Can we decrease the rate of negative sentinel lymph node biopsies? A retrospective study

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Declaration

I, Ismail Cassimjee, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of General Surgery in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Ismail Cassimjee.....on this .....day

of....., 2013

I certify that the study contained in this thesis have the approval of the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa. The Ethics number is M090435

……on this …… day of …… 2013.

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Dr. C Benn (supervisor)	Prof.GP Candy (supervisor)
……	…….
Date	Date

Dedication

To my family:

Sha’ista: For your patience, encouragement and continuous support. To you I am forever grateful.

Suleiman: For making me understand that there is more to life than surgery.

My father: For his commitment and passion to the pursuit of knowledge.

Abstract

The management of breast cancer has changed over the last century, with surgeries becoming less invasive and adjuvant therapies becoming indispensible. Sentinel lymph node biopsies (SLNB) have replaced axillary nodal dissections as a method of staging an axilla in early breast cancer. However, 70% of SLNBs are negative. The aim of this study was to determine if wecould decrease the rate of negative sentinel lymph node biopsies?

A retrospective review over a 10 month period was undertaken. Patients undergoing a SLNB and who had a documented negative axillary ultrasound report were included. One hundred and fifty onepatients were eligible for inclusion. Patients' ultrasound reports and initial biopsy specimen characteristics (ER/PR/Her2-neu, LVI, Grade, Location) were compared to their axillary nodal findings on histology.

An ultrasound was able to predict a pathologically negative axilla in 71.6% of patients. Exclusion of micrometastasis increased the negative predictive value to82.8%. If the ultrasound was negative in a histologically positive axilla, it was likely that only 3 or less nodes were involved. Nodal metastasis could not be predicted based on the tumour characteristics that were reported on the initial tumour biopsy specimens(ER/PR/Her2-neu, LVI, Grade, Location). LVI and DCIS on the initial biopsy specimens were poorly correlated with the final histology specimen findings..

The results show that an ultrasound cannot currently replace a SLNB as an accurate means of evaluating an axilla. A clear limitation is the inability to detect micrometastasis, however the role of micrometastasis in axillary staging is diminishing. Ultrasonographic evaluation of the axilla is currently reported in a non-standardised manner. Classification systems do exist, and if applied to current reporting will increase the negative predictive value of ultrasonography. In the future, the combination of improved reporting standards of axillary ultrasounds, as well as the surgical conservatism with regard to the management of micrometastasis and small volume metastasis in the axilla will hopefully reduce the rate of negative SLNB's.

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Contents

List of abbreviations

ALAMNAC: A randomized Multicenter Trial of Sentinel Node Biopsy Versus Standard Axillary Treatment in Operable Breast Cancer

ALND: Axillary lymph dissection

CpR: Complete pathological response

DCIS: Ductal carcinoma *in-situ*

ER: Oestrogen receptor

FNA: Fine needle aspiration

Her-2: Human epidermal growth factor 2

H&E: Haematoxylin and Eosin

LVI: Lymphovascular invasion

MRI: Magnetic resonance imaging

NCCN: National comprehensive cancer network

PR: Progesterone receptor

SLNB: Sentinel lymph node biopsy

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Chapter 1

1.1 Literature Review

Breast cancer is a major public health care burden. Itis the most prevalent malignancy in women worldwide and has surpassed cervical canceras the most prevalent female malignancy in South Africa (1). Advances in awareness, screening and management have led to a dramatic decrease in mortality from breast cancer(2). The transition to a biological model with the understanding that breast cancer is a systemic disease has led to more directed treatment. Anthracycline based chemotherapy is purported to have reduced the mortality by 38%(3).

The predominant model for breast cancer in the 19th century proposed that breast cancerwas a local phenomenon that spread contiguously along the chest wall then into the lymphatic system and throughout the body, known as the mechanistic model. The Halstedian radical mastectomy had this model in mind, affecting a cure by extensive surgery - removing the breast along with its underlying muscles and including the axillary structures(4). The associated severe morbidity was not unexpected and patients carried the burden of their 'cure' for the rest of their lives(5). In the late 1960's Fischer suggested a biological model in which the disease outcome was based on the presence of metastatic and micrometastatic disease leading to systemic spread(6). This ushered in the modern management of breast cancer – local control of the breast and axilla with an emphasis on systemic therapies to treat metastatic and micrometastatic disease elsewhere in the body.

The Halstedianradical mastectomy was modified to a total mastectomy with an axillary lymph node dissection. Surprisingly at the time, there was no change in mortality(6), but a significant decrease in morbidity was apparent. The National Surgical Bowel and Breast Project –B04 (NSABP –B04) was the first of the randomized breast cancer trials that looked at these endpoints, supporting the biological theory of Fischer (7). The NSABP –B04, confirmed that axillary nodal status was the basis for prognosis and that systemic chemotherapy plays a significant role in the management of the disease(7).

The next evolutionary step in the management of breast cancer was to be more surgically conservative with the breast and breast conservation techniques were applied. Two large randomized control studies investigated breast conservation surgery (lumpectomy) with irradiation, compared to a mastectomy. The results showed that there was no difference in mortality, except the need for irradiation if a breast conserving technique was employed(8,9).

As breast surgeries became less extensive, it was apparent that the axillary nodal status was a key prognosticator of survival in early breast cancer. However, at the same time advances in imaging techniques and screening programs leadto less advanced and *insitu*(DCIS)tumours being detected. Consequently, the positive yield from axillary nodal dissection was decreasing, with the majority of patients having a negative axillary nodal dissection(10). Current statistics suggest that 60 – 70% of newly diagnosed patients with breast cancer have negative axillarylymph nodes(10,11). An axillary lymph node dissection(ALND)has associated complications such as lymphedema, neuropathy, seroma and scarring (12,13). Therefore, decreasing the number of axillary dissections would be beneficial to patients. This advantage provided the impetus for the concept of a sentinel lymph node biopsy (SLNB).

A sentinel lymph node biopsy (SLNB) is a minimally invasive technique of axillary lymph node sampling. It is based on the premise that breast malignancies spread in a predictable manner to the axillary nodal basin, and the initial spread is to a node termed the sentinel node(12). When compared to an axillary nodal dissection, a SLNB has a 95% sensitivity and an almost 100% specificity for nodal metastasis(13).

The rate of negative SLNBsin women with early breast cancer is 70 – 80%(14). In the last decade, ultrasound of the axilla has been used to further stratify patients in order to decrease the rate of positiveSLNBs by preselecting those patients that may proceed directly to axillary nodal dissection. The detection of positive nodes has been further enhanced by the use of a fine needle aspirate (FNA) ora core biopsy technique(10,15).Due to the application of minimally invasive biopsy techniques to exclude positive axillas, 70% of sentinel lymph nodes will be negative in patients with early breast cancer (16).

In summary, there has been a revolution in the management of breast cancer within the last century, from aggressive and debilitating surgeries to less invasive methods.

While substantial progress has been made to date, questions remain with regard to the optimal management of the axilla in the modern management of breast cancer. The aim of this study is to investigate the possibility of decreasing the rate of negative sentinel node biopsies. Using a combination ofaxillary ultrasound findings, tumour characteristics and additional imaging techniquesthe aim is to identify a cohort in whom a ‘negative’ axilla can be safely predicted in a non-invasive manner. This would negate the value of performing a SLNBin the identified cohort, thus taking a stride in the advancement of treating breast cancer.

The breast cancer literature has not focused specifically on this question. In order to better understand the subject, the literature review focused on the following subsections.

1. SLNBs: A comprehensive review on the subject was undertaken.
2. Imaging techniques of the axilla,and its value in predicting malignant lymph node involvement.
3. Tumourcharacteristics and their value in predicting lymph node metastasis.

1.2 Sentinel lymph node biopsies

Sentinel lymph node biopsy has become the standard of care for patients with early breast carcinomaand a clinically benign axilla. The first reported cases stem as far back as 1994. It is a well recognized technique that has been validated by multiple prospective randomized controlled studies. However, although it has been widely accepted, there are many controversies that still exist. Within the aim of this study, if aSLNB is intended to be replacedwith a non-invasive method, a thorough understanding of the nuances of the technique is required. What follows is a detailed review of SLNBs.

1.2.1 Concept of sentinel lymph node biopsyand technique

The SLNB concept is an appealing one, and this in part is fuelled by its simplicity. The ‘sentinel nodes’ are the nodes that the tumour is thought to first spread to. The procedure is facilitated by two techniques: a blue dye injection or by lymphyscintigraphy aided by the use of a radioactive isotope and a gamma probe.

The term ‘sentinel node’ was coined by Gould et al in 1962 in describing a metastatic lymph node of the parotid gland(19). His rationale for using the nodal histology as a precursor to radical neck dissection was similar to the current concepts used in breast cancer. The first critical use of a SLNB was by Cabanas in 1977. He observed that in patients with penile cancer, if their ‘sentinel node’ was negative, their 5 year survival was in excess of 90%. This is in comparison to patients with involved lymph nodes who have a50%5-year survival (20). This led him to the conclusion that those patients with a negative node did not require any further surgery. His study was elegant, however he was erroneous in his presumption that the node was always in a fixed place(20).At the same time, interest was mounting in the potential to avoid elective lymph node dissection in intermediate thickness cutaneous melanoma. The pioneers of the technique investigated patterns of nodal spread in melanomas of the back and around the umbilicus, as the spread of melanomas in these regions wereknown to be variable. In 1977 cutaneous lymphoscintigraphy using vital blue dyes was used to map at risk nodal basins in order to perform a more selective lymph node dissection. The technique was then extrapolated to inguinal nodal dissection, with the deep system only requiring an

exploration if Cloquets' node was positive(21).Cloquets' node is a fairly constant node found in the femoral sheath, just below the inguinal ligament and is the most superior deep inguinal node.The next breakthrough was the use of radioactive colloidal gold for the mapping of cutaneous melanoma, in order to guide the selective lymph node dissection further(21). In the 1980's, with the advent of the S100 protein, the building blocks of the 'sentinel' concept took a leap. The S100 protein had the ability to detect melanoma cells that were not found on standard Heamotoxylin and Eosin (H&E) staining. These occulttumour deposits brought on the realization that only a relatively small number of nodes closest to the primary tumour have metastatic disease.To Morton et al, this meant that the malignant cells must spread to a specific node within the regional basin and then further away to other basins. This in turn was the impetus to find the 'sentinel node'(22).

The clinical technique of dye mapping for sentinel lymphadenectomy was presented to the Society of Surgical Oncology in 1990(23). In their study, Morton et al had237 sentinel lymph node biopsies, in which the sentinel node was detected in 82% of biopsies. More importantly, their false negative rate was less than 1 %(23). This prompted a 5-year phase 3 trial, the Multicenter Selective Lymphadenectomy Trialthat randomly assigned patients with melanoma to wide local excision or wide local excision with lymphatic mapping andSLNB. The trial aimed to recruit 1600 patients, but even before it reached its target, the technique was adopted by clinicians treating melanoma. Preoperative dynamic lymphoscintigraphy was utilized to map the basins as to ensure that no nodes were missed. This evolved into intraoperative lymphoscintigraphy with a radio colloid tracer and a hand held gamma probe as described by Alex et al in 1993(24), which was another breakthrough in sentinel lymph node biopsies. This technique was then adopted in breast surgery with the same group of investigators reporting their technique of lymphoscintigraphy in breast cancer using a gamma probe(25). The validation of this technique in breast cancer began in 1994.

The sentinel node in breast cancer can be found by two methods – blue dye and by lymphatic mapping using a radio-colloid with an intraoperative gamma probe. Within these two methods there are differences in application with regard to location of infiltration of the blue dye and the type of radio-colloid as well as using the methods in

combination or alone. The underlying theme in these varying methods is the understanding of the drainage patterns of the breast. Sappey et al described the communication of the underlying lymphatics of the nipple areolar complex to the breast parenchyma over 100 years ago – Sappey'ssubareolar lymphatic plexus. The understanding was later extended to include the ipsilateral axillary nodes as the drainage area of the breast parenchyma(26). In summary, lymphatic spread from the subareolar plexus drains into the axillary lymph nodes.

