Efficacy of the faxitron biovision x-ray machine in intra - operative determination of metastatic foci in a sentinel lymph node.

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DECLARATION

I, **Dr. George Manyangadze** declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine, Surgery, at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University

. ……21st……day of…January ....., 2020

DEDICATION

This MMed is dedicated to my late mother Pilisiwe Manyangadze, who knew what I could be when I had no idea what life entails.

ACKNOWLEDGMENTS

This research report would not have been possible without the support and contributions from my supervisors, Prof. Carol-Ann Benn, Prof Geoffrey Candy and Dr Pascaline Fru

ABSTRACT

Background

Sentinel lymph node biopsy has revolutionized breast cancer management. Its major benefit of reduced axillary morbidity whilst offering outcomes equivalent to axillary lymph node dissection has been shown in various trials. Multiple trials indirectly highlighted the absence of an intra-operative method or test to identify positive lymph nodes. This study aimed to determine if there was an association between the faxitron biovision x-ray machine (FBXM) results and histology for assessing axillary lymph node (ALND) metastasis intra- operatively.

Methods

To measure the X-ray attenuation of sentinel lymph node biopsy (SLNB) images on FBXM, the intensity of the image generated was measured to give a value, known as the intensity unit and equivalent to the density of the SLN. This value was then used to determine the likelihood of axillary sentinel lymph node metastasis. The mean intensity and maximum intensity units was recorded and the samples retrieved and sent for routine histology. The agreement between the FBXM and standard histopathology was compared using a Chi- squared test and the independent sample T-test was used to assess whether the minimum and maximum intensity differed significantly. The SPSS Statistical software version 20 was used for data analysis.

Results

A hundred and one patient samples were analysed.The FBXM had a sensitivity of 70.8% and specificity of 83.1%. The FBXM had a Positive predictive value of 56.7% and Negative predictive value of 90.1%. FBXM results differed significantly with the gold standard when the Youden index was calculated with the AUC was < 0.6. The p-value was also greater than 0.05 (p-value = 0.148). This meant that there was no significant relationship between the FBXM and the Gold Standard (Histology).

Conclusion

The findings suggest that histopathology remains the standard of care in evaluating SLNs.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ALND	**Axillary lymph nodes dissection**
ACOSOG, Z0011	**American College of Surgeons Oncology Group Z0011 trial**
ASCO	**American Society of Clinical Oncology**
CT	**Computer Tomography**
FBXM	**faxitron biovison x-ray machine**
FNR	**False negative rate**
FNA	**Fine needle aspiration**
FPR	**False positive rate**
IHC	**Immunohistochemistry**
IU	**Intensity Unit**
PPV	**Positive predictive value**
NPV	**Negative predictive value**
PACS	**Picture archiving and communication system**
ROI	**Range of Interest**
ROC	**Receiver Operating Characteristic**
SLN	**Sentinel lymph node**
SLNs	**Sentinel lymph nodes**
SLNB	**Sentinel lymph node biopsy**

CHAPTER 1

1.1 Introduction

Sentinel lymph node biopsy (SLNB) has evolved over the years and multiple studies have validated it as an acceptable standard of care for early node negative breast cancer patients [3]. In the past axillary lymph node dissection (ALND) was the method of choice for managing the axilla in all women with breast cancer. The high morbidity of this procedure included complications such as lymphoedema, nerve injury, shoulder pain and dysfunction necessitating its replacement with SLNB in axillary assessment in early breast cancer. SLNB has developed as a procedure and been refined to achieve similar outcomes to ALND in terms of survival and recurrence rates for early breast cancer with minimal adverse effects. According to ASCO guidelines 2014, women without sentinel lymph node (SLN) metastases should not receive axillary lymph node dissection (ALND). Women with one to two metastatic SLNs who are planning to undergo breast-conserving surgery with whole-breast radiotherapy should not undergo ALND (in most cases). Women with SLN metastases who will undergo mastectomy should be offered ALND. These three recommendations are based on randomized controlled trials [20]

The American College of Surgeons Oncology Group Z0011(ACOSOG Z0011), and the Amaros trials were important randomized controlled trials showing that ALND could be safely omitted in patients with a positive sentinel lymph node after SLNB in clinically node negative early breast cancer, as systemic adjuvant therapy and radiation post breast conserving surgery resulted in decreased axillary recurrence [4]. However, these trials indirectly highlighted the absence of an intra-operative test or method to pick up positive sentinel lymph nodes. The gold standard for pathological assessment of the sentinel lymph nodes (SLNs) is histopathological evaluation in the laboratory. This method limits immediate intra-operative surgical decision due to the time needed from sampling to getting the final histology results leading to delays

and increasing hospital costs. This implies patient have to come back to theatre for a second procedure or definitive surgery should an axillary dissection be needed.

