increased activity because of antigenic stimulus with enhanced radio colloid uptake would occur. The authors also investigated the possibility of visualising axillary lymphatics by injecting into the hand or upper arm and felt that this technique did not accurately reflect the lymphatic impact of a breast tumour. They concluded that

> *"neither the optimum technique for visualising nor the criteria for evaluating the lymphatic components of the axilla have yet been clearly defined"*
>
> *(Ege, 1983)*

Similar studies using the same technique as Ege with alternating bilateral subcostal injections also failed to reliably identify nodal disease related to breast cancer. (Dionne, 1983)

A smaller study of 26 patients investigated the use of peri areolar injection of radiocolloid. In 24 of the 26 patients the lymphoscintographic pattern did not suggest any pathological correlation. (Gasparini, 1987) Interestingly, one group noted that 'axillary' lymphoscintigraphy, bilateral internal mammary and bilateral inter digital injections were approximately as accurate as clinical impression in the assessment of axillary nodal status. As mentioned above this is inaccurate in over a third of cases. (Moffatt, 1992) Increasingly sophisticated techniques to visualise the internal mammary lymphatic chain were carried out (Scatarige, 1990) involving the use of CT scanning, high resolution sonography and magnetic resonance imaging, again with results which contributed little to clinical management.

Until the early 1990's lymphoscintigraphy had a very limited place in clinical practice and was used for such unusual procedures as identifying lymphatic channels in patients selected for surgical lympho-venous anastamoses for treatment of gross postoperative lymphodema (Vaqueiro, 1986).

9.7.2 Application in Melanoma and Breast Cancer

In 1993 reports were published describing the use of lymphoscintigraphy in patients suffering from melanoma (Uren, 1993) and breast cancer (Krag, 1993). In Krag's series of 22 patients a series of injections were made along a 180 degree perimeter of the breast lesion, and scanning was performed using a hand held gamma probe. Results were obtained from one to nine hours preoperatively and this was of assistance in the surgery since in three patients the node highlighted by the lymphoscintigraphy was located lateral

to the breast and because of its inferior location may not have otherwise been removed as part of the standard surgical procedure. It was noted that the lowest level of an axillary dissection is not clearly stated in any description of the procedure, again leading to discrepancies between authors. This study found nodes (either involved or not) in 82% of the patients. Interestingly the authors noted that

> *"the breast tissue does not appear to contain a dense lymphatic plexus as for example, in the skin"*
>
> *(Krag, 1993)*

Also in 1993 a paper from radiologists at the Sydney Melanoma Unit was published looking at lymphoscintigraphy in 209 patients with trunk melanoma. Radio colloid was injected subdermally around the site of the primary melanoma and had a sensitivity of 94% in detecting drainage sites that may contain metastatic disease. The problems which had been discussed with respect to blockage of lymphatic channels were not a significant problem in the study and

> *"in fact, good channels were sometimes seen despite clinically obvious in-transit metastases in the lymph channels."*
>
> *(Uren,1993)*

92% of the patients in this study had drainage to at least one node group, 58% had drainage to two node groups and 10.5% had drainage to three or more node groups. Generally speaking surgical resection was not carried out in patients where there was drainage to more than two node groups and therefore the approach of a targeted biopsy of

a single node from one or more node groups led to a larger number of patients being offered surgery to involved node fields. During the latter part of the study nodes visualised by this technique were marked on the skin and this proved to be of considerable assistance in surgery. The authors conclude that

> *"our study re-emphasises the extreme variability of lymphatic drainage in individuals which makes general rules inappropriate when predicting draining groups clinically."*
>
> *(Uren,1993)*

Similarly using a gamma probe in melanoma patients it was found that

> *"the concept that patients have lymphatic drainage which can be safely predicted from an anatomy text should be laid to rest"*
>
> *(Krag, 1995)*

A group in the Netherlands used the sentinel node biopsy technique in a small group of melanoma patients and found it useful. (VanderVeen, 1994)

The search for an optimum technique and material for lymphoscintigraphy had led to the use of technetium antimony sulphide colloid although some groups (Hung, 1995) have used technetium sulfur colloid which is known to have a larger particle size than antimony. The two substances give similar pictures but there is a faster transport rate to the inguinal lymph nodes and a decreased radiation dose as a result of the use of Sulfur

colloid. It is felt that the optimal particle size for passing through lymphatics is between one and ten nanometres. (Strand, 1979)

In a recent article from the Sydney group (Uren, 1995) breast lymphoscintigraphy alone was used in 34 patients and found to be successful in 25. In contrast to cutaneous lymphoscintigraphy only a small number of patients had lymphatic channels visualised. (20%) and 85% had drainage to the axillary lymph nodes. 32% with entirely inner or outer quadrant tumours had unexpected lymph drainage across the centre line of the breast. It was noted that 79 % of patients had no movement of tracer on early images. Even with their large experience in lymphoscintigraphy this group was unable to visualise channels meeting nodes in the majority of patients which

> *"meant we had to assume that the node we eventually saw in the axilla was the true sentinel node."*
>
> *(Uren, 1995)*

Only one intramammary node was visualised, possibly secondary to 'bloom' from the injection site activity, though internal mammary nodes were seen.

9.8 Sentinel Node Biopsy

9.8.1 General

The term "sentinel node biopsy" was coined by Donald Morton of the John Wayne Cancer Institute, Santa Monica, California in a landmark paper published in 1992

(Morton, 1992, a). He described a technique of targeted lymph node biopsy which he called sentinel node biopsy which had been used in 237 lymphatic basins with successful identification of a sentinel node in 194. The term sentinel node was used to describe

> *"the lymph node nearest the site of the primary melanoma, on the direct drainage pathway. The most likely site of early metastases, the sentinel node can be removed for immediate intraoperative study to identify clinically occult melanoma cells."*
>
> *(Morton, 1992, a)*

Non sentinel nodes were the sole site of metastases in only two of the 194 cases; a false - negative rate of less than 1%. These findings were corroborated in a much smaller study (Reintgen, 1994) in melanoma patients in whom sentinel node biopsy and subsequently full nodal dissection was carried out in patients with intermediate thickness melanoma. No skip metastases were identified, the sentinel node accurately predicted the nodal status in all cases and in many was shown to contain the first evidence of melanoma metastases. Their conclusion was that melanoma metastasis was not a random process, but that sentinel nodes could be accurately mapped. It was felt by these authors that this technique revolutionised melanoma care in that only those patients with metastatic disease in the sentinel node should be subjected to the morbidity and expense of complete nodal dissection.

9.8.2 Blue Dye Marking

The technique of visually locating the sentinel node in melanoma patients was to use a vital dye. In the United States the main agent used is Isosulfan Blue, however in other parts of the world Patent Blue Violet is used. The two substances are effectively very

similar, the former having a specification in the 3rd edition of the colour index (1971) of 42045 and the latter 42051. Methylene blue on the other hand has a specification of 52015.

These were substances which had been used infrequently in clinical practice for years to outline lymphatic channels for lymphangiography. Patent blue dye is an intriguing preparation which has a number of diverse uses in clinical practice and research. The substance is a sulphinated dye compound. It has been used for the dye inactivation of viruses in the preparation of vaccines (Miyamae, 1990); to act as a disclosing agent for plaque in patient education for dentists (Chadwick, 1990); for demarkation of devitalised or necrotic tissues in trauma and sepsis surgery (Kus, 1990) and as a co injectable cell marker for harmlessly tracking cell lines in living cells. The rapid disappearance of the water soluble dye led some workers to investigate incorporation of the patent blue dye into liposomes which acted as carriers for the dye. (Pump 1994)
Patent Blue Dye is marketed in Australia by Rhone-Poulenc Rorer.

9.8.3 Dye Interference with Pulse Oximetry

The peak absorbance of light by Patent Blue Dye at 635 nm (Newton, 1981) is very close to the standard wavelength utilised by oximeters at 660 nm (Morell, 1993) and may lead in some circumstances to apparent desaturation as measured by the pulse oximeter. This effect when injected intravenously is brief, but in intradermal injections such as those proposed by Morton the effect could be prolonged. The effect is not uniform and may

depend upon changes in cardiac output, blood volume and body surface area (Scheller, 1986). The use of local anaesthetics and adrenaline may also have an effect.

In the original article on Sentinel Node Biopsy (Morton, 1992, a) various dyes and various delays were experimented with when injected around the site of a melanoma. The suggestion was made that approximately a five minute delay is optimal. It was felt that intradermal injection of the dye was critical in achieving satisfactory results. In another paper it was noted that the blue dye has a rapid transit time of approximately 20 minutes before the dye dissipates and is no longer visible within the lymphatics. (Krag, 1995)

9.8.4 Application of Sentinel Node Biopsy to the Breast

The application of the sentinel node biopsy technique to the breast was first reported in 1994 (Giuliano, 1994), working with the Morton group in California. In 174 patients sentinel nodes were identified using blue dye alone in 65% of cases. Subsequent axillary dissection showed that sentinel node biopsy (if it could be performed) accurately predicted nodal status in 95.6% of cases. In clinically negative, pathologically positive axillae, the sentinel node was the only involved node in 38% of cases suggesting that the node is indeed the first site of metastatic disease. 18.5% had only level II metastases which would have been missed by sampling. A point strongly made in the paper was that there is a significant learning curve in this procedure and that the false negatives occurred in the first part of the study. In the last half of the study the sentinel node was 100% predictive of axillary status. Interestingly the mean number of positive lymph nodes in

patients in whom a sentinel node was identified was only four whereas in patients with positive lymph nodes in whom a sentinel node could not be identified was nine. The authors suggested that this may indicate some form of lymphostasis or factors in the lymphodynamics which affect sentinel node identification. Nevertheless, this procedure appeared to hold promise in breast cancer as well as melanoma treatment.

In a later paper, (Giuliano, 1995) sentinel node biopsy was shown to have a significantly higher detection rate for axillary metastases on detailed pathology than for standard axillary dissection. Many of these 'extra' cases arose as a result of the presence of micro metastatic disease. This threw up yet another controversy: whether or not the overall survival of patients with micro metastatic disease was poorer than the group of patients with negative nodes. This issue will assume greater importance in the future as tumour size continues to decrease and the rate of node positivity continues to decrease. As one discussant commented in the above mentioned paper,

> *"we must prepare ourselve for the 21st century in breast cancer, when the median diameter of all invasive disease will be 1 cm. or less"*
>
> *(Giuliano, 1995)*

The work of Morton and Giuliano has been substantiated by others in a qualitative fashion.

> *"(we) mapped over 50 patients, the majority of whom have had formal lymphadenectomies following sentinel node biopsy. Our overall sentinel node identification rate is greater than 80% with the axilla being the most difficult region in which to identify the sentinel node. We are presently trying to improve*

this identification rate by performing pre operative lymphoscintigraphy"
(Author's underlining)

(Ross, 1993)

The use of sentinel node biopsy (without lymphoscintigraphy) as an aid to targeted biopsy of potentially involved regional lymph nodes has been used in a number of other settings where the drainage of lymphatics from a region is potentially ambiguous or where it is desirable to know exactly which node group is potentially involved. Such areas include head and neck melanoma (Morton, 1993), carcinoma of the vulva (Levenbach, 1994) and penile carcinoma (Cabanas, 1992).

The term "sentinel node" was somewhat confusingly used in a context different from that described above by a group looking at squamous cell carcinomas of the head and neck (Manelle, 1994) in which a retrospective study "defined" sentinel nodes as those lying within a certain group. The presence of metastatic disease pathologically outside this defined group was taken as a bad prognostic factor. This use of the term "sentinel node" is clearly at variance with the majority of the literature, bears little or no relevance to the term used in the context of this research, and will not be discussed further.

9.9 Lymphoscintigraphy and Sentinel Node Biopsy.

Once Morton's technique of sentinel node biopsy had been shown to be reasonably reliable, and lymphoscintigraphy had been shown to be feasible as a mapping technique, it was a logical step to combine the two procedures in patient management so that the same nodes which were identified on Xray could be removed surgically, thereby limiting the amount of surgery required in patients eligible for lymph node treatment.

An early paper (Krag, 1995) looked at lymphoscintigraphy and sentinel node biopsy in 118 patients (only 44 of whom had blue dye). This group showed that it was possible to identify the same lymph nodes by using lymphoscintigraphy techniques in conjunction with intra operative patent blue dye marking. It was pointed out that the use of a hand held gamma probe helped guide the surgery and was somewhat easier to learn than the lymphatic channel dissection technique described earlier by Morton. The authors claimed an immediate success rate of 98% when using the gamma probe in a variety of clinical settings. By contrast other authors (Morton, 1992) suggested a longer learning curve and felt that 60 cases were required for proficiency in the sentinel node biopsy technique. It was pointed out that mapping using blue dye alone is not an ideal technique for use in locating the lymph nodes to be resected since the surgeon may not know the precise location of where to look for the blue nodes prior to incision. (Krag, 1995) The authors point out that lymphoscintigraphy prior to surgery allows the surgeon to determine where draining lymph nodes are likely to occur and therefore allows more limited surgery. In invited comments on the above article made by members of the Sydney Melanoma Unit, Mc Carthy points out that most nodes visualised on lymphoscintigraphy and blue dye studies are not sentinel nodes since they are not nodes

receiving lymphatic drainage direct from a primary lesion. It was noted that the radionuclide used in the Krag study was Sulfur colloid which had a relatively large particle size and tended to migrate poorly. Mc Carthy claimed that technetium-99m-antimony trisulfide colloid was a better agent to use as the particle size was smaller and more uniform, allowing rapid migration through the lymphatic channels but good trapping and retention by lymph nodes.

In a smaller study of 29 patients with melanoma, lymphoscintigraphy was carried out prior to operative blue dye marking and sentinel node biopsy. It was found that in a third of the cases the clinician could not predict the location of the sentinel node to within 5 cms. of its eventual site. Lymphoscintigraphy however was accurate 100% of the time and was an invaluable aid to the surgeon in identifying all lymphatic basins at risk for metastatic disease. No 'skip' metastases were identified in these patients. (Godellas, 1995)

Although it would appear that the lymphoscintigraphy studies and the sentinel node biopsy studies are identifying the same lymph nodes, up to 1995 only the two articles mentioned in this section (Krag, 1995 and Godellas, 1995) had reported a combination of the two techniques to assist in surgical management and nodal dissection. These studies both involve patients with melanoma and up to 1995 there had been no trial looking at the use of lymphoscintigraphy and sentinel node biopsy using blue dye in patients with breast cancer.

9.10 Micro Metastases.

Certain things in medicine seem immutable and to most surgeons the pathology report fits into this category. As stated by one author, there is a

> *"general feeling of security in the pathologists ability to find disease within a given node."*
>
> *(Wilkinson, 1974)*

The author then performs mathematical calculations which show that to achieve optimum sampling the lymph node must be cut into quarters and oriented in such a way that opposite ends of the lymph node are oriented differently. Since most pathologists simply bisect the lymph node, it is hardly surprising that some lymph nodes which are reported as being free of tumour, do in fact contain metastatic disease when detailed analysis is carried out. Detailed analysis might only involve closer sectioning of the node but could involve the use of immunohistochemistry or monoclonal antibody studies.

The lowest reported incidence of undiscovered micrometastases in lymph nodes was 9% of 921 cases examined (International Ludwig Breast Cancer Study Group, 1990). Other reviews state figures for micrometastases of 12% (Hinsworth, 1993), 14% (Trojani, 1987) and (Cochran, 1988), 22% (Pickren, 1961), 24% (Fisher, 1978), and in one study 33% (Saphir, 1948).

The latter article made the point that in the earliest cases of nodal metastasis, tumour emboli are first noted in the marginal sinus and therefore the most logical way in which to identify these cells is to bisect the lymph node and embed the halves so that the cut surface is sectioned first in one half and the peripheral surface is sectioned first in the other half.

These disturbing figures which suggest significant inaccuracies in one of the foundations on which our staging is based raised the question as to what differences in prognosis are found when one analyses survival data for patients in the light of this knowledge.

It is perhaps reassuring to read articles which suggest that sectioning the lymph nodes at three different levels neither added significantly to the information obtained nor altered the pathological staging of the disease. This view seemed to be borne out by work (Fisher, 1978) and (Pickren, 1961) which suggested that the survival of patients with occult metastases was 'practically identical' to node negative patients. It was felt that other prognostic factors such as tumour characteristics and vascular invasion are more important and which, if present, suggested poorer survival.

The weight of evidence however was against this perhaps comforting view and suggested that micrometastases *are* an important prognostic indicator. The significance of micrometastases was analysed in a number of different ways and expressed in several different ways. From the point of view of survival, micrometastases at level I led to a similar survival to truly node negative patients. Similarly micrometastases at level III

equated in prognostic terms approximately to gross nodal disease at level I. Macro metastases at level III of course led to a much worse prognosis. (Huvos, 1991).

Overall survival for patients with micrometastatic disease compared with truly node negative patients in one study showed a mortality of 41% compared with 21% at follow up ranging from six to twelve years. (Cochran, 1988). In another study (Hainsworth, 1993) a significant decrease in disease free and overall survival was noted if there were occult metastases in two or more lymph nodes. A larger study showed a poorer disease free and overall survival after five years follow up. (The International Ludwig Breast Cancer Study Group, 1990) The presence of micrometastases, vascular invasion and large tumour size were all shown to be poor prognostic factors. It was suggested that detection of micrometastases may identify a 'high risk node negative' population and it was felt that searching for micrometastatic disease should be part of routine pathology.

One group of patients reported in two papers (with different periods of follow up) (Trojani, 1987) and (de Mascarel, 1992) was subjected to multivariate analysis which showed that tumour grade and the presence of micrometastases were both highly significant predictors of disease free and overall survival, but only for ductal carcinoma.

In perhaps the most detailed study yet carried out (Rosen, 1981) patients with truly node negative and micrometastatic node positive disease were compared and significant differences were found for tumours of different sizes and for different periods of follow up. Patients with smaller tumours (2 cms. or less) and six years follow up who were truly

node negative had approximately equivalent survival to micrometastatic disease positive patients and this was significantly better than patients who had macro metastases. At twelve years follow up, however, patients with smaller tumours who were truly node negative had a better prognosis than micrometastatic node positive patients. At this time the micrometastatic disease patient survival was roughly equivalent to patients who had been macro metastasis positive initially. For patients with larger tumours (2.1 - 5 cms.) those with micrometastatic disease had approximately equivalent survival to patients with node negative disease, even at 12 years. Both these groups of patients with larger tumours had a better outcome than patients with macro metastatic disease.

If pathological staging is regarded as important and has also been shown to be inaccurate in a significant number of cases, steps should be taken to correct this problem. Unfortunately the amount of work and expense involved in searching for micro metastases in large numbers of lymph nodes removed from each patient with breast cancer is prohibitive. The general attitude of complacency concerning the accuracy of pathological reporting continues despite the body of evidence to suggest there are serious shortcomings.

The detailed sectioning and analysis of a small number of lymph nodes (such as a sentinel node biopsy specimen) would be feasible even in routine clinical practice and may lead to the acceptance of sentinel node biopsy as a routine clinical procedure.

In a recent paper (Guiliano, 1995) two matched groups of patients underwent either routine axillary node dissection or sentinel node biopsy followed by either routine processing in the first group or detailed pathological analysis in the second group. The number of patients with axillary metastases in the standard axillary dissection group was 29.1% and in the sentinel node biopsy group 42%. Micrometastases were found in 10.3% of the axillary dissection group and 38.2% of the sentinel lymph node dissection group. Both the routine metastasis figures and micrometastasis figures are significantly different for the two groups and with respect to micrometastasis, highly significant.

10 METHODS

10.1 Introduction

Following extended discussions with all participants in the study during 1994 and 1995, application was made to the Human Ethics Committee of the Flinders Medical Centre for permission to carry out the study in 1995. The submission is included in the appendices. (Research application 22/95). This was approved subject to some minor changes with respect to the information sheet and answers to some further questions on radiation dosage. (Approval 3396). The amended information sheet was approved and the dosage information was also accepted. (Approval 3402.12). The information sheet and dosage information are also included in the appendices.

The technique of lymphoscintigraphy combined with sentinel node biopsy to identify lymph nodes to which a tumour would drain was first carried out in melanoma patients with Associate Professor Grantley Gill at the Royal Adelaide Hospital and discussed with a number of consultants from the Sydney Melanoma Unit whose pioneering work in this field has been mentioned previously.

Lymphoscintigraphy is a technique which has not been used in clinical practice to any great degree in recent years and discussions were undertaken with the Nuclear Medicine departments at both Flinders Medical Centre and the Royal Adelaide Hospital. Technical

discussions between the Nuclear Medicine Department at Flinders Medical Centre and the Department of Nuclear Medicine at the Royal Prince Alfred Hospital who carry out lymphoscintigraphy for the Sydney Melanoma Unit took place in relation to various technical aspects of the procedure.

The sentinel node biopsy technique using "patent blue violet" dye was carried out in a similar fashion to the technique used in patients with melanoma and in a manner very similar to the technique employed by the group carrying out sentinel node biopsy in patients with breast cancer at the John Wayne Cancer Institute (Giuliano, 1994).

In order to obtain approval from the ethics committee and patients, it was stipulated that no extra incisons (additional to the routine incisions for their breast surgery) would be made during the procedure. This meant that certain nodes seen outside the normal operative field (such as internal mammary nodes) could not be assessed.

10.2 Patient Selection and Logistics

To qualify for inclusion in the study patients were required to have a proven breast carcinoma, highly likely to be invasive, who were scheduled to have definitive breast resection and axillary dissection at the same procedure and who had either no surgery (or a limited biopsy) which was judged not to have disrupted the breast lymphatics significantly.

Patients with deep lesions and most impalpable lesions were excluded from the study as it was felt that the blue dye biopsy technique may interfere with visualization of the carbon track localisation technique used at Flinders Medical Centre and the Repatriation Hospital, thereby compromising the surgical excision. Patients with superficial impalpable lesions in whom a wide sector resection was to take place and the carbon track was not to be dissected out, were accepted for inclusion into the study.

The performance of a bone scan which would have interfered with the lymphoscintigraphy was a factor which determined when the lymphoscintigraphy could take place in that a three day gap was required between the two procedures. This however did not pose a problem as most patients had a screening bone scan shortly after diagnosis and prior to decisions concerning management. They were therefore available for lymphoscintigraphy in the week prior to surgery which was the preferred time for the study.

Patients who were referred from country regions of the state, taking holidays immediately prior to their surgery or staying with relatives some distance from the hospital and who would otherwise have been eligible generally declined inclusion for logistical reasons. As a result of great cooperation from the Nuclear Medicine Department and the breast clinic, no patient was refused lymphoscintigraphy because of unavailability of an appointment.

After identification of a suitable patient the author contacted the patient , explained the reason for the study and the steps involved should they agree to take part. This was usually carried out by telephone and a consent form and information sheet posted to the patient. When the patient attended for the lymphoscintigram any questions raised by the patient were answered and a further discussion carried out if necessary prior to the scan being performed. Patients were then examined by the author and the procedure was discussed with the radiologist prior to administration of radionuclide injections.

10.3 Lymphoscintigraphy

Matters relating to radiation safety of the proposed radiopharmaceutical were considered by the Nuclear Medicine Department (Dr. John Cormack) and the Human Ethics Committee. The report is included in the appendices.

The radiopharmaceutical used was Technetium-99m labelled Antimony Sulphide Colloid which was initially prepared from kits provided by the Royal Adelaide Hospital Radiopharmacy, but during the latter part of the study was prepared from material obtained directly from the Lucas Heights atomic reactor. (Figure 1, page 69)

The area around the tumour was isolated with a waterproof sheet cut to expose the area of injection and minimise the risk of contamination by inadvertant spillage onto the skin during injection. The use of gloves and careful injection technique to avoid contamination was most important. Universal precautions relating to radiation safety and

Patients were usually in the Nuclear Medicine Department for a period ranging from 2 - 4 hours. Agreeable patients in whom no movement of tracer had been evident were rescanned at longer periods.

