Cape Peninsula
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A REVIEW OF THE SENTINEL NODE IMAGING PROTOCOL AT A TERTIARY HOSPITAL IN THE WESTERN CAPE

by

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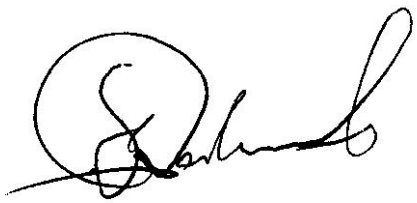
DECLARATION

I, Theodore Russell Geldenhuys, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



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ABSTRACT

Introduction: Breast cancer has an orderly and predictable spread via the lymphatics to the sentinel lymph node (SLN). However, it is not possible to predict which lymph node basins will be involved. Hence, the accurate identification of the SLN is important to evaluate the spread of the primary tumour to the specific lymph node basins. Sentinel node imaging (SNI) involves the administration of a radiopharmaceutical at or close to the primary tumour for the pre-operative imaging, followed by the use of an intraoperative gamma probe during surgery. The current protocol requires the patient to visit the nuclear medicine department twice on the day prior to surgery with a possible third visit 24 hours after injection just prior to surgery. The aim of this study is to identify at which imaging time the SLNs are best identified and to propose possible changes to SNI protocol to reduce the number of imaging times without compromising the number of SLNs identified. The findings will be used to suggest possible changes to the current protocol at the research site, with the view of reducing the number of visits the patient needs to make to the nuclear medicine department.

Materials and methods: This retrospective study used the data from 308 patients who underwent SNI procedures for breast cancer between January 2012 and June 2016. Demographic data were retrieved from the original patient request forms reflecting the date of the study, age of the patient and the site of the lesion in the breast. Histology reports retrieved from the hospital database detailed whether there was metastatic spread to the SLNs identified. Data from the imaging archive and reports included the site of the SLN, the number of SLNs identified and the time of imaging at which a sentinel node was identified. Chi-square analysis was used to find differences in the categorical measurements of the study site data and that of the seminal study. A Student's t-test was performed to estimate the variation among the different imaging times during the SNI procedure.

Results: The study site's imaging protocol identified the SLN in 276 out of a total of 308 patients. Eighty-eight patients had histologically tumour positive SLNs.The results indicated identification of the SLN in 90% of the cases on the delayed 2-4 hours images compared to 27% and 43% on the dynamic and early delayed images respectively.

Discussion: The study site yielded data consistent with that reported in Group B of the seminal study with identification of the SLN in 90% of the cases compared to 94% reported in the seminal study.

Conclusion: The study site's SNI protocol demonstrates the identification of the SLN in 90% of all the patients. The analysis suggests that the SNI protocol can be amended by performing only delayed imaging at 2-4 hours and excluding the dynamic and early planar images, thus resulting in decreasing the number of times that the patient is required to visit the department prior to scheduled surgery.

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DEDICATION

For my family, Jowina, Tarren, Tazwin, Tyla and Thomas

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ABBREVIATIONS

ALND: Axillary lymph node dissection

BMI: Body mass index

CEUS: Contrast enhanced ultrasound

DCIS Ductal carcinoma in situ

EANM: European Association of Nuclear Medicine

FN False negative

FP False positive

ICG: Indocyanine green

LD: Low dose

LIQ: Lower inner quadrant

LOQ: Lower outer quadrant

LN: Lymph node

LS: Lymphoscintigraphy

NIR: Near infrared radiation

NM: Nuclear Medicine

PET: Positron emission tomography

SLN: Sentinel lymph node

SLNB: Sentinel lymph node biopsy

SNI: Sentinel node imaging

SNMMI: Society of Nuclear Medicine and Molecular Imaging

SPIO: Super paramagnetic iron oxide

UIQ: Upper inner quadrant

UOQ: Upper outer quadrant

CHAPTER ONE

INTRODUCTION

The second most common cancer in adults is breast cancer and accounts for more than 20% of cancers in women (Torre et al., 2015:87-108). The first lymph node draining lymph from the primary tumour is the sentinel lymph node (SLN). Thus the SLN would be the first lymph node that a metastatic cell would encounter (Keshtgar & Ell, 1999:65-67).The accurate identification of a sentinel node is important both for the prognosis and the appropriate choice of treatment in a patient with breast cancer (Benson et al., 2007:331-348).
It is important to evaluate the presence of metastases in the axillary nodes. Axillary lymph node dissection (ALND) is commonly performed in patients with breast cancer. Regional tumour control can be obtained by surgically removing the sentinel nodes that may have metastatic spread (Kapteijn et al., 1998:427-430). Cabanas reported that the SLN could be removed by limited surgery and histologically examined to determine if more extensive surgery needs to be performed (Cabanas, 1977:456-466) .

In the 1990's, lymphoscintigraphy (LS) was introduced in combination with sentinel node biopsy. An early study concluded that it is possible to identify the SLN with radioactive tracers and that the SLN could predict the status of the other nodes in the axilla in terms of the presence of metastases (Krag et al., 1993:335-339).

Currently LS and biopsy of the SLN is established practise in the following groups of breast cancer patients (Giammarile et al., 2013:1934-1947):.

1. Stage I and II tumour without axillary lymph nodes
2. Ductal carcinoma in situ (DCIS) with mastectomy
3. Older patients (50 years and above)
4. Obesity
5. Male breast cancer
6. Before pre-operative systemic therapy

A number of studies detailing different techniques reported the following: the choice of radiopharmaceutical, volume of injection, site and depth of injection and imaging protocol (Giammarile et al., 2013:1934-1947; Mudun et al., 2008:262-267; Wilhelm et al.,1999:536-542).
In 2005, this study site (Nuclear Medicine Department)in conjunction with the hospital's breast surgeons embarked on a protocol which was compiled based on the original protocol

devised by Valdes-Olmos (Valdes-Olmos et al., 2000:1500-1506). This protocol was adopted after consultation with the Department of Nuclear Medicine of the Netherlands Cancer Institute who deemed it to be the protocol of choice.

The detailed protocol that was implemented at the study site in 2005, is shown in Appendix A. In summary the protocol requires that imaging acquisition is performed immediately after the intratumoural administration of the radiopharmaceutical whereby by immediate dynamic (flow) images are taken, followed by early planar images at 30 minutes and delayed imaging time at 2 hours, 4 hours, 6 hours with imaging at 24 hours where indicated.

In 2016 the breast cancer surgeons involved voiced their concern regarding the length of the imaging time and number of visits required to the nuclear medicine (NM) department. The length of imaging time was having a negative impact on the patient's pre-operative preparation regarding the scheduling of other procedures required prior to the surgery.

In order to address their concern, a decision was taken to review the data in terms of the number of cases where there was positive and negative identification of sentinel nodes and at which imaging time during the imaging protocol, the SLN was predominately identified. Wang et al., indicates that imaging could be performed only on either the early planar imaging or the later delayed imaging time (Wang et al., 2015:931-934). Thus, by reducing the number of visits to the NM department, more time could be dedicated to the pre-operative procedures required before the patient's surgery.

1.2 Aim of the research

The primary question for this study was: How can the current SNI protocol be optimised in terms of reducing the number of imaging times without compromising the number of SLNs identified?

It was necessary to review the results obtained by the study site using the current protocol in order to compare them to those reported in the literature, using the same protocol.

This information makes it possible to suggest changes to the imaging protocol for SNI at the study site.

The results of this study will be available as a publication and is aimed to assist institutions at a local and national level.

1.3 Objectives of the research

The objectives of the study are to determine whether the SNI protocol can be changed to decrease the number of visits to the NM department without affecting the results. SNI imaging data, surgery results and histology data for the patients referred to the study site for SNI during the period of January 2012 till June 2016 were collected to:

- Establish the results of the current SNI protocol at the study site and determine at which imaging times the SLNs are identified.
- Compare the results of the study site with that which is reported in the literature, using the same protocol.
- Discuss which components of the SNI protocol could be excluded without affecting the results.

1.4 Research Question

Question 1. What does the data from the study site reveal using the current protocol?

1.1 The age of patients
1.2 The site of the primary tumour
1.3 Number of patients in whom the SLN was identified with SNI at the various imaging times
1.4 In which drainage basins were the SLN found
1.5 Number of patients with tumour positive SLN on histology in the axillae

Question 2. Are the results of the study site the same as that reported in the literature in particular compared to the seminal study?

Question 3. What are the implications for excluding various imaging times when identifying SLNs in the axillae and were there any SLNs identified on the early images but not on the later images?

1.5 Limitations of the research

The patient and scintigraphic imaging information available in the department depended on a number of factors such as when the department switched to achieving image data in digital format, and the upgrading of gamma cameras.

- From 2005 until 2010 images were captured as hard copy films. As a result, it is not possible to verify the reports.

- In 2005 the study site made used of a single head gamma camera to acquire images for SNI. These cameras became obsolete at the end of 2007 and were replaced with dual head cameras which were used for scintigraphy.
- At this study site records are stored for a maximum of six years.
- Towards the end of 2016 the study site implemented a new procurement process using a tender basis. As a result, it was not possible to always ensure the radiopharmaceutical was supplied by the same vendor.
- In 2017 the surgical unit received a donation of a probe and magnetic pharmaceutical and as a result the number of referrals for SNI has decreased significantly. No SLN data could be recorded at the study site subsequently.
- Data were excluded from the study for patients who had no histology reports
- Surgical reports indicating the use of blue dye could not be included since 56% of reports were not archived in the patient's files.

1.6 Overview

The following is a brief overview of the chapters that will delineate the process of this study which will address the research objectives and the questions.

Chapter 2 Literature Review

In this chapter, journal articles and published papers of previous and current studies are reviewed. This review includes critical analysis of knowledge and findings in the theoretical and methodology aspects of SNI of patients with breast cancer. The chapter further describes as contextual background: the normal anatomy, lymphatics of the breast, as well as the possible metastatic spread of the tumour. The review proceeds to describe an in-depth analysis of past and present SNI imaging procedures.

Chapter 3 Methodology of study

The research methodology chapter describes the process and procedures used to categorise SNI data, the selection of study participants and the analysis of the collected data. In this chapter the validity and reliability of the study is evaluated. This was a quantitative retrospective study that included all the patients that were referred to the NM department for SNI procedures. Demographic, histology reports as well as data from the NM archive were collected and collated on an Excel data sheet. Data were anonymised and assigned with a

unique code per data set. The data were validated and verified by cross checking scan reports and scintigraphic images were verified by a nuclear physician.

Chapter 4 Results

This chapter presents all the findings derived from the study. Any additional findings are also recorded in this chapter. The chapter describes the different statistical tests that were performed to find the differences in the categorical measurements of the study site and what was reported in the literature using the same protocol. Tests were also conducted to compare the number of SLNs found, the number of tumour positive SLNs and also the variation of the different imaging times when the greatest number of SLNs were identified.

Chapter 5 Discussion

In this chapter the overall findings of the study are interpreted, and the significance is described. The following findings are discussed:

- The age of patients
- The site of the primary tumour
- Number of patients in whom the SLN was identified with SNI at the various imaging times
- In which drainage basins were the SLN found
- Number of patients with tumour positive SLN on histology in the axillae
- Comparing the results of the study site to the literature in particular the seminal study
- The result of excluding various imaging times on the identification of SLNs in the axillae indicate possible changes to the imaging protocol of the study site.

Chapter 6 Conclusion

The focus of this chapter is to resolve the research question regarding the efficacy of the current protocol used at the study site compared to that reported in literature. Based upon the data that was presented and analysed, possible changes to the current SNI protocol is also suggested.

CHAPTER TWO

LITERATURE REVIEW

Breast cancer is the most common cancer in the female population globally as well as most of the countries in Africa (Kantelhardt et al., 2015:108-114). Breast cancer is responsible for one in four cancers diagnosed and one in five cancer deaths in women globally (Ferlay et al., 2010:2893-2917). The anatomical information of the breast, tumour location and tumour spread are required to evaluate the prognosis and possible treatment.

The breast consists predominately of two types of tissues. The milk producing lobes (lobules) and the milk ducts are part of the glandular tissue. The stroma or supporting tissue are made up of fatty and fibrous connective tissues. There is also lymphatic tissue that transports cellular fluids(Sharma et al., 2010:109-126). See Figure 2.1

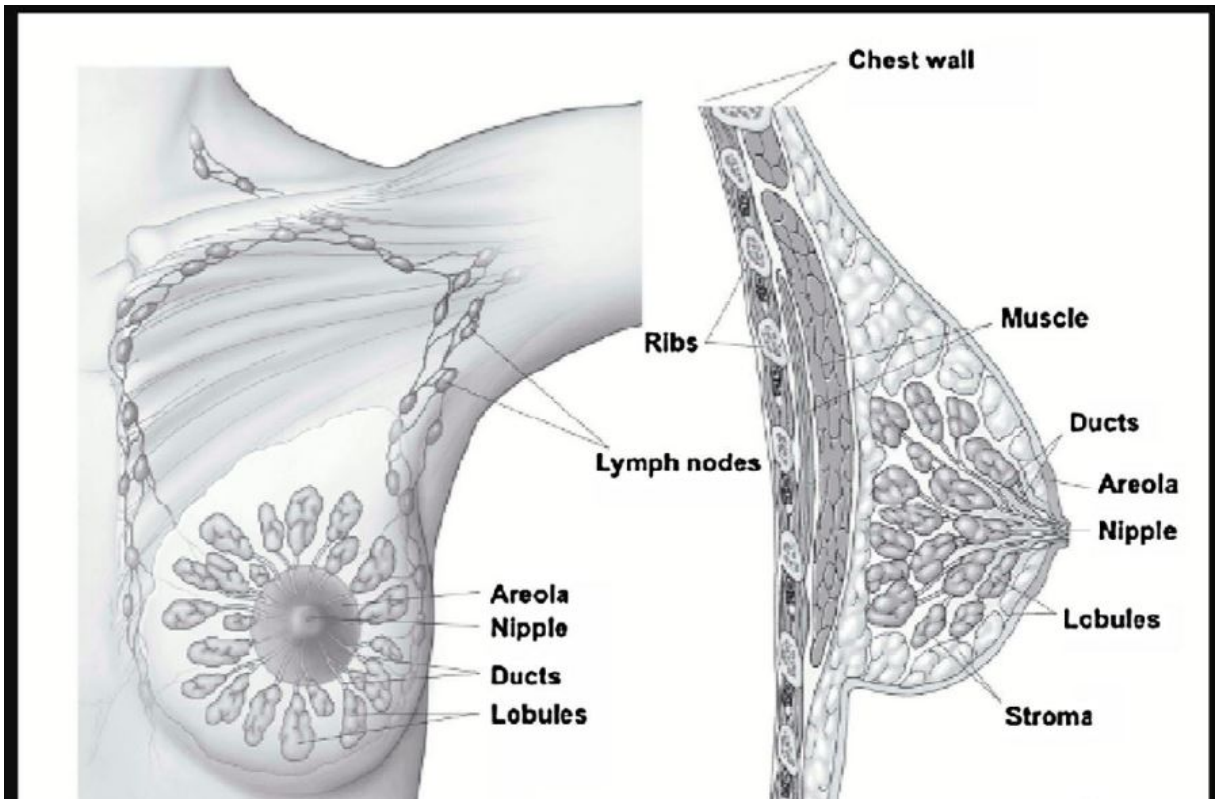

Figure 2.1 Normal breast tissue (Shareef et al., 2016:234)

Breast cancer is classified into two groups namely non-invasive or in situ and invasive. In the case of non-invasive cancer, tumour cells remain within the ducts that form part of the glandular tissue of the breast and do not spread to surrounding tissue. However, in the case of invasive cancer, tumour cells infiltrate the surrounding stromal tissue. Tumour spread through the lymphatic system and its implications for treatment and survival have been extensively investigated and researched. The purpose of this research and ongoing

investigations was to develop an effective diagnostic methodology to enable differentiated treatment choices and better overall management of breast cancer (Sharma et al., 2010:109-126).