The methods of dye/tracer infiltration are subareolarly, peritumorally with intraparenchymal, intradermal or even subdermal injections. The gold standard was thought to be intraparenchymalperitumoralinjection as it would reflect the drainage of the individual tumour. However, when using radio-colloid in the upper outer quadrant of the breast, the technique becomes more challenging as there is considerable 'shine through' whilst using the gamma probe(27). Beitsch et al compared the drainage,as described bySappey'ssubareolar plexus, using blue dye, withtheperitumoral injection of radio-colloid. Their results showed a 94% detection rate of the sentinel node, and in this there was a 99% concordance (blue and radioactive) between the dye and radio-colloid(28). This concordance of subareolar blue dye and sulfur-colloid injected peritumorally has been validated in other prospective studies as well(29). Subareolar injection of dye is also an option in patients with non-palpable tumours. The combined technique of peri-tumoralradio-colloid and subareolar blue dye or *vise versa* is a technique commonly used whilst providing good results(27).

The literature is replete with conflicting studieswith regard to radio-colloid *versus* blue dye or the combination of the two techniques. Kim et al published a meta-analysis on the subject. They included 69 trials with 8059 patients. The results of this meta-analysis favoured the combination of dye and colloid over either procedure alone(30). When the combination is used, the false negative rate decreases to seven percent, as opposed to 10.9% with dye alone or 8.8% for radio-colloid alone ($p = 0.0047$)(30). This is the best evidence available regarding the technique of dye or tracer infiltration and it favours a combined technique. The learning curve of the procedure was highlighted in a multicenter study using the combination technique, showing that the false negative rate

decreased from 13% to 4.3% after the operator had performed in excess of 30 procedures(31).

1.2.2 Advantages of a sentinel lymph node biopsy

The advantage of performing a SLNB is that an ALND can be avoided in SLNB negative individuals. Axillary surgery is commonly associated with the following problems: Lymphedema, pain, paraesthesias and shoulder morbidity (7). Sentinel lymph node biopsy is reported to decrease the rate of lymphedema and sensory loss from 13% and 19% respectively in the ALND group to 5% and 8.7% respectively in the SLNB group (17). The ALAMNAC trial (a randomized Multicenter Trial of Sentinel Node Biopsy Versus Standard Axillary Treatment in Operable Breast Cancer) was a randomized controlled trial that investigated 1031 patients, randomized to two groups (axillary dissection *vs* SLNB) and followed up for 12 months, specifically looking at quality of life outcomes with regard to shoulder and arm morbidity. Their results showed a relative decrease of 0.37 in both lymphedema and sensory loss (18). In addition they had a statistically significant decrease in length of hospital stay, drain usage and time of return to normal daily activities (18). The conclusion from the ALMANAC trial is that the avoidance of an ALND has substantial benefits to the patient, however, the complications are not completely mitigated.

1.2.3 Validation of sentinel lymph node biopsies

In the mid 1990's, as the SLNB technique was being popularized, a number of landmark prospective validation studies were published. In 1998 Krag et al published their pilot study in the New England Journal of Medicine (32) in which they had 443 patients whounderwent a SLNB, using radio-colloid combined with a gamma probe, followed by a completion axillary nodal dissection. The overall rate of identification of the sentinel node was 93%, with a negative predicative value of 96%, whilst the false negative rate was 11% and all surgeons involved had performed an average of 11 procedures(32). In 1994 -1995 Giuliano et al used blue dye alone, to perform a validation study on 113 patients. The identification rate in this study was 93.5%(33). In

1999 Veronesi et al published results on 376 patients in which they used a radio-colloid with an intraoperative gamma probe. They identified the sentinel node 98.5% of the time, with a false negative rate of 6.7%(11). In the conclusion to their paper, they identified the need for a prospective study to validate the omission of an ALNDin a patient with a negative SLNB(11).

In 2003, Veronesi et al published the long termresults of their earlier reported prospective randomized controlled study (11). Having randomized 516 patients into a sentinel only group and a sentinel plus axillary dissection group, they followed the patients up for a mean of 46 months. There were no cases of overt axillary recurrence in their follow up, but the levels of pain and discomfort were lower in the sentinel only group(34).

1.2.4 Indications and contraindications for sentinel lymph node biopsy

A SLNBis indicated in a patient with breast cancer who is clinically node negative(2). This would infer early stage breast cancer, limiting it to a stage 1 or 2cancer.

The learning curve associated with sentinel lymph node biopsies is approximately 30 procedures(31,35).Surgeons who have performed fewer SLNBs, should be proctored or refer their patients to a centre that performs SLNBs. Importantly, an absolute contraindication to SLNB is biopsy proven metastasis in the axilla.

As more women postpone having children until they are in their thirties, the prevalence of breast cancer during pregnancy is on the increase. The use of sentinel lymph node biopsy in pregnancy is not recommended(2). Blue dye is contraindicated in pregnancy due to fears of teratogenicity, and radio-colloid is not recommended although there are retrospective studies that show that there is a low risk to the fetus's(36). If it were to be carried out, waiting until 30 weeks gestation would minimise the risk to the fetus.

Other concerns about SLNBs are raised in patients with multifocal/multicentric disease and in patients who have had previous breast or axillary surgery.

The recurrence rate for breast conserving therapy is reported to be 5% - 15%(37).In patients who have had previous axillary surgery, the likelihood of detecting the sentinel node decreases to between 55% and 75%(37).However it increases to 95% - 99% if the previous surgery was a SLNB or an excisional breast biopsy. The accuracy decreases in proportion to the number of nodes removed, especially if more than 10 are removed. Aesthetic procedures have minimal effects on SLNB accuracy. Radio-tracer should be employed in the mapping as it gives information about extra-axillary sites of malignant spread. The logical conclusion would be that it is safe to perform a SLNB in patients that have had a SLNB or breast conservation surgery with a recurrence, on condition that they have had less than 10 nodes previously removed and the use of radio-colloid tracer is mandatory. Patients should be aware of the possibility of failing to identify the sentinel node, and despite this it is still worth attempting a SLNB. Patients with re-operative breast surgery undergoing SLNB, should be informed that in the case of a sentinel node not be found, an ALND must be performed, preferably at the same setting.

Kim et al investigated the use of SLNB in multifocal/multicentric disease byinjecting radio-colloid and blue dye into the separate lesions, and found that the different lesions had the same drainage patterns (38). The same group had a follow-up study comparing the accuracy of SLNB in patients with multifocal/multicentric disease with unifocaltumours(38). In this study they had 803 unifocal and 139 multifocal tumours that underwent SLNB and were followed up by ALND. The multifocal group showed a 97% identification rate with a 7% false negative rate, which was equivalent to the unifocal group. This finding dispelled the concern over the inaccuracy of SLNB in multifocal/multicenteric disease.

The use of neoadjuvant chemotherapy to downstage larger tumours or patients with involved nodes has created another dilemma. Neoadjuvant chemotherapy is commonly used in treatment protocols for locally advanced breast cancer. From the NSABP-18 trial, we know that it does not offer any survival advantage(39). However, it increases the rates of breast conservation surgery, and theoreticallyit assesses the *in-vivo* response of the primary tumour and metastatic nodes to the chemotherapy(40). Preoperative chemotherapy can completely clear axillary nodes of tumour cells in 23% of node positive axillas, and eradicate micrometastasis to a level of 10% as is seen in ‘node

negative disease' (41). However, does preoperative chemotherapy have any bearing on the sentinel lymph node status? Some of the reasons given as to why pre-operative chemotherapy might be a problem in assessing the true nodal status of patients, is excessive fibrosis of the lymphatics caused by chemotherapy, a blockage of the lymphatic channels by malignant debris, and the fact that patients given neoadjuvant chemotherapy are more likely to have involved nodes(42). A prospective multicenter trial is currently underway in an attempt to answer this question. Indeed, the ACOSOG Z1071 trial will evaluate the effect of chemotherapy on biopsy proven axillary nodal metastasis. The primary objective is to determine the false negative rate post preoperative chemotherapy. There are reports of smaller published trials which also investigated this problem to find an answer to this question. For example, Classe et al enrolled 192 patients over 4 years in 12 centres, evaluating preoperative chemotherapy on rates of SLNB detection. The conclusion of this study was that the results obtained were similar to those inearly breast cancer. The detection rate was 90% and the false negative rate was 11.5%(43). Furthermore, Kuerer et al published a review article involving all available studies that assessed SLNB with neoadjuvant therapy. The resultant identification rate was 83 – 100% in the 12 studies assessed and the conclusion was that these results did not differ from SLNB without preoperative chemotherapy(42). The German SENTINA (SENTInelNeoAdjuvant) trial of the AGO-B (ArbeitsgemeinschaftgynäkologischeOnkologie) is a prospective multicenter study that examines the role of SLNB in the neodjuvant setting. More than 1500 patients will be included. This trial will also help answer this controversy.

In summary, the only contraindications to SLNB are biopsy proven metastasis or a clinically positive axilla. The relative contraindications are pregnancy and previous breast (not aesthetic) or axillary surgery. Importantly, multicentric/multifocal disease is not a contraindication and in patients who have had neoadjuvant chemotherapy SLNB appears safe, however we await the results of the ACOSOG Z1071 trial and the German SENTINA study.

1.2.5 Ductal carcinoma*insitu* and sentinel lymph node biopsies

Controversy remains with regard to the indications for SLNB in patients with ductal carcinoma *insitu* (DCIS). Ductal carcinoma *insitu*is defined as a neoplasm that has no histological features of invasion. It is commonly diagnosed on a core biopsy sample of the breast, and a treatment plan is formulated on these results.Alarmingly, population based screening programs have resulted in an exponential increase in the number of patients with DCIS. The incidence in the USA has increased from 5.8/100000 in 1975to 32/100000 in 2005(44). Pure DCIS has a reported rate of axillary metastasis of between 2% - 13%(45,46). However, core needle biopsies which report DCIS, have invasive cancer in the specimens in 8 – 38% of cases(47-49). Core needle biopsy has obvious advantages over an excisional biopsy but due to the small area sampled, an invasive malignancy may be missed. Ansari et al included 22 studies in their meta-analysis and they found that 7.6% of patients with DCIS on biopsy specimens harboured axillary metastasis. Their conclusion was that it was reasonable to offer a SLNB to patients with DCIS (50).

Moreover, Goyal et al performed a retrospective review of 587 patients with DCIS. Their invasive malignancy rate result on final histology was 38% (51). Multivariate analysis revealed that a mass, on clinical examination or imaging, was the best predictor of invasive disease(51). Of the patients that had an axillary examination, 13.6% had metastasis – all of these patients had invasive disease. In another retrospective study of 110 patients, invasive disease was associated with high grade DCIS on core biopsy in 93.6% of the patients(52). This however is not a consistent finding, and invasion is often associated with intermediate grade DCIS. Predictors of both a positive and negative axilla are inconsistent in DCIS. The general consensus is that a SLNB is required in DCIS if the clinical suspicion for invasive malignancy is high, the patient has a mass or high grade DCIS or if a mastectomy is being performed.If a SLNB is not performed during a mastectomy or lumpectomy for DCIS, and on final histology the specimenshows invasive cancer, axillary staging will subsequently be required.

1.2.6 Controversies in sentinel lymph node biopsy

1.2.6.1 Micrometastasis and isolatedtumour cells

The significance of micrometastatic disease in breast cancer remains a controversy as its clinical relevance is not fully elucidated. The current classification defines axillary metastasis into three categories: as macrometastasis (> 2.0mm), micrometastasis (0.2mm - 2.0 mm) and isolated tumour cells (ITC) (0.01 – 0.2mm) (2). Isolated tumour cells are stained by immunohistochemistry only and are not visible on light microscopy.

The average harvest from a SLNB is 1.5 nodes as opposed to more than 10 nodes in an ALND. As there are fewer nodes to examine, thishas increased the scrutinyofthe lymph nodes and hence, thedetection of micrometastasishas increased. Immunohistochemistry has aided in detecting metastasis which are less than 2mm in size,and these small volume metastasis have led to intense debate.