10.4 Surgery

The blue dye used was "Patent Blue Violet" (2.5% in aqueous solution containing 0.6% sodium chloride and 0.05% disodium hydrogen phosphate; laboratoire Guerbet, Arlney-Sous-Bois, France; distributed in Australia by Rhone-Poulenc-Rorer). (Figure 17, page 77)

After induction of anaesthesia the waterproof plastic dressings covering the marks locating the sentinel nodes seen on lymphoscintigraphy were removed (Figure 15, page 76) and the locations remarked with an indelible marker so that skin preparation during the procedure would not erase the marks. (Figure 16, page 76) The surgical incisions were marked and the tumour location marked. Four quadrantic injections were made around the tumour in a similar fashion to the lymphoscintigraphy injections using a 19 gauge needle. Again protective gloves and careful injection technique to avoid skin and extraneous tissue contamination were essential. Every effort was made during the injection to place the four injections close to but not within the edge of the tumour at the same depth as the tumour. (Figure 18, page 77) Early in the study the full 2 mls. in each ampoule was used but it was found that better visualization could be achieved with

smaller injections and in the latter part of the study a total of approximately 1.2 mls. was used per patient. This matches the technique used in melanoma surgery. For lesions placed laterally within the breast slightly smaller volumes of injectate (approximately 1 ml) could be used however for medial lesions slightly larger volumes (approximately 1.5 ml) were necessary. Breast massage was not used as it was felt that this might stimulate abnormal or non physiologic patterns of drainage.

Immediately the injections had been administered the patient was prepared and draped in the usual fashion. In patients undergoing a partial mastectomy the axillary incision was made first and the skin divided across the axillary tail. The dissection then proceeded very cautiously between the axillary tail and the axillary contents until a lymphatic channel was identified. Rigorous attention to haemostasis was essential to avoid blood obscuring the fine blue stained lymphatic channels. The location of nodes marked at lymphoscintigraphy was carefully noted and dissection took place inferior to the nodes marked. When the lymphatic channel was identified it was carefully followed to the first node on the channel as this entered the axilla. (Figure 20, page 78) If several nodes were seen to be marked with blue dye in the one region around the lymphatic channel all the nodes were removed and sent as the specimen. The lymphatic channel was then followed inferiorly until this entered the breast and the channel was followed a short distance into the breast to ensure that the node identified and sent for examination was indeed the first draining node along the identified lymphatic channel. In cases where a lymphatic channel was not identified but the sentinel node could be identified by following the marks made at lymphoscintigraphy and encountering a blue node, this was accepted as a

sentinel node and sent as the specimen. In patients in whom nodes were not fount to be stained with blue dye, no specimen was sent. The location of the sentinel node was noted with respect to the breast and the axilla and recorded.

For patients undergoing a total mastectomy the lateral portion of the upper flap was incised and mobilised first so that the axilla could be accessed first and the lymphatic channels crossing from the breast into the axilla could be identified in the same manner described above for patients having a partial mastectomy. (Figure 19, page 78) Again the location of the sentinel node in the axilla was noted.

The sentinel node or nodes were sent separately in formalin for histological examination, (Figure 22, page 79) following which the operation proceeded in the standard fashion. In patients having a partial mastectomy, usually the axilla was dissected first, followed by the sector resection. In patients having a total mastectomy usually the breast was mobilised first and the axilla dissected subsequently in continuity. Any other blue nodes within the axillary dissection were noted and removed as a second specimen. This occurred very rarely.

The anaesthetist was advised that following injection of patent blue violet dye the patient's pulse oximetry reading might fall. Patients were warned that some blue green discolouration of the urine might be expected post operatively although most patients did not notice this. All patients underwent routine post operative follow up and were visited to ascertain whether any problems had arisen as a result of the procedure.

10.5 Pathological Processing

Breast resection specimens were usually sent on ice but in the latter part of the study when receptor status could be ascertained on Formalin fixed tissue most specimens were sent in Formalin. All axillary dissection specimens and all sentinel node specimens were placed immediately in Formalin.

The sentinel node was initially examined by the pathologist in the same way as all other routine lymph node specimens. This involved bisecting the node and submitting 5μm sections to Haematoxylin and Eosin staining and examination by light microscopy using X10 objective and X125 magnification lenses. The axillary contents and breast specimens were examined in the standard fashion without the use of clearing agents to identify lymph nodes.

Average sized lymph nodes were bisected and each half placed in a pathology block. For smaller nodes, multiple nodes were embedded per block, whereas for larger or fat replaced nodes, 5mm thick sections would be cut and embedded in multiple blocks.

Tumour and lymph node characteristics were collected for each patient.

10.6 Detailed Pathological Examination

Because of the possibility of missing small metastatic deposits within a sentinel lymph node on routine sectioning, after extended discussions with the pathology department all sentinel nodes which had been reported as negative for metastatic malignancy were subjected to detailed examination of multiple sections.

This involved detailed review of all the previous sections, followed by recutting of the original blocks at 50μm intervals and restaining of sections at 100μm intervals (i.e. every second section), alternately with Haematoxylin & Eosin and Cam 5.2 (an immunohistochemical stain used to identify epithelial cytokeratin, only present in cells of epithelial origin such as breast cancer cells).

Detailed examination of a number of cases in which the sentinel node was successfully identified and subsequently shown to be the only node containing tumour within the axilla ("Bullseye cases") was carried out. It had been intended to subject these nodes to electron microscopy in an attempt to identify technetium within the node, thereby proving that the node seen on lymphoscintigraphy and the node identified as blue are in fact the same node. Ultimately, the logistics of having specimens sent from Adelaide to Perth to an appropriate microscope, as well as the time delay and expense involved proved prohibitive.

Proof of the technique has occurred subsequently, however, as the use of a hand held gamma camera allows the surgeon to identify a sentinel node which is both blue and hot. The need for electron microscopy to prove this is therefore unnecessary.

Highly detailed analysis was carried out on one node as described in the section on pathology results, below.

10.7 Statistical Analysis and Presentation

Data relating to demography, presentation, clinical details, lymphoscintigraphy, surgery and pathology was collected for each patient and stored in a database.

Following discussions with the Epidemiology branch of the South Australian Health Commission who kindly agreed to assist with statistical interpretation, this data was transferred into a DOS file format utilisable by them and from which analyses were constructed. Because of the size and nature of the study the data is presented directly and parametric tests were considered inappropriate.

The database used was '4D first' initially running on a Macintosh LC II, but later as more data manipulation was required on a Power Macintosh 6500. The program used to produce the final thesis document was Microsoft Office 98 for Macintosh having the same file format as Microsoft Office 97 for Windows. This allowed direct transfer of documents between Macintosh and PC environments. Bibliographic data was stored in an

'Endnote' database. Illustrative photography was largely carried out by the department of Media & Illustration at the Flinders Medical Centre and the clinical photography department of the Royal Adelaide Hospital.

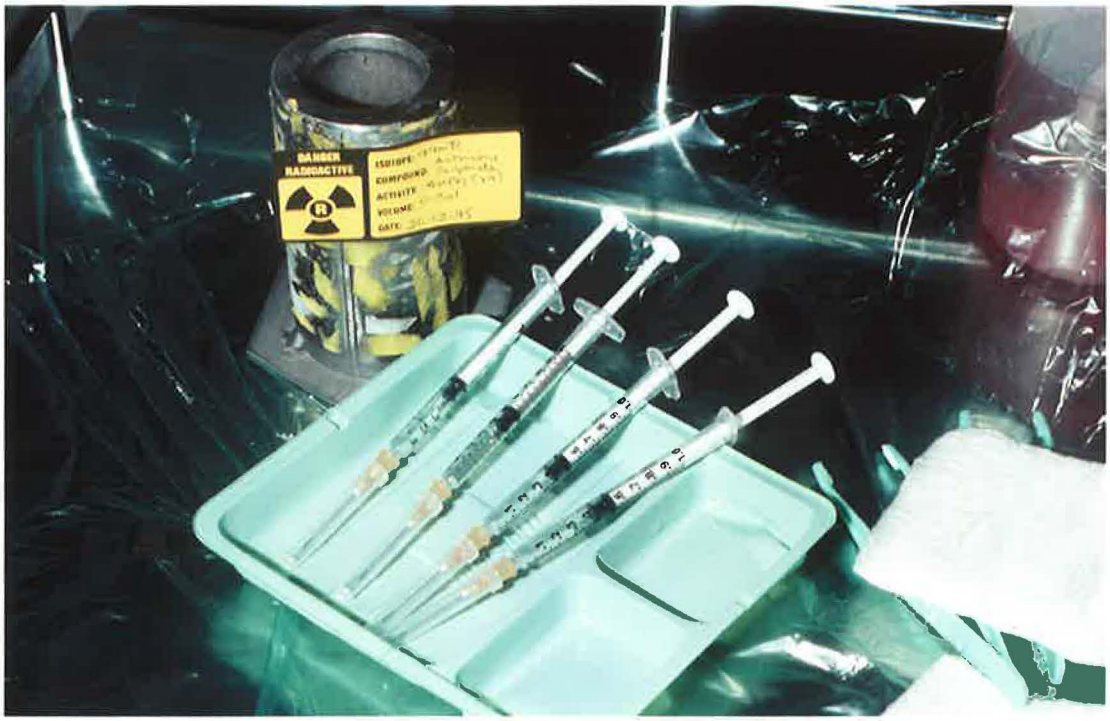


Figure 1: Lymphoscintigraphy; Four aliquots of radiopharmaceutical are prepared

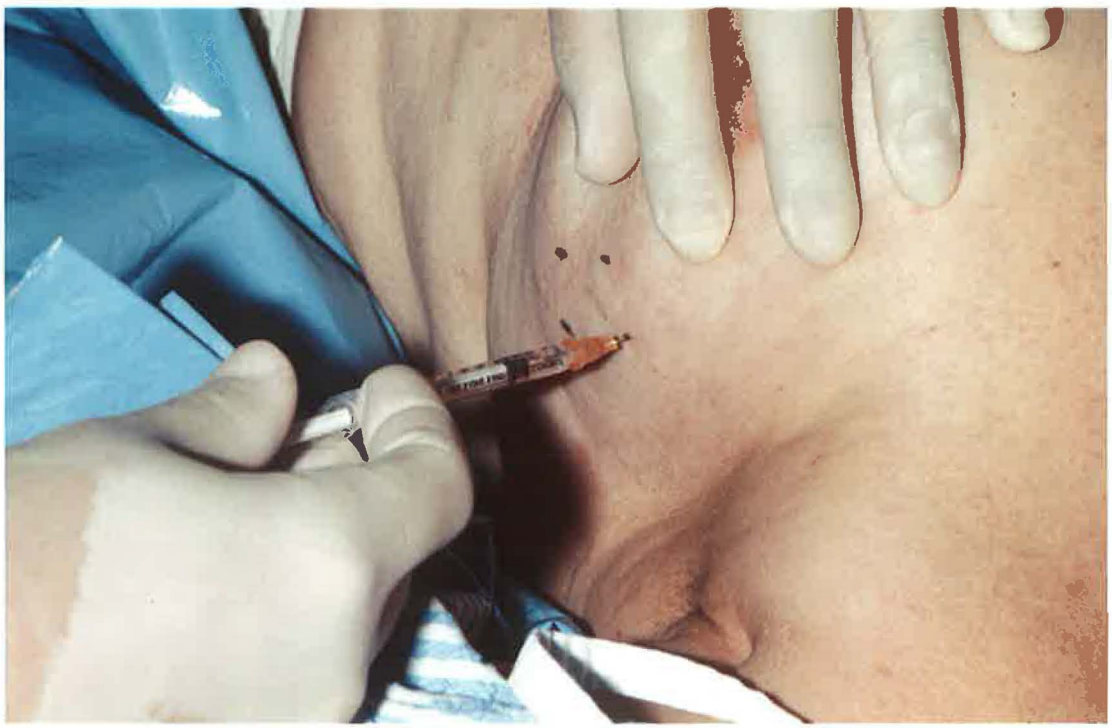


Figure 2: Lymphoscintigraphy; Injections are made around the tumour in four locations

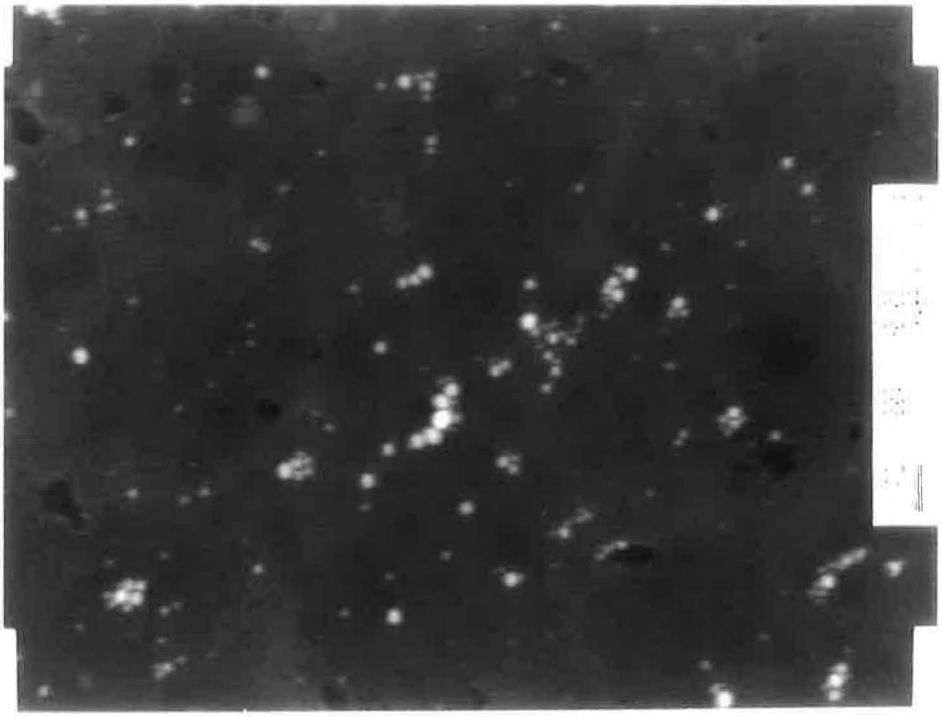


Figure 3: Electron micrograph of unfiltered radiopharmaceutical

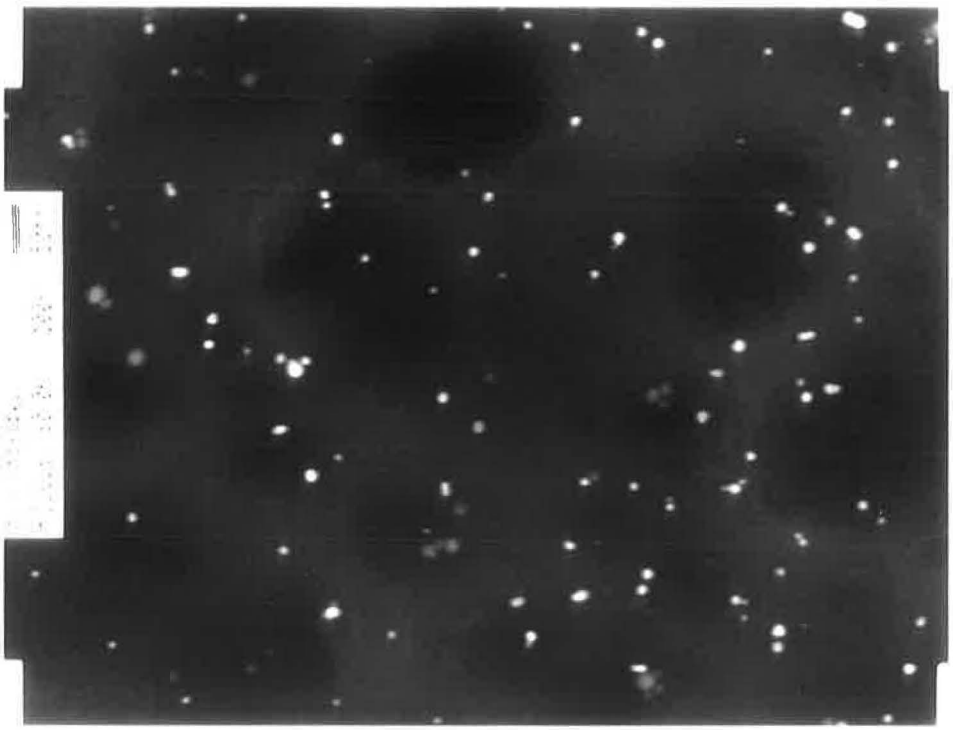


Figure 4: Electron micrograph of filtered radiopharmaceutical

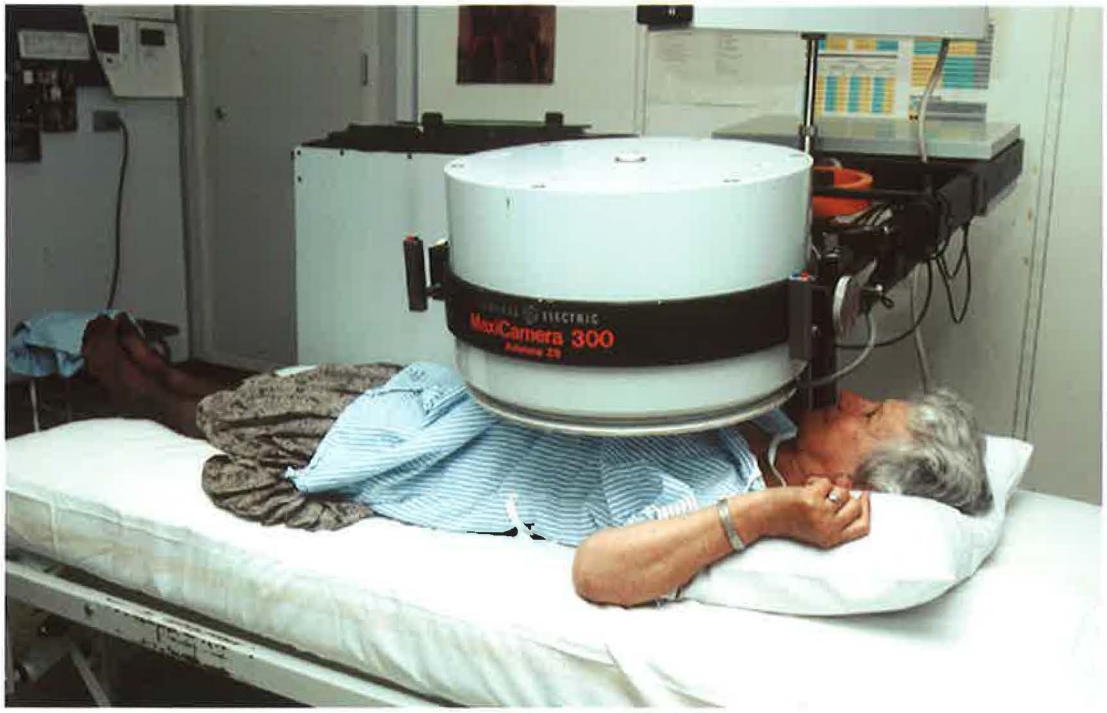


Figure 5: Lymphoscintigraphy; The patient being scanned under the gamma camera

Figure 6: Lymphoscintigraphy; Digital images are captured and stored for printing

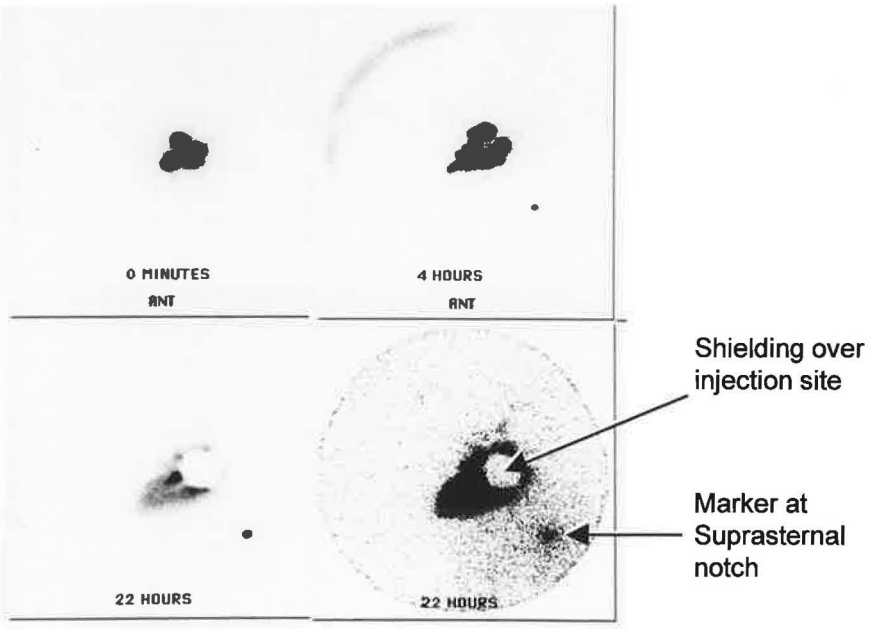


Figure 7: Lymphoscintigraphy; No movement of tracer at 22 hours (unfiltered radiopharmaceutical)

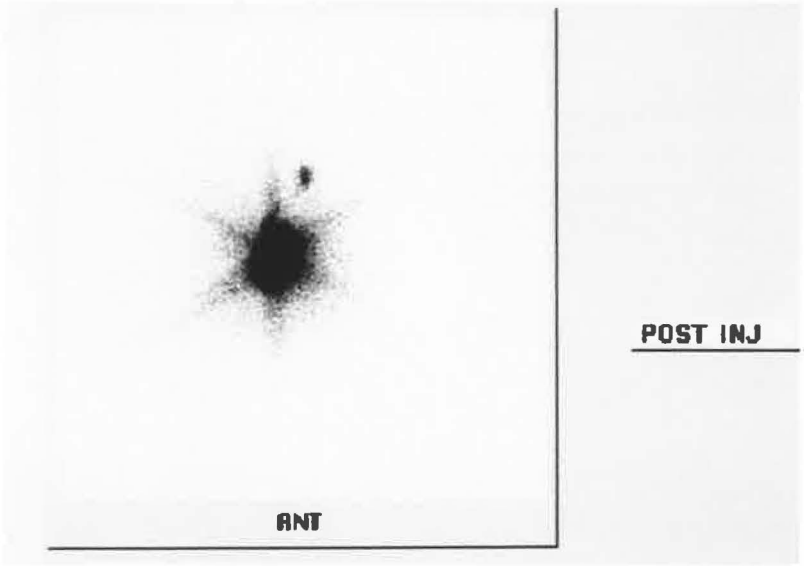


Figure 8: Lymphoscintigraphy; Identification of node without shielding immediately post injection (filtered radiopharmaceutical)

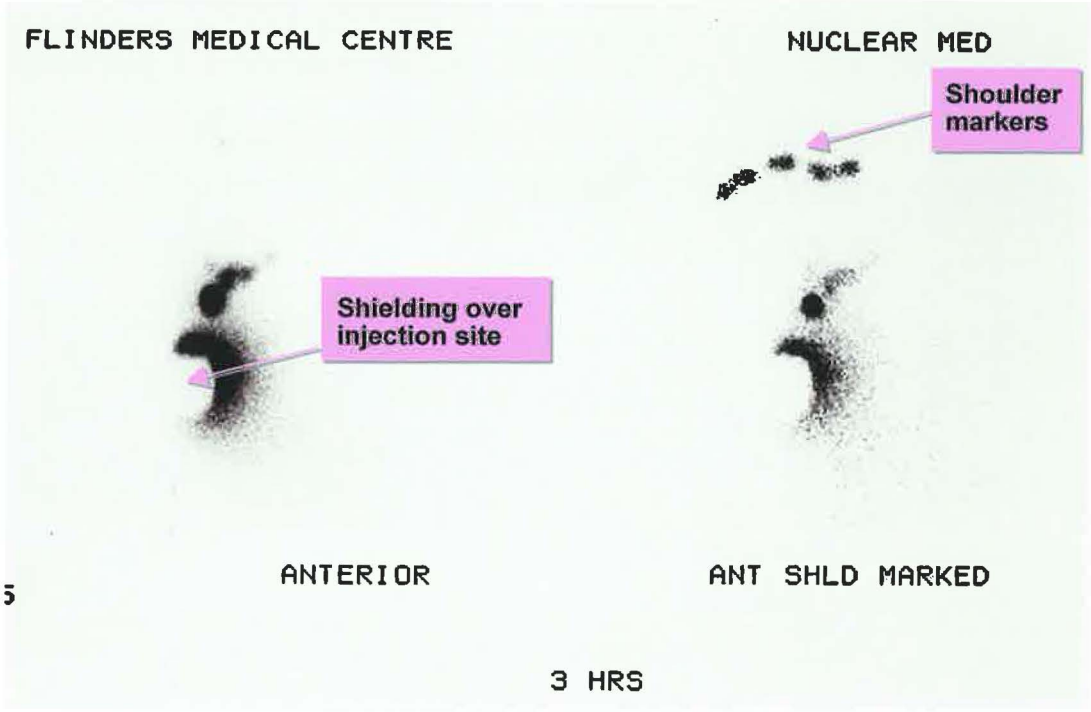


Figure 9: Lymphoscintigraphy; Identification of single sentinel node with other nodes 'downstream'