Aspects such as the imaging of breast cancer have grown significantly in terms of diagnosis and the possible prognosis that can be predicted. The exact anatomical information of the tumour and metastases are required in order to assess prognosis and possible treatment (Alcantara et al., 2014:112). This information includes the anatomical location, the size of the tumour and whether metastases have occurred. Mammography, ultrasound and magnetic resonance imaging are the most commonly used imaging modalities for identifying gross tumour size and location. SNI plays an important role in staging of lymphatic breast cancer and is seen as a minimally invasive technique. SNI is well validated as standard care of stage I and II breast cancer treatment (Alcantara et al., 2014:112; Kaufmann et al., 2010:1184-1191).

2.1 Anatomy of the breast

2.1.1 Lymphatics of the breast

The lymphatic system consists of five categories namely the capillaries, collecting channels, lymph nodes, trunks and ducts and their sizes range from 10 μm to 2 mm in diameter. Lymph fluid is formed when the interstitial fluid is collected through lymph capillaries. The main function of the lymphatic system is to return proteins and excess interstitial fluid to the bloodstream. Lymph flows as a result of the osmotic pressure gradient and the constant change in intraluminal pressure (Swartz, 2001:3-20).

Experimenting with a cadaver of a pregnant woman , the lymphatics of the breast was first described by Cruikshank et al., in 1786 (Cruikshank et al., 1786). Sappey publishing in 1903, described using the same experimental method as Cruickshank which was injecting mercury into cadavers in 1874. He described the lymphatics of the breast as collecting in the subareolar plexus and then draining towards the axilla via the lymph collecting systems (Suami et al., 2008:863-871). This knowledge of the lymphatic drainage anatomy informed SNI injection techniques and thus the subareolar injection technique became the basis for injecting dye and or radioisotopes for SNI (Kern, 1999 :539-545).

Sappey published his results in 1903 as a comprehensive illustration (Suami et al., 2008:868). This illustration is still used in Gray’s anatomy textbook today. It is shown in Figure 2.2

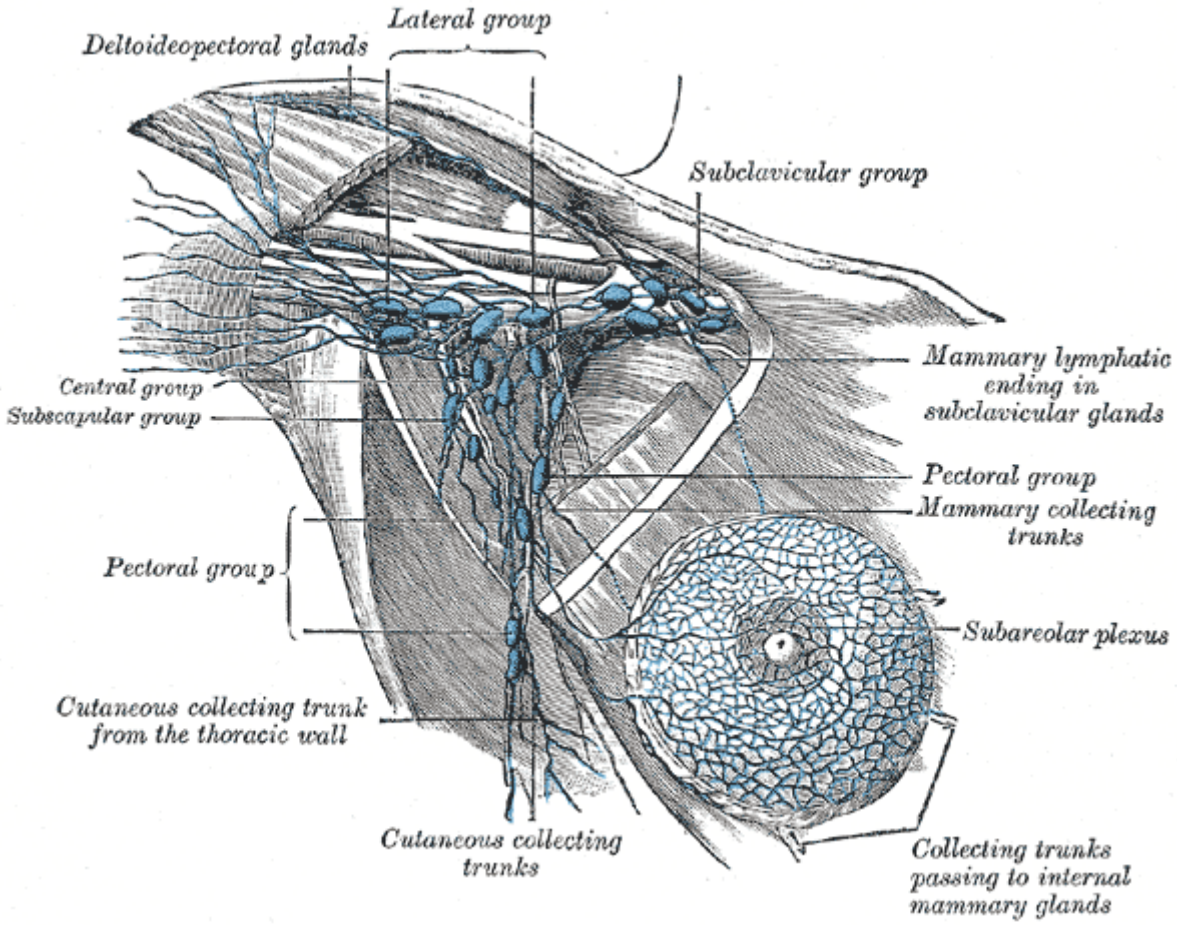

Figure 2.2 Lymphatic drainage of the breast (Sappey, 1903) in Suami et al., 2008:868

Lymphatic drainage plays an important role in the spread of metastatic malignant breast tumours (Tanis et al., 2001 :399-409). Lymph flow from the breast follows the same pathway as the vessels that supply blood to the breast. The three main pathways are as follow:

- along the branches of the axillary vessels to the axillary lymph nodes,
- along the branches of the internal thoracic vessels to the internal mammary chain,
- along the intercostal vessels (Tanis et al., 2001 :399-401).

In 1959, Turner-Warwick found that the lymphatic pathways passed directly from the tumour in the breast to the axillary lymph nodes and bypassed the subareolar plexus. He found that the axillary nodes receive 75% of lymph drainage and the remainder of the lymph drains into the internal mammary chain. Thus, the four quadrants of the breast drain to the axilla and either the internal mammary or posterior intercostal nodes (Turner-Warwick, 1959:574-582). These anatomical and physiological findings are currently used as the basis for SLN mapping.

The superficial lymphatics patterns between males and females are not different and often more than one sentinel node drains the breast (Suami et al., 2008:863-971).

Some routes through or between the pectoral muscles can lead directly to the apical nodes of the axilla. The lymphatics that go through the pectoralis major muscle enter the parasternal or internal thoracic nodes. These connections may lead across the median plane and to the contralateral breast. Lymphatics can reach the sheath of the rectus abdominis and the sub-peritoneal and sub-hepatic plexuses (Tanis et al., 2001:109-112).

2.1.2 Lymph Node Basins

A lymph node basin is the group of nodes that receives and filters lymph that flows from a certain area of the body (Suami et al., 2008 :863-871).

Referring to Figure 2.3, it can be seen that the lymph node basins of the breast consist of the following groups:

- Axillary group, that is subdivided into the following
    - The pectoral / anterior nodes that consist of three to five nodes along the medial wall of the axilla.
    - The subscapular (posterior) nodes consisting of six or seven nodes along the posterior axillary fold and subscapular blood vessels.
    - The humeral (lateral) nodes consisting of four to six nodes along the lateral wall of the axilla, medial and posterior to the axillary vein.
    - The central nodes consisting of three to four nodes situated deep to the pectoralis minor close to the base of the axilla.
    - The apical nodes consisting of six to twelve nodes located at the apex of the axilla along the medial side of the axillary vein and the first part of the axillary artery.

- The parasternal/internal mammary group
- The clavicular group (infra clavicular and supra clavicular)
- Abdominal group (sub diaphragmatic inferior phrenic lymph nodes).

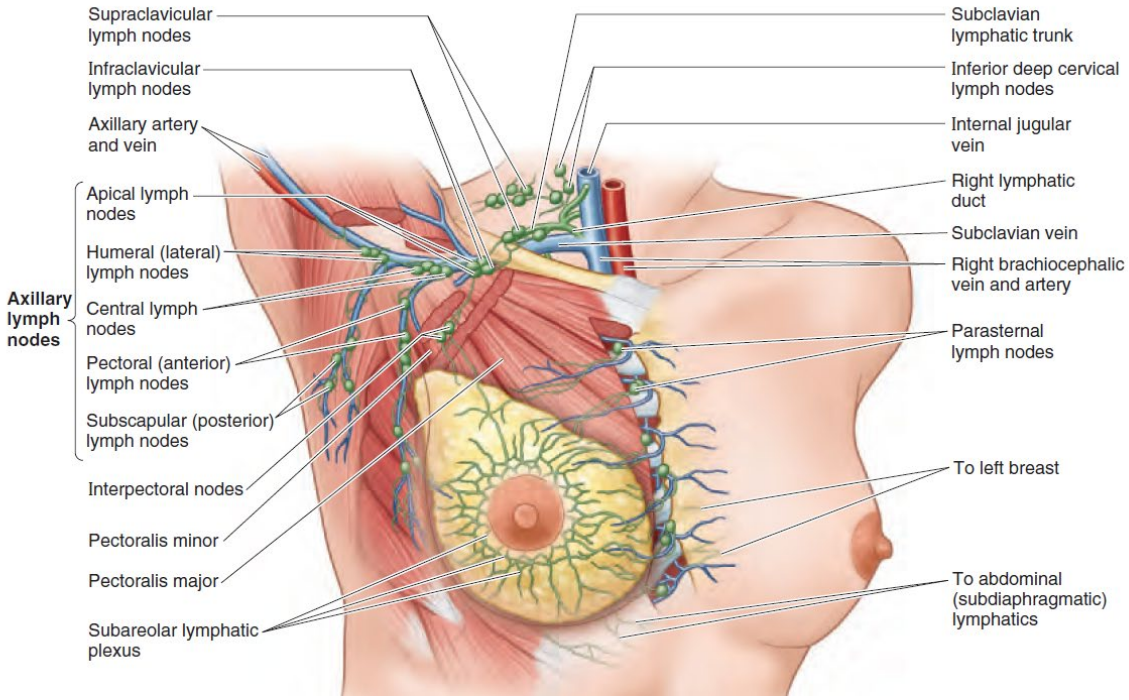

Figure 2.3 Axillary lymph nodes of the breast (Moore et al., 2018:495)

2.1.3 Metastatic spread

In 1980, Christensen detected 'the primary draining nodes' by using breast lymphoscintigraphy (Christensen et al., 1980:667-668). Borgstein investigated the route of metastatic spread through the axillary lymph node filter and indicated that the nodes of the central group are not only most often involved, but also most often exclusively involved (Borgstein et al., 2000:81-89).

According to surgical classification, the breast is divided into four quadrants for anatomical localisation and to describe tumours as illustrated by Moore et al., (2018: 787) in Figure 2.4 shown below.

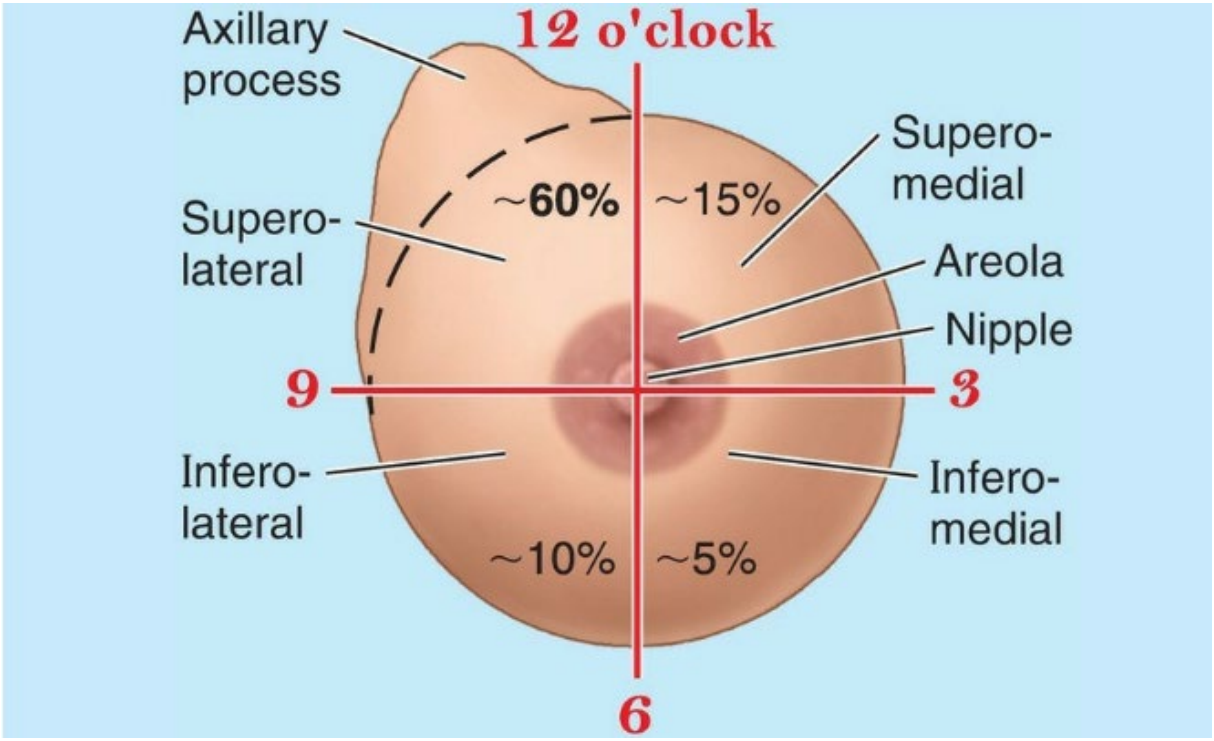

Figure 2.4 Quadrants of the breast (Moore et al., 2018:787)

Upper Outer Quadrant (UOQ)	=Supero-lateral
Upper Inner Quadrant (UIQ)	=Supero-medial
Lower Outer Quadrant) LOQ)	=Infero-lateral
Lower Inner Quadrant (LIQ)	=Infero-medial

Metastatic lymph node involvement is described and classified into three levels (Haagersen, 1972). The levels are based upon their anatomical relationship to the pectoralis minor muscle.

- Level I: lateral to the pectoralis minor muscle involving the external mammary, lateral and scapular groups.
- Level II: behind the pectoralis minor muscle including the central and interpectoral groups.
- Level III: medial and superior to the pectoralis minor muscle including the apical group.