In an effort to quantify the false negative rate of SLNB using standard light microscopy with 0.2mm specimen slices, Weaver et al undertook a study where, they re-examined the negative specimens from the NSABP-32 study(53). Specimens were sliced, from the original 0.2mm slices, into 0.1mm and 0.05mm slices and were examined under light microscopy. They wereaided by a computer assisted cell counter to examine the specimens and found that 64 (27%) of the 236 samples showed metastasis, thirty were detected by light microscopy alone and 34 were detected by the cell counter. Twentyfiveof themetastasis were less than 0.03mm, and 49 were less than 0.1mm.

In their follow-up study the significance of micrometastases, and its impact on overall survival was evaluated. Weaver et al in 2011published a study analysing 3887 patients, with negative sentinel nodes. Women with breast cancer were randomly assigned to a SLNB and ALND, or SLNB alone. The negative specimens were subjectedto further sectioning and immunohistochemistry, resulting in a 15.9% rate of occult metastasis. The results were separated into two groups - a completion ALND or no ALND (SLNB

only). The 5-year overall survival (OS) difference was small at 1.2%. The survival was 94.6%(ALND) and 95.8%(SLNB).However, this was statistically significant (p= 0.03). There were also significant differences between disease free survival and distant disease free survival, but again the absolute numbers were small. The recommendation was that although there are differences in overall survival, further analysis of all negative nodes was not recommended (54).

In contrast, the ACOSOG Z0010 trial, the MIRROR trial and in a review by Billamoria et al, showed that there was no statically significant difference in overall survival between a completion ALND and SLNB alone in patients with micrometastsis(35,81,82).However it was noted that the majority of patients received adjuvant systemic therapy and that it seemed to improve their 5-year disease free survival. One would make the assumption that micrometastasis are smaller metastasis and will grow and spread, however the literature does not support this finding. Indeed, The NCCN guideline on breast cancer recommends basing treatment decisions on H&E staining only and routine immunohistochemistry is cautioned against(2).

In summary, although micrometastatic disease is easily missed on routine H&E staining, the presence of micrometastatic disease does not seem to have an impact on the overall survival especially when adjuvant systemic adjuvant therapy is administered. With reference to this study, it is important, as a clear limitation of ultrasonography would be the detection of micrometastatic disease, however if it were not clinically relevant then, this limitation would be minimised.

1.2.1.6 Isaxillary dissection necessary after a positive *SLNB?*

This is currently the most controversial topic in breast cancer and is also the most relevant to our study. The ACOSOG Z0011 is a recently published prospective non-inferiority trial(56) wherethe aim was to assess if SLNB alone was non-inferior to completion ALND in T1 (< 2cm) and T2 (2 – 5cm) patients with positive SLNBs. All patients had breast conservation therapy combined with whole breast irradiation (not axillary radiation). The trial recruited patients from 1999 – 2004 and was terminated prematurely due to poor accrual.

The results were surprising.No difference was found in local or regional recurrence rates and the 5-year overall survival was 91.8% in the ALND group and 92.5% in the SLNB alone group. The conclusion was that it was safe to omit an ALND in patients with less than three nodes positive on SLNB, in T1 and T2 tumours receiving whole breast irradiation. However, the criticism of this trial is that it was underpowered to answer the question, and we await further evidence to support its findings.

Of note was that 97 of 355 (27.3%) patients in the ALND group had further metastasis on ALND. Extrapolating this incidence to the SLNB group, an assumption can be made on the number of metastatic nodes not operated on in the SLNB group. Importantly, 96% of patients in the ALND and 97% in the SLNB only group received anthracycline-based chemotherapy(57). The chemotherapy decisions were based on the initial tumour biology and characteristics and not on the SLNB findings. This confirms the assumption that irradiation combined with systemic therapy, controlsthe axillary metastasis left behind after a SLNB. So in summary, there is thus evidence for not proceeding to an ALND in well selected patients with a positive SLNB and who are to receive anthracycline based chemotherapy as well as whole breast irradiation.

1.2.1.6What is the role of intraoperative frozen section analysis of sentinel lymph node biopsy?

There are two strategies for dealing with the staging of the axilla. The first is performing an upfront SLNB, and the second is combining it with the definitive surgery. The advantage of combining the procedures is the prevention of a second general anaesthetic with its incumbent morbidity to the patient, but the disadvantage of frozen section is the potential damage to the tissue during sectioning, rendering it inadequate for H&E staining. Combining the procedures makes practical sense in a patient who does not requirea complicated onco-plastic reconstruction or in a patient undergoing a definitive mastectomy.However, the underlying question that remains is: - are the results reliable and does the risk of a second procedure justify the false negative rate of intraoperative frozen section?

The reported accuracy of frozen section is 90% - 93.3%, with a false negative rate of 28.4%(58,59). The converse is that the sensitivity is 66% - 71.6%, and these patients are spared a second operation. Although the false negative rate might appear to be high, only 26% of the false negative patients had macro-metastasis. The overwhelming majority hadmicrometastatic disease – decreasing the false negative rate to an acceptable 7% (59). This highlights the importance of micrometastatic disease and its treatment.

In conclusion, frozen section is a valid technique for decreasing the morbidity of a second anaesthetic, and is accurate in 90% of cases. The associated high false negative rate represents small volume metastasis in the majority of patients and a SLNB alone, is the current treatment for these micrometastasis.

1.3 Ultrasound and its use in the axilla

The use of ultrasound in the evaluation of a patient with breast cancer has become commonplace. The reported sensitivity of clinical examination for detecting a negative axilla is reported to be 38%(66) and patients with a clinically negative axilla are candidates for a SLNB. In an effort to streamline the management algorithm, ultrasound

of the axilla with a FNA is being used to identify positive nodes, and patients with FNA proven metastasis can proceed directly to an ALND or neo-adjuvant chemotherapy.

A high frequency probe is required to perform an axillary ultrasound. Close attention should be paid to inferior aspect of the axilla below the insertion of pectoralis minor, as this is a common place for the sentinel node to be found(60).

Lymph nodes have ultrasonographic features that suggest malignant infiltration.In a normal node, the hilum is hyper-echoic and the cortex is hypo-echoic. Anatomically, lymphatic channels enter lymph nodes at the peripheral cortex and the blood supply is derived from the hilum of the node. Normal lymph nodes have the following features:

1. Oval in shape
2. Fatty hilum
3. Thin peripheral cortex(61)

As the metastatic cells infiltrate the node from the lymphatics into the cortex, the cortex gets thicker and the hilum proportionately smaller, until it is entirely replaced by malignancy. Ultrasonographically, the initial features are a thickened cortex with a normal hilum. The cortex thickens, the node loses its oval shape as the hilum is displaced, and the entire node is replaced by metastatic deposits(62). The hypo-echoic cortex gradually fills the entire node(63).

Table 1.1: Malignant features of lymph nodes

CORTEX	HILUM
Markedly Hypo-echoic	Eccentric
Lobulated	Replaced

Several methods are reported which characterize lymph nodes,however there has not been standardization in the reporting of ultrasound studies. This lends itself to the endogenous bias of user dependency in ultrasonography. In addition, the focus has been placed on predicting positive nodes, with less emphasis on predicting true negatives.

Bedi et al proposed a more objective classification of evaluating nodes for metastasis(64). The proposed classification was based on the cortical morphologic

features of lymph nodes on ultrasound. In their study, inter-observer correlation of positive or negative nodes was 88%. Their classification consisted of Types 1 – 6. Types 1 – 4 were classified as benign, ranging from hypo-echoic with no visible cortex, to thickened hypo-echoic generalized lobulation. Types 5 and 6 were malignant. Combining a FNA with ultrasound can reduce the SLNB rate by 14%(60). Of interest to my current study, the negative predictive value was 96%, and this increased if Type 4 was excluded. Maximum cortical thickness and 'appearance of cortex' are the best predictors of nodal metastasis (60).

The accuracy of ultrasound of the axilla is difficult to ascertain due to the non-standardized manner in which reporting takes place. Alvarez et al conducted a systematic review on the subject, and their findings were very varied and heterogeneous(65). Within the prediction of positivity using the sonographically measured size of the lymph node, the sensitivity ranged between 48.8% and 87.1% and the specificity was between 55.6% and 97.3%. Using morphological criteria for positivity, the sensitivity was 26.4% to 75.9% and specificity was 88.4% to 98.1%. Unfortunately, they did not investigate the accuracy of sonography in predicting a negative axilla.

Nori et al published results of 147 women with breast cancer, in whom ultrasound was combined with core biopsy if the node was suspicious for malignancy. The sensitivity and positive predictive value in this study was 45.2% and 63.1% respectively. The negative predictive value was 77.2% (78/101). Out of these 78 patients, 23 were positive on histology (false negative). Twenty out of the 23 had micrometastases only, and only 1/23 had more than 3 nodes involved(66). We can thus say that ultrasound cannot accurately detect micrometastasis, however a negative ultrasound appears to accurately exclude metastasis involving less than 3 nodes. With respect to the inability to predict micrometastasis, this is probably insignificant, as micrometastasis do not appear to have a clinical significance.These are important results, as they highlight the question of the need for a SLNB in the face of a well performed negative ultrasound.

In the largest study published on this subject, 105 out of 398 patients with a negative axillary ultrasound had positive nodes on SLNB(67). This is a negative predictive value

of 79%. Of the patients who had a positive SLNB and subsequent ALND, 59% had only the sentinel involved and none had more than 3 nodes involved(67). Even though the criteria for negative nodes were not standardized, the negative predictive value of 79%is acceptable. Furthermore, in the ultrasound negative axilla, it is unlikely that more than 2 nodes will be positive. In addition, in another published series,Ragupathy et al showed a sensitivity of 86% for predicting negative nodes(68).

To summarise the role of ultrasonography in detecting nodal metastasis from the above, we can conclude that:

1. A comparison of the literature is problematic as there is no standard method of reporting findings
2. The sensitivity of detecting positive nodes ranges only between 45% - 85%, and when combined with FNA/Core biopsy, it improves the specificity of a positive cytology to 100%.
3. The negative predictive value is underutilised. It predicts a negative axilla on average 75% of the time, and when it is falsely negative the tumour deposits are invariably micrometastasis, or involve less than three nodes.

1.4 Predictors of lymph node metastasis in breast cancer

The management of breast cancer has evolved.Individual characteristics of a tumour prognosticate their behavior, aiding in the determination ofthe need for adjuvant therapy fortumours with borderline indications, bringing us closer to individualizing cancer treatment. The ability to predict lymph node metastasis on the basis of the primary tumour characteristics would allow us to tailor treatment systemically and locally to the axilla.

Patani et al conducted a systematic review on the topic and classified possible predictors into the following categories: - clinical, radiological, pathological and molecular(5). Ultimately the conclusion was that there was insufficient evidence to recommend any specific marker to be used for the prediction of axillary lymph node metastasis(5). Clinical parameters assessed in thestudy were age and palpability of the primary tumour. The hypothesis was that younger age and palpability of tumour were

associated with lymph node metastasis. These lacked any sensitivity across the spectrum of age categories(5). Of interest to this study is that the combination of an ultrasound negative axilla together with other predictors has not specifically been looked at.

The main pathological characteristics are tumour size, tumour grade, multifocality/multicentricity, hormone receptor status and lymphovascular invasion.

With regard to these parameters, a size of less than 10mm correlates with a 10% chance of having axillary metastasis, and a tumour of less than 25mm has a 15% chance of carrying axillary metastasis(69), however,used in isolation this isa clinically unreliable parameter as even small tumours can have extensive metastasis.

In breast cancer the tumour grade is commonly reported by using the modified Bloom and Richardson scale (78). It includes a measure of nuclear pleomorphism, cellular proliferation and tissue differentiation, and grades it out of a total score of nine. There is a well recognized correlation between tumour grade and lymph node metastasis, however, it does not have the sensitivity to be accurateenough(70). The Ki67 index which is a measure of the mitotic activity, is an independent predictor of lymph node metastasis(71). Histologically the following sub-types of ductal carcinoma have been associated with fewer lymph node metastasis – Tubular, Mucinous, Cribriform, Medullary(72).