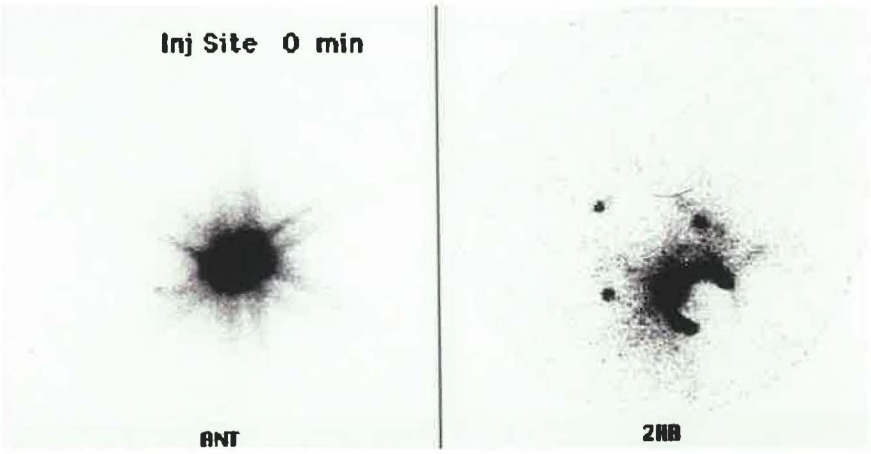


Figure 10: Lymphoscintigraphy; Identification of three sentinel nodes

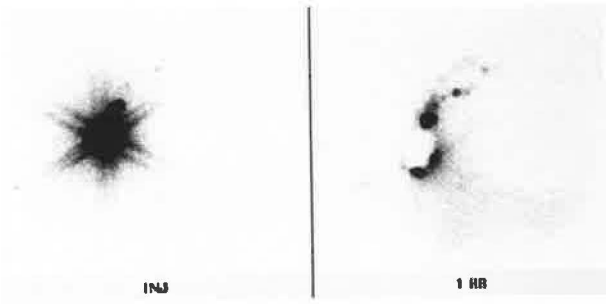


Figure 11: Lymphoscintigraphy; Identification of lymphatic channel and single main sentinel node

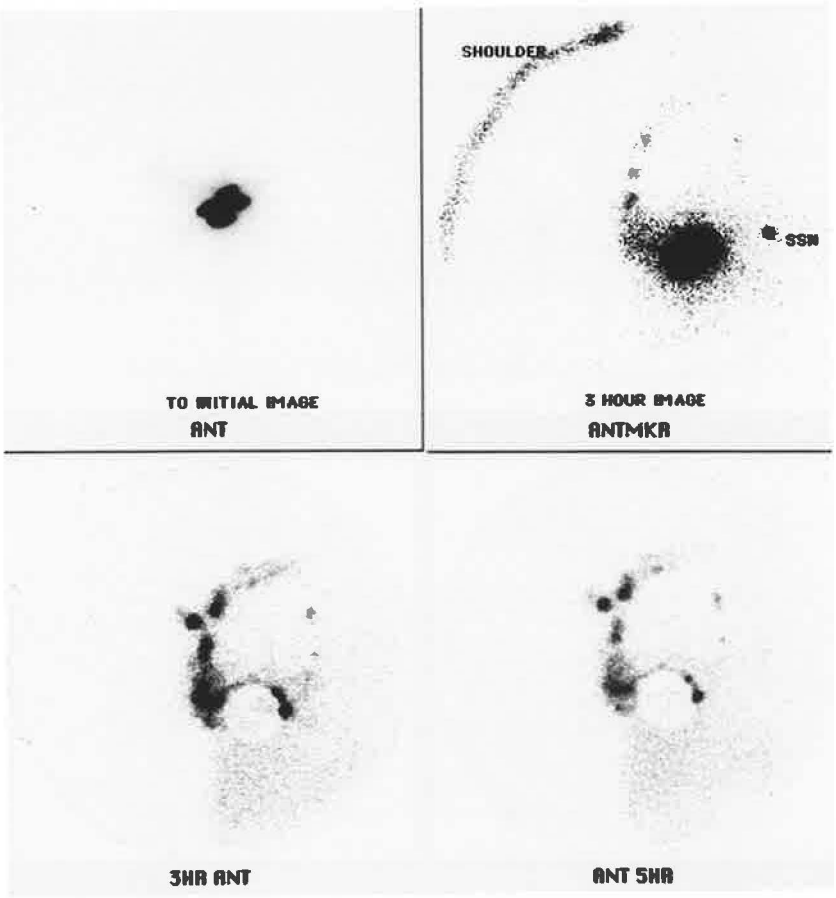


Figure 12: Lymphoscintigraphy; Identification of lymphatic channels and several sentinel nodes within a small area

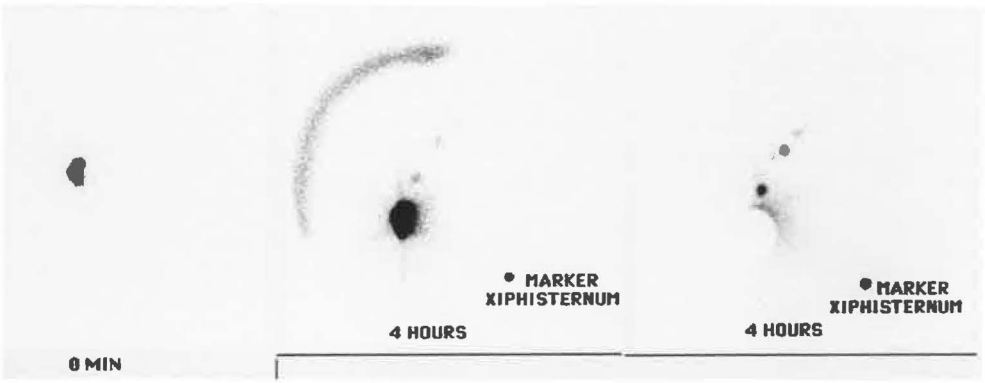


Figure 13: Lymphoscintigraphy; 3 nodes identified

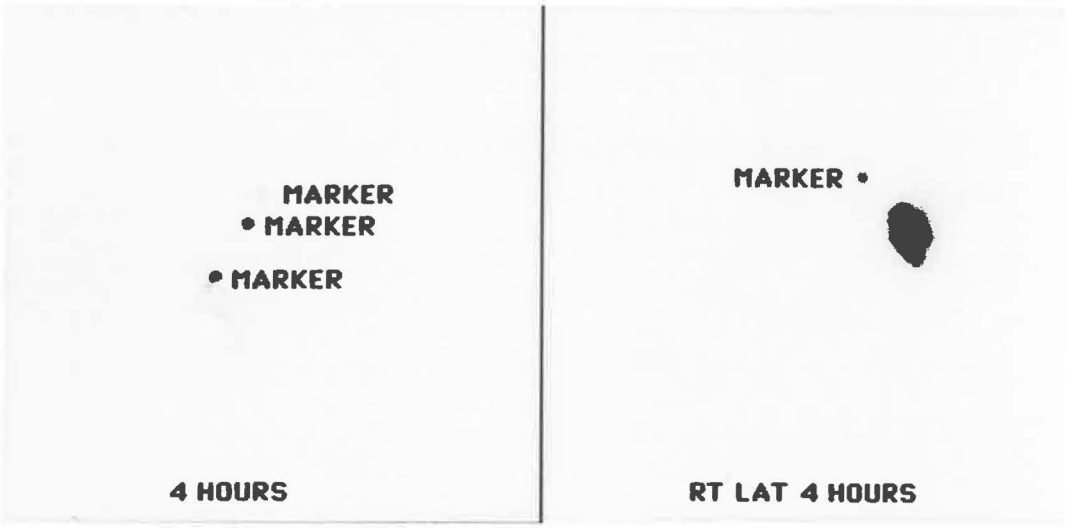


Figure 14: Lymphoscintigraphy; (same case as above)
Nodes marked in AP and lateral planes

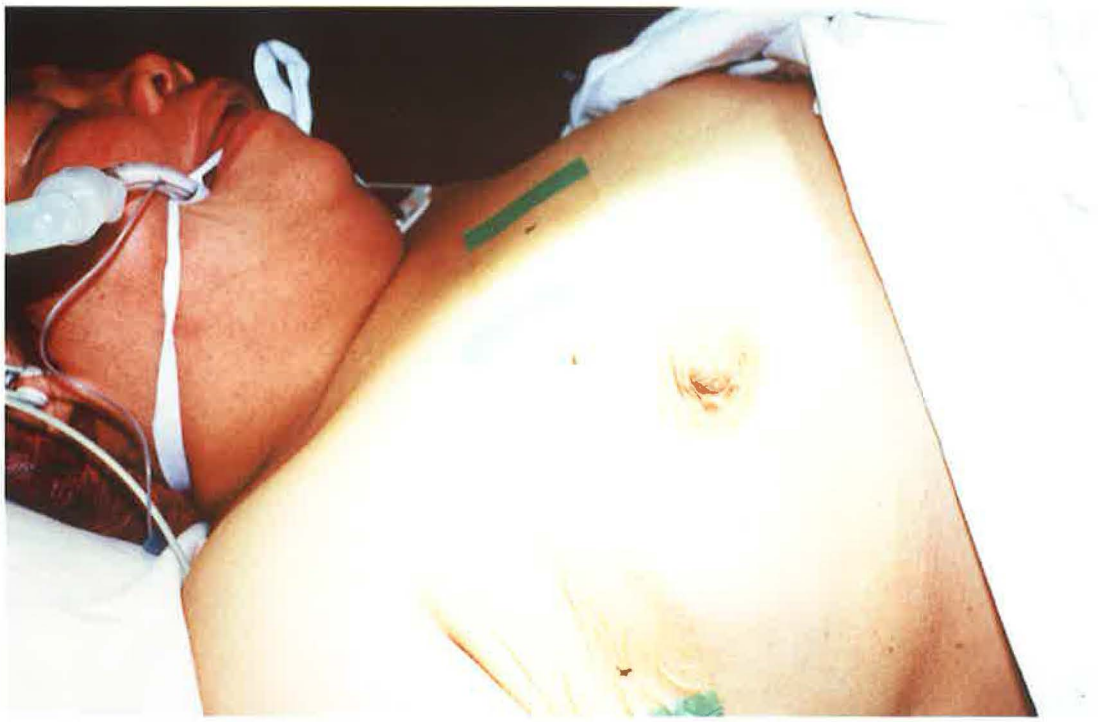


Figure 15: Sentinel node biopsy; sentinel node positions marked following lymphoscintigraphy

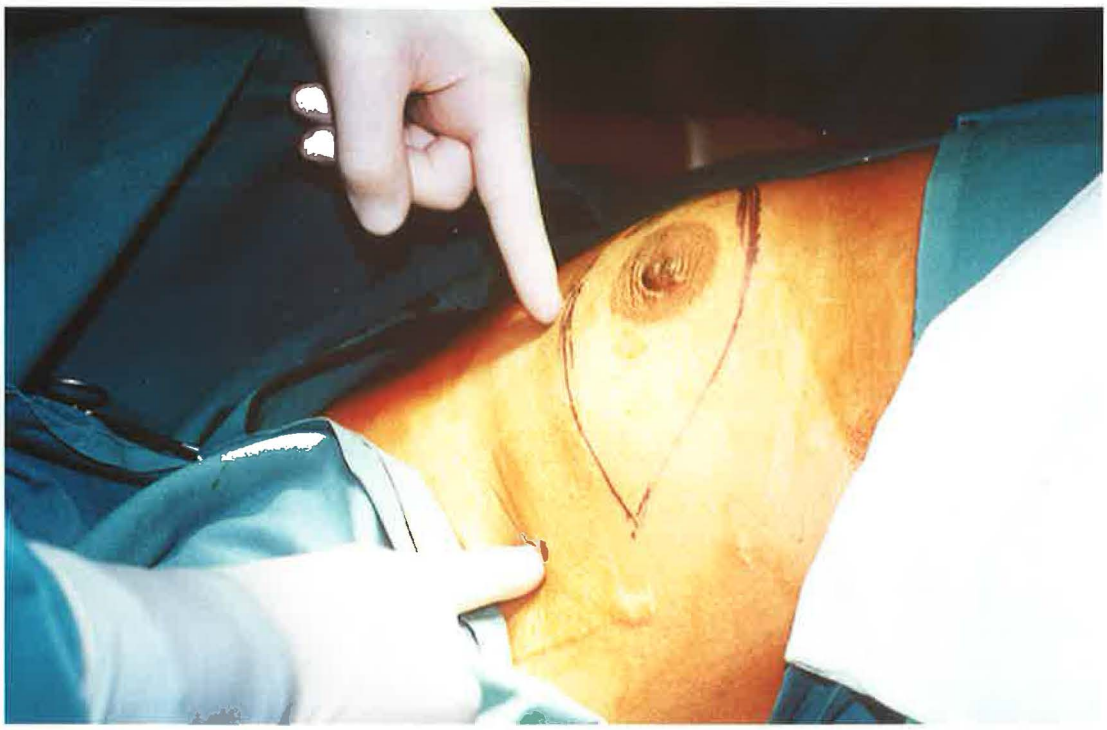


Figure 16: Sentinel node biopsy; sentinel node position marked in AP and lateral planes

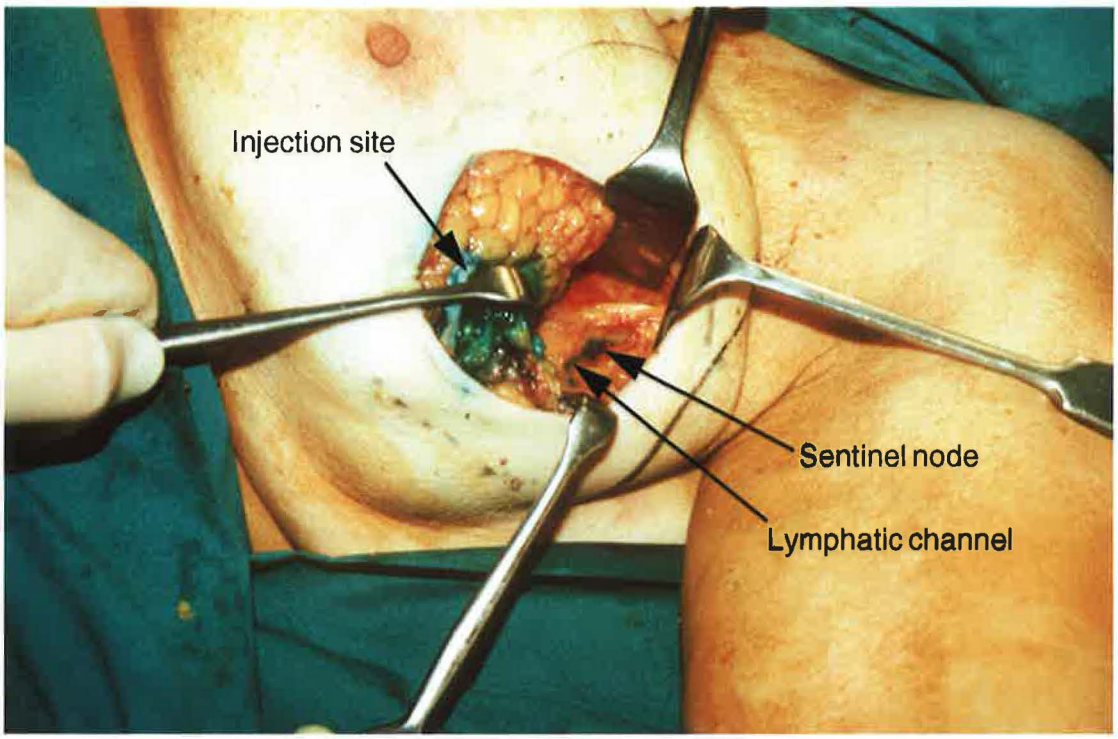


Figure 19: Sentinel node biopsy; Injection site with lymphatic channel and sentinel node identified through single incision

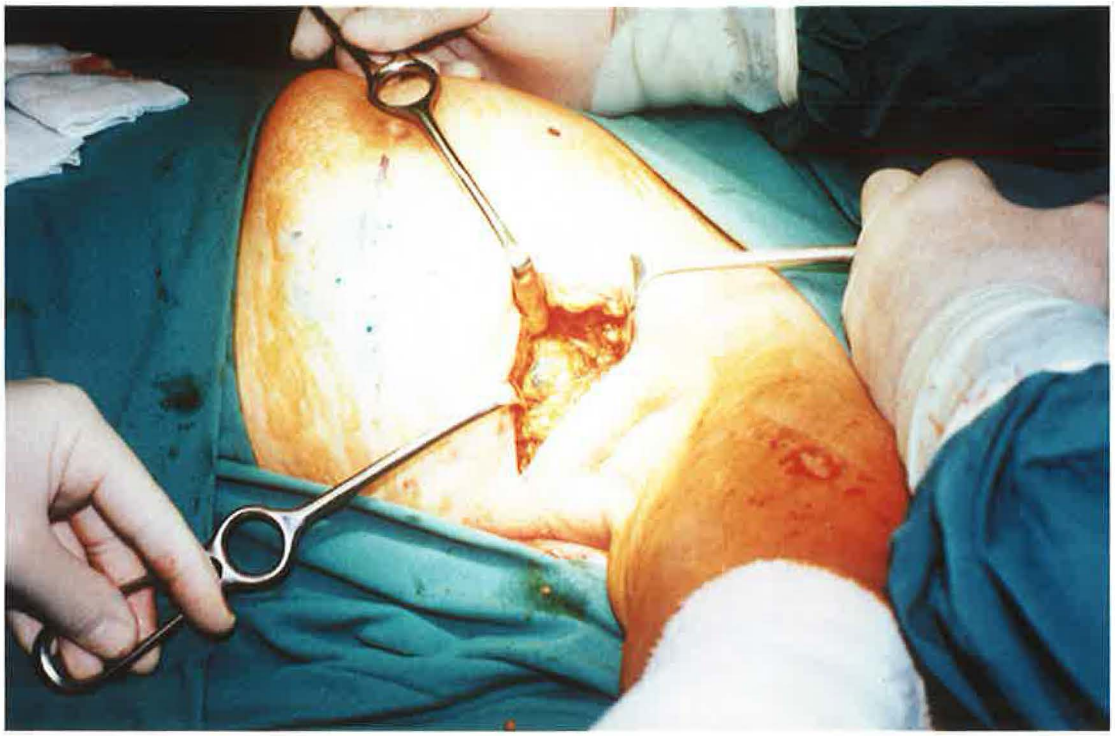


Figure 20: Sentinel node biopsy; Sentinel node dissection remote from injection site, node seen without channels (note accurate location of mark on anterior breast)

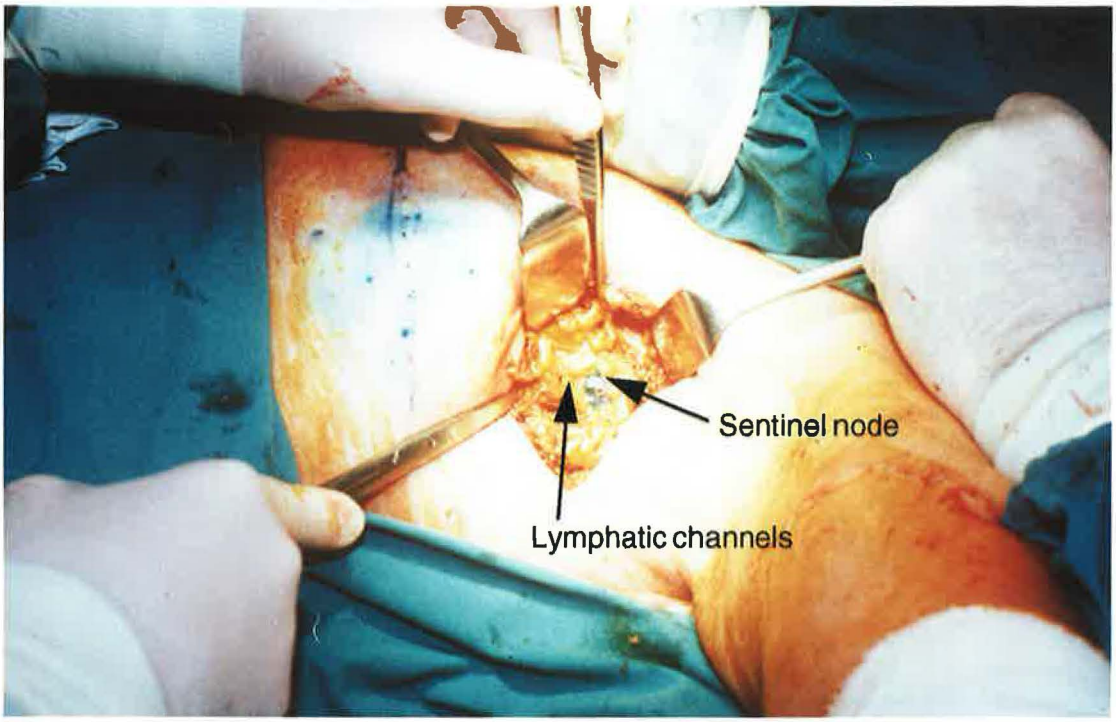


Figure 21: Sentinel node biopsy; Multiple lymphatic channels seen entering lymph node after dissection

Figure 22: Sentinel node biopsy; The resected sentinel node

12 RESULTS

12.1 Overall Results: Demography and Logistics

Of approximately 50 patients who might have been suitable, 30 patients were enrolled in the study and all were women.

Of the 30 cases included in the study, 9 had been referred from the (then) South Australian Breast Xray Service.

The median age of the patients was 59 with a range from 33 to 86.

Most enrolments came from patients in the metropolitan area: 12 from the inner metropolitan area (i.e. excluding the Adelaide hills), 12 from the outer metropolitan area (i.e. within 20 Km of the GPO) and 6 from country areas, all within 100Km of the city.

The average time between patients first being seen in the Flinders Medical Centre Breast Clinic and undergoing surgery was 17.7 days, with a range of 6 to 32 days.

The average time between the Lymphoscintigram and surgery was 2.7 days , with a range of 0 to 8 days. Because of the need to schedule operations on regular lists, no patient had her surgery delayed by the study, which was timed to fit in with the surgery. For country

Tumour Location	Number
Upper Inner Quadrant	5
Upper Outer Quadrant	9
Central	7
Lower Inner Quadrant	4
Lower Outer Quadrant	4

Table 2 Tumour Location

12.2.2 Tumour Size

Because of the exclusion criteria, most tumours were of relatively small size. For very small palpable tumours to be included, these had to be reasonably superficial within the breast. Results are based on measurements of tumour size as assessed by the reporting pathologist.

Tumour Size	Number
Less than 1 cm	2
1 to 2 cm	20
2 to 4 cm	5
Greater than 4 cm	2

Table 3 Tumour Size

12.2.3 Clinical Axillary Lymph Node status

Only 1 patient who had a 3 cm diameter breast carcinoma had a clinically palpable lymph node in the axilla at presentation.