Rahbar et al., illustrate clearly and simply these three levels of metastatic lymph node involvement in Figure 2.5 as shown below (Rahbar et al., 2012:150).

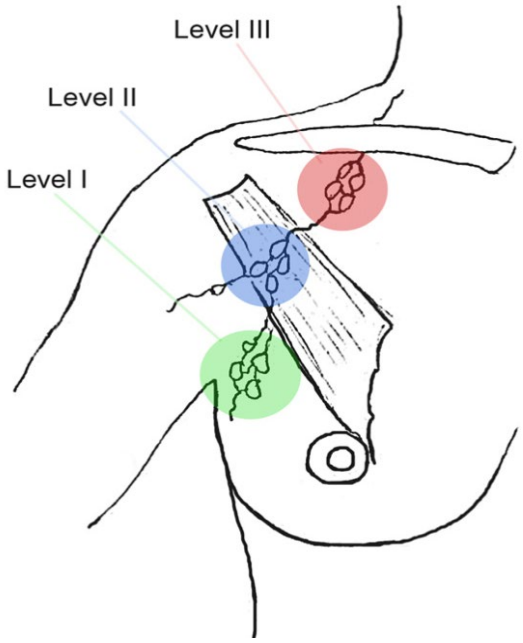

Figure 2.5 Levels of metastatic lymph node involvement (Rahbar et al., 2012:150)

Somashekhar et al., and Rutgers recorded that a positive SLN with SNI and in addition identified with blue dye does not imply metastatic involvement. Histological correlation is mandatory to confirm the presence of tumour cells (Rutgers, 2004:182-186; Somashekhar et al., 2008:111-119). Possible causes of false positive (FP) nodes could be that the tumour cells block the normal lymphatics and that another pathway opens leading to uptake of blue dye and or the radiopharmaceutical in a node that is not metastatically involved. Another explanation of this phenomenon could be the fact that the tumour has two lymphatic drainage path ways (Kataria et al., 2016:396-401).

2.2 Sentinel lymph node mapping

At the end of the twentieth century the concept of lymphatic mapping was introduced. The technique for intraoperative mapping to selectively remove lymph nodes on the direct drainage pathway from the primary tumour was developed (Giuliano et al., 1992:392-399). This sentinel node was considered to be the first site of metastatic disease. The concept of sentinel node biopsy consisted of two principles. The first principle is that of the sequential lymphatic diffusion in an orderly and predictable pattern of lymphatic drainage to a regional lymph node basin, and the second principle involves the entrapment of tumour cells in the first draining lymph nodes (Kapteijn et al., 1998:427-430). Turner et al., provided histological confirmation of the concept of the SLN and concluded in a study that when the SLN is histologically negative, the probability of tumour involvement is less than 0.1% (Turner et al., 1997:271-278).

2.2.1 Lymphatic imaging procedures past and present

The precise diagnosis of axillary metastases requires excision of the SLN and histological confirmation. Axillary lymph node dissection (ALND) was the technique of choice but it meant a higher rate of morbidity compared to a sample technique (Schijven et al., 2003:341-350). SLN mapping was developed over the years and in 1994 Guiliano et al., described blue dye mapping in breast cancer (Giuliano et al., 1994:391-398). The study sample population consisted of patients with potentially curable cancer breast cancer. All patients received an intratumoural injection of 0.5-10 ml of blue dye immediately prior to surgery when on the surgical bed. Following axillary dissection, the first blue lymph node was identified, removed and histologically investigated. The study proved to have value in identifying lymph nodes (LN's) that drain from a specific primary tumour site. This provided the basis for using the LN as a sentinel to predict metastases. It also improved the accuracy of surgical staging. Cases where the SLN appeared outside the axilla could be attributed to the variability of regional lymph drainage (Giuliano et al., 1994:391-398).

In addition to this, radiolabelled colloids with intraoperative detection of the sentinel node using a gamma ray detection probe were introduced and these techniques were applied all over the world (Krag et al., 1993:335-339). A further study by Krag et al., (1998: 941-946) investigated the identification of SLNs with a gamma probe in animals injected with radioactive tracers. This study indicated that the identification of SLNs using the gamma ray probe is just as effective compared to the use of blue dye. As a result of this study, they conducted a multicentre study to test the method of identifying SLNs in patients with breast cancers (Krag et al., 1998:941-946). Patient data were collected between 1995 and 1997. Each patient was injected with technetium 99m (^{99m}Tc) sulphur colloid, 30 minutes to 8 hours before surgery with and mean interval of 2.9 and 1.9 hours between the administration of the radiopharmaceutical and surgery. The imaging time between administration of the radiopharmaceutical and surgery did not influence the identification of SLNs. The radioactive tracer was injected in four areas surrounding the tumour. A handheld gamma probe was used to locate the SLN and subsequently excised and sent for histology examination. The study showed an identification of the SLN of 93%. Factors identified that could influence the rate of detection were the level of experience of the surgeon, the injection technique as well as the patient's age (Krag et al., 1998:941-946). The study recommended increasing the volume of the tracer (Krag et al., 1998:941-946). The high rate of non-visualisation of internal mammary nodes was attributed to the SLN being masked by the overlying injection site. Although the procedure proved to be technically challenging, based on the results of the study, they concluded that it is practical to perform this SLN procedure. This procedure enabled the identification SLNs outside the axilla that would have been missed (Krag et al., 1998:941-946).

In 1998, Cox et al., published guidelines on SLN mapping for patients with breast cancer (Cox et al., 1998:645-653). The status of regional node basins was one of the most important variables in the prognosis of patients with breast cancer. The aim of their study was to show that with the use of lymphatic mapping the spread of the tumour could be predicted with greater accuracy. Patients were injected with ^{99m}Tc sulphur colloid 1 to 6 hours before surgery and isosulfan blue dye immediately before surgery. The dose of the tracer was divided into six parts and injected around the tumour. The SLN was defined as a blue stained node as well as a radioactive node that was confirmed with a gamma probe. All SLNs were histologically examined (Cox et al., 1998:645-653).
The study collected data from 466 patients from 1994 to 1997 and consisted of three phases. Phase one included the training phase where the SLN was removed and the patients underwent a complete ALND. The second phase included patients where the SLN was removed and ALND was only performed in the event that the SLN was found to be histologically positive for metastatic spread. The third phase included patients where the polymerase chain reaction of SLNs was evaluated for submicroscopic metastases (Cox et al., 1998:645-653).

This study was able to identify SLNs in 94% of the total number of patients. A total number of 844 SLNs were removed during surgery. The success rate of identifying the SLN with the radioactive tracer alone, without the blue dye was 68%.
On the basis of these findings the following set of guidelines were drawn up (Cox et al., 1998:645-653):

- Complete axillary dissection: Perform complete axillary node clearance when the SLN is histologically positive for metastases and when lymphatic mapping fails.
- Review protocols at an institutional level: Lymphatic mapping should adhere to the institutional investigational procedures.
- Radiation safety: Appropriate adherence to make use of radio-active substances in a safe manner.
- Training: All members of staff involved with SLN mapping should undergo sufficient training.
- Data Collection: Data should be collected to validate SLN procedures.
- Self-credentialing: Collected data to be used to verify and identify SLN involvement in patients prior to chemotherapy.
- Anatomical considerations: The identification of the SLN could be compromised when located close to the tumour site. SLN involvement in the intermammary chain was also an important consideration that influenced the accuracy of lymphatic mapping.

The study concluded that the SLN could be accurately identified using radioactive tracer and blue dye staining (Cox et al., 1998:645-653).

Recent studies performed discovered the need for Single-photon Emission Computed Tomography/Computed Tomography (SPECT/CT) imaging in combination with the standard lymphoscintigraphy (Wagner et al., 2013:191-202). The combination of SPECT and CT imaging called hybrid imaging was developed using a single imaging device. The additional value of this combined method is that the exact anatomical position of the tumour is located. SPECT/CT images also yielded additional nodes as well as nodes that were not visualised on the planar images of lymphoscintigraphy. Hybrid imaging resulted in minimal or no patient movement occurring between imaging, and that the fused images (SPECT/CT images combined) made interpretation easier (Navalkissoor et al., 2015:203-215). SPECT/CT imaging also appeared to have a higher detection rate of lymph nodes (LN) in the interpectoral and intramammary locations. The low dose (LD) CT provides more information regarding the abnormal lymph draining basins. The value of SPECT/CT is highly regarded by surgeons as providing them with the correct anatomical location for incision and it seems to add more importance to the “see and open“ approach for surgeons (Valdes-Olmos et al., 2014:491-504) .

The integration of SPECT/CT into SLN mapping was reported as an important pre-operative imaging component and that it complemented planar lymphoscintigraphy. The added value of SPECT/CT appears to be the anatomical localisation of SLNs already visualised on planar images, the detection of more SLNs in other drainage basins, and the assistance with the visualisation of SLNs in patients where no SLN is visualised on the planar images (Valdes-Olmos et al., 2014:491-504).

A further development was the use of molecular imaging which facilitates the visualisation, characterisation, and quantification of biologic processes at cellular and molecular levels. Positron emission tomography (PET) with 2-deoxy-2-[fluorine-18] fluoro- D-glucose (^{18}F-FDG) is used to evaluate the primary tumour and one of the major advantages of PET is the ability to detect distant extra axillary LN metastases. Yararbas et al., reported that lymph nodes that were detected after surgery with PET/CT using ^{18}F-FDG may affect the management in patients with isolated metastatic axillary lymph nodes (Yararbas et al., 2018: 72-79).

Various new techniques for SLNB have been developed that involve non-ionising investigations. These investigations include indocyanine green (ICG), super paramagnetic iron oxide (SPIO), and contrast enhanced ultrasound (CEUS).
ICG which fluoresces in the near infrared (NIR) part of the spectrum is injected directly into the breast tissue and the SLNs are then identified by the fluorescent imaging system. The

advantage of ICG is that it is inexpensive, but the major disadvantage is that ICG cannot be used in patients which are allergic to iodine. Papathelemis et al., (2018:468) conducted a study evaluating the use of ICG in combination with ^{99m}Tc nanocolloid. They concluded that ICG is as effective as ^{99m}Tc nanocolloid and that the combined use of these two methods yields a higher number of SLNs identified. However the leakage of ICG into the lymphatic system during surgery could obscure the area of surgery when accurate identification of SLNs is necessary (Papathemelis et al., 2018:468). Together with the previously mentioned disadvantage is that ICG cannot be used in patients who are allergic to iodine (Xiong et al., 2014 :843-849).

In 2014 Thill et al., investigated the use of a magnetic tracer, SPIO to localise the SLN. This method involved the use of the magnetic tracer in conjunction with a probe/magnetometer. The tracer is injected in the subareolar tissue at least 20 minutes before surgery. The magnetic properties of SPIO allow for rapid accumulation in the SLN due to the high activity of phagocytosis by the macrophages. The particle size (60 nm) of the magnetic tracer is similar to the radiopharmaceutical used for lymphoscintigraphy and follows the same lymphatic pathway to the SLN (Thill et al., 2014:175-179).

At the time of this study, SNI with radioactive tracer was the investigation of choice and considered as the most accurate investigation. Some of the advantages of using the magnetic tracer method were that the surgeons could inject the tracer 20 minutes prior to surgery instead of the more lengthy imaging procedure for SNI. As a result, more time is available to book patients for surgery (Thill et al., 2014:175-179).

The study concluded that the magnetic tracer technique compared well with SNI and the identification of the SLN per patient equalled the identification of the SLN of SNI. Some of the advantages include the safe use of the tracer without the risk of radiation exposure as well as the increased schedule of patients for surgery (Thill et al., 2014:175-179).

However, a similar study by Shiozawa et al., in 2013 using a different magnetic tracer and magnetometer, only reported a 77% identification of the SLN (Shiozawa et al., 2013:223-229). Disadvantages of this method include the following: the probe does not reach the same depth as a gamma probe; it cannot be used in patients who are hypersensitive to iron or dextran compounds and patients with pacemakers or metal implants (Ferrucciet al., 2018: 405-417). It was recommended that further studies be done to evaluate the efficacy and consistency of the use of this technique (Thill et al., 2014:175-179).

The CEUS microbubble technique is based on the use of dispersion with sulphur hexafluoride gas that is injected intradermally around the areolar area. The lymphatic drainage is visualised by using CEUS. Advantages of this method are the real-time imaging for SLNs, equipment and contrast agents are relatively inexpensive and readily available. The patient is not exposed to radiation and the microbubble does not contain iodine for patients that might be allergic to it. The identification of SLNs compares well to standard SNI procedures. Disadvantages however include the facts that the procedure involves a long learning curve as CEUS is operator dependent. The CEUS technique is still to be recognised as standard practice in the management of breast cancer. Randomized controlled trials have been suggested to evaluate the new techniques against the standard technique of practice (Ferrucci et al., 2018: 405-417).

Ferrucci et al., report that SLNB is still a recognised standard practice in the management of breast cancer and that randomised controlled trials should be done to evaluate the new techniques against the standard technique of practice (Ferrucci et al., 2018: 405-417).

The uses of non-ionising techniques as an alternative for using radiocolloids prove to detect a higher number of SLNs. However the higher detection rate may lead to more intensive surgical procedures to remove these nodes. Krag et al., indicated that the false negative (FN) rate increase with the higher number of SLNs identified and submitted for histological examination (Krag et al., 2007:881-888). The major disadvantage of these methods is the removal of nodes that are not affected by tumour spread. The purpose of SLNB is to reduce extensive surgery and to reduce the number of normal nodes removed during surgery (Papathemelis et al., 2018:468).

Hellingman et al., from the Netherlands Cancer Institution where the seminal study was conducted, did a study to evaluate whether pre-operative factors could be associated with the non-visualisation of SLNs. A decrease in the identification of the SLN was noted over recent years which could not be explained. They reported that the method of injection is currently still debatable and that their method of choice is the deep intratumoural injection to allow identification of the intramammary LN’s. The identification of these nodes could possibly influence the need to additional radiotherapy (Hellingman et al., 2019:421-429).The use of SPECT/CT and an additional second injection of the radiopharmaceutical could increase the identification of the SLN (Hellingman et al., 2019:317-324).

Hellingman et al., speculated that the non-visualisation could be due to the increase in the number of non-palpable tumours, the increase in the number of patients with locally advanced tumours as well as SLNB after neoadjuvant treatment (Hellingman et al.,

2019:317-324). Other factors influencing the identification of the SLN could be higher age, BMI, the size and location of the tumour (Hellingman et al., 2019:421-429).

2.2.2 Radiopharmaceutical

^{99m}Tc -based agents are commonly used for radioguided SLN biopsy in breast cancer. Colloidal particles such as antimony trisulphide, nanocolloid albumin, sulphur colloid and a novel receptor-targeting small molecule were widely investigated and special procedures are used when using sulphur colloid (Wilhelm et al., 1999:536-542). These particles have to be filtered with a 0.22 μm filter in order to ensure more uniform, smaller colloidal particles. Michenfelder et al., also reported that different heating times can reduce the particle size but in their study, it showed only a slight significance from the standard heating time (Michenfelder et al., 2014: 283-288).