The presence of a multifocal/multicentrictumour has been shown to be predictive of axillary nodal metastasis(73). Lymphovascular invasion has also been associated with lymph node involvement and in their study, Viale et al showed a 5.1 fold increase in lymph node metastasis when lymphovascular invasion (LVI) was present(72). D2-40 (Podoplanin) is a pathological stain and a marker of lymphangiogenesis. When positive it is widely used as a surrogate for lymphovascular invasion (78).

Hormone receptor status (Er/Pr) has not been identified as a specific marker of lymph node involvement. Her-2 (Human epidermal growth factor 2) over expression has been associated with a more aggressive type of breast cancer, however, over expression has

also not consistently being found to be associated with increased lymph node metastasis (80).

The location of the tumour in relation to the breast has a bearing on lymph node metastasis. In a prospective study of 135 patients by Susini et al specifically included axillary ultrasound into their algorithm. A Ki67 > 10%, a positive ultrasound and a tumour in the right upper quadrant (RUQ) were consistently associated with nodal metastasis. Conversely, inner quadrant tumours with a Ki67 < 10% and a negative ultrasound predicted negative nodes(74).

The breast research group at Memorial-Sloan Kettering recognized the factors alluded to above as risk factors for metastasis, but also understood the cumbersome nature of applying them within a clinical context(75). They included 3786 patients in their database and developed a nomogram to predict nodal metastasis. Multivariate analysis showed a statistical significance for the following variables: histology, size, multifocality/multicentricity, location, and ER/PR status. Thedeveloped nomogramwas adapted for use on smartphones and personal computers making it clinically applicable (www.mskcc.org/nomograms).Although admitting that their nomogram is not perfect, it can be used as a tool to educate patients on their risk of nodal metastasis (75).

1.5 Summary of the literature review

Significant strides have been taken in the management of breast cancer over the last century. A level twoALND remains the gold standard for staging an axilla. However, there is a clear benefit to a SLNBand there has been a shift towards performing SLNBs in early breast cancer. In this literature review we have taken a journey through the history, have examined the validation studies as well as evaluated the evidence for the indications, the controversies and thecomplexities of this technique. A SLNB has been proven to be equivalent to an ALND in the appropriately selected patient.

However, as shown, the majority of SLNBs (70%) are negative (14 – 16). This is the motivation for this study. Can we safely predict which axilla will be free of metastasis using non-invasive means?

Included in the literature search is a review on the utility of ultrasonography in staging an axilla. Much of the focus in the literature has been on predicting positive nodes, and this has been met with mixed success. However, the sensitivity for predicting a negative axilla approaches 75% (66). This is not accurate enough to replace a SLNB. So, the data was scrutinised, focusing on the false negatives within the cohort of Nori et al and it was discovered that the majority of false negatives were patients with micrometastatic disease. An ultrasound will never be able to detect micrometastatic disease, but examining the literature led me to question the significance of micrometastatic disease. I discovered that an ALND is not mandated after finding micrometastatic diseasein an axilla(35, 81, 82), and this is counter-intuitive. Purported reasons for this are the use of chemotherapy and irradiation to the breast. Also, from the literature it became apparent that there was no widely adopted consensus on what constituted a malignant or benign node, highlighting the user dependency and thus fallibility of ultrasound as a screening tool.

Other predictors of axillary lymph node spread that are readily available were reviewed, so as to determine if they could be combined with ultrasonography, to better predict a negative axilla. Again, the results were mixed, with most studies concentrating on predicting positive nodes, and not negative ones.

Frommy review of the literature, I conclude that a SLNB is currently the best method of staging an axilla, however, there are a significant number of negative examinations. An ultrasound in combination with other markers would be the most appropriate method of staging an axilla non-invasively, but in order to achieve this we would need to understand and overcome the shortcomings of an ultrasound study, notably its inability to detect micrometatstatic disease and the lack of standardization in the reporting of studies. In an effort to decrease these negative examinations, this is an important preliminary study to conduct.

Chapter 2

2.1 Methods and measures

This is a retrospective review of patients who underwent a SLNB between December 2008 and November 2009.The University of Witwatersrand ethics committee granted ethics approval for the study (M090435/2009). All SLNBs performed between December 2008 and November 2009 were screened for eligibility.

The inclusion criteria were all patientsthat: -

- Had a documented negativeaxillary ultrasound (no suspicious nodes) or
- A negative FNA/core biopsy if one was carried out, and
- Fulfilled the criteria for a SLNB as per the indications proposed in the NCCN breast cancer guidelines(2)
    - Early breast cancer and
    - Clinically negative nodes or
    - A negative FNA/core biopsy of suspicious nodes

The exclusion criteria were: -

- Advanced breast cancer
- Patients without an axillary ultrasound report
- Inadequate documentation of findings on ultrasound
- Patients with an ultrasound report suspecting a metastatic node
- Recurrent breast cancer

2.2 Patients – inclusions and exclusions

In total, 183consecutive patients were screened, of these 151 were included and 32 did not meet the inclusion criteria.

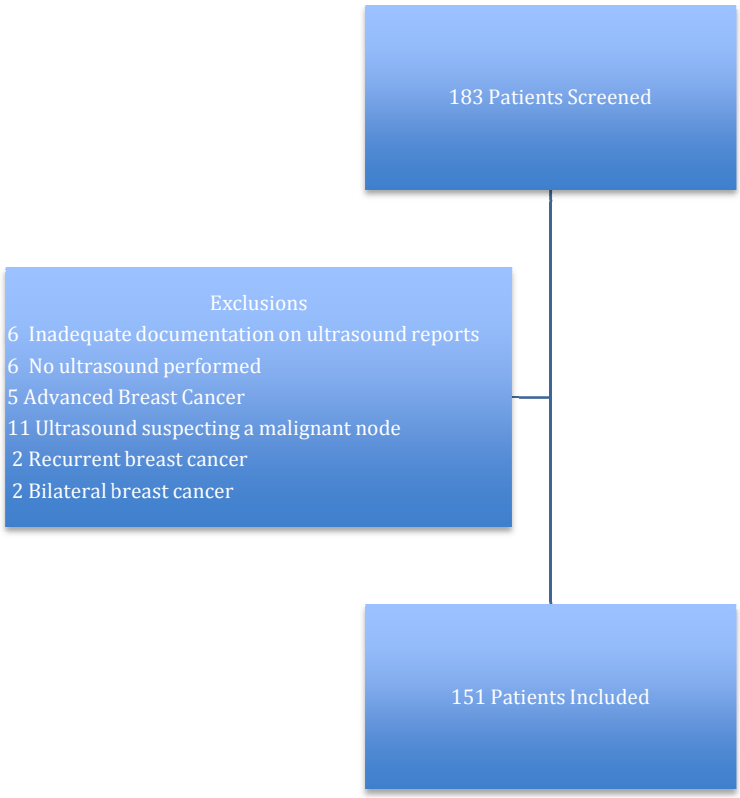

Figure 2.1: Flow diagram showing inclusion and exclusion criteria

2.3Tumourcharacteristics

The following characteristics of the patients and their tumours were documented on a Microsoft Excel (2011) spreadsheet.

The demographics of the patients, their age and race were captured.

I used the initial tumour biopsy specimen results to document the following: - histological type (ductal, lobular, DCIS), tumour grade, hormonal receptor status(Er/Pr/Her2-neu), lymphovascular invasion. The initial biopsy was used, as it represents the information available before a SLNB is preformed, and thus is more relevant than the final tumour histology when aiming to predict axillary metastasis. For the purposes of comparison I grouped the receptor status of patients into Luminal A, Luminal B, Triple negative and Her2-neu. A similar classification was initially proposed by Perou et al in 2000 (76). Triple negative was used as the broader term to encompass basal-like tumours as we could not specifically test for the basal phenotype.

To calculate the size, location and T-stage, the patient'sultrasound reports were used. The ultrasound reports were recorded as being normal or reactive. A reactive node is a histological change in a benign node in response to anon-malignant stimulus, however it is a histological diagnosis and cannot be determined on ultrasound.Interpreting the ultrasound reports with this in mind, the assumption made was that reactive nodes were benign. As ultrasonography is user dependent, I thought it important to document the ultrasonographers identity.

The multicentricity/multifocality of a tumour was determined primarily on the mammogram findings, however a subset of patients had an MRI performed as well, and some had multicentricity/multifocality diagnosed on MRI.

The number of sentinel nodes harvested and the presence of metastasis were documented. This was further subdivided into macro and micrometastasis. The patients with micrometastasis were further separated into those that had isolated tumour cells only.

When available, the final histology specimens were used to record the following variables: the size, histology, hormonal characteristics, lymphovascular invasion and axillary lymph node histology and nodal yield. The aim was to compare the final histology characteristics to the initial biopsy specimen, to gain an indication of the reliability of initial specimens.

Statistica version 9 was used to analyse the collected data. Univariate analysis was used to detect the significance of prognostic factors in predicting lymph node metastasis. P-values were calculated using both Pearson and M-L Chi squared tests for each set of recorded variables.

2.4Surgical practice

The study was conducted at the Netcare-Milpark Breast Centre wheremanagement decisions are made by a multidisciplinary team that is lead by a specialist surgeon whose area of interest is the breast. She runsa referral based hospital practice in Johannesburg that deals exclusively with breast health care.

2.5 Multidisciplinary involvement

Although there is a radiologist within the multidisciplinary team at the Netcare-Milpark hospital breast care centre, the majority of referrals are from other radiology practices. Thus, the reporting of ultrasounds is not standardised. The pathologist in the multi-disciplinary team is responsible for reporting on all SLNB specimens, but outside referraltumour biopsiesare reported by various pathology laboratories around the greater Johannesburg area. It is not economically feasible to repeat all imaging and biopsy reporting.

2.6 Method and technique of sentinel lymph node biopsy

A single surgeon with the relevant expertise performed all the SLNBs at the Netcare-Milpark breast care centre. The unit's policy is to perform all SLNBs as a separate procedure using H&E staining as well as immunohistochemistry, in order to identify micrometastatic disease and isolated tumour cells. Patients with positive nodes had a completion axillary lymph node dissection as a separate procedure, combined with their definitive oncological breast procedure.

The SLNBs are performed in accordance with standard recommendations. A combined dye and radio-colloid technique is employed. The ^{99m}Tc-labelled nano-colloid is injected via the peritumoral route in the nuclear medicine department within the hospital. Whenever possible, the patients are injected with ^{99m}Tc-labelled nano-colloid on the morning of the operation, and their procedures are carried out in the afternoon.

In theatre, prior to the surgeon scrubbing, Patent V blue dye is infiltrated subareolarly. Intraoperatively an axillary crease approach is used, and a gamma camera is used to detect the ^{99m}Tc-labelled nano-colloid. All blue nodes, as well as all nodes with a reading greater than 10% of the maximal nodal reading on the gamma camera are removed.

Chapeter3.

Results

3.1 Demographics

The average age of presentation of the group was 53.3 years (27 – 82), with the majority being between 40 and 70 years old. The predominant ethnicity of the cohort was Caucasian (87.6%).