12.2.4 Tumour Type

As expected, most of the tumours were Ductal in type, however lobular carcinomas were also seen. Also included in the study were; 1 'Inflammatory' ductal carcinoma, 1 Mucinous variant of Ductal carcinoma and 1 Ductal Carcinoma In Situ with multiple areas of invasion.

Tumour Type	Number
Ductal carcinoma	26
Lobular carcinoma	3

Table 4 Tumour Type

12.2.5 Tumour Grade

A wide variety of tumour grades was found in the study. Grading is based on the widely accepted "Bloom & Richardson" system.

Tumour Grade	Number
Grade I	7
Grade II	10
Grade III	12

Table 5 Tumour Grade

12.2.6 Axillary Lymph Node Status

The **final** pathological status of the axillary lymph nodes after full analysis is set out below:

Axillary Lymph Node Status	Number
Node Negative	15
Node Positive	14

Table 6 Axillary lymph node status

12.3 Lymphoscintigraphy: Results

12.3.1 Success Of Lymphoscintigraphy

Lymphoscintigraphy successfully demonstrated a sentinel lymph node in 23 of the 29 patients.

The principal reason for failure of the technique in these 6 cases was a failure of the radionuclide to travel away from the injection site.

One of the 6 patients left after 2 hours, despite being advised as to the probable length of time the study would take. At this stage, no movement of tracer had been observed. The following results of lymphoscintigraphy include therefore only the 28 patients who had a complete lymphoscintigram.

As mentioned above, serial filtering of the colloid and the use of slightly larger volumes of injectate led to a very high success rate in the latter half of the study. In addition, the filtered colloid appeared to travel to the sentinel node much more rapidly than unfiltered colloid, regularly giving good quality films within 1 hour of injection.

12.3.2 Study Duration

The median time taken to complete the study in the first half of the series was 2-3 hours, whereas for the last half was 1-2 hours. One patient in the early part of the study who had no movement of tracer from the breast agreed to return for repeat scanning the following day (18 hours post injection) and still no movement of tracer was seen. Later in the study with the techniques mentioned above, images were obtained much more rapidly and in several patients it was possible to clearly identify a sentinel node within 10-15 minutes.

Please refer to Figure 25, page 90.

12.3.3 Lymphatic Channels

Lymphatic channels were only seen in 5 patients, although this did not prevent the identification of sentinel lymph nodes which appeared on gamma scanning independently of activity in lymphatic channels.

12.3.4 Sentinel Nodes

Sentinel nodes were identified in 23 of the 28 patients. The number of sentinel nodes identified by the lymphoscintigram study is illustrated in Figure 26, page 90.

12.3.5 Variable Drainage

As had been noted in previous studies, patterns of lymph node drainage were quite variable, though most patients did have drainage to at least some part of the axilla. Nodes described on the lymphoscintigram as being 'intramammary' were often in the sub pectoral region, or at the periphery of the breast in the low axilla. Figure 27, on page 91 illustrates where the nodes were located as assessed by the radiologist performing the Lymphoscintigram.

In this study no drainage was seen directly to the upper axilla.

12.3.10 Lymphoscintigraphy success rates versus nodal involvement

The success of lymphoscintigraphy was independent of whether the axillary nodes contained tumour, with roughly equal numbers of successful cases in both groups. The unsuccessful cases were predominantly in the node negative group where tumour replacement of the node as a possible cause for failure is eliminated.

Success	Node negative	Node positive
Lymphoscintigraphy possible	11	12
Lymphoscintigraphy not possible	4	1*

*NB does not include 1 patient who failed to complete the study

Table 7 Lymphoscintigraphy success rates related to final nodal status

Please refer also to Figure 32, page 93.

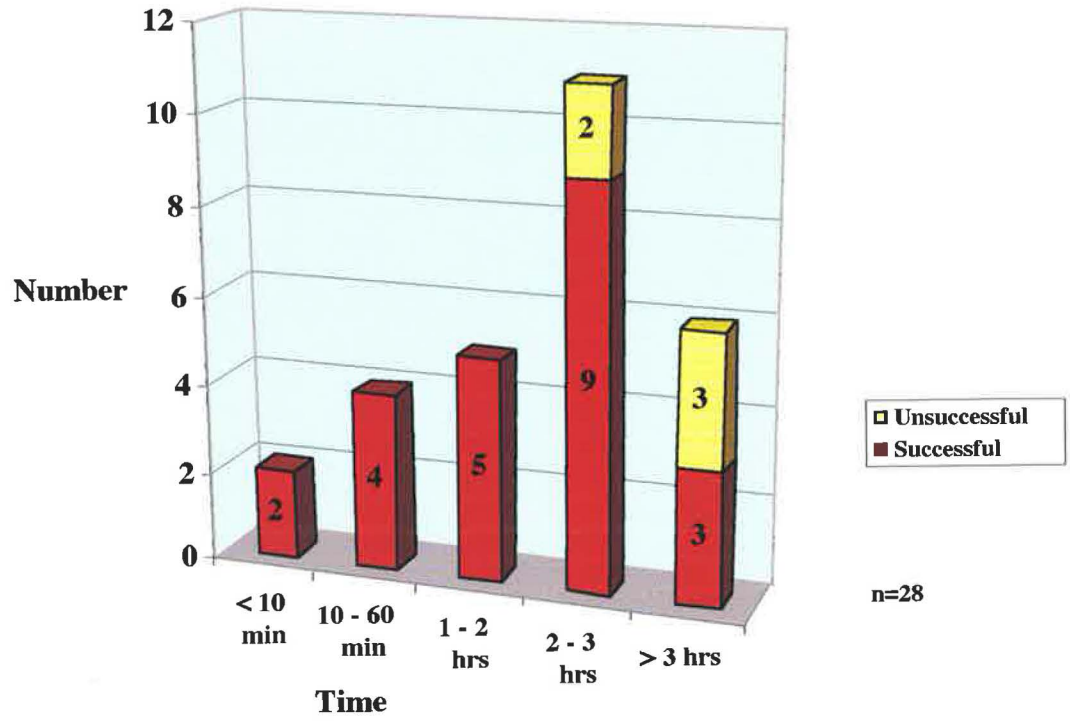


Figure 25 **Duration of lymphoscintigraphy studies**

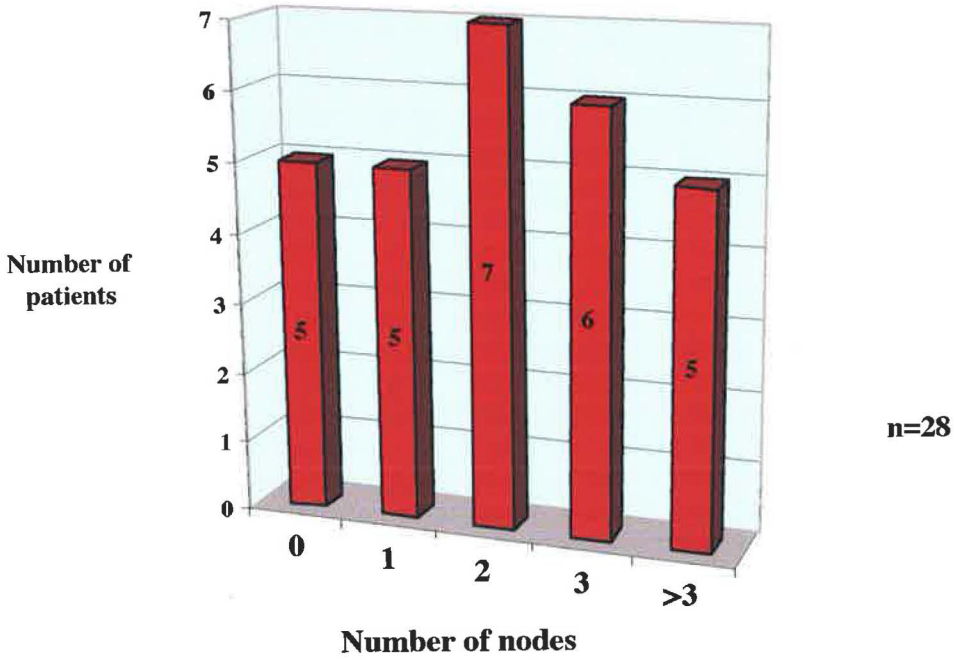


Figure 26 **Number of nodes seen at lymphoscintigraphy**

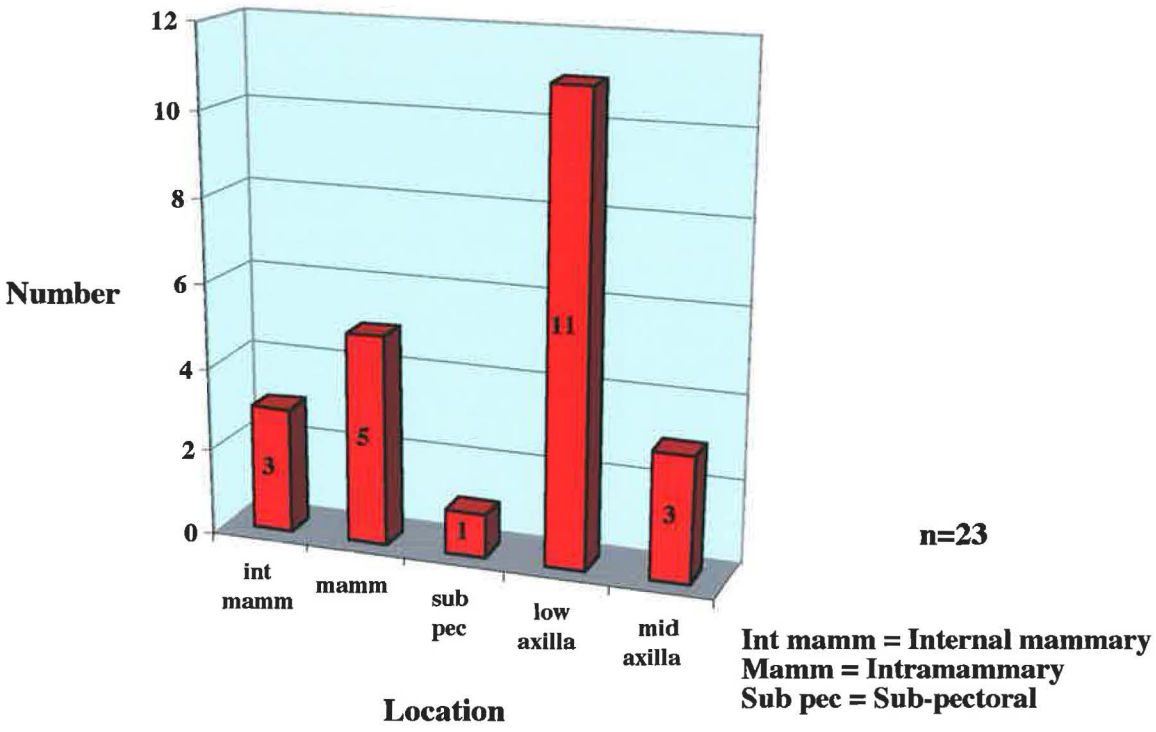


Figure 27 **Location of nodes at lymphoscintigraphy**

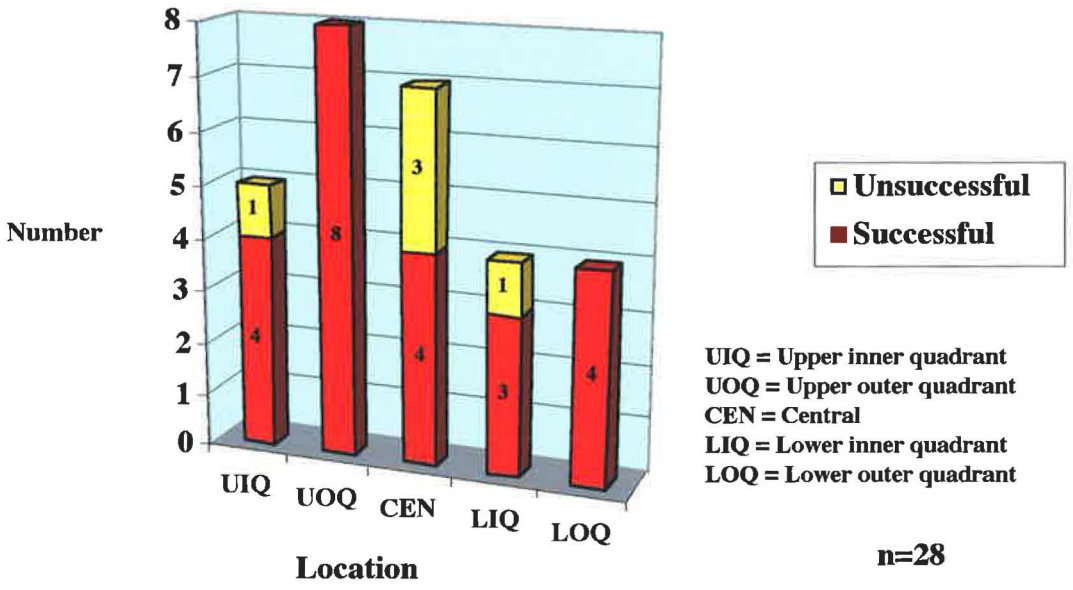


Figure 28 **Success of lymphoscintigraphy by tumour location**

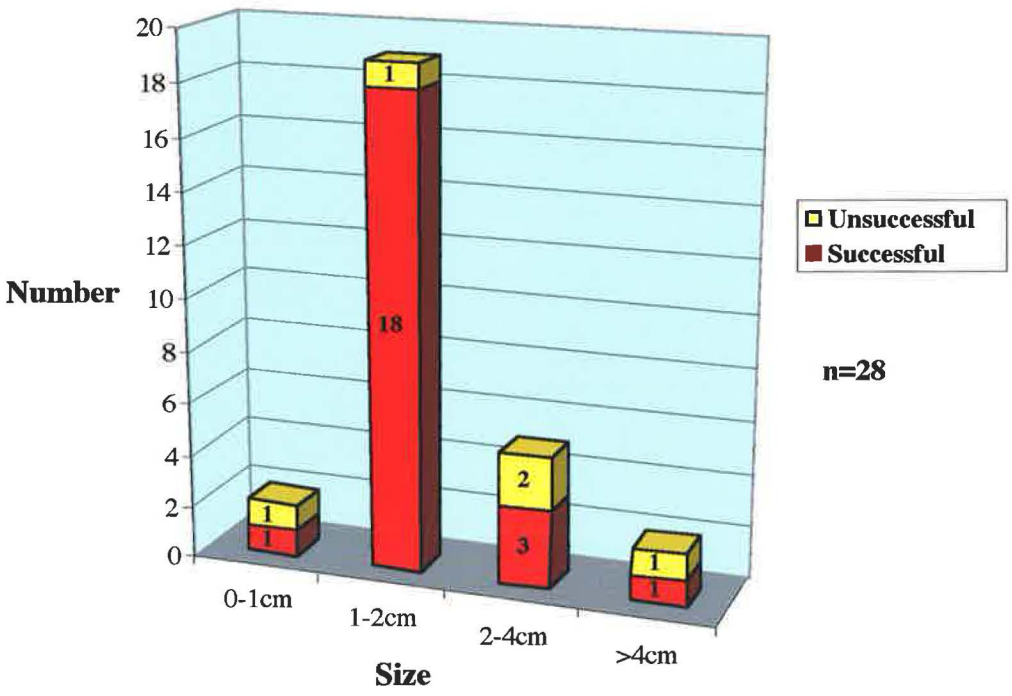


Figure 29 **Lymphoscintigraphy results by tumour size**

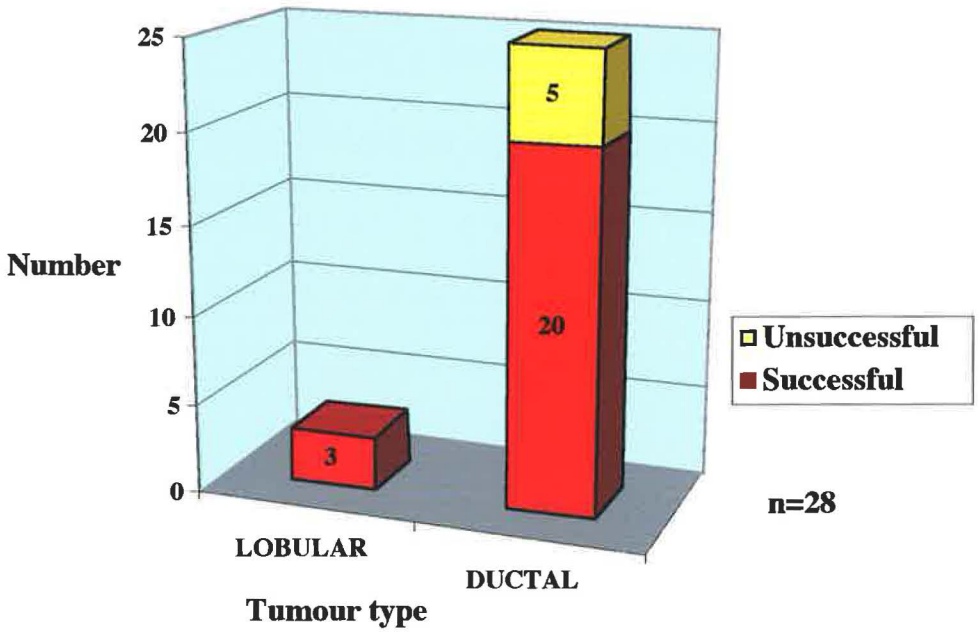


Figure 30 **Lymphoscintigraphy results by tumour type**

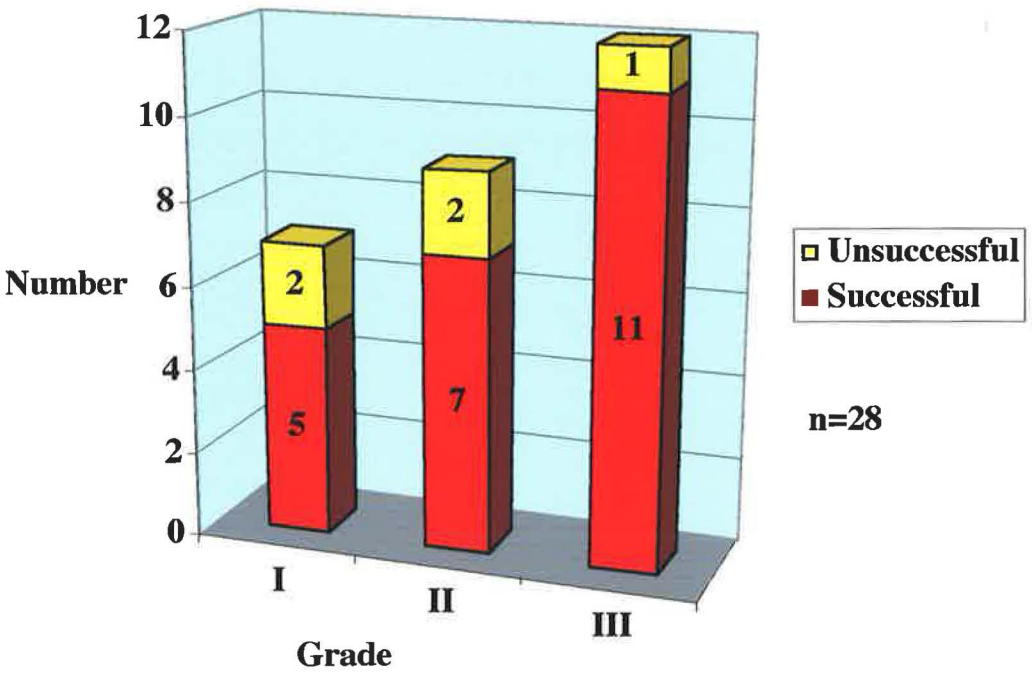


Figure 31 **Lymphoscintigraphy results by tumour grade**

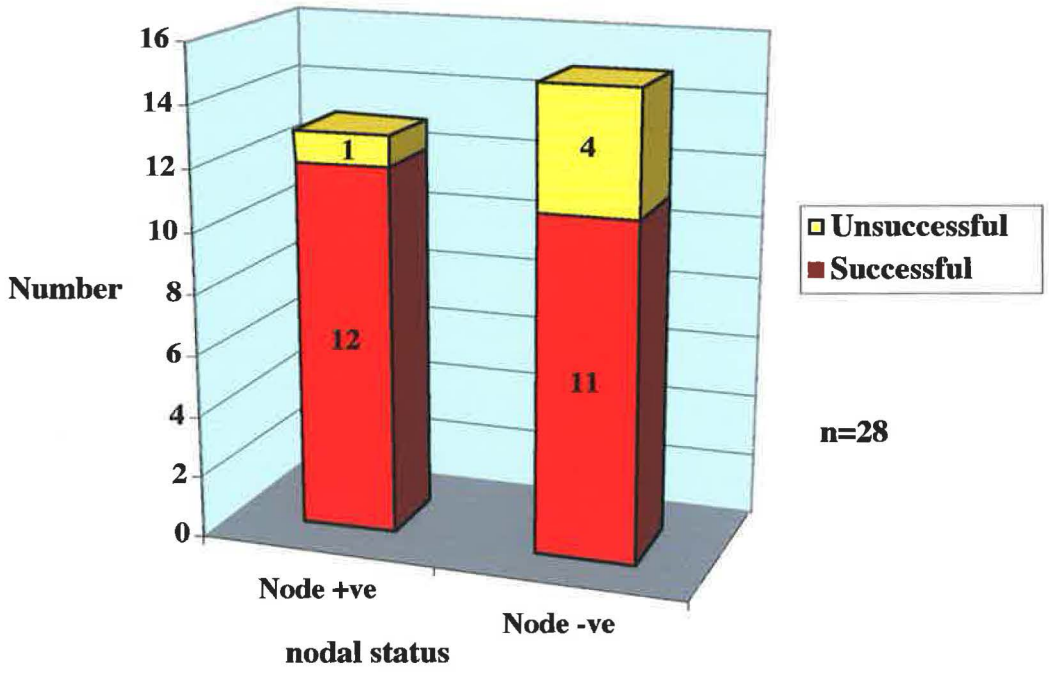


Figure 32 **Lymphoscintigraphy results by nodal status**

12.4 Dye Localisation and Biopsy: Results

12.4.1 Overall Results

The standard technique described above was used for all patients. With the exception of 1 case which was carried out by one of the senior consultants on the Breast and Endocrine unit because of logistical difficulties, all cases were personally carried out by the author. Although minor apparent desaturation was observed in a small number of patients after injection of the blue dye, this was not a concern, once recognised.

The first procedure in this series was carried out on 29th March 1995 and the final procedure on 31st January 1996.

A sentinel node (or nodes) was identified in 21 of the 29 patients.

12.4.2 Sentinel Node Biopsy Duration

As expected, the time taken to complete the biopsy became shorter as the study progressed. More confident and accurate markings made at lymphoscintigraphy were most helpful, and as the technique became more familiar, the incidence of being unable to identify a sentinel node decreased. It had been decided prior to the study that if a sentinel node had not been identified at 30 minutes, the study would be terminated. Overall results are presented in **Figure 33**, page 98.

12.4.3 Location of Node at Operation

Following the protocol, the first draining node on the lymphatic pathway was accepted as the sentinel node, and in no case was this found to be intramammary at operation.

The locations of nodes found at sentinel node biopsy are illustrated in **Figure 34**, page 98.

12.4.4 Number of Sentinel Nodes identified at operation

Generally only one blue staining node was found, although occasionally 2 or even 3 nodes, in close proximity to each other, often encased within a single capsule all stained blue. In such cases all blue staining nodes at the same level were regarded as sentinel nodes.

If "second order' blue nodes were found, further along a lymphatic pathway from the first blue staining node, these were not regarded as sentinel nodes, though they were removed and sent for separate analysis.

The number of nodes found at sentinel node biopsy is shown in **Figure 35**, page 99.

12.4.5 Sentinel node biopsy success rates versus tumour location

The location of the tumour within the breast did not affect the success of the procedure markedly, although there appeared to be a slightly higher failure rate for medially placed tumours in this series. A graphical representation of the success rate of dye localisation related to tumour location is presented in **Figure 36**, page 99.