The choice of a radiopharmaceutical depends on the drainage, distribution and clearance of the radiopharmaceutical. The ideal radiopharmaceutical should have a good compromise between fast lymphatic drainage and optimal retention in SLNs (Lyman et al., 2005:7703-7720). The characteristics of ^{99m}Tc nanocolloid with a particle size of 5-100 nm, is widely used due to the rapid transit to the sentinel node with a longer retention time (Giammarile et al., 2013:1932-1947). Leidenius et al., reported that the frequency of the visualisation of the sentinel node is significantly higher for ^{99m}Tc nancolloid compared to other pharmaceuticals with a larger particle size (Leidenius et al., 2004:233-238).

However there are studies which have shown that the frequency of identification of axillary SLNs is not significantly affected by the size of the particles (Mariani et al., 2001:1198-1215; McCready et al., 2005:185-194).

As such the selection of radiopharmaceutical is based more on its availability rather than on the ability to detect SLNs and it was recommended that ^{99m}Tc nanocolloid be used as the radiopharmaceutical of choice when available (Yararbas et al., 2010:805-810).

2.2.3 Injection techniques

The site of injection, as to which will provide optimal SLN identification, still varies at different institutions. Different studies conducted have indicated that there is no significant difference in identifying the SLN when using either deep or superficial injection techniques (Klimberg et al., 1999:860).Various tumour and breast injection sites for SNI in breast cancer are used. The technique of injection depends on where the radiopharmaceutical is to be deposited. There are seven different injection techniques in use namely, intradermal, subdermal,

periareolar, subareolar, peritumoural, subtumoural, and intratumoural.These injection techniques are classified into two categories namely deep (intratumoural, subtumoural and peritumoural) and superficial (periareolar, subperiareolar,subdermal and intradermal) (Nieweg et al., 2004:153-156).

Deeper injections including peritumoural or intratumoural injections yield an increase in the amount of internal mammary nodes seen. Periareolar injection techniques at the border of normal breast skin and the areolar, yield a higher success rate in the visualization of axillary sentinel nodes and is of value when the sentinel node is close to the primary tumour.The intratumoural injection can be safely and accurately administered into the tumour. In practise the advantage would be that the resistance of the tumour can be felt when inserting the needle and thus the chance of injecting the radiopharmaceutical adjacent to the tumour is minimised. However, intratumoural injections are also known to have a slower transit time which is evident when comparing the imaging times of when most SLNs are identified (Nieweg et al., 2004:153-156).

The major difference between the various injection techniques is the ability to visualise the axillary nodes exclusively rather than other lymph nodes. Intratumoural injection techniques identify more nodes outside the axilla, in particular the internal mammary nodes. Periareolar injection sites yielded a higher success rate in the visualisation of axillary sentinel nodes. This technique showed its value when the sentinel node is close to the primary tumour. The disadvantage of this technique was the FNs and that it was less sensitive in cases where there was nodal involvement in the internal mammary chain (Nieweg et al., 2004:153-156).

Further studies performed using the periareolar injection technique described a more specific area of the injection site, namely the cutaneous-areolar junction. This is the site at the border of normal breast skin and the areolar. This method showed increased uptake and concentration of the radiopharmaceutical in the SLN (Krynyckyi et al., 2003:97-107). Studies have confirmed that the combination of both periareolar and intratumoural injection techniques provided more LN identification both in the axilla as well as the internal mammary chain (Argon et al., 2006:795-800; Hindie et al., 2011:405-414).

Furthermore, studies by Argon et al., have confirmed that the combination of both periareolar and intratumoural injection techniques provided more LN identification both in the axilla as well as the internal mammary chain (Argon et al., 2006:795-800; Hindie et al., 2011:405-414). Guidelines by the EANM and SNMMI for LS and SN mapping suggest that the combination of both deep and superficial injections may improve SLN detection and decreased FN findings. Dynamic imaging should be started immediately after injection with

early 15-30 minute imaging, followed by 2-4 hours imaging and 18-24 hour images when required. (Giammarile et al., 2013:1932-1947; Kapteijn et al., 1998:427-430).

2.3 Seminal SLN imaging study used for study site protocol in 2005

In the early 2000's, the study site investigated an imaging protocol for SLN identification for patients with breast cancer. After consultation with the Department of Nuclear Medicine of the Netherlands Cancer Institute the current SLN imaging protocol was introduced as the imaging protocol of choice (Valdes-Olmos et al., 2000:1500-1506).

The protocol used a single intratumoural injection of the radiopharmaceutical as this had proved to be effective for lymphatic mapping. Sentinel node localisation was performed the day before scheduled surgery. Imaging commenced immediately after the administration of the radiopharmaceutical. A dynamic (flow) set of images were acquired for 20 minutes with a low energy high resolution collimator on a dual head gamma camera with energy window of 15% (±5%) centred over the 140 kilo electron volt (keV) photopeak of ^{99m}Tc. These images were followed by planar static images acquired at 30 minutes, 2 and 4 hours. Cobalt-57 (^{57}Co) was used as a transmission source. Valdes-Olmos et al., found this method effective for the visualisation of the SLN but was dependent on the patient's age, as well as the proximity of the SLN to the injection site and intramammary node involvement (Valdes-Olmos et al., 2000:1500-1506).

2.3.1 Results of Valdes-Olmos studies:

Data were collected and evaluated from 150 patients at the Nuclear Medicine department of the Netherlands Cancer Institute (Valdes-Olmos et al., 2000:1500-1506). All patients were injected with ^{99m}Tc nanocolloid intratumourly and SNI was performed a day before surgery. The patients were divided into two groups. Group A consisted of 100 patients injected with a mean dose of 61.6 MBq for the validation phase of the study, and Group B, 50 patients after the tracer dose was increased to a mean dose of 90.8 MBq. The following information was recorded:

- Age
- Number of patients in which LN's were visualised
- Quadrant of the breast in which the tumour was localised
- Number of foci
- Site of identified LN
- Tumour positive SLNs

Group A:

In Group A LNs were visualised in 83% of the patients. The mean age of this group was 53 years. Of these patients (83%), only 1 node was visualised and in 61%, 2-8 nodes. Early lymphatic flow was seen in 41% of the patients and later visualisation at 2-4 hours was seen in 59% of the patients. A total number of 97 basins could be identified consisting of 83 axillary, 14 internal mammary chain and 3 clavicular regions. The total number of sentinel nodes identified was 152 (Valdes-Olmos et al., 2000:1500-1506).

Group B:
The visualisation rate in this group was 94% with a mean age of 54.1 years. In 57% a SLN was seen on early images and in 43% SLNs was seen on later images (2-4 hours after administration of the radiopharmaceutical). A total number of 53 basins could be identified consisting of 45 axilla and 8 internal mammary chains. The total number of sentinel nodes identified was 94 (Valdes-Olmos et al., 2000:1500-1506).

Results of Group A:
Rate of identification: 83%
Axillary LN identified: 96%
Non axillary LN identified: 19%
Tumour positive SLNs:
Axillary SLNs: 40%
Non axillary SLNs: 25%

Table 2.1: Results of Group A of the seminal study

Rate of identification: 83%	Axillary SLNs identified: 96%	Non axillary SLNs identified: 19%
Tumour positive SLNs:	Axillary SLNs: 40%	Non axillary SLNs: 25%

Results of Group B:
Rate of identification: 94%
Axillary LN identified: 96%
Non axillary LN identified: 17%
Tumour positive SLNs:
Axillary SLNs: 32%
Non axillary SLNs: 25%
Table 2.2: Results of Group B of the seminal study

Rate of identification: 94%	Axillary SLNs identified: 96%	Non axillary SLNs identified: 17%
Tumour positive SLNs:	Axillary SLNs: 32%	Non axillary SLNs: 25%

2.4 The significance of dynamic (flow) imaging

Dynamic imaging is a set of consecutive images taken at a set amount of time per frame over a specified period. These images are taken immediately post administration of the radiopharmaceutical. The advantage of dynamic imaging is the visualisation and differentiation between SLNs and second echelon nodes. Second echelon nodes do not drain directly from the primary tumour but rather via the SLN (Zarifmahmoudi et al., 2016:130-135).

Various studies have been performed to evaluate the feasibility of dynamic imaging. Martinez-Rodriguez et al., performed a study in 2013 and this indicated that dynamic imaging improved the identification of the SLN by 10.5%. They recommended that dynamic imaging become part of the standard protocol (Martinez-Rodriguez et al., 2013:296-300). However, Doting et al., indicated in their study that the only advantage of dynamic imaging was the identification of second echelon nodes and since there seemed to be a low incidence of these nodes, dynamic imaging was not recommended (Doting et al., 2007:469-475).

Zarifmahmoudi et al., conducted a study in 2016 using a radiopharmaceutical with a very small particle size of 20 nm. Radiopharmaceuticals with a smaller particle size in the range of 5-100 nm, are widely used because of the rapid transit to the sentinel node with a longer retention time. In this study a SLN detection rate of 86.6% was reported on the dynamic images. It was further reported that when using radiopharmaceuticals with larger particle size with dynamic imaging, the detection rate of SLNs would have limited success due to a slower transit time from the injection site. They concluded that when using a radiopharmaceutical with a smaller particle size, dynamic imaging is feasible and that the advantage of this would be the ability to differentiate between SLNs and second echelon nodes (Zarifmahmoudi et al., 2016:130-135).

2.5 Time of lymphoscintigraphy

The time period after the patient had a biopsy of the breast tumour should be taken into consideration when performing SNI as this could lead to non-visualisation of the SLN (Aliakbarian et al., 2011:199-202). Sadeghi et al., reported that the one day protocol vs the two day imaging protocol had similar results but that the one day protocol made it more

difficult to schedule surgery times.Their study recommended a two day protocol but recomended using radiopharmaceuticals with a larger particle size (Sadeghi et al., 2009:507-510). However, if SLNs are identified on the earlier images, then delayed imaging would not be required. This could ultimately decrease the overall length of SNI with an additional advantage of more imaging time available for other nuclear medicine imaging procedures (Higashi et al., 2004:1-4).

2.5.1 Early imaging vs delayed imaging

In 2017, Zarifmahmoudi et al., (2018: 30-34) performed a study to determine at which point after administration of the radiopharmaceutical, SNI does not yield any further diagnostic information and can be terminated. Imaging was performed at 5, 10, 30, 45, 60 and 90 minutes after the periareolar administration of ^{99m}Tc-phytate. Phytate has a larger particle size compared with other pharmaceuticals such as ^{99m}Tc nanocolloid with a particle size of 5 -100 nm and would therefore have a slower transit time from the injection site. In the study, the SLN was identified in 40% of the patients after 30 minutes. The 45 minutes imaging reported an identification of the SLN in 91% of the cases. Imaging beyond 45 minutes did not yield any additional diagnostic information. The study concluded that imaging should be performed up to 45 minutes in cases where the SLN is not visualised and imaging beyond this time seemed unlikely to yield further information regarding identification of SLNs in the axilla (Zarifmahmoudi et al., 2018:30-34).

2.5.2 Summary of literature review

The literature cited describes various techniques and methods to identify the SLN. Studies were performed to evaluate the choice of radiopharmaceuticals, the injection technique and at which imaging time the SLN was most likely to be detected. The choice of radiopharmaceuticals was reported to depend on the particle size and the availability of the radiopharmaceutical. In the majority of cases the injection technique was determined by the local surgical team. It was found that imaging times depended on visualisation of the SLN and the injection technique as to whether it was deep or superficial. When deeper injections (intratumoural, subtumoural and peritumoural) were done, imaging times tended to be longer with further delayed imaging. With superficial injection techniques (periareolar, subperiareolar,subdermal and intradermal), the overall imaging time could be shortened. It was noted that the EANM also provides comprehensive guidelines regarding injection techniques and the imaging times post injection of the radiopharmaceutical.
The literature cited also describes the use of non-ionising methods in the identification SLNs. These methods have proved to be of value in detecting the SLN, but the efficacy of these methods has not been conclusively determined.

CHAPTER THREE

METHODOLOGY OF STUDY

This chapter describes the process of the methodology of this study; ethics process and permissions needed; sample used; data collection, production and analysis; data verification and validation. The limitations of the study are also outlined.

3.1 Ethics

The World Medical Association Declaration of Helsinki stipulates that research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications (World Medical Association, 2013: 2191-2194). Clause 24 of the declaration states clearly the criteria that need to be adhered to, to ensure that the research using patient data and details is ethical. The privacy and confidentiality of the patients must be maintained. All data should be anonymised and all identifying data removed. Patients should be allocated a unique study number in order to achieve this. All results should be saved in an electronic format in a safe password protected desktop computer. The desktop computer must be kept in a safe and secure access controlled office. Access to the data should be limited to the principal researcher, the supervisors and statistician. These criteria were all adequately adhered to in this study.
Ethics approval process for the research study was obtained and granted by:

- Research Ethics Committee(REC) of the Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology: REC Approval Reference No.: CPUT/HW-REC 2019/H1 18th January 2019 – see Appendix F1
- Research committee, Groote Schuur Hospital 23rd January 2019 – see Appendix F3
- Human Research Ethics Committee, University of Cape Town – HREC REF: 099/2019 05th March 2019 – see Appendix F2

Permission for the researcher to access departmental patient data for research purposes at the study site was given by Head of the Division – see Appendix F4.

3.2 Participant researcher

The research was performed by a qualified Nuclear Medicine Radiographer as the principal researcher. The researcher is involved with developing imaging protocols, scintigraphic imaging acquisition and imaging record keeping. All of the scintigraphic images were evaluated and reported by a qualified nuclear medicine physician on the day of the scan. The scan reports (imaging results) were reviewed against the actual images on the nuclear medicine archive by a nuclear physician. The data that was collected by the researcher was verified by a nuclear medicine physician as the clinical supervisor of the research study.

The validity and verification of data were done by cross-checking the demographic data in the nuclear medicine imaging archive, reports, request forms and histology forms. The data were verified by means of by creating two sets of the same data set i.e., the data were entered twice on an excel spread sheet and the two copies were compared to see if the data sets matched.

3.3 Methodology

3.3.1 Population

The population consisted of all patients who underwent SNI from January 2012 until June 2016.

Inclusion criteria:

- Images that were available on the Nuclear Medicine Imaging archive in electronic format
- Studies performed with intratumoural injection
- Studies where the histology reports were available

Exclusion criteria:

- Studies that were performed using the periareolar injection technique (periareolar injections are only performed in cases where the primary tumour is not palpable)
- Studies performed where the radiopharmaceutical was administered at the scar site. (This scar site is used when the patient has had the primary lesion removed.)

3.3.2 Data collection and production process

A search was conducted on the nuclear medicine imaging and reports archives between January 2012 and June 2016 to determine which patients had undergone a SNI investigation. SNI identifying filters were applied to display only the SNI procedures. From this set of data all the patients who had SNI for breast cancer, were identified. From the second set of data all patients who had intatumoural injections were identified.

This data sheet was used to retrieve the original patient referral forms, the hospital folder and histology results.

Demographic data were retrieved from the original patient referral form. From the referral form the following were recorded:

- The date of study
- Age
- Site of the primary lesion in the breast
    - Left vs Right
    - Quadrant

Data retrieved from the imaging archive and reports included:

- The site of the SLN
    - Left vs Right
    - Axillary region
    - Intramammary region
    - Clavicular region
- At which time the SLN was identified
    - Immediate dynamic images
    - Early static images at 30 minutes
    - Delayed static images at 2-4 hours
    - Delayed static images at 24 hours

From the histology reports the following information was extracted:

- Whether there was metastatic spread evident in the SLN

Following the above, all data were anonymised and assigned a unique identifier.