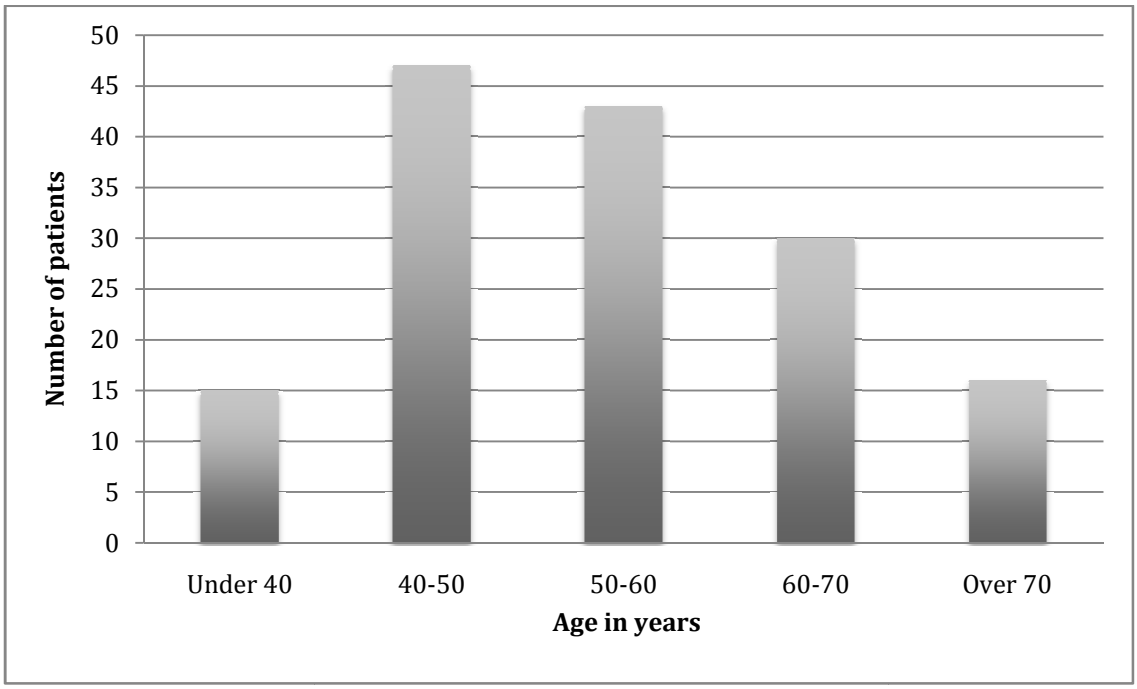

Figure 3.1: Bar graph representing the age distribution of the patients in the study(N=151)

3.2 Nodal metastasis

Of the 151 SLNBs performed, 43 (28.4%) had evidence of nodal metastasis on axillary nodal histology.Twenty-six(17.2%)of the 43 had macrometastasis (>2mm) and the remaining 17 (11.2%)had micrometastatic disease (< 2mm). The significance of differentiating out the micrometastatic disease becomes relevant when deciding on the

management strategy. If we treat micrometastatic disease as a negative axilla, then this brings the negative predictive value of an ultrasound to 82.8%.

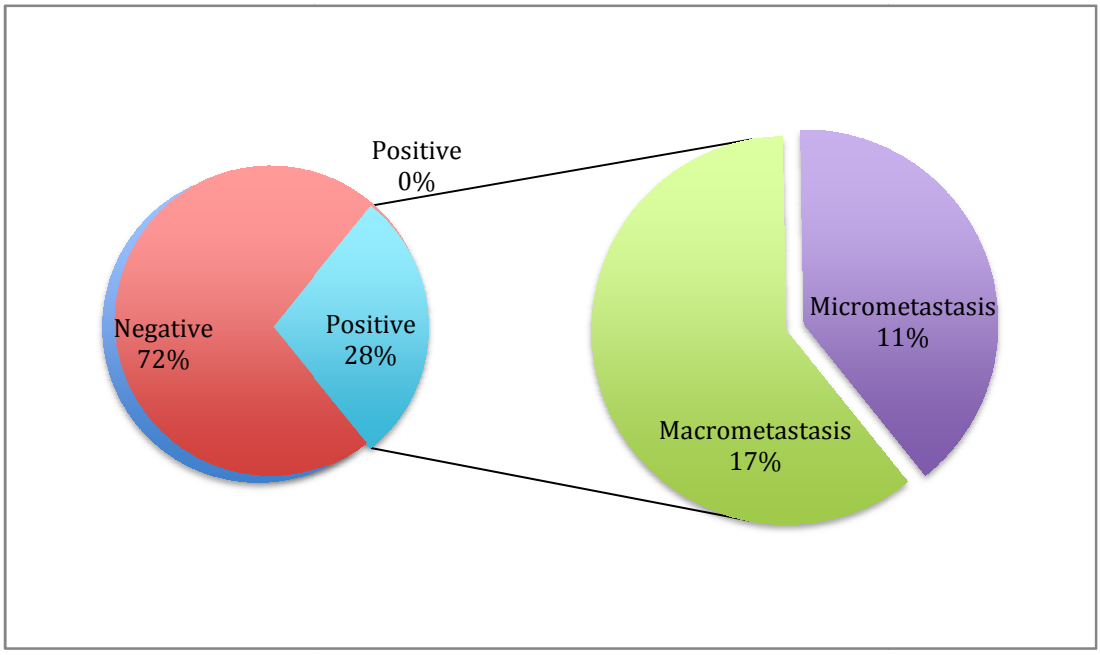

Figure 3.2: Pie chart representing the distribution of positive and negative nodes as percentages (n = 151)

3.3Tumourhistology

The initial biopsy histology was categorized into a DCIS group and an invasive group. The invasive group was subdivided into the following groups: - ductal, lobular and other (mucinous, papillary, medullary, adenocarcinoma).

There was a significant association with DCIS and an absence of nodal metastasis when compared to the invasive subtypes (p = 0.0203). There were 17 patients in total with DCIS. Ductal carcinoma *in situ*alone was reported in 14 of the biopsy specimens, and the other three had DCIS in combination with another histological feature. Two had DCIS and lobular cancerisation and one patient had DCIS and Paget's disease.

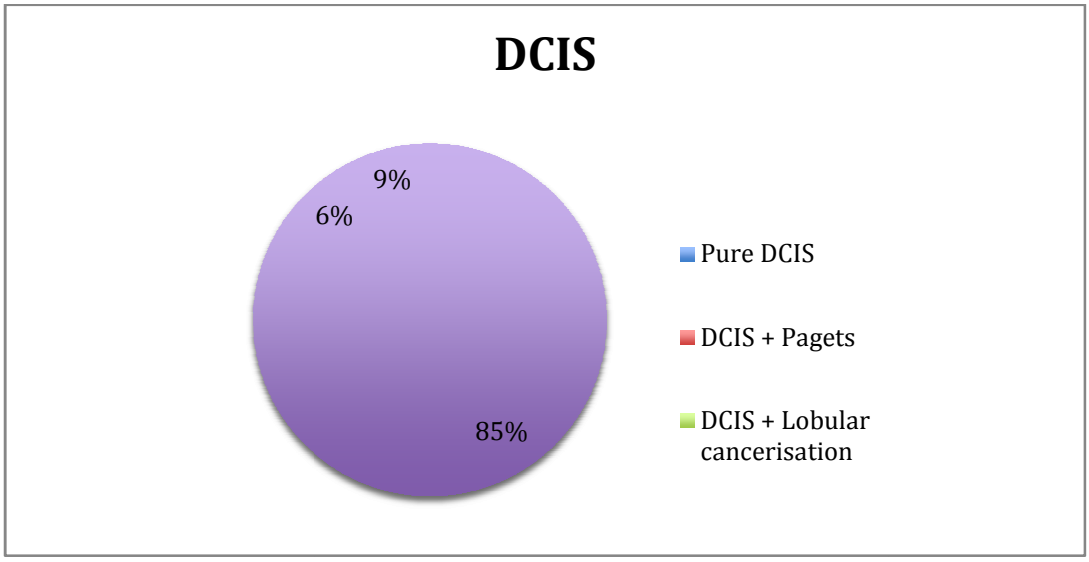

Figure 3.3: Pie chart showing the various DCIS groups (N = 17)

Of the17 patients with an initial diagnosis of DCIS, six ultimately had an invasive malignancy on their definitive histology.This is a rate of 35.2%, and is on the upper limit for invasion on core biopsy specimens that is reported in the literature (47).

In the invasive group, there was no correlation between the histology type of the biopsy specimen and the nodal status.

3.4 Size and T stage

The average diameter of the tumourswas 16.7 mm ± 9.2mm (range: 0.9 – 48 mm) and the T stage of the tumour was calculated from this using the ultrasound measurements. The T1 group had 74 patients in total, and they make up the majority. Thirteen patients had microcalcifications detected on imaging only (T1mi). The study cohort represents a typical group of early breast cancer patients (Figure 3.4).

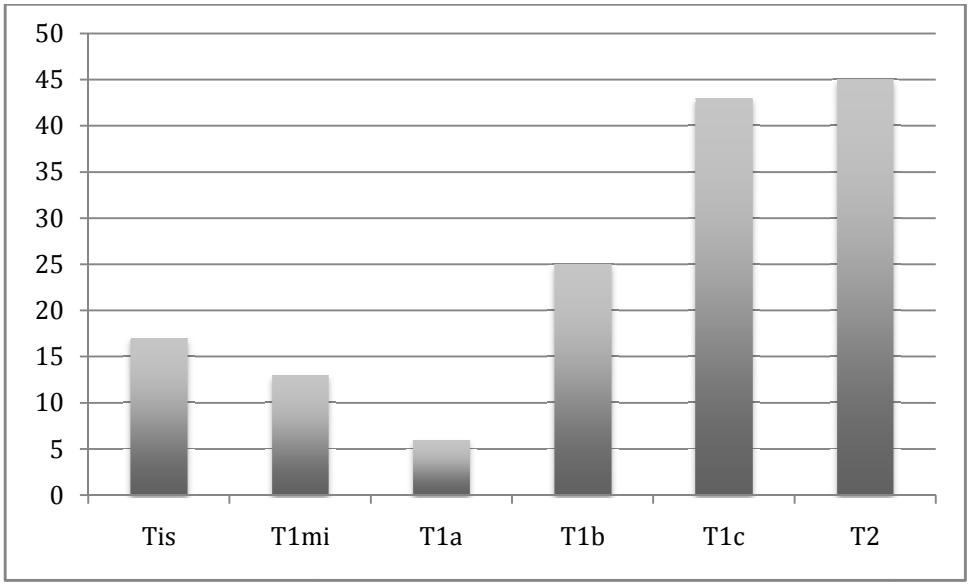

Figure 3.4: Number of patients separated out into their T stage groups Categories as per the AJCC7thed(N=151)

T stage as predictor of nodal metastasis in invasive breast cancer was not statistically significant. A good example illustrating this was that six of the 13 patients in the microcalcification group had nodal metastasis (Figure 3.5), where intuitively one would expect a lower rate of metastasis. Only the DCIS (Tis) group was predictive forno nodal metastasis, where only one of 17 patients had a metastasis and this was a micrometatstasis.

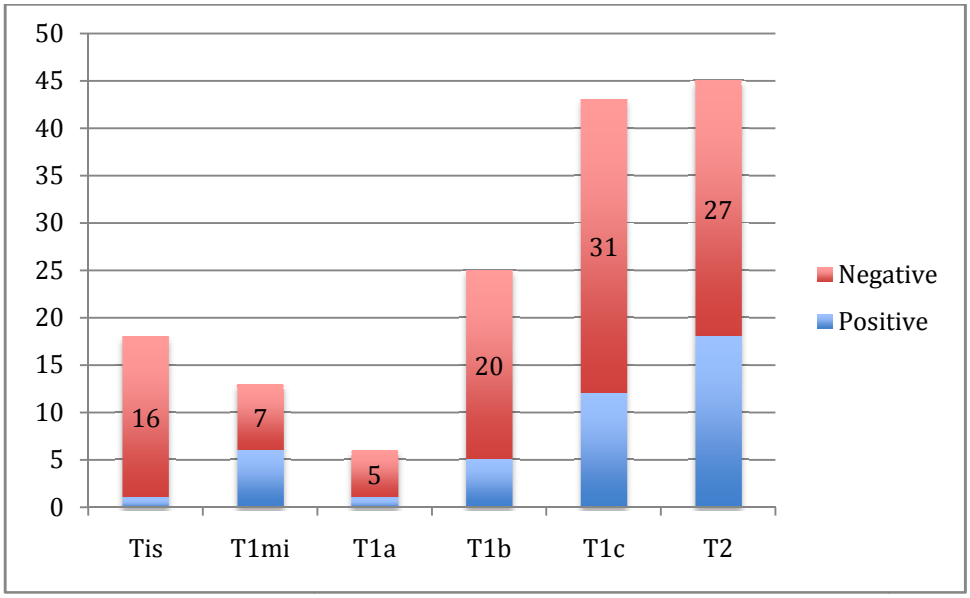

Figure 3.5: The number of nodal metastasis separated into their T stages

3.5Multicentric/multifocal disease

Although patients withunifocal disease had significantly more negative axillary nodes (p = 0.0002), 17.4% of patients with unifocal disease had a positive node (Figure 3.6). Thus, the negative predictive value of unifocality in predicting a node negative axilla is 82.6%.