12.4.6 Sentinel node biopsy success rates versus tumour size

As previously mentioned, most of the tumours were relatively small. The larger tumours appeared to be just as successfully dealt with using this procedure as the smaller tumours. Please refer to **Figure 37**, page 100.

12.4.7 Sentinel node biopsy success rates versus tumour type

Most of the tumours were ductal in type, with various subtypes of ductal carcinoma seen in this series, all of which were successfully studied. Successful biopsies were also carried out on the few lobular carcinomas also seen in this study, with failures in both the ductal and lobular groups. This is shown in **Figure 38**, page 100.

12.4.8 Sentinel node biopsy success rates versus tumour grade

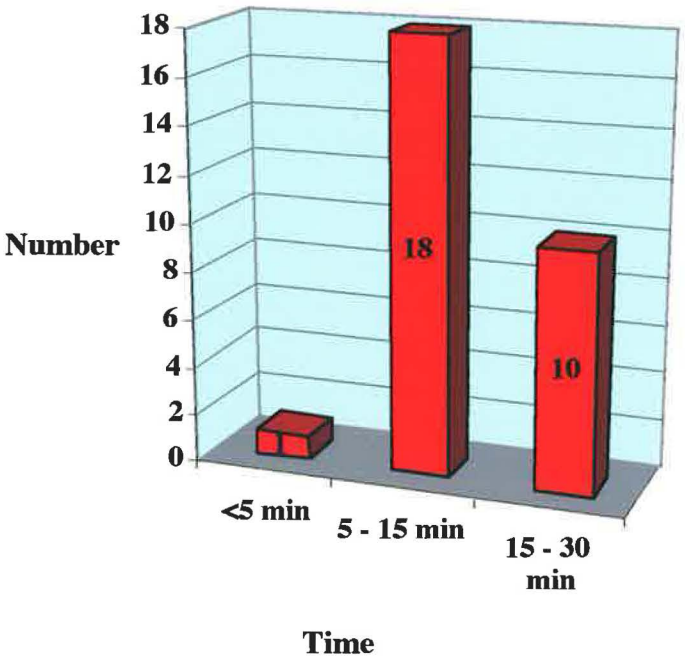
A wide variety of tumour grades was seen in the series, and appeared to have no bearing on the success or failure of the technique. This is shown in **Figure 39**, page 101.

12.4.9 Sentinel node biopsy success rates versus nodal involvement

The procedure was successful in patients who had both positive and negative nodes at pathology, with numbers distributed relatively evenly in both groups. As in

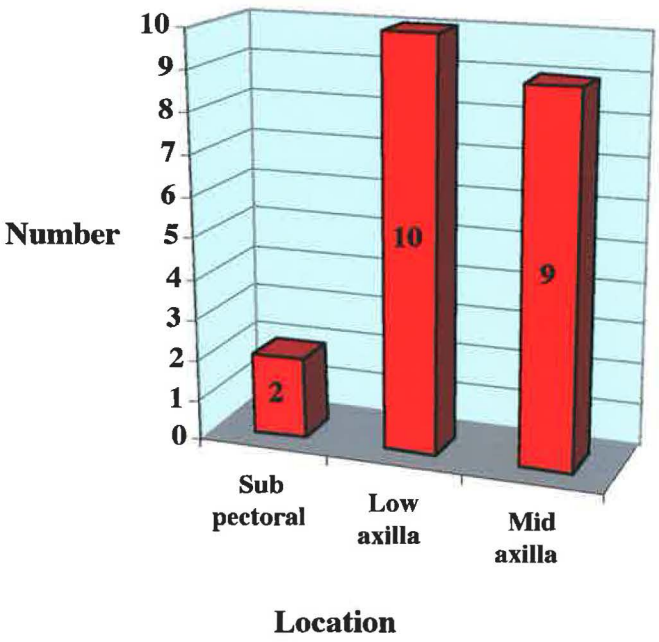
lymphoscintigraphy, most of the unsuccessful cases occurred in the node negative group, where tumour replacement of the node as a possible cause for failure is eliminated.

This is illustrated in **Figure 40**, page 101.



n=29

Figure 33 **Duration of dye localisation procedure**



n=21

Figure 34 **Location of node at dye localisation**

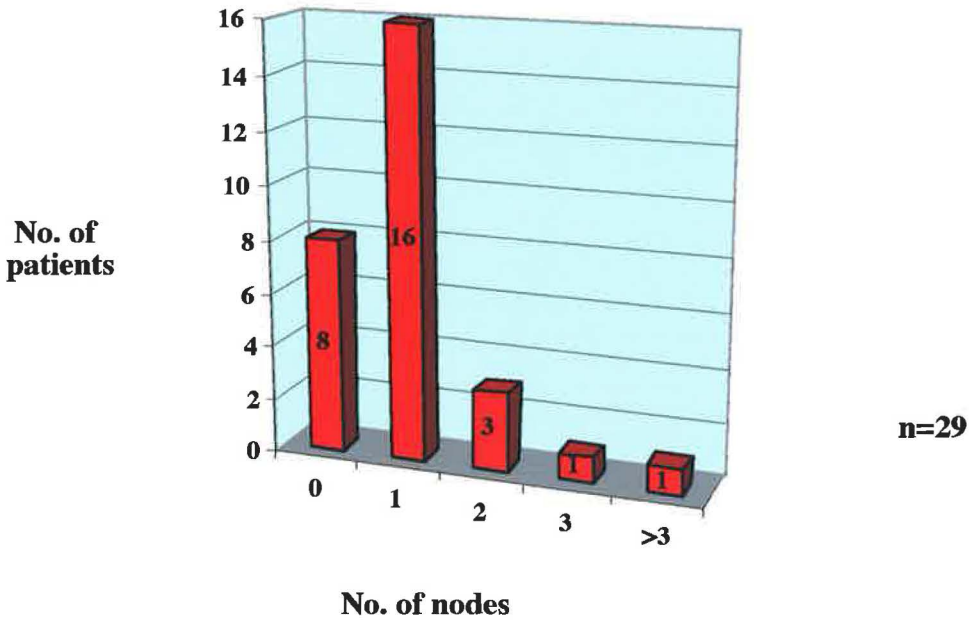


Figure 35 Number of nodes seen at dye localisation

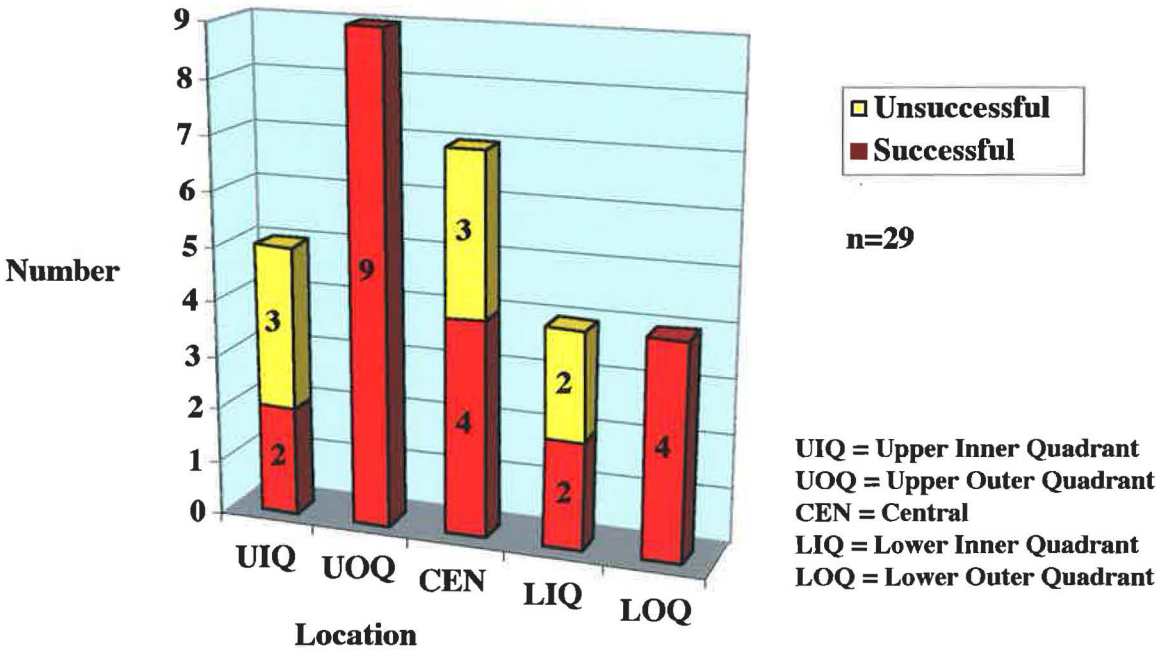


Figure 36 Dye localisation results by tumour location

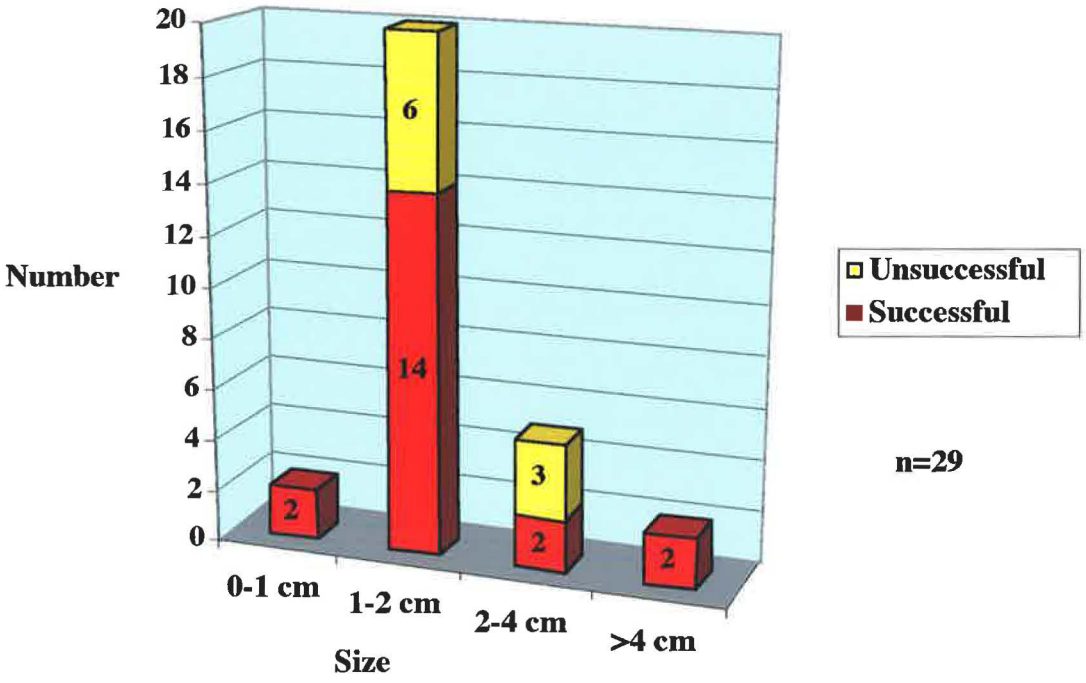


Figure 37 **Dye localisation results by tumour size**

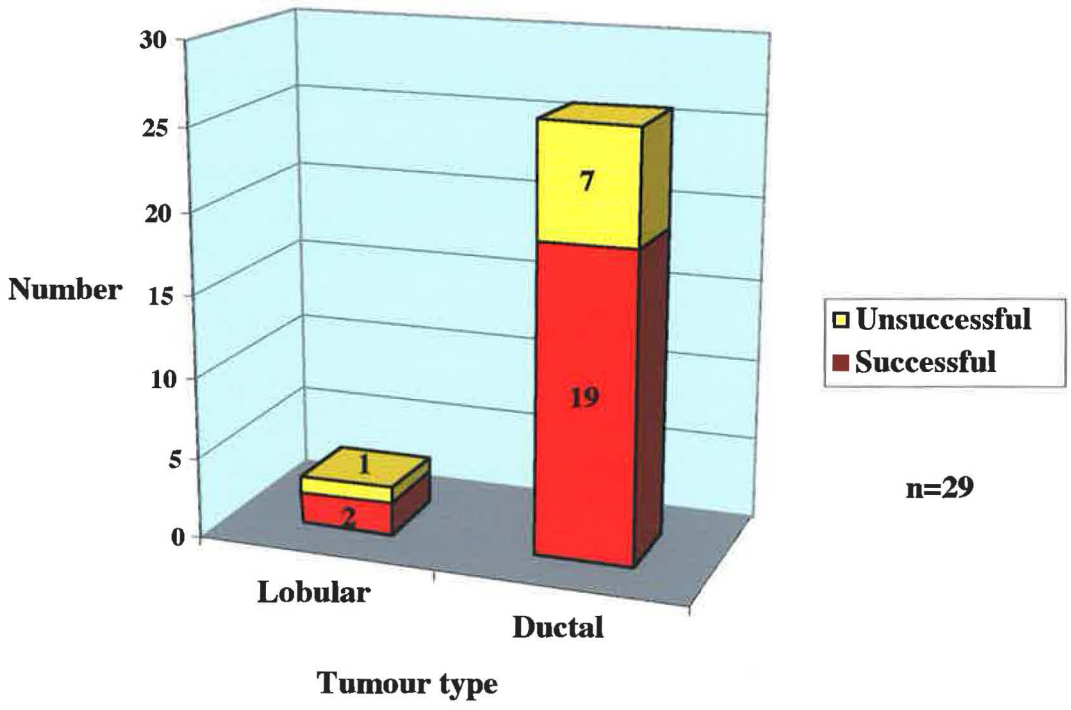


Figure 38 **Dye localisation results by tumour type**

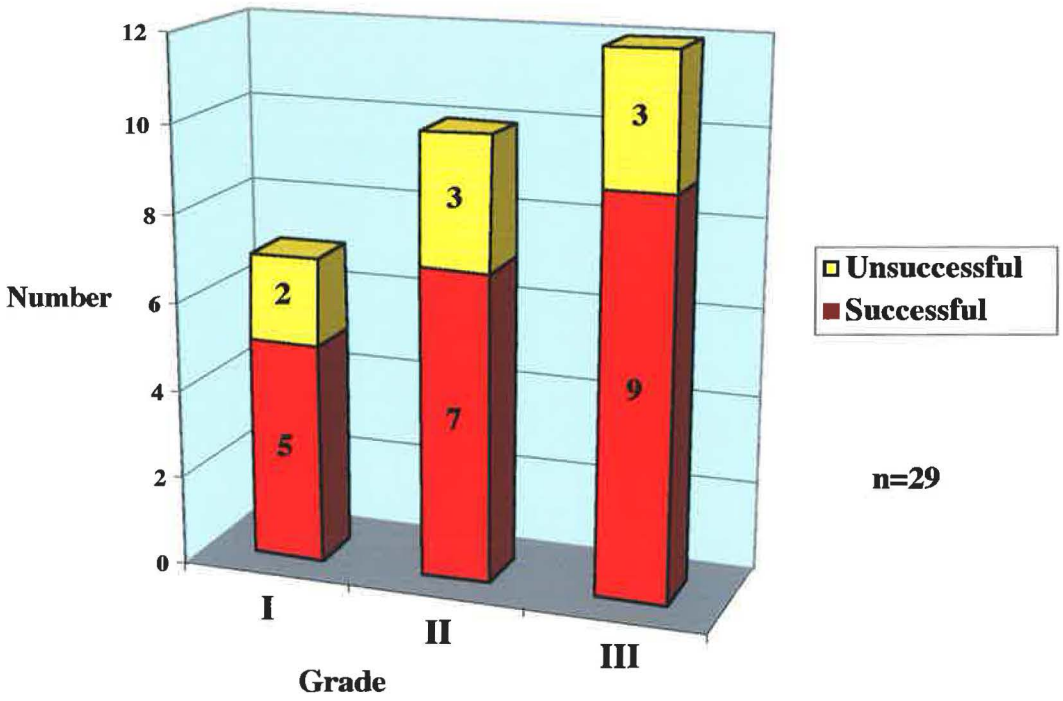


Figure 39 **Dye localisation results by tumour grade**

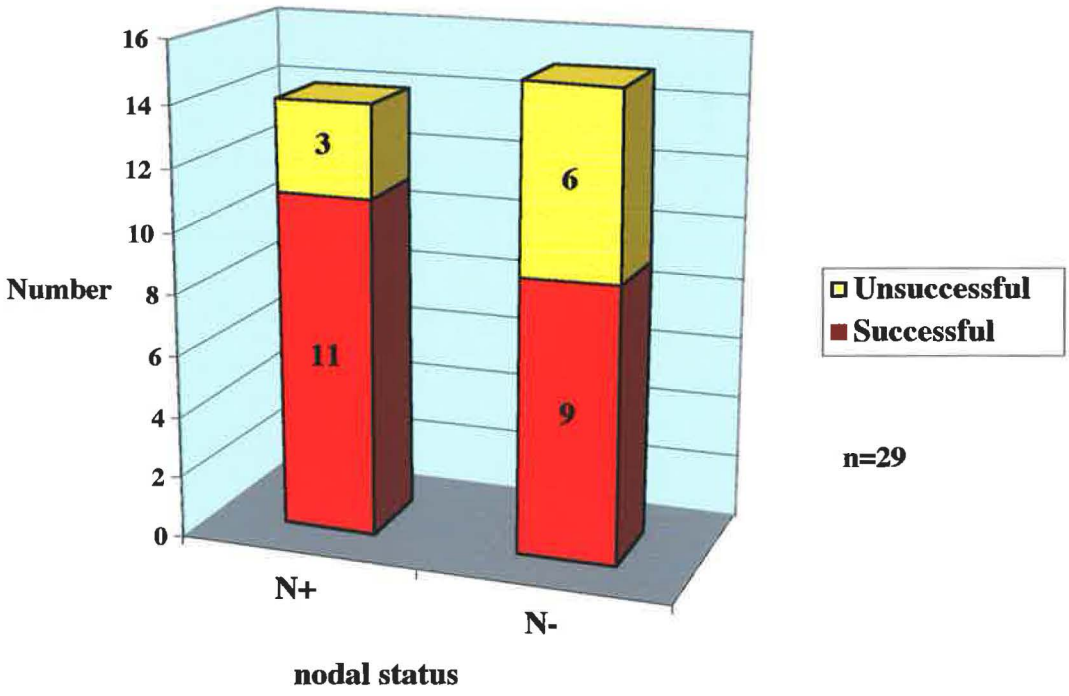


Figure 40 **Dye localisation results by nodal status**

12.5 Combined Results

In the majority of cases the Lymphoscintigraphy and the Sentinel Node Biopsy were both successful.

In a small number of cases one was 'successful' whilst the other was not.

In only a few cases was neither modality successful. A representation of the overall success of lymphoscintigraphy and dye localisation is shown in **Figure 41**, page 103.

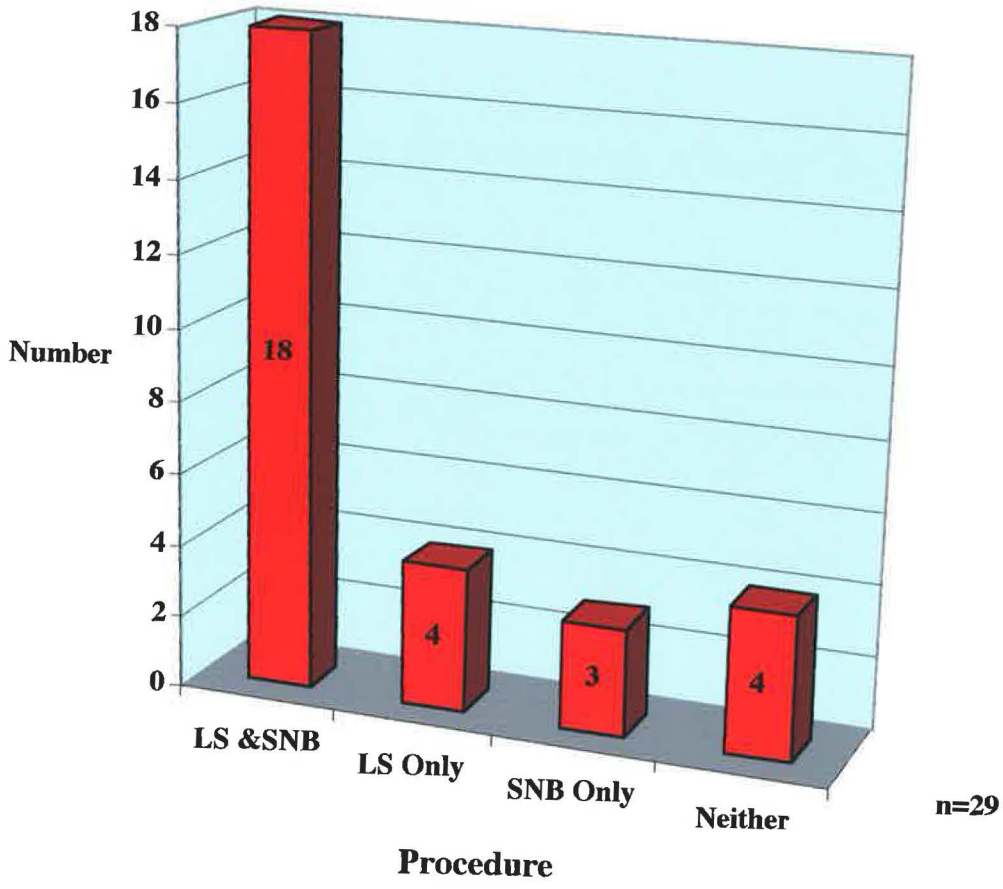


Figure 41 **Overall procedure success rates**

13 PATHOLOGICAL ANALYSIS

13.1 Routine Analysis

Axillary Lymph Node Status

The Sentinel node specimen as well as the breast and axillary node dissection specimens were subjected to 'routine' histopathological processing as described above. Pathologists did not report any difficulty assessing the specimens as a result of the presence of dye, which is water soluble and washed out during processing. The fact that a node or nodes had been removed from the axillary dissection specimen, also posed no problems.

The initial pathological processing yielded the following results:

Axillary lymph node status	Number of patients
Positive	12
Negative	17

Table 8 Initial Pathological results

Sentinel Lymph node Status

Accuracy of the sentinel node in the assessment of the axillary status was examined, and after routine pathology alone the following results were obtained:

Sentinel node status	Number of patients
Sentinel node correct, N+ (True positive)	**11**
Sentinel node correct, N- (True negative)	**9**
Sentinel node incorrect, N+ (False negative)	**1**
Sentinel node incorrect, N- (False positive)	**0**

Table 9 Accuracy of sentinel node on the basis of routine pathology

13.2 Detailed Analysis

Following these results, the sentinel nodes classified as negative on routine histology were subjected to detailed analysis using the technique described above. Of the 11 sentinel nodes identified in node negative patients further examination involved examination of 16 lymph nodes and 220 slides.

This revealed unsuspected metastatic disease in 2 cases.

Photomicrographs illustrating this finding are shown in Figure 42 and Figure 43 on page 111.

Amended pathology reports were issued for these patients and their cases re-presented to the multidisciplinary breast unit meeting in the light of the findings to decide whether these patients required any adjuvant treatment.

The final results after this analysis are as follows:

Nodal Status	Routine pathology	Sentinel node
Correct (true positive)	**12/12**	**11/11**
Correct (true negative)	**15/17**	**9/10**
Incorrect (false negative)	**2/14**	**1/14**
Incorrect (false positive)	**0**	**0**

Table 10 Final results comparing sentinel node and routine pathology

This table outlines perhaps the most important finding of the study and bears some further explanation.

Pathological analysis is such that if a node is reported as positive, either after routine surgery and pathology, or sentinel node biopsy and detailed pathology, the result is by definition correct, hence the true positive rate is 100% and the false positive rate zero. Perhaps the most important statistic is the **true negative** rate. Translated into clinical terms, what this means is "If the procedure indicates that the node is disease free, how sure can one be that this is correct?"