3.3.3 Data validation and verification process

The validity of the data were performed in a stepwise process as detailed in the flow chart below.

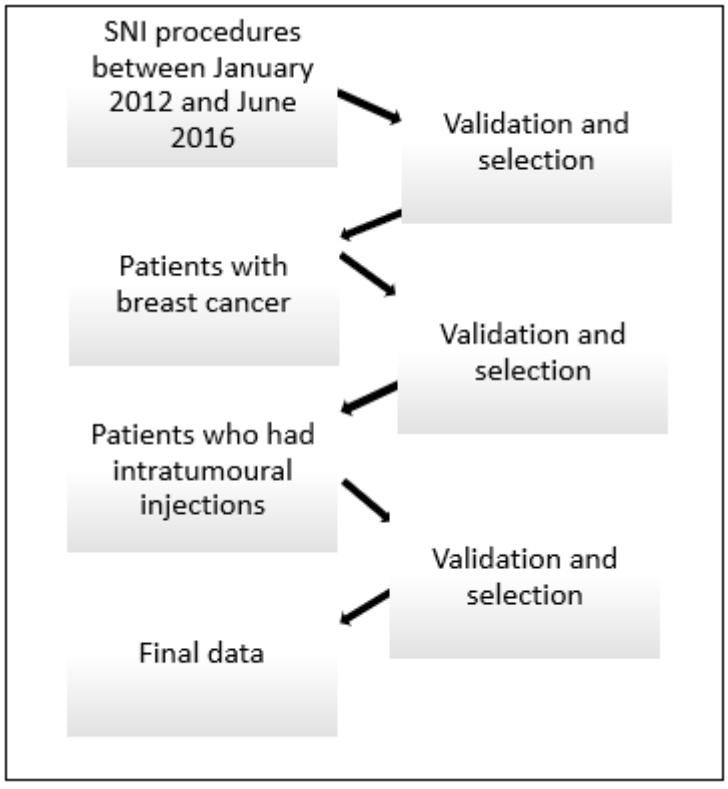

Figure 3.1 Data validation flowchart

Verification of the data were done by cross-checking the data in the nuclear medicine imaging archive, reports, request forms and histology forms. In addition, the data were cross checked by creating two sets of the same data set i.e. the data were entered on two separate excel spread sheets and the two copies were compared to see if that data sets matched. See appendices B, C and D.

3.4 Limitations

The research study encountered various limitations with regards to patient and scintigraphic image information not being available.

- Limited availability of surgical reports making it impossible to comment on the added value of using blue dye.
- From 2005 until 2010 images were captured as hard copy films. These had not been archived and saved in the department and as a result, it is was not possible to verify those particular reports.
- In 2005 the study site made use of a single head gamma camera technology to acquire images for SNI. These cameras became obsolete at the end of 2007 and were replaced with dual head gamma cameras.
- At this study site records are stored for a maximum of six years.
- Towards the end of 2016 the study site implemented a new procurement process using a tender basis. As a result, it was not possible to always ensure the radiopharmaceutical was supplied by the same vendor.
- In 2017 the surgical unit received a donation of a probe and magnetic pharmaceutical, as a result there have been hardly any SNI done since. Therefore, no SLN data were available at the study site subsequently.

3.5 Data Analysis

This study was a retrospective quantitative study. The study consisted of numerical data. Data were entered into an excel spreadsheet. Excel (Microsoft Excel for Office 365 MSO (16.0.10730.20264) and Statistical Package of the Social Sciences (IBM SPSS Version 25) package was used for the statistical analysis.

Descriptive statistics as well as inferential statistics were used in the following:

- The age of patients
- The site of the primary tumour
- Number of patients in whom a SLN was identified with SNI at the various times of imaging

- In which drainage basins were the SLNs found
- Number of patients with tumour positive SLN on histology in the axillae
- Comparing the results of the study site with Group B of the seminal study
- The result of excluding various imaging times on the identification of SLNs in the axillae indicating possible changes to the imaging protocol at the study site.

To determine the measures of central tendency of the data in terms of skewness, Pearson's 2 skewness Sk_2 coefficient table was used (Doane & Seward, 2011: 1-8).
The significance of differences between groups were measured using *p*-values. A *p*-value of less than 0.05 was considered to be statistically significant (Rana & Singhal, 2015:69-71).

Table 3.1 Range for sample skewness (Doane & Seward, 2011: 8)

n	Lower Limit	Upper Limit	n	Lowe Limit	Upper Limit
25	-0.726	0.726	90	-0.411	0.411
30	-0.673	0.673	100	-0.391	0.391
40	-0.594	0.594	150	-0322	0322
50	-0.539	0.539	200	-0.281	0.281
60	-0.496	0.496	300	-0.230	0.230
70	-0.462	0.462	400	-0.200	0.200
80	-0.435	0.435	500	-0.179	0.179

T-tests are used to determine the probability of significant differences in the identification of the SLN between 2 groups. It indicates the difference between the mean values from each data set, the standard deviation of each group, and the number of data values of each group (Kim, 2015:540-546). In this study it was used to describe the frequency of the left and right breast involvement as well as in which quadrants the primary tumour was present.
In order to compare the number of SLNs identified with the imaging technique to the number of SLNs which were found during surgery and positive histologically for metastatic spread, the Student's t-test was performed. The identification of the SLN by SNI vs the identification by surgery was evaluated to establish whether the SLNs identified by SNI were positive for metastasis.

Student's t-tests were also performed to estimate the variation among the different times of imaging during the SNI procedure to calculate the time post injection when the greatest number of SLNs could be identified.

The Chi-square test is used to measure whether there is no association between two groups and also to measure if the distribution of data fits the distribution of the expected data(Rana & Singhal, 2015:69-71). The data of the study site and seminal site were recorded in a

contingency table for this purpose. Chi-square analyses were used to find differences in the categorical measurements of the study site data and the data of the seminal study.

The p value is also known as the observed significance level and was used to test the hypothesis. A value of 5% meaning that there is less than a 1 in 20 chance of being wrong has been used conventionally for many years (Greenland et al., 2016:337-350). A p-value of less than 0.05 was used as the cut off for significance of the results for this study.

3.6 Comparison to the seminal study

The design of the study took into consideration that it should be comparable for homogeneity i.e. measuring the same sample characteristics, must be able to demonstrate similar concepts of other research performed in the field and be able to demonstrate compatibility to what is described in theory. Criteria of the study needed to be comparable to other studies that measured the same or a similar research question (Heale & Twycross, 2015:66-67).

The results at the study site were compared to the results obtained by the seminal study of Valdes et al. (2000). The seminal study evaluated the effectiveness of the injection technique as well as the effect of injecting a higher dose. Group B of the seminal study was injected with the higher dose and the data collected from this group is comparable with that of the study site.

The following were collected to be compared with Group B of the seminal study:

- Quadrants involved
- Comparison of identification of the number of patients in whom a SLN node was identified at the different imaging times
- Comparison of the identification of the SLN

CHAPTER FOUR

RESULTS

A total number of 335 patients were identified on the nuclear medicine archive of the study site. In 25 patients the primary tumour could not be palpated, and therefore the radiopharmaceutical could not be administered intratumourally. These patients were excluded from the study. The histology reports of two patients could not be found and as a result these patients were excluded. The results of the remaining 308 patients were analysed for this study.

Table 4.1 Patient demographics

	Total number of patients
Number of SNI procedures identified for study	335
Primary tumour not palpable (periareolar injection)	25
Patients without histology reports	2
Study site's population	308

4.1 The age of patients

The data were statistically analysed for skewness and were found to be slightly skewed to the left. The skewness value falls within the range of normal skewness. As such, we can assume that the data for age and age range are approximately normally distributed in terms of skewness. The mean is used to measure the central tendency of the data. See Table 4.2 and Figure 4.1

Table 4.2 Age statistics

N	308
Mean	52.31
Skewness	-0.174
Std. Error of Skewness	0.139
Minimum	24
Maximum	78

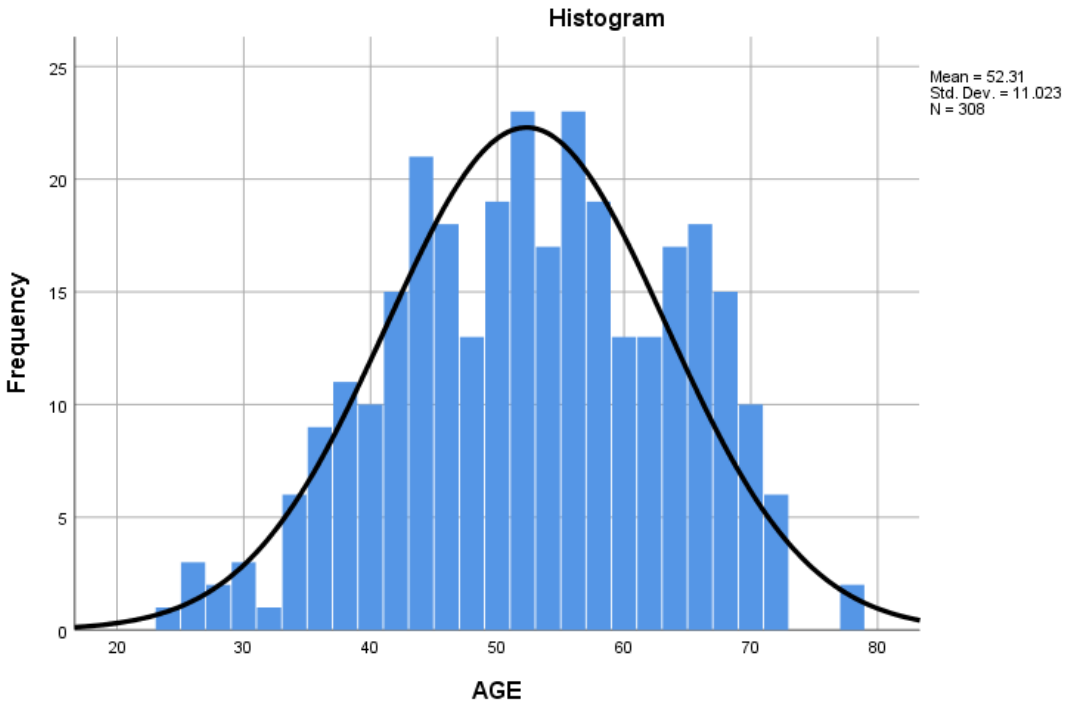

Figure 4.1 Age distribution

The ages of the patients ranged between 24 and 78 years with a mean age of 52 years. A total number of 166 patients were above the age of 52, and 142 patients below the age of 52. An independent samples t-test was performed to calculate whether there were any significant differences in the occurrence of breast cancer in the groups above and below 52 years of age. The result of the sample t-test was, t (308) = 0.109, *p* = 0.461, indicating that there is no significant difference between occurrence of breast cancer in the age groups above and below 52 years. See Table 4.3

Table 4.3 Mean age of patients above the age of 52 years and below 52 years

Age	N	Mean	Std. Deviation	Std. Error mean
Above 52 years	166	60.73	6.017	0.467
Below 52 years	142	42.45	6.383	0.536

The mean age of the patients that were found to be negative with LS was 54 years and 52 years for those that had a positive SNI procedure.

The group of positive studies consisted of 147 patients above the age of 52 years and 129 patients below the age of 52 years. The group of negative studies consisted of 19 patients above the age of 52 years and 13 patients below the age of 52 years. A Pearson's Chi-square test was conducted to evaluate whether there was a relationship between the age of the patient and the identification of the SLN by SNI. The results were found to be, x^2 (1, n=308) = 0.431, p = 0.511. The results indicate that there is no statistically significant difference in the ability to detect the SLN by SNI for the groups above and below 52 years. See Table 4.4

Table 4.4 Age and identification of the SLN by SNI

	Positive	Negative	Total
Above 52 years	147	19	166
Below 52 years	129	13	142
Total	276	32	308

4.2 The site of the primary tumour

The primary tumour was found in 171 patients in the left breast and in 137 in the right breast. A Pearson's Chi-square test was conducted to evaluate whether there was a relationship between the occurrence of breast cancer in the left breast compared to the right breast. The results were found to be, x^2 (1, n=308) = 197.697, $p < 0.001$, indicating a statistically significant difference in the occurrence of breast cancer in the left breast (56%) compared to the right breast (46%).

The occurrence of the primary tumour in the upper two quadrants of the breast was observed in 216 patients and the lower two quadrants in 92 patients.
To evaluate the occurrence of the tumour in the upper two quadrants of the breast compared to the lower two quadrants of the breast, a Pearson's Chi-square test was conducted. The results of the test were, x^2 (2, n=308) = 99.844, $p < 0.001$. The results indicate a statistically significant difference in the occurrence of breast cancer in the upper quadrants of the breast (70%) compared to the lower quadrants of the breast (30%).

The occurrence of the primary tumour in the two outer quadrants of the breast was observed in 211 patients and the two inner quadrants in 97 patients.
To evaluate the occurrence of the tumour in the two outer quadrants of the breast compared to the inner two quadrants of the breast, a Pearson's Chi-square test was conducted. The results of the test were, x^2 (2, n=308) = 84.389, $p < 0.001$. The results indicate a statistically significant difference in the occurrence of breast cancer in the two outer quadrants of the breast (69%) compared to the two inner quadrants of the breast (31%).

The primary tumour was observed most often in the left UOQ in 78 patients.