Interestingly, the use of MRI in detecting multicentricity/multifocality,changed the operative decision in eight of the patients.

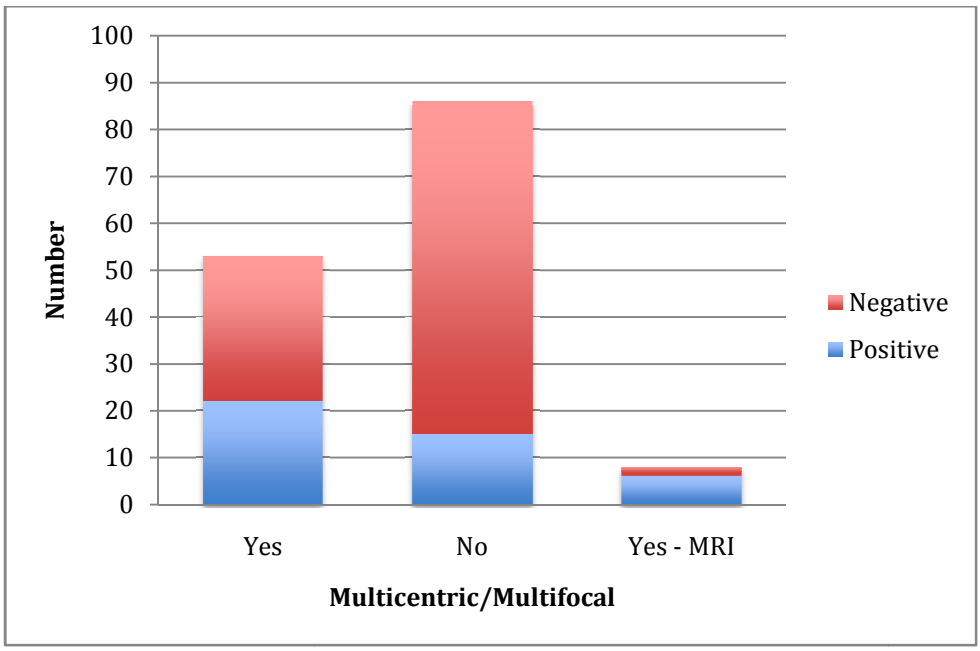

Figure 3.6: Multicentricity and its relationship to nodal metastasis (N=147)
Yes – MRI: MRI was used to establish or refute the diagnosis

3.6Tumourlocation

The tumours were categorized based on the quadrants from which they originated. The categories were: - upper outer quadrant (61), upper inner quadrant (22), lower outer quadrant (12), lower inner quadrant (4), central (44) and multiple quadrants (7).

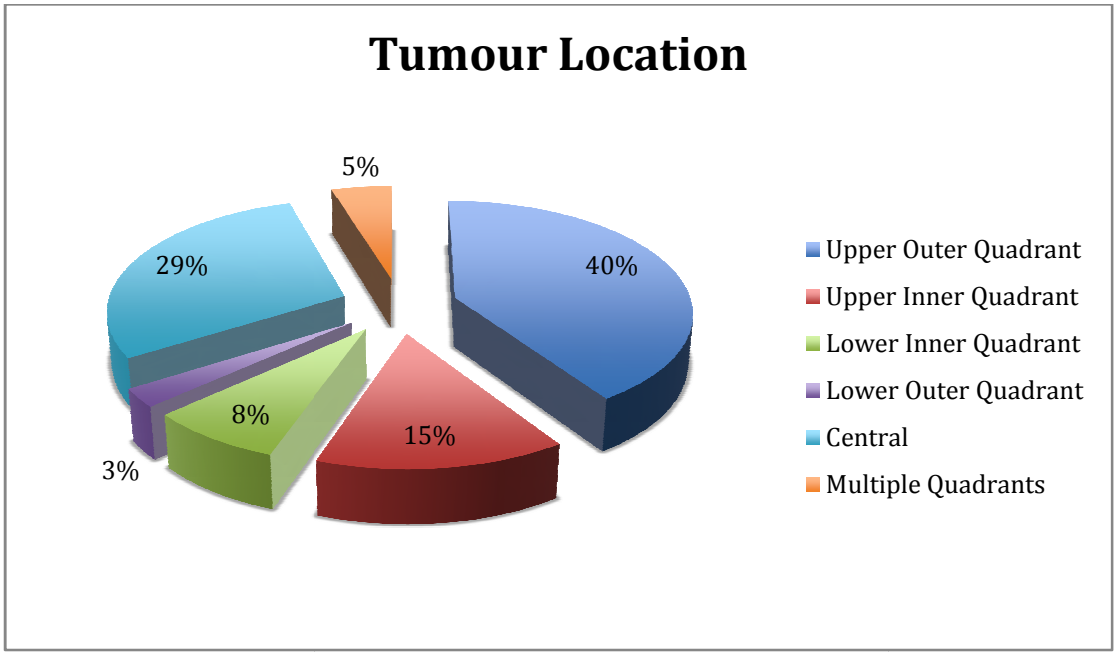

Figure 3.7: Percentages of tumourlocationsrepresented as quadrants of the breast

There was no association between nodal metastasis and tumour location in this study. Tumours in the lower inner quadrant had no associated metastasis. However there were only 4 tumors in that location, and the sample size is therefore too small to draw any meaningful conclusion (figure 3.8).

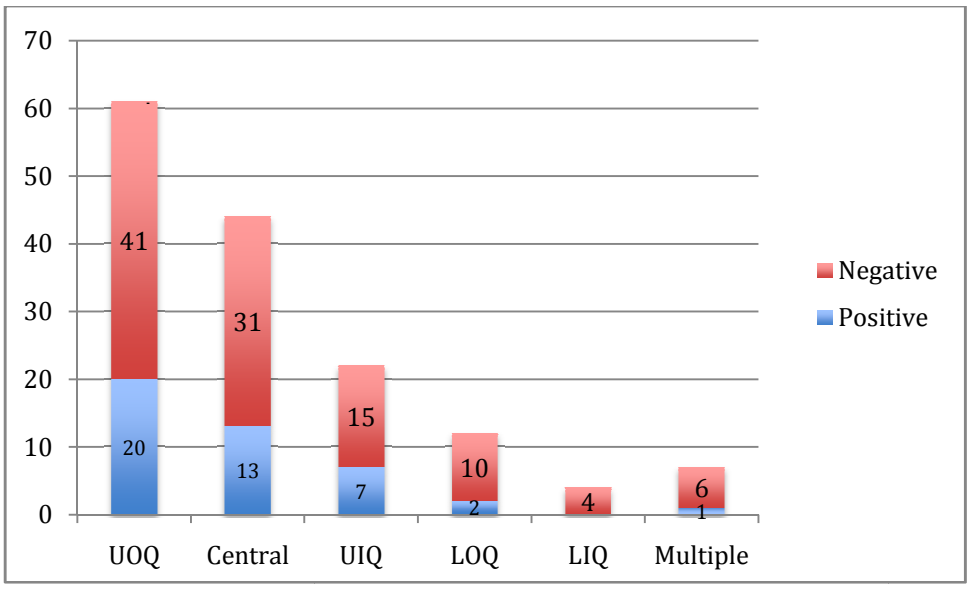

Figure 3.8: Tumourlocation and its relationship to nodal status

3.7Tumourgrade

The initial biopsy samples graded the malignancies, using the Bloom and Richardson grading system. Biopsies were graded into the following categories: -low, intermediate and high. Twenty-one of the biopsy specimens had no comment on tumor grade and this accounted for 13.9% of the specimens.Interestingly, twenty-two percent (10/44) of the high grade specimens had nodal metastasis, and twenty-five percent (7/28) of the low grade specimens had associated nodal metastasis (Figure 3.9). There was no statistically significant association between the nodal status and the grade of the tumour.

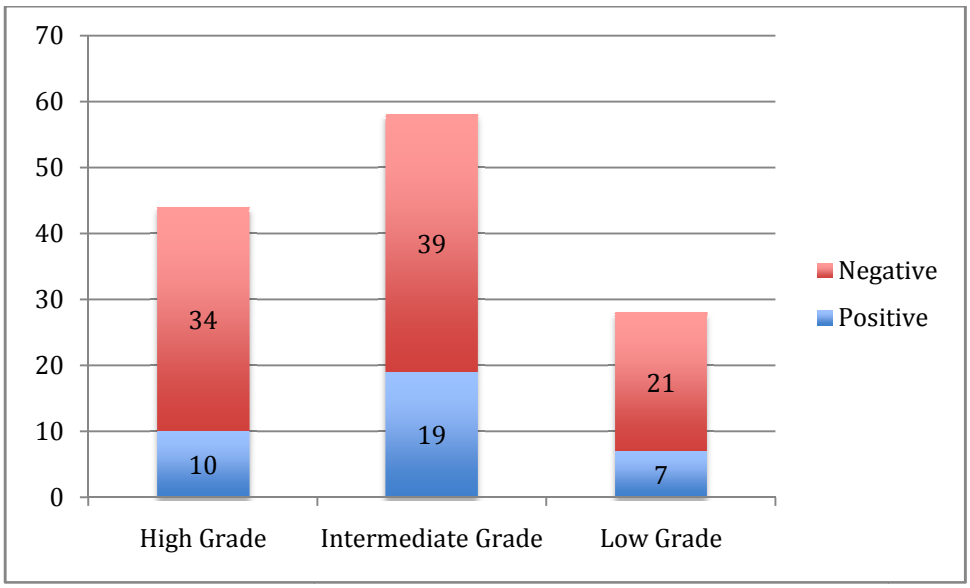

Figure 3.9: Tumor grade and its relationship to nodal status

3.8 Hormonal markers

Traditionally, hormonal markers in breast cancer were limited to oestrogenreceptors (ER) and progesterone receptors (PR). Over the last 15 years, and with the advent of Trastuzumab (Herceptin) as an effectivemonoclonal antibody which targets the Her-2 oncogene, Her2-neu has been included as part of the reporting process.

As discussed above, the initial biopsy results were utilized for the data analysis. Out of the 151 patients, twenty-nine had incomplete data.There was no statistically significant

association between the subtypes and nodal metastasis. The largest subtype was the luminal B (60/122), followed by Her2-neu, triple negative and luminal A. I expected the Her2-neu group to have a greater proportion of patients with nodal metastasis, but they did not (8/26) (Figure 3.10).

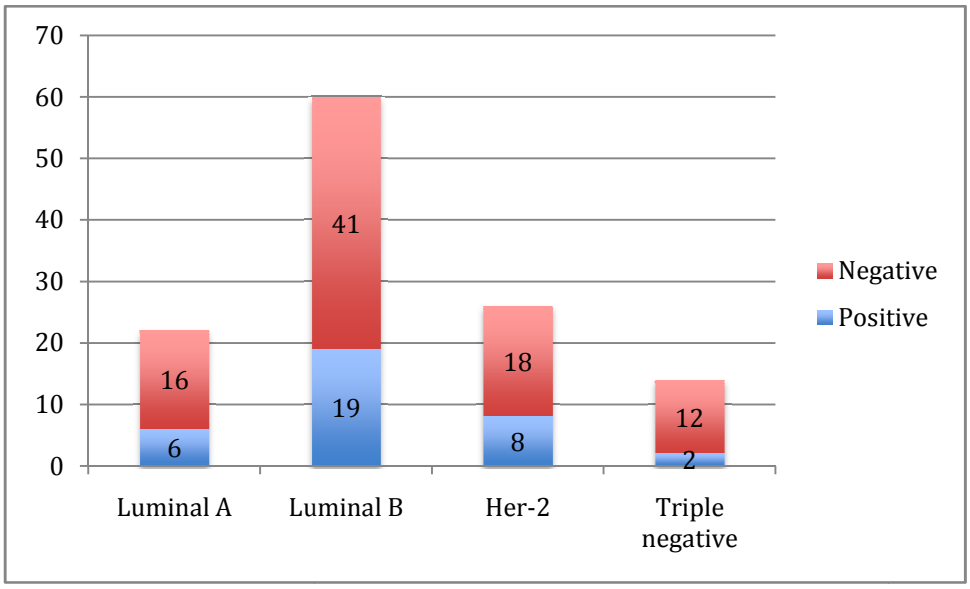

Figure 3.10: Hormonal patterns and its relationship to nodal status(N=122)

3.9Lymphovascularinvasion

Eighty-nine of the 151 patients had pathological documentation of the absence or presence of lymphovascular invasion (LVI) in their biopsy specimens. In thegroup who were negative for lymphovascular invasion(LVI), 19/69 (27.5%) had nodal metastasis. Conversely, only 5/20 (25%) with LVI had nodal metastasis. Sixty-two (41%) of the patients did not have any comment on their LVI status in their initial biopsy pathology reports (Figure3.11). Lymphovascular invasion was found to be an unreliable marker of nodal status. Furthermore, there was a lack of consistency between the final histology specimen and the initial biopsy with respect to LVI (Figure 3.12).