In this series there was one false negative sentinel node i.e. 1 node said to be free of disease when the axilla did contain disease, giving **a true negative rate of 9/10 or 90%** for the successful sentinel node biopsy cases which were truly node negative.
On the other hand, of the 29 cases subjected to routine histology of a complete axillary dissection, 17 were said to be free of disease, when the sentinel node later showed disease in 2 cases (i.e. 2 false negatives), giving a **true negative rate of 15/17 or 88%**.
Another way of stating the success of the procedure is to say that 20/21 sentinel node biopsies were correct (95%) compared to only 27/29 (93%) of routine pathology cases. Sentinel node biopsy therefore is more accurate at staging the axilla than routine pathology, provided that detailed pathology is performed.

13.3 'Bullseye' sentinel node cases

Described below, these cases are perhaps the most impressive finding of the study and tend to suggest that the technique is accurate.
As has been seen in many studies of sentinel node biopsy both in melanoma and breast cancer, there are some patients who at presentation only have disease in one node. In 7 of the 8 patients who had a single node involved, sentinel node biopsy was possible, and in all 7 cases that node was the sentinel node. In other words in this study **7 of the 14** patients who truly had metastatic disease in the axilla, **disease was only found in the sentinel node**.
These cases were spread throughout the series, and most had large numbers of lymph nodes identified. In these 'bullseye' cases, of 112 nodes removed, Sentinel Node Biopsy correctly identified the 7 nodes containing tumour.

Case Number	Total number of axillary nodes retrieved in the case
2	12
3	21
8	14
9	8
23	22
25	14
30	21

Table 11 Occurrence of 'Bullseye cases' and total node numbers

In cases where the sentinel node was positive, but was not the only node involved, a range of numbers of positive nodes was seen, suggesting that the procedure is not affected by the presence of disease in the nodes.

Case number	Number of involved nodes
7	3
10	9

13	15
18	2

Table 12 Number of nodes involved in successful sentinel node biopsy cases where the sentinel node was *not* the only node involved.

13.4 Highly Detailed Analysis

Because of the occurrence of a false negative sentinel node, this node was subjected to highly detailed analysis to exclude as completely as possible a histological false negative in which the node contained a micrometastasis which had been missed even on relatively detailed analysis.

The case was the 22nd in the series, therefore failure could not be attributed to unfamiliarity with the technique and the surgery was carried out by the author. This was the patient who refused to complete the lymphoscintigraphy, and thus only blue dye was used to locate the node, but as mentioned above, blue dye alone was used in 2 other cases of successful sentinel node biopsy.

The case was not unusual in any other respect: the patient had a 1-2cm diameter tumour located in the upper outer quadrant with a clinically negative axilla and no previous surgery. The biopsy took between 5 and 15minutes and 1 node was located in the mid axilla. Pathology demonstrated a Grade II Ductal cancer and 3 axillary nodes involved with tumour.

The specimen was subjected to sectioning at 25μm intervals and alternate sections were stained with H&E and Cam 5.2. This amounted to 75 slides of the lymph node, and took 2 hours of the pathologist's time to examine.

Despite this extremely careful examination the sentinel node in this case was confirmed as negative for malignant cells.

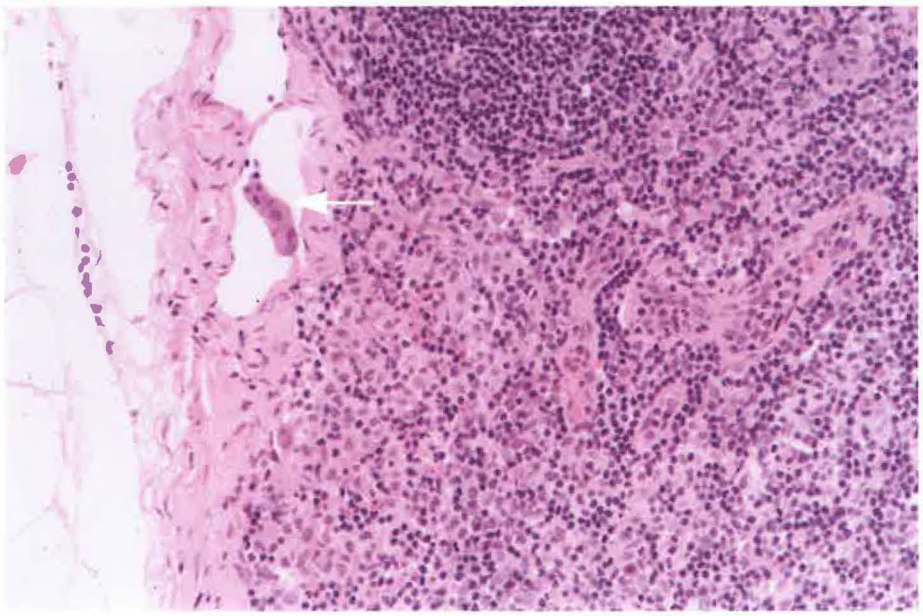


Figure 42 Photomicrograph: tumour embolus in the sentinel node (H&E)

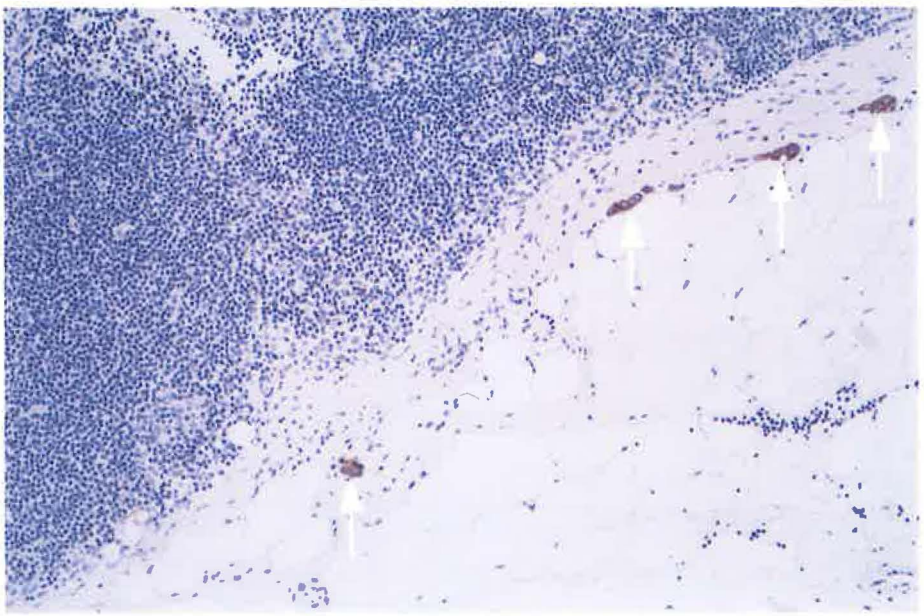


Figure 43 Photomicrograph: small groups of tumour cells in the sentinel node (Cam 5.2 Immunohistochemical stain)

14 DISCUSSION

14.1 Successes

The study was successful in enrolling a significant number of patients with breast cancer who, despite relatively tight exclusion criteria represented a broad range of tumour parameters. All locations within the breast were represented, tumours of varying sizes were found although as expected most were small and both ductal and lobular carcinomas were seen. Perhaps surprisingly, given the apparent early presentation of most of the tumours, there were large numbers of patients with both high grade and node positive tumours. Although it would have been possible to relax the exclusion criteria for the study and to accrue more patients, it was felt that exclusion of patients who had either very large tumours or who had been subjected to a previous open biopsy would lead to 'cleaner' data which would be more useful for the purposes of the study.

The relative ease with which the lymphoscintigraphy studies were scheduled to fit in with the patients surgery and the willingness of patients to undergo the procedure for what had to be presented to them as 'experimental work' was pleasing. Especially in the early part of the study when the time taken to achieve reasonable lymphoscintigram pictures amounted to three hours or more in some cases, it is perhaps surprising that only one patient left before the studies were completed. Toward the end of the series, the more rapid studies, often being only one to two hours in duration were much more in line with

The location of sentinel nodes in this study did not yield any unexpected results, although there were three patients who had drainage only to the internal mammary nodes and not to the axilla as well as two patients who had drainage to sub-pectoral nodes which would certainly not have been removed in a standard axillary dissection. At least some of the nine patients who had drainage to a mid axillary node would not have had this node removed in an axillary sampling procedure although a routine level II dissection would have removed all the true axillary nodes found in this series.

Analysis of the combined results of lymphoscintigraphy and dye localisation suggests that in only a very small number of cases will it not be possible to identify a sentinel node using one or other or a combination of the two techniques. It would clearly be of benefit to be able to use the hand held gamma camera intra-operatively in order to assist in sentinel node localisation so that this could be achieved using radioactive tracing as well as blue dye marking. This of course requires the lymphoscintigram to be carried out within 24 hours of surgery or requires a second set of radionuclide injections shortly before surgery. Other results from centres carrying out sentinel node biopsy both for melanoma and breast cancer since this study has been carried out suggest that blue dye alone is successful in locating approximately 75% of sentinel nodes whereas with the addition of a gamma camera this identification rate can be increased to over 90%.

It was very pleasing that even in this early series, 20 of 21 successful sentinel node biopsies were accurate in assessing the axillary nodal status, with only one false negative result. The fact that detailed analysis of the sentinel nodes which were thought to be free

of tumour on routine histology disclosed two patients who had metastatic disease in the sentinel node was also pleasing.

The most impressive statistic for clinicians though is that the procedure was able to identify the *only* node containing metastatic disease in the axilla in half of the patients who had axillary nodal involvement.

14.2 Difficulties

The study did highlight difficulties in carrying out this procedure although after a few procedures had been performed, it did become much more routine throughout the hospital.

14.3 Recruitment

Causes for not being enrolled in the study included specific exclusion criteria as mentioned in the study design, or logistical difficulties such as because of where the patient lived.

Recruitment of cases was somewhat difficult, especially if coming in for the scan did not fit in with patients' plans or in cases where the patients were nervous about being 'experimented upon'. Later in the study when it could be said that numbers of these procedures had been performed and that the technique might help in identifying tumour which would be difficult to find under normal circumstances, many patients were more

accepting of the study and agreed to be included. More streamlined scheduling of the lymphoscintigraphy which could be performed quickly and often on the day of surgery, was certainly a factor in recruiting some patients who would not have otherwise agreed to take part.

14.4 Impalpable Lesions

With the large number of patients who present with 'impalpable' or very small lesions, the potential benefit of this procedure could be expected to be greater than in patients with larger tumours who might need a complete axillary dissection in any case, though for the purposes of this study such patients were excluded. One of the challenges in development of this procedure will be to devise a reliable and reproducible technique in which it is possible to inject both the radio nuclide and the blue dye in the same place and in the correct peritumoural location. In South Australia where carbon track localisation for impalpable lesions is a popular technique, the blue dye completely obscures such a track and would eliminate this type of patient. Use of a hook-wire might obviate this particular problem, and the availability of ultrasound or stereotactic mammography might help with placing the injections.

14.5 Large Tumours

Patients presenting with a large tumour or multifocal tumours present a particular difficulty for this procedure in that the tumour itself may have already disrupted the lymphatic drainage to such a degree that injection around the tumour in widely separated locations does not give the type of images and localisation found in this study. In such

patients, however, the possibility of lymph node metastasis is high and both total mastectomy and complete axillary dissection is likely to be necessary in any case. It may be that sentinel node biopsy in contraindicated in such patients.

14.6 Previous Biopsy

The problem of dealing with a patient who has had a previous open biopsy which has shown carcinoma is one which is apparently more common in the United States than it is in Australia. High quality fine-needle cytology or core biopsy histology which is improving steadily, will, in most cases remove the need for open biopsy prior to definitive surgery. Whilst in some breast surgery and in most melanoma surgery injections are made around a biopsy cavity, it remains to be shown that this is as accurate a method as sentinel node biopsy where the lymphatics have not been disrupted by surgery.

14.7 Unresolved Problems

As is often the case with research or new techniques, some questions are answered but others are thrown up. This procedure is no exception and perhaps raises more questions than it answers.

14.8 Technical Failure

Complete technical failure of the procedure for unexplained reasons was seen in this study. Other centres report the same phenomenon in both melanoma and breast surgery. The reasons for this are unclear. Whether this is an idiosyncratic reaction to the chemicals used in the procedure or whether this reflects some aberration of lymphatic anatomy or drainage is unclear and further work needs to be carried out in this area. The differences between Lymphoscintigraphy and Sentinel Node biopsy in which one fails where the other succeeds in the same patient also remains unexplained.

14.9 False Negative Results

The "false negative" rate of this procedure has occurred in all reported series of sentinel node biopsy and again the causes for this are as yet unclear. Whether arborisation of lymphatics occurs in which some lymphatics travel to what is thought to be a sentinel node whereas other branches bypass the sentinel node and drain into a different node is not clear although collateral circulation with respect to arteries and veins is of course well known. Another explanation for the phenomenon might be that tumour cells travelling along the lymphatic pathway traverse the node and are not captured by it so that they deposit in second order nodes leaving the sentinel node free of disease. Whatever the explanation, the phenomenon does not appear to be due to a technical failure of the radiologist, the surgeon or the pathologist and remains an enigma.

What is quite clear is that although the procedure does indeed have a small false negative rate, there is a very definite and much larger false negative rate applicable to routine histological analysis of a complete axillary dissection specimen and an accepted false negative rate of up to 20% is not unusual. Many studies have confirmed this as has been mentioned earlier. In this series the false negative rate of routine histology was twice that of sentinel node biopsy.

14.10 Micrometastasis

As mentioned earlier, much has been written about micrometastatic disease previously and sentinel node biopsy has re-ignited this debate in that metastatic disease can now be identified at a single cell stage.

Initially a number of 'false positive' results were reported and shown to be due to benign naevus cells located within lymph nodes, this apparently being a well known and recognised phenomenon. With immunohistochemical stains and refined histological techniques though there is no doubt that single malignant cells can be confidently identified in sentinel lymph nodes. Studies mentioned earlier have shown that patients with micrometastatic disease do not have the same life expectancy as patients who are truly node negative although the time course of their relapse is usually much longer than patients with macro-metastatic disease.

Whether the identification of abnormalities in a lymph node can be extended to the use of biochemical means such as RT-PCR (reverse transcriptase polymerase chain reaction)

rather than a histological analysis remains to be seen. Even with serial sectioning of a sentinel node, it is possible that a micro-metastasis might be missed and some feel that RT-PCR would identify single cell abnormalities which might be missed by histology. Others feel that the biochemical abnormalities picked up by this sensitive technique might be found in normal or merely dysplastic cells and give false positive results. At this stage it would seem prudent to restrict analysis to the use of proven histological and immunocytochemical techniques until the questions surrounding newer techniques have been resolved.

14.11 Refinement Of The Technique

As might be expected 'teething' problems were encountered with the techniques, none of which had ever been carried out at Flinders Medical Centre previously. Considerable experience and expertise was built up over the duration of this study both in performing lymphoscintigraphy, and blue dye marking.

14.12 Lymphoscintigraphy

Radionuclide must be fresh, and preferably prepared onsite on the day of use.
Arranging for several patients to have procedures on the same day is ideal, if possible.
The radiopharmaceutical appears to give better images after filtration, because of clumping of particles.
The volume of injectate used should be around 1.5-2 mls in total, with larger volumes being better than small volumes.
Isolation of the injection site with waterproof drapes is important.

Breast massage following injection gives better images, and should be carried out in a large circular motion for 3-5 minutes, based on subsequent personal communication with members of the Sydney Melanoma Unit.

Marking of sentinel nodes in the AP & Lateral planes is important, especially if a hand held gamma camera is not to be used during surgery.

Discussion about the study prior to theatre between the radiologist and surgeon is most important.

A dedicated team of radiological staff is important, since the procedure is quite operator dependent. Motivation to make the procedure work, and to persist when tracer movement is slow is an advantage.

14.13 Dye Localisation

Patent Blue Violet dye is very helpful in locating nodes.

The dye should be injected approximately 5-10 minutes before the biopsy procedure is to begin, and care must be taken not to contaminate surrounding areas with the dye.

The axilla must be dissected first, otherwise dye may wash out before channels are identified.

Careful haemostasis is most important, to avoid obscuring the operative field.

Careful dissection of the fine lymphatic channel below the axilla must be the starting point of the procedure.

It is important to follow the channel back into the breast to ensure that the node identified is the first draining node on the lymphatic pathway.

If several nodes are found to be blue staining and in close proximity to each other, all should be removed as sentinel nodes.

If nodes are found downstream from the sentinel node, but on the same lymphatic pathway, these should be sent separately as 'second-order nodes' since they can not be regarded as true 'sentinel nodes'.

14.14 Use Of A Gamma Camera

Whilst not used in this study, subsequent research and work from other centres has shown that being able to identify a radioactive node in the axilla (or elsewhere) has the potential to make the procedure considerably easier. When a 'Hot and blue' node is identified, one can be confident that a true sentinel node has been found.

In some cases lymphatic channels and blue nodes may not be found and in such cases a gamma camera may make the difference between a successful and unsuccessful procedure.

Similarly, one should not rely on the gamma camera alone, because as shown in this series, blue dye tracing is sometimes successful when lymphoscintigraphy (and hence gamma probe tracing) is not.

14.15 Non-Axillary Biopsy

Whilst in order to obtain approval to carry out this study it was necessary to state that lymph nodes in areas other than the axilla would not be biopsied, it is felt that where the sentinel node biopsy procedure indicates that the primary draining lymph node from a tumour lies outside the axilla, this should be biopsied in exactly the same way as an

axillary node and for the same reasons. This is certainly the practice in melanoma surgery and there is ample data to support this as a reasonable policy in breast surgery.

As mentioned above locating such a node with blue dye and if possible a gamma camera should prove reasonably straightforward. It should be remembered that this is a biopsy only and not a node clearance, hence one level of internal mammary nodes, or one supraclavicular node (if that is all that is shown by the procedure) is adequate surgery. It is felt that this should carry minimal morbidity, and considerable prognostic value.

Subsequent treatment if the non axillary sentinel node proves positive for tumour remains to be clarified, but will depend on the location and patient as well as tumour factors.

14.16 Pathological Processing

It has become clear in the course of this study that accurate results depend on the quality of the histology, as well as the quality of lymphoscintigraphy and sentinel node biopsy. Serial sectioning, multiple staining and examination of many sections of one lymph node in addition to routine histology of multiple lymph nodes from an axillary dissection specimen requires an enthusiastic pathologist and involves significant extra cost. There is currently debate as to how many sections should be taken of a lymph node and to which stains the sections should be subjected. Arguments about whether the more expensive immunohistochemical stains which are quicker to examine and therefore cheaper from the point of view of the cost of analysis are cheaper overall than routine H & E sections which take longer to analyse have yet to be answered.

14.17 Clinical Management

Anatomical teaching tells us that breast lymphatics drain to lymph nodes not only in the axilla but also in the supraclavicular regions, the internal mammary regions and in some cases to the opposite axilla as well as the upper abdomen. In the light of this it seems almost negligent that breast surgery involves itself only with the axilla. There has been a vogue in past times for internal mammary lymph node biopsy which was a difficult, dangerous and low yield procedure. For these reasons the procedure has been abandoned. It will be interesting to follow patients who have scintigraphic evidence of drainage only to the internal mammary nodes to see whether disease relapse occurs in this location when the nodes remain untreated. One use of sentinel node biopsy which indicates that the sentinel node lies outside the axilla might be to prove or disprove the presence of metastatic disease in these regions so that adjuvant therapy such as radiotherapy might be used with therapeutic effect.

It would seem logical that if we are prepared to accept the high false negative rate of routine axillary dissection and routine pathology which currently exists, replacing this with a minimally invasive technique, with presumably a lower complication rate and a much lower false negative rate would seem to be a logical step. Most data would suggest that in the 'worst case scenario' in which a patient suffers an axillary nodal disease relapse after false negative sentinel node biopsy, adequate control may still be achieved by therapeutic axillary dissection at a later stage followed by adjuvant therapy.

Clearly serial sectioning of a single lymph node may well be a cheaper and more acceptable alternative for the pathologist than routine axillary dissection and routine pathology as it is currently practiced. Clinical management is driven by well conducted research but increasingly it would seem that clinical management is dictated by medico-legal considerations, cost considerations and public opinion or patient wishes.

If it is found that, as would appear to be the case, sentinel node biopsy with detailed pathology of the sentinel node is a more accurate method of staging the axilla than the way in which this is currently carried out; it is cheaper than the current technique; patients ask for it and refuse to undergo complete axillary dissection, then surgeons will be forced to adopt the technique. If the courts judge that the risks of lymphodema or other complications of axillary dissection outweigh the potential risk of a false negative study, we may also be forced to adopt the technique. The procedure would seem to have such popular appeal that it may well be adopted for purely emotional reasons in much the same way that laparoscopic cholecystectomy was under a decade ago with clinical validation of the technique following some time later.

14.18 Cost Considerations

The cost of enrolling a patient in this type of research is not high and can be absorbed into the consultation costs which normally occur when a patient attends a breast clinic. If the person carrying out the study is not the person seeing the patient in the clinic, a second visit may be necessary. If and when such a technique becomes incorporated into clinical practice, these costs disappear.

Lymphoscintigraphy involves expensive radio tracers which must be shipped from the point of manufacture to the site where they are used with very tight time constraints which can be costly. Highly expensive imaging cameras and processing technology, as well as photographic costs are involved. In this study the nuclear medicine department agreed to absorb these costs within a clinical and research context so that the patient was not charged for the 'extra' procedure. As mentioned, the cost of commercial rate parking at the hospital was covered for patients by a grant from the 'Flinders 2000' foundation for this purpose which was used to purchase parking vouchers at reduced rates.

Theatre time, whilst an expensive commodity even in Australia is still available for this type of research, and was absorbed as part of the cost of the hospital stay for the patient. The cost of routine reporting was part of the patient's routine admission costs.

Pathological processing is a skilled, labour intensive and therefore expensive part of the process. The author paid almost $2000 towards labour costs for a laboratory technician's time involved in the preparation of sections for detailed analysis carried out in this study. The pathologist's time was given free of charge for this study.

Whilst these might be important considerations for the researcher, the cost which is most important to health administrators is that of the procedure, should it be introduced into clinical practice.

The monetary cost of the procedure is quantifiable, though the ultimate cost to the patient of inaccurate staging can only be guessed at.

A notional cost of the Sentinel Node Biopsy procedure is set out below, and compared with that of routine axillary dissection, the need for which might be significantly reduced if the procedure is really as successful as it appears to be.

Item	Axillary dissection	Sentinel node biopsy
Lymphoscintigram	0	$500
Radiopharmaceutical	0	$50
Patent Blue dye	0	$40
Theatre time @ $800 /hr Axillary dissection 45 min Sentinel node biopsy 15 min	$600	$200
Wound drain	$100	0
In hospital stay @$300 / day Axillary dissection 6 days Sentinel node biopsy 2 days	$1800	$600
Postoperative visits @ $50 Axillary dissection: 4 Sentinel node biopsy: 1	$200	$50
Pathology $20 / normal slide, $30 special Axillary dissection: 1 section of 15 nodes Sentinel node biopsy: 6 normal, 6 special	$300	$300
TOTAL	**$3000.00**	**$1740.00**

Table 13 Notional costings for Sentinel Node Biopsy and standard axillary dissection compared

15 CONCLUSIONS

15.1 General

A number of conclusions can be drawn from the data which go some way to answering questions set out in the original aims of the research.

15.2 The Patient

The procedure was well tolerated by the patients in this series and no specific complaints were received, except for the length of time taken to carry out some of the early lymphoscintigraphy studies.

The concept of the procedure was readily understood by most patients, except that many expected the procedure to discern whether metastatic disease was present or not, in much the same way as a bone scan does.

15.3 Lymphoscintigraphy

Lymphoscintigraphy can be successfully applied to the breast and this shows sentinel lymph nodes in just the same way as in melanoma surgery.

Lymphoscintigraphy localises both normal and pathological axillary lymph nodes. Use of lymphoscintigraphy with marking of the sentinel lymph nodes is extremely valuable in the surgery and sentinel node biopsy. It would appear that use of a hand held gamma

camera to highlight nodes seen on lymphoscintigraphy which are occasionally not seen with blue dye marking would also be useful.