To evaluate the occurrence of the tumour in the left UOQ of the breast, a Pearson's Chi-square test was conducted to calculate the significance. The results of the test showed, x^2 (2, n=308) = 6.042, p = 0.109. The results indicate that there is no statistically significant difference in the occurrence of breast cancer in left UOQ of the breast compared to the other quadrants of the breast. See Figure 4.2 which summarises the site of the primary tumour

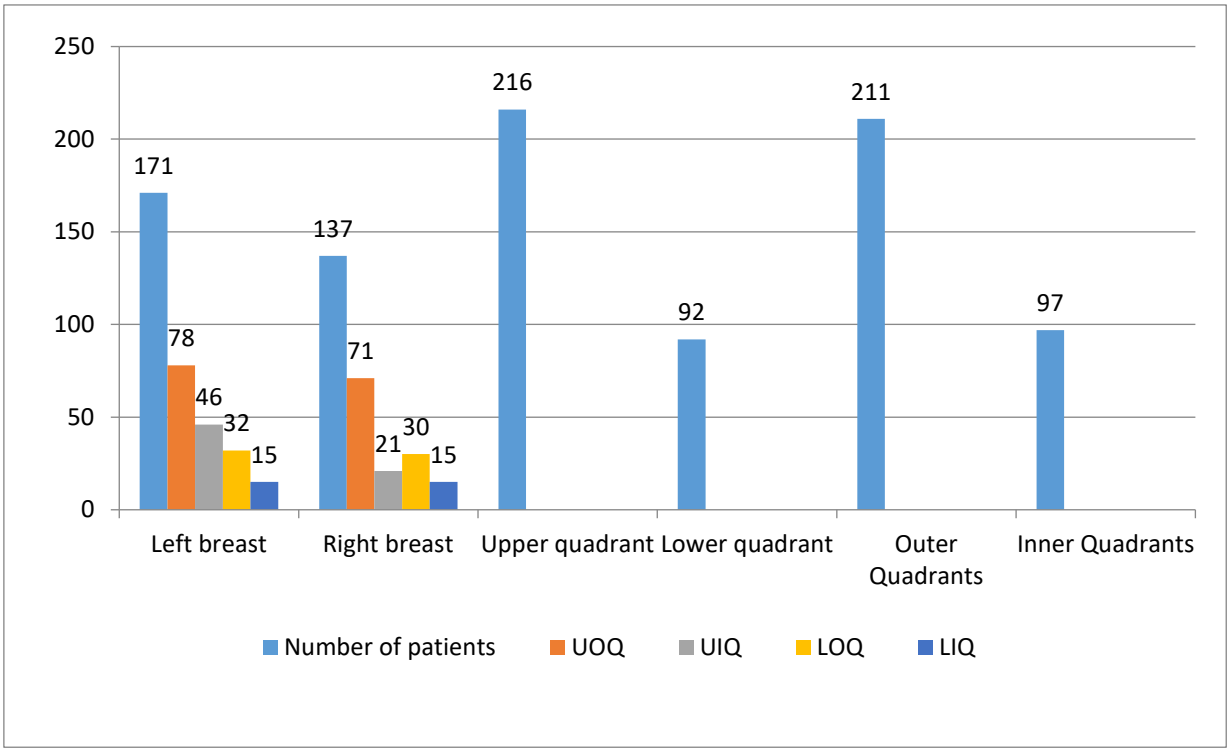

Figure 4.2 Site of primary tumour

4.3 Number of patients in whom the SLN was identified with SNI at the various times of imaging

In all 308 patients the data were analysed to compare the number of patients in which the SLN was identified with SNI. In 276 patients (90%) the SLN was identified with LS compared to 32 patients (10%) where the SLN could not be identified. See Figure 4.3

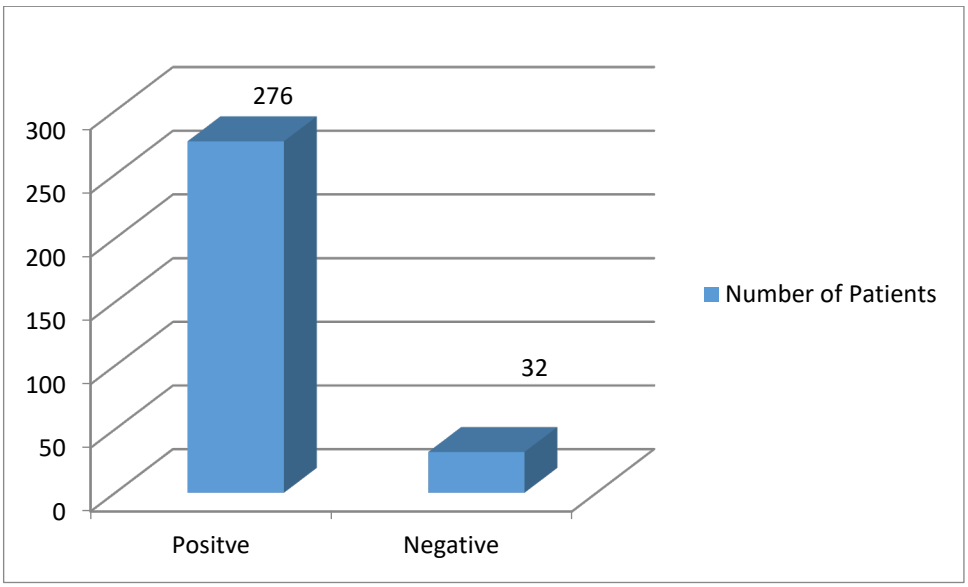

Figure 4.3 Number of positive studies vs negative studies

The SLN (276) was identified in 27% (*n*=83) of patients on the immediate dynamic images. On the early delayed 30 minutes images, the SLN was still visible in these patients and the SLN was identified in an additional 50 patients. The SLN was identified in 43% (133) on the delayed 30 minutes images. On the delayed 2- 4 hours imaging, the SLN was still visible in the patients that were positive on the immediate dynamic images and early delayed 30 minutes images with the SLN identified in an additional 143 patients. The greatest numbers of positive imaging studies were found at the delayed 2-4 hour imaging with identification of the SLN in 90% (*n*=276). Delayed 24 hour imaging was performed on 2 patients that were negative at the delayed 2-4 hour imaging, and these patients were still negative for SLN identification at 24 hours post radiopharmaceutical injection.

These findings are demonstrated in Table 4.5 and Figure 4.4

Table 4.5 SLN identified at different imaging times

	SLN still present	New SLN	Total SLN
Dynamic	0	83	83
Early 30 minutes	83	50	133
2-4 hours	133	143	276
24 hours	0	0	0

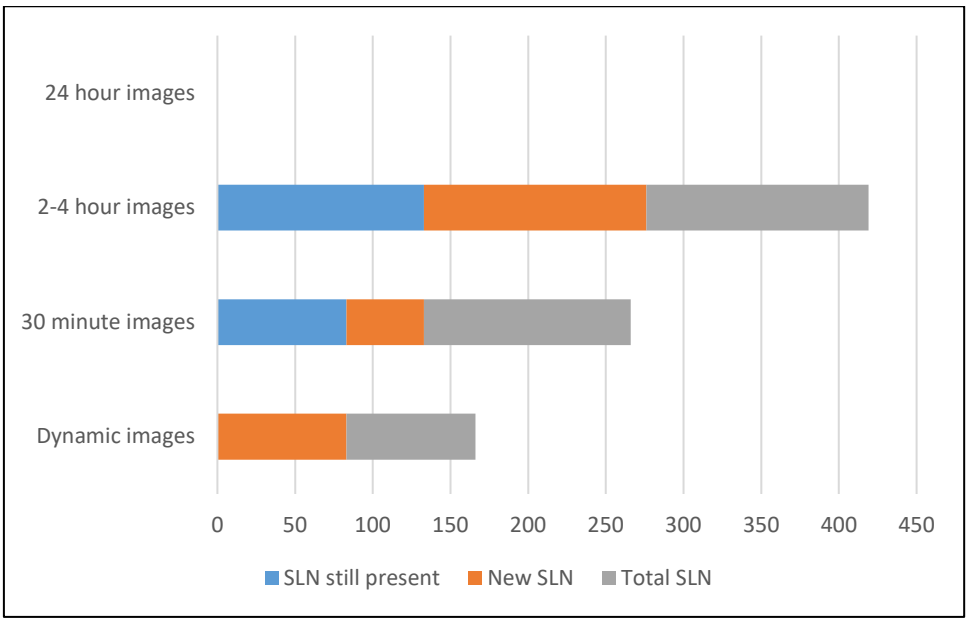

Figure 4.4 Number of patients in whom the SLN was identified with SNI at the various imaging times

4.4 Lymph node drainage basins where the SLN was identified

Lymph node drainage was observed in 270 axillary, 58 internal mammary and 39 clavicular basins respectively. See figure 4.5 and 4.6

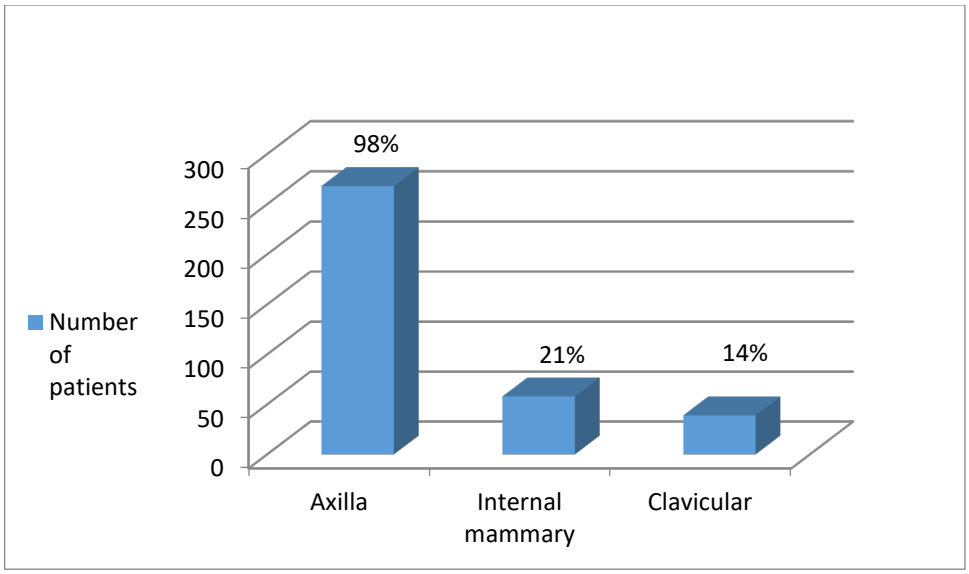

Figure 4.5 LN drainage basins

In 41% (*n*=126) of the patients, multiple drainage basins were seen. See Figure 4.6

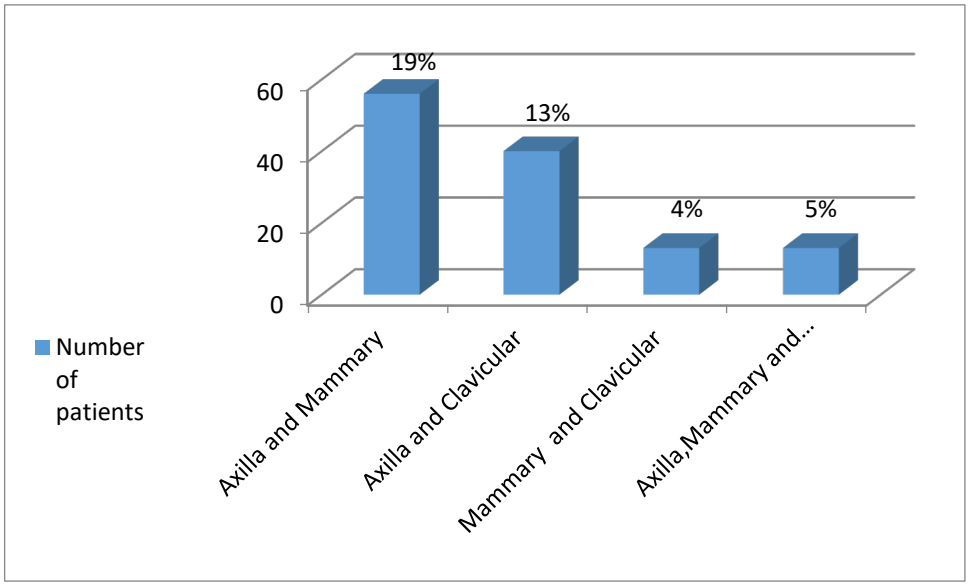

Figure 4.6 Combined LN drainage basins

4.5 Number of patients with tumour positive SLN on histology in the axillae

Of the 276 patients in whom the SLN was identified 270 were in the axillae. When these nodes were examined histologically, 29% (*n*=79) were positive for metastases. There were 3% (*n*=9) patients with positive histology in whom the SLN had not been identified on SNI.

See Figure 4.7

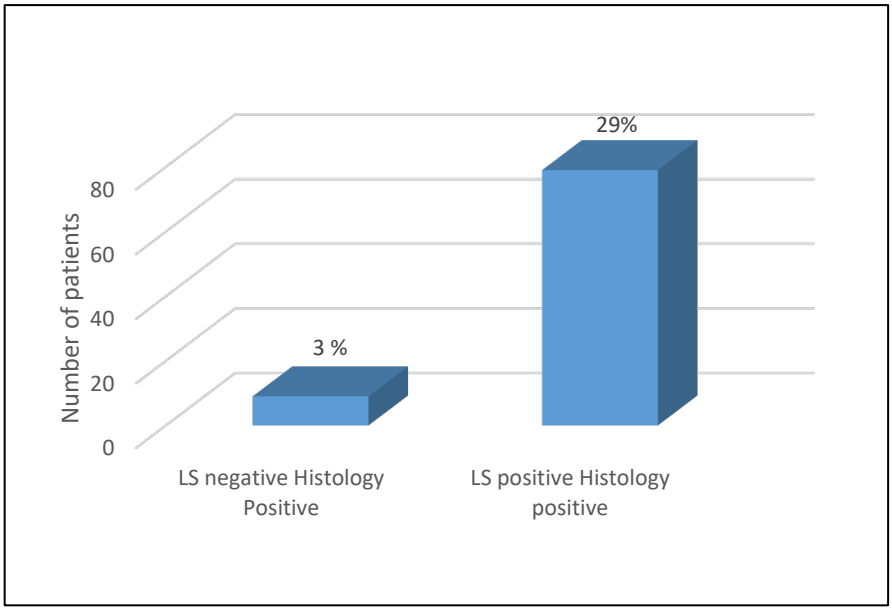

Figure 4.7 Number of patients in which a tumour positive SLN was identified with LS and histology

4.6 Comparison of results

Group B of the seminal study used a similar pharmaceutical dose as that of this study site, and therefore these results were able to be compared.

4.6.1 Quadrants involved at study site compared to quadrants reported to be involved in the seminal study

To evaluate and compare the location of the primary tumour in the breast, an independent-samples t-test was conducted and Levense's Test for Equality of Variance was used to evaluate and compare the quadrants of the study site to the quadrants identified by the seminal study. Results of the study site (mean=1.91, SD =1.035) was compared to the seminal study (mean=1.81, SD = 1.014). The *t* score was reported as *t* (308) =0.642, *p*=0. 521.These results suggest that there is statistically no significant difference between the quadrants identified by the study site compared to the quadrants identified by the seminal study. See Table 4.6

Table 4.6 Quadrants of study site vs seminal study

	Study site	Seminal study
UOQ	149	25
UIQ	67	10
LOQ	62	8
LIQ	30	4
Total	308	50

4.6.2 Comparison of different imaging times of study site vs seminal study

In the seminal study there was no differentiation between the dynamic images and the early 30 minute images. Both these sets of images were combined in a grouping as early images. In order to evaluate and compare at which time of the SNI procedure most SLNs were detected, an independent-samples t-test was conducted and Levense's Test for Equality of Variance was used to evaluate and compare the times most SLNs were detected by the study site compared to the times most SLNs were detected by the seminal study. Results of the study site (mean=1.30, SD =0.458) were compared to the seminal study (mean=1.43, SD = 0.500). The *t* score was reported as *t* (308) = -1.745, *p* = 0.082. These results suggest that there is statistically no significant difference between the identification of the SLN of the study site and seminal study, at the different imaging times of the SNI procedure. See Table 4.7

Table 4.7 Comparison of time phases between study site vs seminal study

	Study site	Seminal study
Early imaging	133	27
Delayed 2-4 hours	276	47

4.6.3 Comparison of the drainage basins seen in this study site vs seminal study

A sample *t* test was performed to calculate if there was a significant difference between the data from the study site compared to the seminal study regarding the location of the SLN. The results of the sample *t* test were, t (4) = 5.387, $p = 0.033$, indicating that there is no statistically significant difference between the study site's data compared to the seminal study. See Table 4.8

Table 4.8 Drainage basins comparison between study site and seminal study

	Study Site	Seminal Study
Axillary basin	270	45
Non axillary basin	97	8
Total	367*	53

*In 126 patients multiple drainage basins were identified.

4.6.4 Comparison of percentage of SLN identified in the study site vs seminal study

To compare the results of the study site with group B of the seminal study, Pearson's Chi square analysis was performed. The Pearson's Chi square was calculated as $x^2 = 0.940$, (1, n=308), $p = 0.332$ indicating no significant difference between the results of the study site and the seminal study.