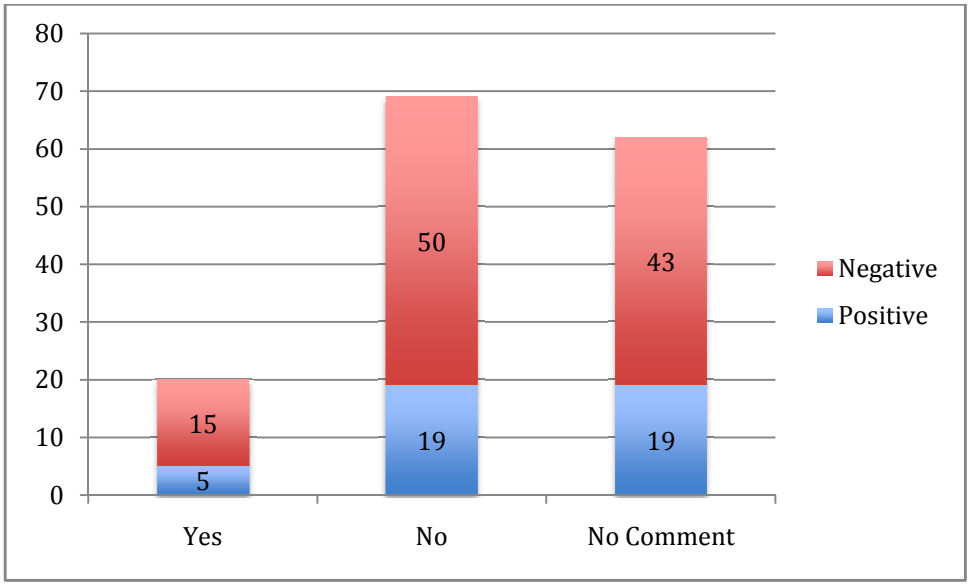

Figure 3.11: Lymphovascular invasion on the initial biopsy specimens and itsrelationship to the nodal status (N = 151)

Of the 17 specimens that were LVI positive, only seven remained positive in the final histology specimens (41%) (Figure 3.12), and of the 55 patients that were initially negative for LVI, eleven were positive for LVI on final histology (20%) (Figure 3.12).This inconsistency brings the reliance of LVI as a marker of nodal disease into question.

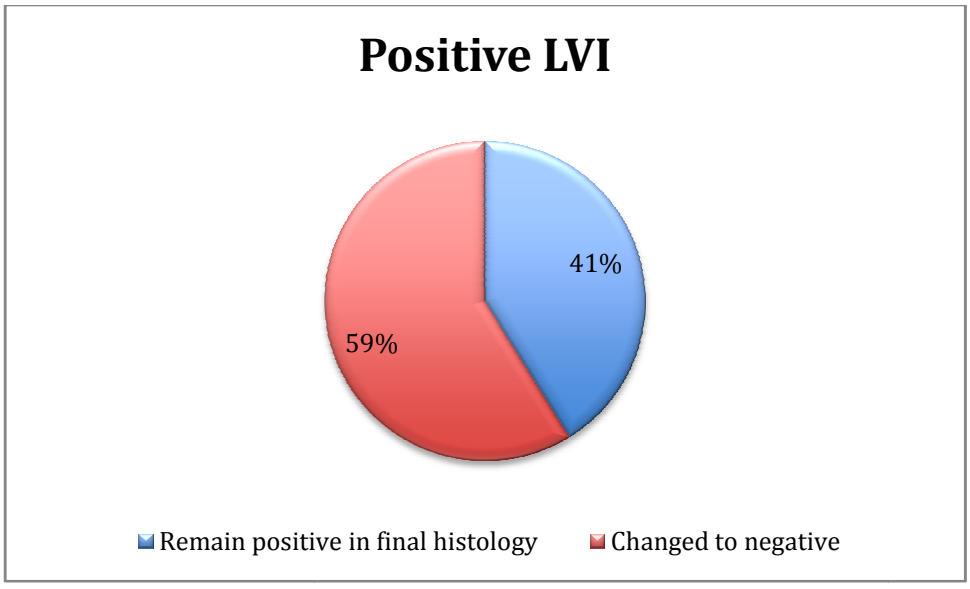

Figure 3.12: The change in LVI in patients who were initially LVI positive.

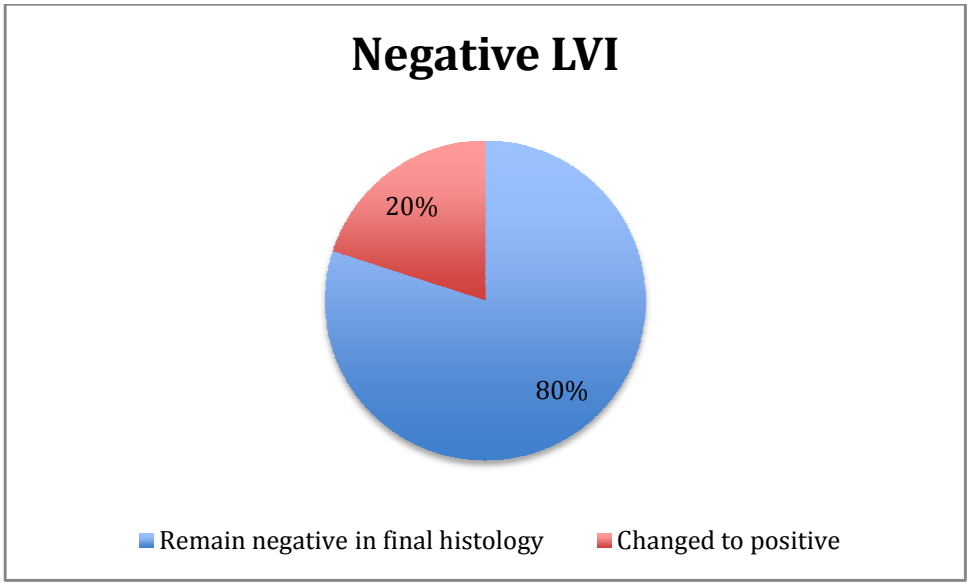

Figure 3.13: The change in LVI in patients who were initially LVI negative.

3.10 Sentinel lymph node data

The average sentinel lymph node yield in the cohort of 151 patients was 3.75 nodes per patient. Of the 43 patients with positive sentinel nodes, 32 (74.4%) had only one node involved, five (11.6%)had two nodes involved, five (11.6%) had three nodes involved and only one(2.3%) had 5 nodes involved. From the 32 with one node involved, 17 (53.1%) had micrometastasis.

Of the patients that proceeded to ALND, only oneof 23 had a non-sentinel lymph node metastasis. This patient had two of two nodes positive on SLNB. From the patients that had at least one node negative and only one node positive, none had a positive node on final histology. However, all of these patients had chemotherapy between their SLNBs and ALNDs. In addition, there was a complete pathological response of tumours, in six of the 23 patients who had an ALND.

3.11 Ultrasonographer and ultrasound results

Of the 151 ultrasounds performed, 72 were performed by four radiologists, and the other 79 were performed by 44 radiologists. We grouped the four radiologists with more

than 10 procedures each together, and the remaining 44 were grouped together. We expected that the more experienced radiologists would be more accurate in their assessment, however this did not hold true. Our data showed that the choice of ultrasonographer had no bearing on the results when analysed in this manner.

3.12 Magnetic Resonance Imaging

Onehundred and eight patients, in the cohort of 151 patients, had an MRI of the breast. Theirnodes were categorized as positive on MRI, negative on MRI or nodes visualized but probably benign (Figure3.13). Magnetic Resonance Imaging was an inconsistent predictor of nodal metastasis in breast cancer. An MRI was anticipated to have a good negative predictive value, but of the 72 negative MRI's, 16 had positive nodes (22%) and of the MRI's that suggested positive nodes, eighteen of 32 were negative (56%).

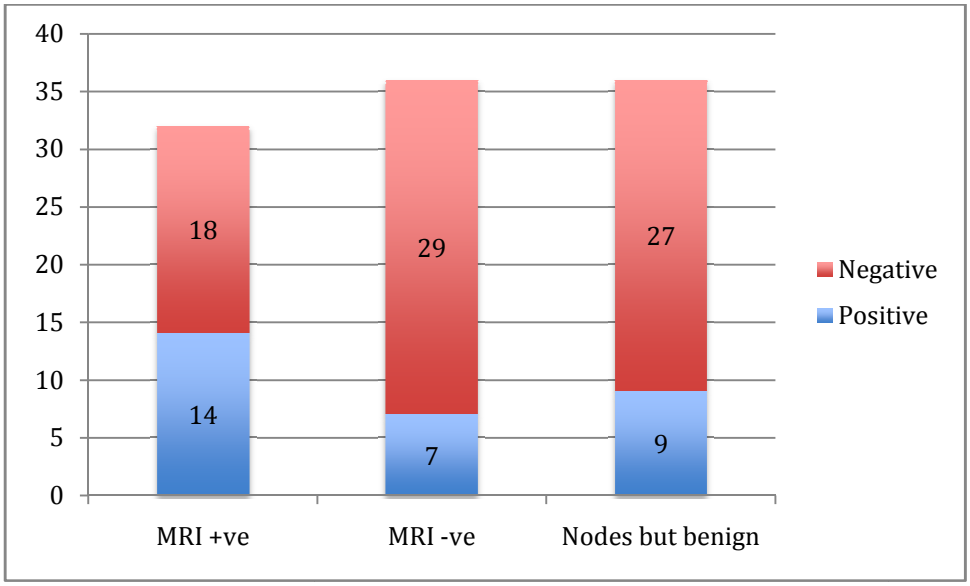

Figure 3.14: MRI as a predictor of nodal metastasis in breast cancer (N=108)

Chapter 4.

Discussion

Progress within the last 50 years has revolutionized the way breast cancer is treated. Improved imaging and screening programs have changed the face of the disease.Cancers that present at an early stageare treated more effectively and have better outcomes. However, new treatments create new challenges, and the rate of negative SLNBs is one such challenge. The rate of negative SLNBs is 70% - 80% (14 - 16)and the possibility of decreasing this, needs to be investigated.

In order to accomplish this, the question that arises is, can we safely and non-invasively predict which axillas will be free of metastasis and thus decrease the amount of negative sentinel lymph node biopsies performed?

In order to answer the question, the problem needs to be contextualised. Seventy to eighty percent(14 – 16)of SLNBs are negative, and if we extend this to includemicrometastatic disease, my results show a negative (non significant) predictive value of 82.8%.This impliesthat as a test for negative nodes, a SLNB is not specific enough. A negative SLNBadds to the morbidityof a patient. When performed as a separate procedure, it exposes patients to the risks of a general anaesthetic. Theyalso have associated shoulder discomfort, paraesthesias, lymphedema(17) andthen there is the psychological burden of an added procedure which is often forgotten. However, omitting a SLNB may result in residual disease, which may impact on the mortality of patients.

Therefore the risk of not performing a SLNB may be summarised as follows: preventing morbidity in the majority at the expense of mortality in the minority,while, even onedeath is unjustifiableas it is entirely preventable.

4.1 Aims and methods

The aim of my study was to improve prediction patterns of nodal spread with a view to decrease negative SLNBs. I retrospectively studied the records of patients who had undergone a SLNB over a ten-month period. Only those who had a documented negative ultrasound or a negative FNA (if it was done) were included in my study, resulting in a total of 151 patients.