Injections carried out for lymphoscintigraphy studies do not upset patients.
The procedure is well tolerated generally, provided scanning times are kept below three hours. Once it is possible to confidently reassure patients that the procedure has significant benefit for them, it will probably be acceptable to most patients.

15.4 Dye Localisation

Intraoperative dye localisation of lymph nodes can be applied to the breast. Difficulties arise if too large a volume of blue dye is used, if the delay between dye injection and biopsy is too long or too short or if blue lymphatic channels cannot be identified. Apparent oxygen desaturation occurs following injection of dye in some patients.

The procedure is applicable to both total and partial mastectomy.

15.5 Lymphoscintigraphy and Sentinel Node Biopsy Combined

Lymphoscintigraphy does prove useful in identification of sentinel lymph nodes although even in cases where lymphoscintigraphy has not been successful, blue dye marking can sometimes prove effective by itself. The blue dye is critical when a gamma camera is not used since the lymphoscintigraphy can only mark the approximate area of the lymph node which may be quite difficult to identify amongst the axillary fat. There do not appear to be any surgical complications of the procedure.

The procedure may not be applicable to patients with large or multifocal tumours since it would be expected that more than one lymphatic channel would drain the area, leading to an increased probability of a false negative study, thus caution must be exercised in applying the procedure to such patients.

15.6 Pathology

Blue dye does not interfere with tissue processing in the standard fashion.

The well researched and proven false negative rate of routine pathology is an aspect of breast cancer management which seems to be accepted and about which many clinicians are quite complacent.

Assessment of the sentinel node in addition to an axillary dissection is more time consuming than routine histology, but detailed pathology on only a sentinel node specimen may save the pathologist time. The exact number of sections which should be carried out on each node and what processing each section should be subjected to is now

being debated by pathologists and surgeons. Some proposals currently under discussion are included as an appendix.

Although there is a definite failure rate and a false negative rate of the procedure, when sentinel node biopsy can be performed it is a more accurate predictor of axillary status than traditional techniques if detailed pathology is carried out on the sentinel node.

15.7 Further Research

In the same way in which the sentinel node was subjected to serial sectioning and special staining in this study, it would be ideal to examine all the non sentinel nodes in the same fashion. Only then could one truly assess whether the sentinel node is as good or better than more detailed analysis of specimens taken in the traditional fashion.
This, of course would require a huge amount of work and research funding which is beyond the reach of any individual, or even many departments of surgery and pathology. Such a study has been carried out since this study was performed and the results suggest that metastasis occurred to non sentinel nodes in 0.09% of cases. (Turner, 1997)

15.8 The Future

Sentinel node biopsy appeared at the time this study was carried out to have a place in the management of breast cancer and in the relatively short time since this study was carried out the procedure has become one of the hottest topics of breast cancer surgery sweeping the world.

Sceptics still question the false negative rate, the time and expense involved in the procedure and the availability of the technology and expertise necessary for such a procedure, especially in remote areas where breast surgery may be carried out. This study has shown that the procedure can be carried out without lymphoscintigraphy immediately prior to surgery and without an intra-operative hand held gamma camera which is an expensive item of equipment. Patients from remote areas could travel to the nearest nuclear medicine facility to have a lymphoscintigram carried out in the week before surgery, returning to have their surgery (using blue dye alone) closer to home.

For reasons outlined above it seems inevitable that sentinel node biopsy alone will replace routine axillary dissection, at least in certain circumstances. This has certainly been the experience in melanoma surgery. Clinical management based on the finding of micrometastatic disease, seen as a result of sentinel node biopsy is much less clear cut and will probably remain a controversy after the arguments about sentinel node biopsy have been laid to rest.

If patients are to be spared an axillary clearance when the sentinel node is negative, but to have one if the node is positive, there will be increasing pressure on pathologists to give an answer immediately, so that patients may be spared a second anaesthetic for the axillary clearance. At present frozen section is not capable of providing this type of information, especially with respect to micrometastasis, and further developments will need to be made in this area if current practice is to change. One could expect patients to readily accept the need for a delay and careful processing of the specimen in much the

same way as for thyroid malignancies where frozen sections are not favoured because of difficulties assessing these, even on formalin fixed tissue.

Perhaps one of the most important conclusions from this study is that breast surgery can only move forward with the help of a multi-disciplinary team in which the patient, the radiologist, the surgeon, the pathologist, the oncologist and the radiotherapist all work closely together. The accuracy and strength of the procedure will be dictated by its weakest link.

The answers to many outstanding questions raised by this technique will need to await the results of larger trials of careful design and detailed analysis.

16 APPENDICES

16.1 Ethics Committee submission

1. TITLE : SENTINEL LYMPH NODE MAPPING & BIOPSY IN BREAST CANCER.

2. INVESTIGATORS

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3. LOCATIONS

The project will take place at Flinders Medical Centre, and may later include patients at other Adelaide hospitals.

4.5. PROJECT DETAILS

Introduction

Breast cancer is one of the commonest malignancies affecting women of all ages. Current surgical treatment involves surgery for the primary lesion and surgery to the axillary lymph nodes of varying types. The axillary surgery has been accepted for its staging and prognostic value, and, some would argue, its therapeutic value. The extent of lymph node dissection of the axilla remains controversial, and in patients who have a routine axillary clearance, there is a risk of lymphoedema.

Background

Several decades ago when it was first realised that breast cancer spread via the lymphatics mainly to the axillary lymph nodes, surgeons excised the breast and axillary lymph nodes in an attempt to cure the disease surgically. In order to remove as many lymph nodes as possible, radical surgery which included removal of the pectoralis major and minor muscles together with the axillary lymph node field was the accepted procedure. The rate of development of lymphoedema following this type of procedure was high, and was one of the most feared complications of surgery.

With improvements in adjuvant therapy including radiotherapy, chemotherapy and hormonal manipulation it has been realised that this form of radical surgery is inappropriate. It was also realised that in low risk malignancy full axillary dissection is not necessary and axillary lymph node sampling has replaced formal complete axillary dissection in these cases. Similarly, patients with malignant melanoma considered to be in the low risk group for metastatic disease would not normally have block dissection. In patients with "intermediate thickness lesions", however, there is controversy about whether complete block dissection is beneficial. For lesions on the lower extremity the risk of lymphoedema after routine block dissection is significant. Statistically approximately two-thirds of patients with intermediate thickness lesions will not have evidence of malignant disease on block dissection. This was an incentive to try and separate from the group of patients with intermediate thickness lesions a sub group in whom block dissection would be beneficial[1].

Interest then focused on the procedure of lymphoscintigraphy to try and identify lymph drainage pathways, this being particularly useful in lesions of the trunk where it was not immediately apparent which region lymphatics from a particular area would drain to. When this was performed, there was some evidence to suggest that the lymphatics draining to a particular region would selectively go to one (or a small number) of lymph nodes which became known as the sentinel node (s).

It has been hypothesised that if malignant cells had travelled via the lymphatics and were to be found in the lymph nodes, then it would be most likely that they would be found within the sentinel node. In order to identify the sentinel node at operation, blue dyes have been injected around the site of the primary lesion to supplement the information from the lymphoscintigram in which sentinel nodes were marked for position and depth beneath the skin surface. Early reports suggest that the blue dye method of identification is reasonably reliable. Early reports of the usefulness of the sentinel node biopsy technique suggest that after a learning period, "sentinel" nodes can be identified and removed reliably in a high proportion of cases and that in patients with malignant disease in the lymph nodes the sentinel node is almost always involved. In many cases, the "sentinel node" is the only lymph node involved. The false negative rate (negative sentinel node but positive lymph nodes elsewhere in the region) was extremely low and may be less than 1%[1].

In a recent paper[3] axillary sentinel node biopsy was carried out in a number of patients with breast cancer using only the blue dye technique for localising sentinel nodes. After a "learning curve" the authors found that sentinel nodes identified in the last 87 of their procedures were 100% predictive of axillary nodal status with no false negatives in that number.

These early reports suggest that sentinel node biopsy is an attractive technique which may be reliable enough to use as a basis for staging the axillary nodes in the first instance and selecting a small sub group of patients who would then benefit from formal axillary dissection. The number of patients thus exposed to formal axillary dissection would be reduced, and the risk of lymphoedema should be significantly reduced or perhaps eliminated for patients having only a sentinel node biopsy. Before this technique could become part of regular clinical practice, further studies of the technique related to breast surgery in an Australian setting need to be carried out.

Study Proposed

It is proposed to study a group of patients with palpable breast cancer (possibly later studying a group with visualisable but non palpable lesions) scheduled to have a routine axillary dissection as part of their surgical management. Patients who have had a previous limited breast biopsy would be eligible for inclusion, however patients who have had a partial mastectomy or previous axillary surgery would be excluded from the study.
Between 50 and 100 patients would be required for this study and it is envisaged that this will take between 1 and 2 years to complete.
Because information will be available virtually immediately the pathology results are obtained, lengthy follow up periods will not be required before conclusions can be drawn, and data will be analysed immediately.
Patients may be withdrawn from the trial if clinical management would be compromised by inclusion in the study, and of course may elect to withdraw at any time.

6. DRUGS & THERAPEUTIC AGENTS

In an attempt to maximise the information available to the surgeon and to study the drainage patterns of lymphatics from the area of the breast lesion both lymphoscintigraphy and the blue dye node localisation methods will be used.
Blue dye to be used will be either isosulfan blue (1% in aqueous solution; Zenith Parenterals, Rosemont Ill), or patent blue-V (2.5% in aqueous solution containing 0.6% sodium chloride and 0.05% disodium hydrogen phosphate; Laboratoire Guerbet, Aulney-Sous-Bois, France) as suggested in reference 1.Both are known to be non toxic in vivo and will be injected as provided by the supplier, using a 25-gauge spinal needle.

Radiopharmaceutical to be used will be Technetium-99m-antimony sulphur colloid (^{99m}Tc-Sb2S3), prepared onsite for each patient. It is anticipated that particle size will vary from 3 to 12 microns, and that several small volume injections given using ultrasound guidance with a dose of 5-7 MBq will result in a radiation dose of approximately 0.2-0.5 Sv to the injection site. (Refer ref. 1) This will be carried out under the direct supervision of Dr. A. Wycherley, head of Nuclear Medicine, FMC, in accordance with hospital and departmental protocols. Lymphoscintigraphy of various types is a long established investigational modality and no new methods are to be employed in this study.

7. PROCEDURES

Several days prior to surgery the patients having given informed consent for the procedure would attend the nuclear medicine department where an injection or small number of injections would be made surrounding the palpable lesion in the breast under ultrasound guidance. The volume of the injections would be extremely small (less than 1ml of fluid in total). The patient would then be scanned using a gamma camera visualising the lymphatic channels and sentinel node(s) which would then be marked on the skin with felt pen and the patient discharged to be admitted for surgery in the usual fashion. This procedure has been outlined in the references [2].

After results of the lymphoscintigram are available these will be reviewed jointly by the radiologist and surgeon to allow preoperative planning.

Studies from the Sydney Melanoma unit where this procedure has been carried out in melanoma patients suggest that some unexpected results are received with respect to the lymphatic drainage, nodes occasionally being identified in areas not normally regarded as part of a lymph node field - the so-called "interval node". Information gained from the lymphoscintigraphy which may suggest drainage from the breast to lymph node regions other than the axilla will not add to the surgery in the early stages of this study, but will clearly be useful information and may suggest further areas of study and perhaps some changes to the standard current surgical management.

At the time of surgery after induction of anaesthetic the patient would have blue dye injected into the region of the primary breast lesion in such a way that the lymphoscintigram and blue dye injection sites would be excised with a skin ellipse together with the primary breast lesion. The axillary surgery would then proceed, identifying the sentinel node first and sending this off for separate histopathological evaluation. The axilla would then be dissected in the usual fashion, marking the site from which the sentinel node had been removed in order to evaluate its position with respect to the remainder of the axillary nodes.

8. ASSESSMENT OF PATIENTS

Patients will be monitored whilst in the nuclear medicine department in the routine manner by radiographers and nurses as well as medical staff, and under anaesthesia by an anaesthetist and surgeon. In the unlikely event of

adverse reactions the investigation will be halted, appropriate therapeutic measures undertaken and this will be reported to the committee.

8a. ASSESSMENT OF RESULTS

The results will then be analysed to determine the usefulness of the lymphoscintigram and ease of identification of the sentinel node both radio graphically and surgically. The rate of positivity of the sentinel node would then be compared with the rate of positivity of the axillary nodes and correlated with the size, type and location of the primary breast lesion.

This information will be most useful in its own right, but may well point the way to future studies. It is anticipated that the results will be published in an appropriate national or international journal.

Potential exists for large scale multi centre trials of sentinel node biopsy through the ANZ Trials Group and this will be investigated.

9. ADMINISTRATIVE ASPECTS

Funding for the project will be sought from appropriate granting bodies but initially may be carried as part of routine management of these patients, the increased cost attributable to the study being justified as part of acceptable clinical management.
Data sheets for each patient's results will be kept in the surgical oncology clinic where these patients would normally attend for management. No special facilities will be required for the study.
Theatre and radiology facilities would be used by these patients in any case thus no special pemission should be required, but the heads of the breast unit and nuclear medicine units are co-investigators.

10. CONSENT

Informed consent will be obtained by both Radiologist and Surgeon for the respective procedures using the standard FMC form.

11. PATIENT INFORMATION SHEET

Please refer to attached sheet.

12. ETHICAL CONSIDERATIONS

Benefits anticipated from the study are as set out in (4,5) above.
Risks are assessed as being minimal, if any, since the blue dye is known to be non toxic, and the radiopharmaceutical is in common clinical use.
Dr. Collinson will be working closely with the consultants on the breast and endocrine unit who will be primarily responsible for clinical management of the patients.
Patients will be recruited from the Breast Oncology clinic on a voluntary unpaid basis.
No advertising is planned.
As stated in the patient information sheet, all personal information will remain confidential, as will the patient's medical record, and no information which could lead to identification will be released or used in publications.

13. NH & MRC GUIDELINES

All research and clinical work will be carried out in accordance with NH & MRC guidelines on human experimentation (1992).

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2. Uren RF et al. Lymphoscintigraphy in High-Risk Melanoma of the Trunk; Predicting Draining Node Groups, Defining Lymphatic Channels and Locating the Sentinel Node. *J. Nuc. Med. 1993;* 34 (9): 1435 - 1440.

3. Giuliano AE et al. Lymphatic Mapping and Sentinel Lymphadenectomy for Breast Cancer. *Ann.Surg. 1994;* 220 (3); 391 - 401.

16.2 Patient information sheet and consent forms

PATIENT INFORMATION SHEET
BREAST OPERATION STUDY

You are invited to take part in a study being carried out looking at the type of surgery which is carried out in patients who have breast cancer.
The aim of this study is to see whether it is possible to localise the first lymph gland in the arm pit to which tumour cells may drain using x-rays and inert dyes. By examining this lymph gland and comparing it with the remaining lymph glands in the arm pit, which would be removed in a standard operation, we hope to find out whether just removing this first gland alone would give us the information presently obtained by removing all the glands in the arm pit.
If this is so, then ultimately it may be possible (at some future date) to restrict the surgery to removing this gland alone, in the first instance.
The study involves two steps which will add to your treatment for breast cancer.

(i) The first step is a special x-ray in the Nuclear Medicine department in which an injection of a very small amount of radioactive tracer around the area of the tumour, will allow special x-rays to be taken which will show the first lymph gland to which breast fluid (and tumour cells) may drain. This will then be marked for the aid of the surgeon. This procedure may take around an hour or so, most of which will be spent lying under the special x-ray camera. This will be carried out several days prior to your planned surgery. This procedure which is known as lymphoscintigraphy is a routine clinical procedure and has been performed safely for many years. Although it has been used in the breast on some occasions, this particular x-ray is not a routine in the management of breast cancer as yet.

(ii) The second part of the study will be carried out under the anaesthetic for your planned surgery. This will involve the surgeon injecting a small amount of blue dye again around the area of the tumour so as to locate the first lymph gland in the arm pit and confirm the findings of the x-ray studies. The area in which the injections were made for the x-ray and the dye will be removed along with the tissue routinely removed as part of your operation. There will be no extra incisions and the routine incisions will not be any longer than would otherwise have been the case. It is expected that this particular procedure will not prolong your operation by any more than 10 or 15 minutes at the most. The first lymph gland will be sent for separate evaluation in the hospital laboratory and the remainder of the lymph glands in the arm pit will be removed in the standard fashion.

Apart from attending for the x-ray (which may be scheduled at the same time as other x-rays you are having) the study will not involve any extra time or any restrictions on activities.
We do not believe that there are any significant risks or adverse effects of either the x-ray or the dye localising method used in the operation. The actual amount of radiation you will receive from the x-ray is approximately equivalent to 2 standard chest x rays. The risk involved is the same as smoking 4 cigarettes, drinking 2 bottles of wine or travelling 500 km in your car. The blue dye has been reported to cause allergic reactions but this is rare, and can be readily controlled with medications given by the anaesthetic doctor who will be monitoring you during the operation. The dye may cause slight blue coloration of the tissues which may persist for 8-10 days before disappearing, but the tissues involved will be removed by the operation.
We will be seeing you after the operation at regular intervals as is our standard practice.

Your involvement in this study is entirely voluntary, and your non participation will not affect your treatment at the Flinders Medical Centre in any way. Should you decide to withdraw from the study, you may do this freely and without prejudice to any future treatment at Flinders Medical Centre.
All records containing your personal information will remain confidential as do the rest of your medical records, and no information which could lead to your identification will be released. This trial is not being sponsored by any drug or medical company and is being carried out as part of ongoing research at Flinders Medical Centre.

Should you require further details about this study, either before, during or after the study, you may contact Dr. T. Collinson at Flinders Medical Centre, telephone 204 5511.
This study has been reviewed and approved by the Clinical Investigations (Ethics) Committee at Flinders Medical Centre. Should you wish to discuss the study with someone not directly involved in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Secretary of this committee, Ms C Hakof (204 4507).

FLINDERS MEDICAL CENTRE

CONSENT TO PARTICIPATION IN RESEARCH

Ward
Unit No.
Surname
Other Names
D.O.B./Sex
Address

I, ... request and give consent to
(first or given names) (surname)

my involvement in the research project ".....
(short title of research project)
....."

I acknowledge that the nature, purpose and contemplated effects of the research project, especially as far as they affect me and (.....N/A.....) have been fully explained to
(my [foetus] baby, if applicable)

my satisfaction by ..... and my consent is
(first or given names) (surname)

given voluntarily.

I acknowledge that the detail(s) of the following procedure(s)

.....
LYMPHOSCINTIGRAPHY
BLUE DYE LOCALISATION
.....
.....
.....

has/have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time and the frequency with which the procedure(s) will be performed.

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that my involvement in this research project and/or the procedure(s) may not be of any direct benefit to me and that I may withdraw my consent at any stage without affecting my rights or the responsibilities of the researchers in any respect.

I understand that any payment made to me is simply an expression of gratitude for assistance in this research project.

I declare that I am over the age of 18 years.

Signature of research subject : ..... Date: .....

Signature of Witness: .....

Printed Name of Witness: .....

I, ... have described to .....
the research project and the nature and effects of the procedure(s) involved.
In my opinion he/she understands the explanation and has freely given his/her consent.

Signature ..... Date .....

Status in project: .....

11/94

16.3 Approvals

Flinders Medical Centre
Bedford Park South Australia 5042

Telephone (08) 204 5511
International 618 204 5511

Committee on Clinical Investigation
Extension 4507
AV:CMH

Facsimile (08) 204 4006 International
618 204 5450

22 March 1995

MEMORANDUM

TO: Dr. T. Collinson, Dept. of Surgery

FROM: Dr. A. Vedig, Chairman, Committee on Clinical Investigation

TOPIC: **Research Application 22/95**

I am pleased to advise that the Committee on Clinical Investigation has approved your research application in accordance with the following extract from the Minutes of its meeting held on 13 March 1995.

3396 RESEARCH APPLICATION 22/95 - DR. T. COLLINSON
Sentinel lymph node mapping and biopsy in breast cancer.
Reviewer: Dr. M. Cochran

This application was approved subject to the following amendments to the information sheet:

- Re-produce in larger print.
- List any side effects/risks that could occur in relation to blue dye and technetium, even though minor.
- Provide a radiation dosage with comparisons, and possible effects as per legislative requirements.

If conditional *('subject to' or 'in principle')* approval is granted, research involving human subjects may proceed only after written acceptance of the conditions of approval (including a copy of the modified research protocol) has been received by the Committee.

This approval is for a period of one year. Application for re-approval must be made annually. Please note that if this trial involves normal volunteers it will be necessary for you to keep a record of their names and you will be required to supply this list with your annual report.

You are reminded that the Committee on Clinical Investigation must approve the content and placement of advertisements for the recruitment of volunteers.

The Committee must be notified and approve any changes (e.g. additional procedures, modification of drug dosage, changes to inclusion or withdrawal criteria, changes in mode and content of advertising) in the investigational plan particularly if these changes involve human subjects.

The safe and ethical conduct of a trial is entirely the responsibility of the investigators. While the Committee on Clinical Investigation takes care to review and give advice on the conduct of trials, approval by the Committee on Clinical Investigation is not an absolute confirmation of safety, nor does approval alter in any way the obligations and responsibilities of investigators.

It is the duty of the chief investigator to give prompt notification to the Committee on Clinical Investigation of matters which might affect continued ethical acceptability of the project, including:

1. Adverse effects of the project on subjects and of steps taken to deal with these.
2. Other unforeseen events.
3. A change in the base for a decision made by the Committee, e.g. new scientific information that may invalidate the ethical integrity of the study.

If patients are involved the chief investigator is also responsible for the process of notification, seeking approval or permission of Departments, Divisions or individual consultants.

A. Vedig
Intensive Care Unit
Extension 5206

Flinders Medical Centre
Bedford Park South Australia 5042

Telephone (08) 204 5511
International 618 204 5511

Committee on Clinical Investigation
Extension 4507
AV:CMH

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18 April 1995

MEMORANDUM

TO: Dr. T. Collinson, Breast Oncology Unit, FMC

FROM: Dr. A. Vedig, Chairman, Committee on Clinical Investigation

TOPIC: **Research Application 22/95**

Your attention is drawn to the following extract from the Minutes of eting held on 27 March 1995.

3402.12 Research Application 22/95 - Dr. T. Collinson
Sentinel lymph node mapping and biopsy in breast cancer.
Reviewer: Dr. M. Cochran

Amended information sheet was received and approved. However, another version rewritten by some of the Committee members is forwarded to the Investigator for his consideration. This information sheet also is approved by the Committee.