Table 4.9 Results of study site vs seminal study

	Study Site	Seminal Study
Total number of patients	308	50
Patients with SLN identified	276	47
Patients with SLN not identified	32	3
Percentage SLN identified	90 %	94 %

4.6.5 Comparison of number of patients with tumour positive SLN on histology in the study site vs seminal study

Table 4.10 Number of patients with tumour positive SLN on histology in the study site vs seminal study

	Study Site	Seminal Study
Total number of patients with SLN identified	276	47
Patients with tumour positive SLN	88	15
Patients with tumour negative SLN	188	32
Percentage patients with tumour positive SLN	32 %	32 %

4.7 Summary of the results

Our study found a higher incidence of breast cancer in patients in the age group above 50 years and that the UOQ was mostly involved as the primary site of the tumour. The axillary basin was found to be involved in most of the cases and the number of axillary basins are comparable to that of the seminal of the study. The study confirmed that the majority of positive imaging occurred on the later delayed imaging and that the results of the study site were found to be similar when compared to the results of the seminal study. SLNs that were identified on early imaging remained visible throughout the study and additional SLNs were identified on later imaging. When compared to the seminal study the same percentage of patients with histologically positive nodes were identified.

CHAPTER FIVE

DISCUSSION

SNI is widely used to evaluate the presence of metastases in lymph nodes and is commonly performed in patients with breast cancer. The SLN identified can be removed by limited surgery and tested for the spread of tumour cells to determine whether further surgery is necessary. The current protocol requires the patient to visit the nuclear medicine department twice on the day before surgery with a possible third visit 24 hours after the initial visit just before the surgery. The primary question for this study was: How can the current SNI protocol be optimised in terms of reducing the number of imaging times without reducing the number of SLNs identified at the study site?

5.1 Results of SNI studied at the site

The first question asks, “What are the results at the study site using the current protocol?”

- The age of patients
- The site of the primary tumour
- Number of patients in whom the SLN was identified with SNI at the various imaging times
- In which drainage basins were the SLN found
- Number of patients with tumour positive SLN on histology in the axillae

5.1.1 The age of patients

The risk of breast cancer increases for patients above the age of 50 years and accounts for 87% of cancer related deaths (Cheng et al., 2011:562-575). The age of the patients at the study site ranged between 24 and 72 years with a mean age of 52 years. Patients were classified into two groups namely, below and above 52 years. A total number of 54%(*n*=166) patients were above the age of 52, and 46% (*n*=142) of patients below 52 years, thus indicating a tendency of a higher number of patients in the older group diagnosed with breast cancer, although this was not found to be statistically significant in our study. The mean age of those above 52 years of age was 61 years, and the mean of those below 52 years of age was 43 years. See Table 4.3. The incidence of breast cancer is commonly higher in older patients as compared to younger patients. Dobi et al., reported that there is an increasing number of younger patients being diagnosed with breast cancer resulting in the decrease of the ratio of older to younger patients diagnosed with breast cancer. Their ratio is similar to the ratio found in our study (Dobi et al., 2011:425-428).

The ability to identify the SLN with LS was found not to be related to the age of the patient in this study.

The slightly higher number of patients in the age group above 52 years could be due to decreased lymphatic flow as a result of more fatty tissue in older patients and or the inability of the lymph nodes to retain the radiopharmaceutical (Krag et al., 1998:941-946). Non-visualisation could be the result of the SLN being obscured by the injection site and or retention of the radiopharmaceutical at the site of injection as well as the absence of lymphatic flow from the tumour (Krag et al., 1998:941-946).

5.1.2 The site of the primary tumour

The presence of the primary tumour in the left or right breast is described as the laterality ratio. Breast cancers are in general diagnosed more in the left breast than the right breast. Sughrue et al., indicated that laterality can be influenced by the age of the patient. In our study, the primary tumour was present in the left breast for 56% (*n*=171) of patients, and right breast involvement was observed in 46% (*n*=137) patients. The difference is statistically not insignificant and can be described as similar to that reported in the literature (Sughrue & Brody, 2014:8).

Tumour involvement was identified in all four quadrants of the breast. The primary tumour was observed in 219 patients involving the upper two quadrants compared to 92 patients in the lower two quadrants. When comparing the outer two quadrants to the inner two quadrants, the primary tumour was observed in 211 patients for the outer two quadrants and 97 patients for the inner two quadrants. These differences were found to be statistically significant when comparing the upper vs lower quadrants, and outer vs inner quadrants. See Table 4.6. A study conducted by Lee confirmed the findings of our study and indicated that the higher incidence of the primary tumour in the upper breast is due to the fact that the upper quadrant consists of more breast tissue (Lee, 2005: 151-152).

The site of the primary tumour was observed most often in the left UOQ seen in a total of 78 patients. See Figure 4.2. Although being recorded as the highest occurrence in the left UOQ, there is no statistical difference when compared to the other quadrants of the breast. These findings are consistent with results reported by Cheng et al., (2011:562-575).

5.1.3 Number of patients in whom the SLN was identified with SNI at the various imaging times

The imaging times involved are immediate dynamic images, early static images at 30 minutes, delayed static images at 2-4 hours; with possible delayed static images at 24 hours.

Early SLN identification was seen in 27% (*n*=83) patients on the dynamic images compared to 43% (*n*=133) patients where the SLN was identified on the early 30 minutes images. The greatest number of positive SLNs were identified on the delayed 2-4 hour images. The 2-4 hour images yielded 90% (*n*=276) patients in which the SLN was identified. The higher rate of identification on the delayed 2-4 hour images could be attributed to the fact that the radiopharmaceutical was administered intratumorally which is known to have to slower transit time from the injection site to distant SLNs. Our study was also able to detect SLNs on the early dynamic as well as the early 30 minutes images although to a lesser extent when compared to the later images. Early visualization could be due to the chemical characteristics ^{99m}Tc nanocolloid with a particles size of 5-100 nm. These characteristics of the radiopharmaceutical allows for rapid transit and thus the ability to detect more SLNs. In our study, the SLNs identified on early images remained visible on the later images. The study confirmed that the majority of positive identification imaging occurred on the 2-4 hour delayed imaging and would therefore consider excluding early dynamic and early 30 minute imaging from the SNI protocol.

The number of positive studies at the various imaging times was compared to those of the seminal study. The results indicated that there was no significant difference (p=0.082) between the two studies, with both studies identifying more SLNs at the delayed 2-4 hours imaging time. Both the radiopharmaceutical and the injection technique can influence the time at which the SLN is identified. Imaging at a later imaging time (2- 4 hours) appears to be more efficient in terms of identifying the SLN. This is illustrated in Table 4.5 and Figure 4.4.

Imaging was only performed in two patients at 24 hours. These two patients remained negative for the identification of the SLN by SNI and no metastasis were present in the nodes removed. Both patients fell within the category of patients above 50 years and non-visualisation could be the result of retention of the radiopharmaceutical at the site of injection and/or the absence of lymphatic flow from the tumour (Krag et al., 1998:941-946).

5.1.4 Lymph node drainage basins where the SLN was identified

Our study showed overall axillary drainage in 98% (*n*=270) of the patients, followed by internal mammary drainage in 21% (*n*=58) and clavicular drainage in 14% (*n*=39) of patients. See Figure 4.5.

In 46% of patients, multiple drainage basins were seen. Drainage to the axillary and the mammary basin was seen in 19% patients whereas drainage to axilla and clavicular was reported in 13% patients. In 4% patients the mammary and clavicular basins were involved.

Drainage that involved all three basins, axillary, mammary and clavicular was seen in 5% patients. See figure 4.6.
These numbers are similar to those described in the seminal study with the axillary basin most often involved, followed by intramammary basin involvement and then clavicular basin involvement (Valdes-Olmos et al., 2000:1500-1506).
Several factors influence the drainage pattern from the tumour and these include the radiopharmaceutical, injection technique as well the SNI protocol. Mariani et al., described that the drainage patterns are more or less comparable to the 3 main arterial blood supply and that most of the drainage occurs to the axillary basin (Mariani et al., 2001:1198-1215).
Chen et al., (2005: 251-257) described 88% axillary drainage in their study.
The number of axillary and intramammary basin involved for the study site and seminal study showed no significant difference. However, when comparing clavicular basins, a significant difference was noted, our study showed a greater number of clavicular basins. Brenot-Rossi et al., (2003: 1232-1237), indicated that normal variations in the anatomy of the lymphatics and various flow patterns could explain our findings.

5.1.5 Number of patients with tumour positive SLN on histology in the axillae

Out of the 276 patients that the SLN was identified by SNI the SLN was identified in the axilla in 98% (*n*=270) patients. In 2% (*n*=6) of patients, non axillary nodes were exclusively identified. Histological evidence of metastases was seen in 32% (*n*=88) of these SLN. This results were similar to those of the seminal study reported as 32% (*n*=15) (Valdes-Olmos et al., 2000:1500-1506).

At the study site, surgeons make use of the intra operative probe as well blue dye to detect SLNs. The combination of SNI with a gamma probe and blue dye increased the overall identification of the SLN and this is recommended practice (Giammarile et al., 2013:1932-1947).
The axillary basin appears to be the primary basin from the breast with direct drainage to the axillary lymph nodes (Brenot-Rossi et al., 2003:1232-1237).

5.2 Comparison of the results to Group B of the seminal study

The second question asks, “Are the results of the study site the same as that of the seminal study?”
The seminal study investigated the effectiveness of intratumoural injection and the effect of administering a higher dose on the identification of the SLN. Our study administered a higher dose similar to group B of the seminal study.

5.2.1 Quadrants involved

The UOQ was involved in 54% (*n*=149) of the patients at the study site compared to 53% (*n*=25) in the seminal study. Results for the UIQ were 24% (*n*=67) and 21% (*n*=10) for the study site vs the seminal study respectively. Similar results were found when comparing the LOQ with 22% (*n*=62) at the study site vs 17% (*n*=8) at the seminal study. The LIQ involvement was recorded as 11% (*n*=30) vs 9% (*n*=4) for the seminal study. These results show that there is statistically no significant difference between the quadrants identified by the study site compared to the quadrants identified by the seminal study. See Table 4.6
The laterality ratio of the study site was calculated as 1.25 and is comparable to 1.14 of the seminal study (Valdes-Olmos et al., 2000:1500-1506).

5.2.2 Comparison of different imaging times

Early images were able to identify the SLN in 43% (*n*=133) of the cases compared to the seminal study reporting 54% (*n*=27). Delayed imaging showed similar results with 90% (*n*=276) for the study site and 94% (*n*=47) for the seminal study.
No statistical differences in the numbers of patients in whom the SLN could be identified was demonstrated in both the early and later delayed images.

5.2.3 Comparison of number of patients with tumour positive SLN on histology in the axillae

The SNI protocol at the study site was able to identify the SLN in 90% of the patients and is similar to the results of Group B (94%). There is no significant statistical difference when comparing the results with p=0. 332. See Table 4.9. The combination of SNI with a gamma probe and blue dye increase the overall identification of the SLN and this is recommended in practice (Giammarile et al., 2013:1932-1947).
The findings suggest that using a similar injection technique, radiopharmaceutical and imaging protocol it is possible for the study site to achieve similar results.

5.3 Optimising the SNI protocol of the study site.

The third question asks, “What is the result of excluding various imaging times on the identification of SLNs in the axillae and were there any SLNs identified on the early images but not on the later images?

The majority of positive SNI studies were observed at the delayed 2-4 hours imaging (90%) compared to the early dynamic imaging (27%), early static imaging at 30 minutes (43%) and delayed 24 hours (0%). This difference was statistically significant with $p < 0.001$.

The study furthermore demonstrates that the SLNs identified on the early dynamic and early static images at 30 minutes were still visible on the later delayed images at 2-4 hours. This suggests that the exclusion of early dynamic and early static imaging at 30 minutes would not affect the number of SLN identified in the axillae.

With dynamic imaging the practice is to inject the patient on the camera bed. If the dynamic images were excluded it would not be necessary to inject the patient on the camera bed. This would improve camera utilization and prevent possible contamination.

5.4 Current studies from the seminal site

The Netherlands Cancer Institute, where the seminal study was conducted, recently conducted studies to evaluate predictive risk factors for non-visualisation of SLNs (Hellingman et al., 2019:421-429). A further study was conducted by this institute to evaluate the association of non-visualised SLN with higher nodal metastases (Hellingman et al., 2019:317-324).

Both studies used ^{99m}Tc nanocolloid injected intratumourally with the planar imaging times at 15 minutes and 3-4 hours post injection. In the first study additional SPECT/CT images were acquired after the non-visualisation of the SLN and the patients were reinjected. The identification of the SLN was 87% with planar imaging which increased to 92% with the SPECT/CT and/or reinjection of the radiopharmaceutical (Hellingman et al., 2019:317-324). This study described several factors that could possibly cause non-visualisation of the SLN. These factors were older age, high body mass index, mantle field radiation therapy, large tumours, nonpalpable tumours and tumours that were located medially. In addition to these findings, they recommended using periareolar injections in patients identified with limited prognosis in relation to internal mammary SLNs identification (Hellingman et al., 2019:421-429).

In the second study SPECT/CT images were required after non-visualisation on the 3-4 hour planar images. The identification of the SLN of this study was 87% with planar images and the overall identification of the SLN after SPECT/CT images increased to 91%. The second study concluded that the non-visualisation of the SLN after SPECT/CT imaging is not associated with higher nodal metastases (Hellingman et al., 2019:317-324).

Both studies indicated a high identification of the SLN on both planar and SPECT/CT images. It is noted that the Netherlands Cancer Institute did not perform dynamic or 30 minute images but rather 15 minute and the delayed images at 3-4 hours compared to the our current protocol at the study site. The change in their protocol was confirmed in

correspondence between the researcher and the head of nuclear medicine of their institution. Refer to appendix F.
The studies conducted did not indicate whether the SNI procedure was positive or negative on the early 15 minute images. The identification remains high for planar imaging (87%) at the Netherlands Cancer Institute.

5.5 Proposed research at the study site

Wagner et al., (2013: 191-202), indicated the need to use SPECT/CT imaging in combination with standard lymphoscintigraphy known as hybrid imaging. This type of imaging allows for the fusing of SPECT/CT images and thus makes it easier for interpretation of the anatomy in a three-dimensional view. It would also appear to have a higher detection rate of lymph nodes in the interpectoral and intramammary locations. From a surgical point of view, namely the "see and open" approach, the additional value SPECT/CT is the ability to locate the exact anatomical position of the tumour (Wagner et al., 2013:191-202).
At our study site the new hybrid imaging technique only became available at a later stage and thus no SPECT/CT SNI has been performed at the time of this study.
Based on the guidelines and literature and the availability of hybrid imaging (SPECT/CT) at the study site at present, it is recommended that further studies be performed to evaluate the SNI protocol using the dual injection method as well as SPECT/CT.

The use of the dual injection technique (deep and superficial) could result in the detection of more SLNs on early images. The guidelines of the EANM suggested the use of a combination of both deep and superficial injections may improve SLN detection and decreased FN findings. For deep injections a volume of 0.5-1.0 ml is indicated and for superficial injections a smaller volume of 0.05-0.5 ml is recommended (Giammarile et al., 2013). Kim et al indicated a volume of 0.5 ml for periareolar injections would cause minimum discomfort to the patient (Kim et al., 2004: 1597-1599).