My results showed that based on the information from the initial biopsy (ER/PR/LVI/grade/histology) and radiology (size, location) I was unable to predict nodal involvement. Patterns of spread appeared to be random, for example, even small tumours with 'favourable' characteristics had nodal metastasis.

However, my study showed that a non-standardized ultrasound of the axilla can predict that if axillary metastasis are present, three nodes or less will be affected in 97% of the cases. This is an important finding considering the findings of the Z0011 trial (Axillary dissection versus no axillary dissection in women with invasive breast cancer and sentinel lymph node metastasis), which showed that a SLNB is equivalent to a completion ALND in patients with one or two sentinel lymph nodes positive (56).

4.2 Radiological results

Importantly, I showed that ultrasound predicted a negative axilla in 71.6% of the patients included in the study. This concurs with international literature(14). Following the results of the NSABP-32(77) and the follow up study by Weaver et al(54) on occult metastasis and its management, the recommendation by the NCCN was to not proceed with a completion ALND in patients with occult metastasis(2). In this study, when I exclude the patients with occult metastasis (17/43) the predictive value for an axilla with non-significant metastases improved to 82.8%.

In the recent past, the major body of work done on ultrasound of the axilla and its use in breast cancer has tried to improve the positive predictive value. In order to achieve this, biopsy techniques have been employed with a view to avoid a SLNB and proceed directly to an ALND in patients with a positive FNA or core biopsy of their axillary nodes. This has reduced the rate of SLNBs by 14%(60).

Yet, there has been no coordinated effort by the breast care community to predict negative lymph nodes and reduce the rate of negative SLNBs. In my study, there was no standardization of ultrasound reporting and the reports were from multiple ultrasonographers, with varying levels of experience. A lack of standardization in reporting is the major impediment to progress in this arena.

However, Bedi et al(64) proposed a classification for characterizing lymph nodes. Types 1-4 were benign, Type 5 and 6 suggested malignancy. Their negative predictive value for Types 1-4 was 96% and this improved if Type 4 was removed. The incorporation of this validated classification in reporting would allow us to objectively stratify grades of negative axillas, and in that way identify a true negative axilla more accurately.

If, for example, there are 100 patients with early breast cancer, we can assume that 70 of these patients will not have axillary metastasis. If we were able to classify 30 of these into Types 1-3, we could accurately rule out metastasis in these nodes and thus sparing patients a SLNB. I would like to see us clinically validate this classification within a trial setting.

4.3 Tumour characteristics

In the literature, there are conflicting results as to which markers accurately predict nodal metastasis. However, the majority of publications find significance in one of their studied parameters(69,72,74,75) and this may represent a publication bias.

I studiedtumour size, T stage, multicentricity/multifocality, grade, hormonal markers, LVI, tumor location and histology and found a significant association with nodal metastasis in only two of the studied parameters.

I found that multicentricity/multifocality predicted for nodal metastasis. However, 17% of the patients with unifocaltumours had a positive node. Therefore, as a marker, it lacks the accuracy required to make a firm recommendation.

4.4 Ductal carcinoma*insitu*

I found tumour histology to have a significant associationwith nodal metastasis,in particular DCIS predicted a negative axilla. Initial biopsy specimens were utilized instead of the final histology, as the aim was to recognise factors that influence nodal metastasis, with a view to change surgical strategy. With this in mind, of the 17 patients that had an initial diagnosis of DCIS on biopsy, six had an invasive malignancy on final histology. This is an invasive malignancy rate of 35.2%, which is on the upper limit for invasion of DCIS core biopsy specimens. The reported rate in the literature is between 8-38%(47).

Furthermore, oneof the patients that had a positive SLNB had a 0.75 mm micrometastatic deposit in her node. Her initial tumour on preoperative imaging was thought to be 13mm by 8mm (T1). The final primary tumour size was 8mm and completion ALND yielded no additional nodal metastasis. Interestingly, this was an intermediate grade tumour, not a high grade or a palpable DCIS lesion.

Although I was limited by an extremely small sample size, my data suggests negligible benefit in performing a SLNB in DCIS, however our rate of invasion on final biopsy specimens is high and thus the suspicion of invasive malignancy remains high. Thus, in thisparticularbreast care centre, the practice of SLNBs in DCIS should continue. However, in patients with DCIS,I found no evidence for performing a separate staging SLNB as the risk of metastasis is small, and a combined breast procedure with frozen section analysis would have been more appropriate. These conclusions were not part of the aims of this study, and need to be further investigated.

4.5 Metastatic axillary nodes

In the group of patients with positive nodes, only one patient had more than three nodes involved (1/43). Another, very interesting finding was that of the patients that had an ALND, only one had an additional node involved (1/23). Twenty-two of the twenty-three had a non-therapeutic ALND. All these patients had preoperative chemotherapy, and this may explain the results. In addition, six of the twenty-three patients had a complete pathological response to their primary tumour (CpR).

In the ACOSOG Z0011 (56) trial, patients with a positive SLNB were randomized to ALND or observation. Their patients had less than three nodes involved on H&E staining and had breast conservation therapy with whole breast irradiation (not axillary radiation). Interestingly, twenty-seven percent of the ALND group (120/446) in the Z0011 trial had additional metastasis and if we extrapolate this to the group that had a SLNB alone, they would have had a similar rate of extra-sentinel lymph node metastasis.

Why is it that the two groups in the Z0011 trial had the same outcomes, when the SLNB group had metastasis that was not surgically removed? Could it be that 96% percent of the patients received adjuvant systemic therapy and all the patients had a lumpectomy and breast irradiation? The inference is that the radiation and chemotherapy must confer protection over smaller volume axillary lymph node metastasis and thus render them inert. Furthermore, if this assumption is correct, then the question is, why the need for an axillary staging procedure at all?

The question that this data raises, in conjunction with the Z0011 data, is whether aSLNB is mandatory in a patient that is ultrasound negative, and is receiving the combination of breast conservation therapy, radiotherapy and adjuvant chemotherapy. My results show that a negative axillary ultrasound screened out axillas with threeor less positive axillary nodes in 96% of cases, and tying this in with the Z0011 trial, where the inclusion criteria for randomization was the presence of less than three nodes that were positive, this would infer that patients with a negative ultrasound will be cured by a SLNB.

Our understanding of cancer biology and patterns of nodal spread are incomplete. The disease free survival and mortality after an ALND as compared to SLNB are equivalent (58, 59). However, their sensitivities vary, anda problem that is inherent to SLNBs is their false negative rate. When performing SLNBs using paraffin section, the sensitivity is 90-93% (30), while utilizing frozen section, this percentage drops to 71.6%(table 4.1)(58,59). My data concluded that an axillary ultrasound has an82.8%negative predictive value to detect negative nodes in an axilla. At first this may appear to be unacceptably low, but when compared to a SLNB using a frozen section (71.6%), it is superior.

Table 4.1: A comparison of ALND, SLNB and axillary ultrasound

	ALND	SLNB paraffin	SLNB frozen	Ultrasound
Outcomes	Equivalent	Equivalent	Equivalent	N/A
Level of invasiveness	Maximum	Less invasive	Less Invasive	Non-Invasive
Sensitivity	100%	90% - 95%	71.6%	82.8%

I am not suggesting replacement of SLNBs with an ultrasound, based on this single centre retrospective review, however, it does highlight imperfections in our understanding of cancer spread. I believe that as our understanding improves, the management will change and axillary ultrasounds will become increasingly utilized in decision-making.

A first step towards this is a prospective, randomized study where patients with a negative ultrasound (who are to receive chemotherapy and radiotherapy) would be assigned to either a SLNB or an ALND. This could potentially provide more insight into whether a negative ultrasound is equivalent to a SLNB.

4.6 Lymphovascular invasion and the reliance on the initial biopsy

Lymphovascular invasion (LVI) is reported in the literature to be a marker of nodal metastasis(72). In this study initial biopsy resultswere used to understand the association betweenlymphovascular invasion and nodal metastasis. The results from my study do no not support the link between the two. However, a weakness of my study is that a significant proportion (61/151) of the biopsy results did not test for it.

Unfortunately, the sample set is too small generalise, however, there is no association between LVI and nodal status in this data. This is possibly because I used the biopsy results and not the final histology results. Final pathology results are under greater scrutiny than initial biopsy specimens, and therefore, probably have a higher yield of positive LVI.

Ialso assessed the accuracy of initial biopsy results when compared to the final histology specimens, in particular lymphovascular invasion. The correlation was only 20%, with a significant proportion of results changing when reviewed in the final histology. The proportion of patients in the DCIS group that had invasive malignancies, in conjunction with the poor LVI correlation, brings into question the reliance that is placed on core biopsy results.

4.7 Limitations of this study

The limitations of this study are that this is a retrospective review and we were reliant on information stored in files that were not designed to measure our outcomes. There was a significant amount of missing data for most of the studied parameters, and this weakened the robustness of the data calculations. Moreover, as this is a private practice, and there were various pathology laboratories and radiology practices involved, each had their own reporting standards, and this led to inconsistencies in documentation. In retrospect, our sample size was also inadequate to answer this question, especially the DCIS subset.

Chapter 5.

Conclusion and recommendations

My research concluded that in early breast cancer, neither an ultrasound nor various tumour characteristics were able to accurately predict metastatic nodal spread to an axilla. Furthermore, it questioned the reliance on initial biopsy specimens, as the association with final tumour specimens was poor. This was particularly marked for DCIS and LVI.

Our understanding of cancer biology and patterns of spread have progressed substantially in the last century, yet our knowledge is incomplete. Breast cancer research has provideda template for research in the surgical domain and the NSABP trials have been aninvaluable repository of knowledge to turn to in order to make evidence based management decisions. SLNBs have been validated through this body of research and are an integral part of current practice.

A SLNB is a minimally invasive method of staging an axilla, and is the procedure of choice in patients with early breast cancer and a clinically benign axilla. The issue is that the majority (70%) of these SLNBs are negative.It would be incorrect to equate a negative SLNB to an unnecessary one, as it remains a vital staging procedure, however, it remainsan invasive procedure. Thus, in asking the question“Can we decrease the rate of negative SLNBs”, we are exploring whether we can replace a SLNBwith a non-invasive method to accurately stage an axilla in a select group of patients.

With regards to an ultrasound of the axilla, my data accurately predicted a negative axilla in 71.6% of patients, and this improved to 82.8% when micrometastasis were excluded. This is not accurate enough to replace a SLNB. However, when we compare these results to the sensitivity of a frozen section SLNB, the accuracyis similar. In this study a negative ultrasound could predict that an axilla, when positive would have three or less lymph nodes involved.

A frozen section SLNB has equivalent outcomes to an ALND in terms of disease free survival and 5 year mortality (58,59). This highlights the imperfections in our understanding of disease patterns, as an ALND and a SLNB have varying sensitivities, yet their outcome measures are the same. I suspect that if we compared a negative ultrasound without subsequent axillary surgeryagainst these outcome measures, the results will be similar.

Another key finding was that ultrasounds were not reported in a standardized manner or classified according to an objective and validated method. The emphasis thus far has been on predicting positive lymph nodes, and not negative ones. Scoring systems for lymph nodes do exist. For example, an excellent scoring system has been proposed by Bedi et al (64). This classification groups nodes into types 1-6, where, Type 1 is definitely benign and Type 6 is definitely malignant. Using a classification similar to this will screen the negative axillas more objectively, and provides a starting point for future research on this topic. I believe this classification would allow us to identify a sub-group within the group of negative ultrasounds that will definitely be benign. This would be a significant step forwards in the treatment of breast cancer.

Insights from this study suggest that these issues could be further examined as follows: -

- A prospective study evaluating a classification system for reporting axillary nodes with particular emphasis on predicting negative nodes is required.
- If the classification system is validated, it should be used to evaluate ultrasound negative axillas in two randomized groups. One group to have a SLNB, and the other to be observed. (Similar protocol to Z0011)

This will add to the body of knowledge and clarify the role of an ultrasound in early breast cancer.

In addition, DICS patients have a low rate of positive nodes, and I recommend that their SLNBsare combined with their definitive surgery with the use of intraoperative frozen section.

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