A. Vedig
Intensive Care Unit
Extension 5206

16.4 Radiation dosimetry report and radionuclide particle size analysis

DOSIMETRY REPORT Monday, March 6, 1995 2:27 PM

Protocol: SENTINEL LYMPH NODE MAPPING
ID: FMC00594
Date: Thursday, March 2, 1995
Median Age: 45 years
Median Weight: 70 kg

Radionuclide Tc-99m **Activity** : 30 MBq
Chemical form: Labelled small colloid (eg antimony sulphide colloid)
Physiological Characteristics: Interstitial injection for lymphoscintigraphy

Referring MO	Collinson
Nuc Med Physician	Wycherley
Nucleographer	Daryl Peter
Hospital/Practice	Flinders Medical Centre
Purpose of Study	Lymph Node mapping

Comments

Protocol: SENTINEL LYMPH NODE MAPPING

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Radionuclide Tc-99m **Activity** : 30 MBq

Chemical form: Labelled small colloid (eg antimony sulphide colloid)

Physiological Characteristics: Interstitial injection for lymphoscintigraphy

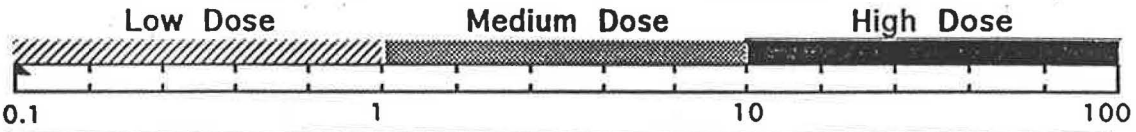
Doses in millisieverts * Dosimetry derived from adult data

Adrenals	9.9e-2	Lungs	5.4e-2	#Lymph nodes	3.2e+2
Bladder Wall	9.9e-3	Oesophagus	2.7e-2	#Inject. site	2.5e+3 D
Bone Surfaces	7.8e-2	Ovaries	2.4e-2		0.0e+0
Breast	2.4e-2	Pancreas	1.2e-1		0.0e+0
Stomach Wall	6.0e-2	Red Marrow	1.5e-1		0.0e+0
Small Intestine	4.2e-2	Skin	2.7e-2	1 dose (marked D) exceeds deterministic limit	
ULI Wall	5.4e-2	Spleen	7.8e-1		
LLI Wall	1.8e-2	Testes	4.8e-3		
Kidneys	9.6e-2	Thyroid	6.9e-3		
Liver	7.5e-1	Uterus	1.8e-2	Other tissues	2.7e-2

Effective Dose: 0.10 mSv

The above Effective Dose is equivalent to:

2	Chest X-rays (0.05 mSv each)
0.04	Lumbar spine X-ray examinations (2.2 mSv each)
0.04	times the average annual dose from natural background (2.4 mSv per year)

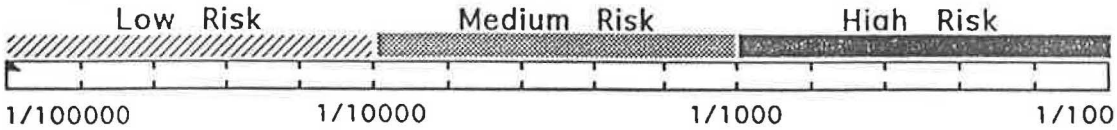


Lifetime cancer mortality risk as a result of this dose	0.00055 %
Average loss of Life Expectancy as a result of this dose	0.02 days
Average loss of lifetime per attributable cancer death	10 years

* Risks and LLE's derived from data for a 45 year old

The above risks are equivalent to:

Travelling 550 km by car(death from a car accident)
days of being a 60 year old!
Spending 0 days climbing mountains
Smoking 4 cigarettes over a period of time (lung cancer risk)
Drinking 2 bottles of wine over a period of time (cirrhosis, cardiovascular disease)



Protocol: SENTINEL LYMPH NODE MAPPING
ID: FMC00594
Date: Thursday, March 2, 1995

Median Age: 45 years
Median Weight: 70 kg

HEREDITARY RISKS

Dose to ovaries: 0.024 mSv

Hereditary risk associated with the above dose is 0.000074 %
This risk is weghted for severity and includes all successive generations

Note Only applicable to patients who are likely to have children
For large doses (>100 mSv),there is some evidence to suggest that risk may be minimised (where appropriate) by delaying conception for around six months.
Temporary sterility may occur at doses of 150 mSv or greater, permanent sterility at between 3500 and 6000 mSv.

CLOSE CONTACT DOSES

Children being nursed or cared for by the patient will receive some exposure from the radioactive material within him/her. Estimated doses and dose rates from the administered activity are as follows:

Maximum dose rate (0.1 m): 9 μSv per hr

Maximum cumulative dose: 0.019 mSv

** No avoidance of close contact required at 1 mSv level

** No avoidance of close contact required at 5 mSv level

STAFF DOSE RATES AND CONTAMINATION HAZARD

Maximum dose rate received by staff from this patient: **0.6 μSv per hr**
(at one metre)

Contamination Hazard (Annual Limit of Intake, ALI): **1000 MBq**

COMMENTS RELATING TO DOSIMETRY

Doses to the interstitial injection site and lymph nodes may be quite high, but generally, for administered activities below 200 MBq, this will be below the level generally accepted to be be the threshold for deterministic effects (20,000 mSv). Stochastic risk from the dose to these sites will be small because of the small volume of tissue involved. The main stochastic risk will arise from activity leaching through to the bloodstream from the lymphatic system - this has been estimated to be a maximum of 33% of the injected activity[Cormack, 1992], based on worst possible case assumptions..

Protocol: SENTINEL LYMPH NODE MAPPING
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DOSE/RISK TO DEVELOPING EMBRYO/FOETUS (if applicable)

Effective Dose to embryo/foetus: 0.01 mSv

Placental Transfer: No

Risks associated with the above dose are as follows:

NOTE *Based on worst possible case of exposure at 12 weeks post conception*

Malformations (threshold of 100 mSv): 0 %

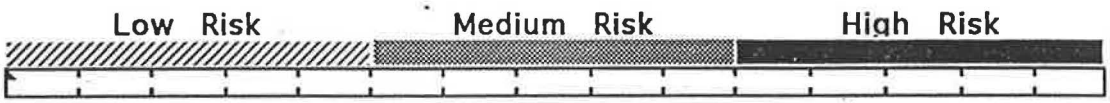
Severe mental retardation: 0.00072 %

NOTE the above risk for severe mental retardation is based on a linear extrapolation to zero dose. A threshold of around 100 mSv MAY exist in the 8 to 15 week post-conception period, rising to at least 200 mSv in the 16 to 25 week post-conception period.

Average IQ points lost: 0.00054 IQ points

Lukaemia in first 10 years of life: 0.00005 %

Cancer in later life: 0.00022 %



NOTE Termination of pregnancy soley on the grounds of a diagnostic radiation dose is very rarely, if ever, justified. As a "rule of thumb", termination should not even be contemplated unless the radiation dose to the foetus exceeds 100 mSv at a critical stage of the pregnacy.

ADVICE TO NURSING(breast feeding)MOTHERS (if applicable)

Maximum dose to infant from ingested breast milk: 0.45 mSv

The infant should be fed just prior to administration of the radioactive substance. If the dose from breast feeding does not exceed 1 mSv, breastfeeding may resume normally 4 hours after administration. If the dose exceeds 1 mSv, suspension of breast feeding for a given time may be necessary (see below).

** Breast feeding may resume normally from 4 hours after administration of the radioactive susbstance

* The general ICRP 52 recommended suspension period for breast feeding is 12 hours to avoid significant transfer of this radiopharmaceutical through breast milk. This is based on activities typically administered for DIAGNOSTIC procedures. If an alternative value is indicated above (see **), this should be used; this has been specifically calculated for the activity administered and is likely to be more accurate

* Note also comments made on previous page relating to close contact doses.

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REPORT CHECKED AND VERIFIED BY:

NAME OF RADIATION SAFETY OFFICER: John Cormack

DATE: 7/3/95

RADIATION SAFETY OFFICER
FLINDERS MEDICAL CENTRE

SIGNATURE:

FAX

Date

Numbe

TO: Dr Charles Evill
FMC

Phone 204 4224
Fax Phone 204 5450

CC:

FROM: Prof. John Thomas
Light Scattering & Materials Science
Applied Physics
University of South Australia
The Levels, SA 5095

Phone (08)302 3206
Fax Phone (08)302 3389

REMARKS: ☐ Urgent ☒ For your review ☐ Reply ASAP ☒ Please Comment

Dear Charles:

As discussed briefly on the telephone on Monday (24/4), I have made particle size measurements on your antimony sulphide sol sample using dynamic light scattering. The measurements were straightforward and required only ~5 minutes each.

The sample as supplied by Roger Smart was measured directly and several repeat runs gave an average size of ~37nm. Distribution analysis indicated a main peak around 36nm with a small peak near 6nm. See following 2 pages of print out which show results of simple (cumulants) analysis which gives an average size, and distribution analysis (NNLS V3.0). Note that these results were quite reproducible.

The sample was diluted ~4 fold with Milli-Q/Milli-Ro water and measurements repeated. The results were similar

The diluted sample was sonicated for ~2 mins and re-measured. The results were unchanged.

The sonicated sample was filtered through 0.2 micron filter and re-measured and the results were unchanged (some material retained in filter).

Clearly there is a lot of material present in the size range 35-40nm.

Please call me if you want to pursue or discuss further.

Regards,

John

Auto Correlation
24 channels

B12030AI

04-24-1995
15:05:19

CR ~ 33Kcps

Run #: 6
Status: STOP
Prescale A: 0
Prescale B: 0
Time(***) = 0
Del.Time(usec): 7.5 E 0
Tau2 = 7.5
Tau3 = 7.5
Tau4 = 7.5
Duration(usec): 3 E 8
Rate = 33.51 KHz.
Angle(degrees): 90
Samples = 4E+07
A Total = 1.001696E+07
B Total = 1.001696E+07
Overfl = 0
S/N = 0.15
M. Base = 156888
C. Base = 156788.6

----Data(***) = ----------Auto----------Base Sub----------Serial-------

***** Cumulant Analysis *****

Sb2S3 sol neat

	Amp	Diam(nm)	Gamma	Poly	Skew	Kurtosis	RMS Error
Linear	1.187	39.8	4027.6				1.000E-03
=>Quadratic	1.191	36.7	4276.8	0.102			6.650E-04
Cubic	1.190	37.8	4241.2	0.073	-2.86		6.680E-04
Quartic	1.191	36.2	4337.4	0.199	3.67	13.23	6.680E-04

RAll. 1

===BI2030AT===

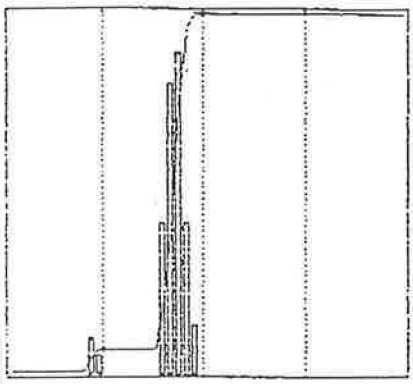
NLS V3.0

04-24-1995
15:06:28

ercent	Lower	Upper
Inten.	10	90
Weight	95	5
Number	100	0

Mean = 35nm
Var. = 0.000
Skew = -1.439
RMS = 6.61E-04

Sb2S3 sol neat



d/nm	G(d)	C(d)	d/nm	G(d)	C(d)
3	0	0	41	81	86
3	0	0	46	42	96
4	0	0	52	14	100
5	0	0	62	0	100
6	7	2	71	0	100
→ 6	15	6	82	0	100
7	7	7	95	0	100
8	0	7	110	0	100
9	0	7	127	0	100
11	0	7	146	0	100
13	0	7	169	0	100
15	0	7	195	0	100
17	0	7	225	0	100
19	0	7	260	0	100
22	0	7	300	0	100
28	42	18	dust	0	100
32	81	39			
→36	100	65			

reproducible

16.5 Patent Blue Dye: product information

Patent Blue V

Rhône-Poulenc Rorer Australia Pty Ltd

Composition Deep blue sterile, isotonic solution containing 2.5% w/v of Patent Blue V.

Indications Colours the lymph vessels so that they can then be injected with an X-ray contrast medium.

Warnings Before administration it is essential to enquire if there is a previous history of allergy or intolerance. In such circumstances it is advisable to administer a corticosteroid or antihistamine premedication.

It would also seem prudent to test for hypersensitivity by injecting a very small volume of the solution and waiting a few minutes to ascertain if there is a reaction.

Adverse Reactions Patent Blue V solution can provoke allergic reactions of varying degrees of severity. These reactions are rare and can be rapidly controlled with a corticosteroid. They occur immediately or a few minutes after injection of the dye.

Nausea, hypotension and generalised muscle tremors have also been reported; laryngeal spasm, if it occurs, requires the use of a muscle relaxant and intubation. Rare but more serious allergic reactions include circulatory failure with a state of shock, dyspnoea and oedema of the glottis.

Dosage and Administration 2 mL of Patent Blue V injection solution is diluted with an equal volume of sterile normal saline (an alternative method advocates dilution with 1% lignocaine hydrochloride solution) and 0.5 mL of the diluted dye is injected into the subcutaneous tissue of each interdigital web space. When the lower extremities are being examined, 1 mL is also injected below the tip of each malleolus. The lymphatics should then be visible through the skin and it is important that there should be no accidental spillage of the dye. Patent Blue V may be used for children at the surgeon's discretion.

Note. After injection of the dye a bluish colouration of the skin occurs, which normally disappears within 24 to 48 hours. More persistent colouration, which should not be confused with cyanosis, may occasionally be seen in cases of lymphatic stasis or circulatory disorder; this persistence may be avoided by using the minimum quantity of dye. An area of blue colouration can persist around the injection site for 8 to 10 days.

Presentation Ampoules, 2.5% w/v, 2 mL: 5's.

Storage Protect from light.

Poisons Schedule S4.

16.6 Presentations and timing

The work carried out in this research has previously been presented by the author at the following meetings:

Royal Australasian College of Surgeons Annual Scientific Congress, Sydney, May 1998. (Verbal presentation)

Australasian Sentinel Node Biopsy workshop, Adelaide, November 1998. (Verbal presentation)

Sentinel Node Biopsy Pathology workshop, Adelaide, December 1998. (Coordinated by the pathologist involved in this study.)

A Journal article has been prepared for submission to the Australian & New Zealand Journal of Surgery.

A number of factors contributed to delays in the preparation of this thesis.

Through Professor Villis Marshall at Flinders Medical Centre and employment at the Repatriation General Hospital as the Chief Resident in Surgery for the year 1995 the research was carried out without any special research funding for the project. The clinical work extended over the full year.

A further year was spent in 1996 as the Laparoscopic Fellow on the Minimal Access Therapy Training Unit (MATTU) in Guildford, Surrey, with Professor Michael Bailey. This is one of only 3 such units in the UK and was set up by the RACS. The position involved Laparoscopic teaching at the RACS headquarters in London and clinical work at the Royal Surrey County Hospital. This was a busy year, not allowing much work to be done on a project on the other side of the world.

After returning to Adelaide work continued on the project and it was realised that detailed pathology work would greatly add to the value of the research. This particular aspect of the work on an emerging technique is unique to the study and similar work has only been carried out in one or two centres overseas subsequently. The pathologist who had been involved in the project during 1995 was on maternity leave, and when she returned, considerable time elapsed whilst the original pathological blocks were recut and re-examined. This was funded by the author and had to be carried out after hours by one of the laboratory technicians and the pathologist, leading to further delays.

By early 1998 considerable interest in the technique had been seen internationally and it was felt that the work should be presented to the Royal Australasian College of Surgeons Annual Scientific Congress in Sydney in May 1998, preparation for which slowed work on the thesis itself. In November 1998 an Australasian symposium on the topic was convened in Adelaide which again took some further preparation.

16.7 Proposals for Pathology Processing

As a result of this study and its findings, the pathologist involved has put forward some proposals for assessment of Sentinel nodes. At present, of course, these are under discussion.

Cut standard section and submit to H&E section. If positive for malignancy, no further action

If negative, section the node at 100 μm intervals

Stain alternate slides with H&E and Immunohistochemical stain (Cam 5.2) (~ 10-12 sections)

Retain intervening slides for suspicious but non diagnostic cells on other slides.

A more recent paper on the topic has suggested the following regime:

3 sections of each node, with a slide from each side i.e. 6 slides per node

2 slides for Frozen section and H&E staining

2 slides for Standard paraffin fixed specimen and H&E staining

2 slides using an 'antibody cocktail'

(Turner, 1997)

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18 GLOSSARY

Adjuvant treatment Any therapy directed against a malignancy, other than Surgery, including Chemotherapy, Radiotherapy, Hormonal therapy or immunotherapy.

Axillary Dissection / Axillary Clearance Removal of all the lymph glands from the armpit

Axillary lymph node A lymph gland found in the armpit

Bioimpedance A technique for measuring the electrical resistance of the skin, so that changes caused by diseases such as lymphoedema may be compared

Breast biopsy Removal of a piece of breast tissue, usually not of a particular anatomical configuration ranging in size from a very small piece of only a centimetre or so in diameter, to something much larger, up to the size of a partial mastectomy

Carbon localisation A method of permanently marking the position of an impalpable breast lesion, by carrying out stereotactic mammography, and injecting liquid carbon in the area of the lesion and gradually withdrawing the needle so that a track is 'laid' out to the skin which can subsequently be followed by the surgeon when removing the lesion. This has the advantage that the procedure can be carried out at any time and does not need to be done just before surgery, as in the case of a hookwire. Usually carried out by a radiologist.

Clearing agents Chemicals used by the pathologist to 'dissolve' the fatty tissue in a surgical specimen, making lymph nodes easier to identify.

Core biopsy A procedure usually performed as an outpatient to obtain tissue rather than cells (as in a FNA) so that histology rather than cytology can be carried out. This is used when FNA has been non diagnostic, or in order to confirm a probable diagnosis of carcinoma, so that definitive surgery can occur in one operation, rather than subjecting the patient to a surgical biopsy followed by a definitive operation. The needle used has an outer cylindrical sheath so that a tiny cylindrical specimen of tissue is obtained.

Cytology Examination of pathology slides containing single cells or groups of cells from a fine needle aspirate, rather than pieces of intact tissue such as from a biopsy specimen.

Ductal Carcinoma In Situ (DCIS) Breast cancer at its earliest stage, i.e. confined to the breast ducts themselves and not invading into the breast tissue.

Epithelial cytokeratin A protein component found in cells of epithelial origin such as breast ducts and skin. Tumours arising from these tissues usually contain this also, but lymphatic tissue arising from non epithelial tissue (mesenchyme) does not. Lymph nodes which contain this substance therefore contain metastatic disease.

False negative result Analysis of a sentinel node biopsy specimen in which no malignancy is found, but where the lymph node region does contain tumour.

Fine Needle Aspiration (FNA) Removal of cells from the breast using a small needle (e.g. 19 gauge) and suction so that cells can be placed on a microscope slide, stained and examined by cytology.

Formalin fixed specimen Tissue which is placed into the preservative fluid formalin, to be later embedded into a paraffin base for sectioning and pathological staining. This process takes considerably longer than frozen section.

Frozen Section (FS) Technique of freezing a tissue specimen using liquid nitrogen rather than fixing in formalin and embedding in paraffin, so that the specimen can be sectioned, stained and examined in a matter of minutes, giving answers during an operation. The definition and clarity of frozen section is inferior to traditional sections.

Gamma camera / Gamma probe Fixed camera or hand held camera which detects gamma radiation emitted by the radionuclide injected for the lymphoscintigram.

Haematoxylin & Eosin (H&E) A standard histological staining technique using blue and red stains

Hookwire A fine wire placed into the breast under stereotactic mammographic control into or adjacent to a lesion in the breast which is impalpable, to guide the surgery. When the wire is in the correct place, the outer sheath is withdrawn exposing a small hook which secures the wire in position. Some wires have a thickened portion at a specified distance from the hook to assist in depth judgements.

Immunohistochemistry Special pathological staining technique utilising a stain or fluorescent dye attached to an antibody; used to detect certain cell characteristics and differentiate cells from one another which might appear similar under other staining techniques.

Internal mammary lymph node A lymph node located inside the chest somewhere along the chain of nodes running with the internal mammary artery, just lateral to the edge of the sternum from the region of the clavicle to the inferior costal margin.

Intramammary lymph node A lymph node which may be variable in location somewhere within the breast tissue.

Isosulfan Blue A Vital Dye used for identifying lymphatics. Used mainly in the USA. Almost identical colour and specifications to Patent Blue-V

Lymphoedema Swelling of the arm or leg caused by impairment of lymphatic drainage of the limb. This may be congenital or acquired as a result of infection, surgery, radiotherapy or other conditions affecting the lymphatics. In contrast to swelling caused by cardiac failure, lymphoedema is non pitting.

Lymphoscintigram A nuclear medicine Xray study in which a small amount of radioactive tracer is injected around a breast tumour and which then travels along the lymphatic pathway , identifying the first lymph node on the pathway to drain fluid from the area of the tumour

Mammogram / Mammography (MMG) An Xray of the breast, standard views comprising a vertical view and an oblique view. Suspicious areas may be subjected to views from other angles or magnified views.

Metastasis Malignant tumour which has spread to another part of the body from the area in which it has arisen, either by transport through the lymphatic system, or the venous system

Micrometastasis metastasis of only one or a few cells

Oximeter / Pulse oximeter An electrical and optical measurement device placed over a part of the periphery such as the finger or ear which measures the colour of the blood within the part, and estimates the oxygen content of the blood, based on the colour, since this is proportional to the colour.

Partial mastectomy Removal of a larger part of the breast than in a breast biopsy. The segment removed is usually a 'pie slice' or an ellipse, disposed radially with respect to

the nipple in order to remove the ducts draining a tunour which run radially towards and away from the nipple.

Patent Blue Violet A vital dye which is used to identify lymphatic channels within the body.

Pathology block A small piece of material, usually paraffin wax, in which pieces of tissue to be examined are embedded. Approximately 20 X 15 X 5 mm in size. Slices of tissue and wax are shaved from the block, mounted on a glass microscope slide, stained and preserved for examination

Plethysmography A technique for measuring changes in volume of a part of the body, for instance using volume displacement

Quadrantectomy An older term, strictly speaking implying the removal of a quarter of the breast tissue.

Radionuclide / Radionucleotide / Radiopharmaceutical / Radiocolloid A molecule such as sulphur, sulphide or albumin, to which particles of radioactive material such as Technetium are attached to carry out a lymphoscintigram. The molecule is taken up in the body, the tracer indicating its location when scanned with a gamma camera.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) A biochemical technique in which enzymes are used to amplify and detect small amounts of material from certain cells in a specimen which may not be identified on any other pathological test.

Section / Pathology section one incision made through a paraffin embedded or frozen section block, from which may be taken two or more slides which are then subjected to histological analysis.

Sentinel node The first node along a lymphatic pathway draining an area of the body. The sentinel node is the most likely site of metastasis from ythat area if metastasis has occurred.

Sulfur Colloid A molecule used fro lymphoscintigraphy, predominantly in the United States.

Skip metastasis Metastases in which tumour spread has occurred to a more distant lymph node when the lymph node which appears more likely to receive lymphatic drainage from the area first, is spared.

Slide / pathology slide one shaving made from a paraffin embedded or frozen section block, which is placed on a glass microscope slide, subjected to staining and histological analysis.

South Australian Breast Xray Service / BreastScreen SA A governmental body set up and funded by the South Australian Government whose aim is to screen with mammography as many women in the 50-69 year target age group within the state as possible. Women of other ages are also accepted. The service is free and accepts women only if they do not have any palpable abnormality. For routine screening a clinical examination does not take place. Women are screened every 2 years.

South Australian Cancer Registry A Government funded state wide registry of all cancer diagnoses in the state, referred directly from the reporting pathologist.

Stereotactic mammography An Xray carried out to identify the location in three dimensions of a lesion seen within the breast. Xray pictures are taken from 30 degrees off vertical on each side and compared. The parralax error is used to calculate the exact location , and fine needle or core biopsy can be carried out under guidance using an attachment to the machine. It is also useful for placing a hookwire.

Supraclavicular lymph node A lymph gland situated above the collar bone in the lower part of the neck.

Tonometry A technique for measuring the elasticity of the skin, or a body part, for example with calipers.

True negative result A pathology result in which the sentinel node is free of tumour and the remaining lymph nodes in the field are also truly free of disease.

19 INDEX

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P

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CORRIGENDA

The comments below are made following examination of the thesis:

1. The term "Patent Blue Violet" used in this thesis and by others (Pump, 1994) referring to the vital dye is not generally used and many authors currently refer to the dye as Patent Blue V where "V" represents the roman numeral "5".

2. Although blue dye can be used for mapping alone, cases in which the sentinel node cannot be identified by blue dye occur, as they did in this study, and therefore preoperative lymphoscintigraphy is to be regarded as an integral and essential part of the technique of sentinel node biopsy.

3. Although not available for this study, a hand held gamma probe would have been useful for intraoperative localisation of a sentinel node which had been identified by lymphoscintigraphy, but could not be identified with blue dye. The use of an intraoperative hand held gamma probe is also to be regarded as an integral part of sentinel node biopsy.

4. Whilst encouraging, especially in view of the significant number of cases in which the only involved node was the sentinel node, the results of this study must be regarded as preliminary since authors with considerable experience in sentinel node biopsy for melanoma feel that there is a learning curve of around 50 cases before a surgeon can be regarded as proficient in this technique.

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