Further research using the dual injection technique would be relatively easy to perform and would not require specialised equipment. The possibility of a decrease in FNs with early visualisation would further decrease over all imaging time and improve results.

At the time of this study, the SPIO method was introduced at our study site as the method of choice for identifying SLNs and hardly any SNI were performed on patients. The data of this research can be used to compare the identification of SLNs to that of the SPIO method.

CEUS and molecular imaging are not used at our study site as the method of choice for identification of SLNs and when this becomes available to the study site it should be investigated to evaluate the efficacy and consistency of the use of these techniques.

5.6 Limitations of the study

The research study encountered limitations regarding patient and scintigraphic image information not being available.

Surgical reports that indicate the findings of the SLN by means of blue dye were only available for 135 (56%) patients out of the 308 and were excluded from the study. Schafer indicates that when missing data is equal or larger than 5% of the population, results could be biased whereas Bennet indicated a 10% or larger as being biased data (Bennett, 2001:464-469; Schafer, 1999:3-15). As a result of the large number of surgical reports that were not available, the study did not report on the identification of the SLN with blue dye.

From 2005 until 2010 images were captured as hard copy films and as a result, it was not possible to verify the reports. In 2005 the study site made used of a single head gamma camera to acquire images for SNI. These cameras became obsolete at the end of 2007 and were replaced with dual head cameras which were used for scintigraphy. At this study site records are stored for a maximum of 6 years before being destroyed.

Towards the end of 2016 the study site implemented a new procurement process using a tender basis. As a result, it was not possible to always ensure the radiopharmaceutical was supplied by the same vendor.

In 2017 the surgical unit received a donation of a probe and magnetic pharmaceutical, as a result there have been few SNI done since. No SLN data could be recorded at the study site subsequently.

This was a quantitative retrospective study that included all the patients that were referred to the NM department for SNI procedures. Disadvantages of a retrospective study consist of obtaining information of previous recorded data. The researcher relies on the fact that those records were recorded accurately. Retrospective studies are also exposed to misrepresentation of the association between the independent and dependent variables, bias, and in the selection of control groups (Sedgwick, 2014:1072).

CHAPTER SIX

CONCLUSION

Accurate identification and location of the SLN are imperative in terms of the prognosis and management of patients with cancer of the breast.

In 2005, the study site compiled a protocol which was based on the original protocol devised by Valdes-Olmos (Valdes-Olmos et al., 2000:1500-1506). After consultation with the Department of Nuclear Medicine of the Netherlands Cancer Institute, the protocol was implemented and has been used by the study site from 2005. This protocol involves several visits to the department for dynamic (flow) images, followed by early planar images at 30 minutes and delayed imaging time at 2-4 hours and at 24 hours where indicated.
Then in 2016 the breast cancer surgeons at the study site stated their concern about the number of visits required to the nuclear medicine (NM) department. The length of time required for imaging was having a negative impact on the patient's pre-operative preparation with regards to the scheduling of other procedures that patients require prior to the surgery.

In order to address this, the study site reviewed the results of SNI done over five years with the aim of determining if it was possible to exclude any of the imaging times. It was important to be able to demonstrate that the same number of SLNs in the axillary drainage basins would still be identified and that no SLNs seen on early images could not be seen on later images.

Our study found a higher number of patients in the older group (above 52 years) diagnosed with breast cancer and confirmed the findings in literature that the incidence of breast cancer is commonly higher in older patients as compared to younger patients.
The identification of SLNs by SNI for both the age groups below and above 50 years showed similar results and, in our study, the ability to identify the SLN with LS was found not to be related to the age of the patient.

The primary tumour was mostly present in the left UOQ at this study site which is similar to the findings in the seminal study with no statistical difference. The occurrence of the primary tumour location between the left breast and the right breast had a laterality ratio of 1.25. This ratio is similar to that of the seminal study of 1.14.

Early images were able to identify the SLN in 43% of patients compared to the seminal study with 54% whilst delayed imaging showed similar results with 90% for the study site and 94%

for the seminal study. There was no statistical difference in the identification of the SLN in either group.
Histological evidence of metastases was seen in 32% of the SLN identified at the study site with the same results as the seminal study (32%). The findings suggest that when using a similar SNI protocol, it is possible for the study site to achieve similar results.

We confirmed with this study that the majority of the SLN were identified on the later delayed imaging at 2-4 hours. Importantly we showed that there were no SLN seen on the earlier images that were not seen on the delayed images. These findings suggest excluding early dynamic and early 30 minute imaging from the SNI protocol. By excluding early imaging this will reduce the number of times the patients are required to visit the department from a possible 3 times to a possible 2 times. Most patients will only have to visit the department once as opposed to twice. The added benefit would be more imaging time available for other nuclear medicine imaging procedures.

SNI imaging was only performed on two patients at 24 hours and these did not reveal any additional SLNs. Even though these numbers are small it is deemed unlikely that additional images performed at 24 hours will significantly improve SLN identification. The additional visits to the department for the patients and possible delay on the day of the surgery are also factors which should be taken into consideration.

More recently, The Netherlands Cancer Institute conducted a study to evaluate predictive risk factors for non-visualisation of SLNs. SPECT/CT images were required after non-visualisation on the 3-4 hour planar images. The identification of the SLN increased from 87% on the planar images to 91% with SPECT/CT images (Hellingman et al., 2019:421-429). The study site now has SPECT/CT capability, and this could be performed if the 2-4 hour delayed images are unable to identify the SLN.

Periareolar injection techniques should be investigated as per the guidelines of the EANM and SNMMI for LS and SN mapping which suggest that the combination of both deep and superficial injections may improve SLN detection and may result in decreased FN findings.

Furthermore, the use of molecular imaging should be investigated to evaluate the detection, characterization, and quantification of biologic processes at cellular and molecular levels. Yararbas et al., (2018: 72-79), indicated that PET imaging with ^{18}F-FDG is known to evaluate the primary tumour and one of the major advantages of PET is the ability to detect distant extra axillary LN metastases as well as micro metastases. They reported that lymph nodes that were detected after surgery with PET/CT may affect the management in patients with

isolated metastatic axillary lymph nodes (Yararbas et al., 2018:72-79). At our study site no studies were done as yet to evaluate and to validate the use of PET imaging as standard procedure for patients with breast cancer.

At present, the SPIO method is the method of choice for identifying SLNs at the study site and it could be of value to compare the identification of SLNs of our study to the SPIO method.

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APPENDICES

APPENDIX A: CURRENT PROTOCOL AT STUDY SITE

Surgeons and Nuclear Medicine Physicians consult to do a pre-operative assessment to validate if the patient would qualify for LS. Hardcopy images are provided to the surgeons on the day of the surgery and assisted with a gamma probe to locate nodal involvement. This technique is used in combination with blue dye injected subcutaneously (periareolar) on the day of surgery. This imaging protocol was implemented after it was found that the study was a valid and reliable method in identifying SLN.

Radiopharmaceutical, dose and volume:

The radiopharmaceutical is reconstituted with 500 MBq pertechnetate in a volume of 1 ml. This is added to Amersham NANOCOLL kit.

Activity of 80MBq -100MBq is drawn up in a volume of 0.2 ml with a 1ml syringe attached to a 26 gauge needle.

The administered dose is calculated as follows:

Activity drawn-up – Residual activity = Injected Dose

The injection is administered intratumorally.

Imaging:

Instrumentation:

A dual head Gamma camera with Medium energy all-purpose collimators set at an energy window of 10% centred over 140 keV photo peak of ^{99m}Tc in 256 x 256 matrix. The ^{57}Co transmission source is used to outline the body and a point source of 2 -5 MBq pertechnetate is used as a marker for the SLNs. A point source is a small cylindrical shaped capsule wherein the radioactive material is sealed.

Acquisition:

The patient is positioned supine with the arm on the side of affected breast raised above the head for lateral and anterior images. The position of the arm would be the same position as it would be during surgery. This arm is then moved out at the side and placed on a trolley for the anterior images.

Routine images are acquired immediately (flow/dynamic images), 30 minutes, 4 hours and occasionally 24 hours static images, over the area of interest.

The dynamic images are acquired for 15 seconds per frame for 15 minutes on a 128 x 128 matrix size, in an anterior position. Static images are acquired for 200 seconds on 256 x 256 matrix size, in the anterior, as well as lateral (of the affected side) position.

After the final set of images the scan is reviewed by the clinician and if a sentinel node is seen, it is marked on the patient's skin with a permanent marker with the aid of a radioactive point source marker. This mark corresponds to where the sentinel node position is visualised on the image monitor. These markings are confirmed and count rate documented using the intraoperative gamma probe.

This will conclude the NM imaging and the patient leaves the department.

APPENDIX B: DATA COLLECTION TABLE

Age of patient ☐	Identified on: Dynamic(flow)-immediate ☐ Early planar-30 minutes ☐ Delayed planar- 2 to 4 hours ☐ Delayed planar- 24 hours ☐
SLN identified: Yes ☐ No ☐	Site of SLN Axilla ☐ Internal mammary ☐ Clavicular region ☐
Number of foci 1 ☐ 2 ☐ 3 ☐ >3 ☐	SLN positive for malignancy Yes ☐ No ☐
Tumour Quadrant Upper outer quadrant ☐ Upper inner quadrant ☐ Lower outer quadrant ☐ Lower inner quadrant ☐	Surgical findings Radioactive node ☐ Blue dye node ☐

APPENDIX C: DEMOGRAPHIC DATA COLLECTION EXCEL SHEET

	A	B	C	D	E	F	G	H	I	J	K	L	M
1		Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No		
2	Age	SLN Identified	Identified on Dynamic	Identified on early planar	Identified on late planar	Axillary basin	Internal mammary basin	Clavicular basin	Histology Positive	Radioactive node (Surgery-Gamma Probe)	Blue dye positive	Number of Foci	Tumour Quadrant
3													
4													
5													
6	Table :												
7	Yes=1	Dynamic = immediate											
8	No=2	Early planar=30 minutes											
9		Delayed planar= 2 to 4 hours											
10													

Yes=1

No=2

Dynamic = immediate

Early planar=30 minutes

Delayed planar= 2 to 4 hours

APPENDIX D: COMPLETED DATA COLLECTION EXCEL SHEET

	Numerical Number	Yes=1/No=2	Numerical Number
PATIENT IDENTIFIER	AGE	SLN IDENTIFIED LS	NUMBER NODES HISTOLOGY
NM1	47	1	2
NM2	52	1	3
NM3	44	1	1
NM4	55	1	3
NM5	68	1	5
NM6	51	1	2
NM7	63	2	1
NM8	69	1	3

APPENDIX E: ETHICS APPROVALS

Appendix E1: Ethics Approval (Cape Peninsula University of Technology)

Cape Peninsula
University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

18 January 2019
REC Approval Reference No:
CPUT/HW-REC 2019/H1

Dear Mr Theodore Russel Geldenhuys

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to Mr Geldenhuys for ethical clearance. This approval is for research activities related to student research in the Department of Medical Imaging and Therapeutic Sciences at this Institution.

TITLE: **A Review of the sentinel node imaging protocol at a tertiary hospital in the Western Cape**

Supervisor: Ms B Wyrley Birch, Ms C Lackay and Dr T Kotze

Comment:

Approval will not extend beyond 19 January 2020. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Dr. Navindhra Naidoo
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Appendix E2: Ethics Approval (Human Research Ethics Committee, University of Cape Town)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

05 March 2019

HREC REF: 099/2019

Dr T Kotze
Department of Nuclear Medicine
J-Block, GSH

Dear Dr Kotze

PROJECT TITLE: A REVIEW OF THE SENTINAL NODE IMAGING PROTOCOL AT A TERTIARY HOSPITAL IN THE WESTERN CAPE (MASTER OF SCIENCE RADIOLOGY: MR T GELDENHUYS)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 March 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Mr Theodore Geldenhuys will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

Appendix E3: Ethics Approval (Research Committee, Groote Schuur Hospital)

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
E-mail : Bernadette.Eick@westerncape.gov.za

Mr T. R. Geldenhuys
CPUT – Health & Wellness Sciences

E-mail: theodore.geldenhuys@uct.ac.za

Dear Mr. Geldenhuys,

RESEARCH PROJECT: A Review Of The Sentinel Node Imaging Protocol At A Tertiary Hospital In The Western Cape

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until **19 January 2020.**

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No additional costs to the hospital should be incurred i.e. Lab, consumables or stationary.
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
f) Confidentiality must be maintained at all times.
g) **Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).**
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) **Kindly submit a copy of the publication or report to this office on completion of the research.**

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 23 January 2019

C.C. Mr. L. Naidoo
Dr H. Aziz

G46 Management Suite, Old Main Building,
Observatory 7925
Tel: +27 21 404 6288 fax: +27 21 404 6125

Private Bag X,
Observatory, 7935
www.capegateway.go.v.za

Appendix E4: Data Collection Permission Approval (Nuclear Medicine Department, Groote Schuur Hospital)

GROOTE SCHUUR HOSPITAL
C3 Nuclear Medicine
It 0214044169

To:

CAPE PENINSULA UNIVERSITY OF TECHNOLOGY: HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE

Date: **3 August 2018**

To whom it may concern,

I hereby give Mr Theodore Russell Geldenhuys (Course: MSc Radiography

Student Number: 192056247) permission to access departmental patient data for the purpose of research for his thesis titled:

A Review of the sentinel node imaging protocol at a tertiary hospital in the Western Cape.

Yours sincerely

Rachelle Steyn (Dr)
Acting Head of Nuclear Medicine Division

Appendix F: Correspondence with Netherlands Cancer Institute

From: Theodore Geldenhuys
Sent: Friday, 21 June 2019 09:58
To: e.rutgers@nki.nl
Subject: Sentinel Imaging protocol
Importance: High

Dear Sir

I am a radiographer at Groote Schuur Hospital (Nuclear Medicine) currently busy with a Masters degree. My thesis are based on reviewing the current protocol for sentinel breast imaging. We introduced an imaging protocol after a visit early 90's by Prof Hoefnagel. The aim of the research is to see whether we can propose changes to the protocol by looking at when we observe the most nodes and then only do imaging at that time interval. At the moment we do early dynamic, early 30 minute and delayed 2-4 hour imaging.
Is it possible for you to send or describe your current nuclear medicine imaging protocol(Radiopharmaceutical, injection technique and imaging times, planar/SPECT/CT) in order for me to compare to our protocol.

Thank you
Kind Regards

Theodore Geldenhuys

From: m.stokkel@nki.nl
Sent: Thursday, 27 June 2019 08:23
To: Theodore Geldenhuys; e.rutgers@nki.nl
Subject: RE: Sentinel Imaging protocol

Dear Theodore,
I have read your mail and hereby send you my answer:
In former year we did: we did early dynamic, early 30 minute and delayed 2-4 hour imaging.
Nowadays it is: early imaging 15 min p.i. followed by a scan at 3 hr p.i.. However, we are justifying the approach of only a scan at 2 hrs p.i.

Kind regards,

Marcel Stokkel

Nuclear Medicine Physician
Head of the dept. of Nuclear Medicine
Head of the Division of Diagnostic oncology

t:0031205122283
e:m.stokkel@nki.nl

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

The Netherlands Cancer Institute | Plesmanlaan 121 | 1066 CX AMSTERDAM | www.nki.nl