This current study was aimed at evaluating the potential accuracy of a radiological intra-operative method of identifying positive lymph nodes. Although other methods such an intra-operative frozen section, scrimp cytology have been reported, these are not addressed in this study. Here, a comparison between intra-operative radiological assessment of the sentinel and final pathology results which could potentially alleviate the challenges associated with waiting for histology results was done. Potential benefits to the patient are that no secondary axillary procedure maybe needed and decisions on need for adjuvant therapy are made quicker. This will lead to an improvement in results turnaround time, enhancing the efficiency and cost effectiveness of managing breast cancer. For example in a patient with a positive SLN, completion ALND could be performed as an option intra-operatively and the patient referred for adjuvant therapy without the need for the patient to return for a second axillary procedure which will often be challenging because of fibrosis in the previously operated field and increasing the risk of axillary morbidity. This study was aimed at assessing the ability of the faxitron biovision x-ray machine in identifying positive sentinel lymph nodes intra-operatively in a clinically node negative axilla in breast cancer.

1.2 Background

Breast cancer is the number one ranked malignancy commonly diagnosed across all population groups in South Africa according to the 2014 National Cancer Registry. In 2014, the lifetime risk of developing breast cancer was 1 in 33 South African women [(1)].

Breast cancer can be histologically categorized into in-situ or invasive components and 80% of invasive malignancy is ductal carcinoma. The existence of receptors (or lack thereof) further guides treatment and prognostication of patients. These receptors respond deferentially to adjuvant therapy. These are the progesterone (PR), eostrogen (ER) and human epidermal growth factor receptor 2 (HER2). Amplification of HER2 and the absence of all three of these nuclear receptors HER2, PR and ER, so called triple negative are some of the possible scenarios[(2)].

Sentinel lymph node biopsy as a procedure results in less morbidity when compared to axillary lymph node dissection. It is now the preferred standard of therapy for the management of clinically node negative axilla in early breast cancer. Its major benefit is reduced axillary morbidity whilst offering improved survival outcomes and reduced recurrence equivalent to ALND as shown in the NSABP protocol B32 trial [(3)]. The overall survival, disease free survival and regional control in this trial were statistically equivalent between groups and validated SLNB as a procedure to manage early breast cancer in node negative axilla. Findings from the trial further showed that when a sentinel lymph node is negative, SLNB surgery alone with no further ALND is appropriate, safe and an effective therapy for breast cancer patients with clinically negative nodes [(3)].

The implications of this trial are broad and of importance. If SLNB offers similar outcomes to ALND in terms of disease free survival and regional control whilst concurrently achieving much fewer arm morbidities, utilizing SLNB and having a systematic method to determine metastatic foci of a SLN intra-operatively would be far more beneficial to a patient. It will go a long way in improving breast cancer management.

As such, instead of waiting for histopathological results and bringing the patient back to theatre for a definitive procedure, surgical decisions could be made intra-operatively and performed immediately if an accurate method to identify positive sentinel lymph nodes existed.

The American College of Surgeons Oncology Group Z0011trial [(4)], randomized 891 patients with pathological sentinel nodes to axillary dissection or SLNB only. The study closed prior to meeting accrual goals but the results obtained showed that SLNB was adequate as a procedure to manage axillary breast cancer in clinically node negative axilla. The study highlighted the absence of an intra-operative method or test to identify positive lymph nodes. The study met the expected objective of showing that SLNB as a procedure has less arm morbidity and produced comparable results to ALND. The implication of the findings was that if an accurate method to identify all positive sentinel lymph nodes intra-operatively could be established, surgical decision making could be made instantly.

The AMAROS trial, another randomized controlled trial looked into comparing ALND to SLNB with radiation therapy and no further ALND. The aim was to assess whether axillary radiotherapy provided comparable regional control whilst achieving less morbidities. This randomized, multicentre trial enrolled patients with T1-2 breast cancer and without palpable axillary lymph nodes. Randomization was done by a computer generated allocation and assigned (1:1) to receive either ALND or axillary radiotherapy if positive SLN. The primary

outcome was 5 year axillary recurrence. ALND and radiation to the axilla after a positive lymph node offered excellent and comparable axillary control in patients with T1 –2 breast cancer without palpable axillary lymph nodes without the added disadvantage of arm morbidity [(4)]. Ipsilateral arm lymphedema was noted more commonly after ALND than after axillary radiation. This trial re-affirms our belief of the need to have an accurate intra-operative method to determine positive sentinel lymph nodes. If SLNB offers comparable outcomes to ALND, intra-operative determination of positive lymph nodes would harness the benefits of reduced morbidity whilst reducing the overall cost and emotional burden of operative management of primary breast cancer.

De Groef et al. (2015)[(5)] in a major follow up study to investigate the prevalence rate of arm lymphoedema, pain, impaired shoulder range of motion and shoulder function one year after SLNB for breast cancer showed that the most feared side effect after SLNB, namely lymphoedema is the lowest with only 8% one year after SLNB. The drawback with this study was the short follow up period although their results were similar to those of other studies which also alluded to the lower rate of lymphoedema with SLNB when compared to ALND.

In 2009, Bilimoria et al.[(6)] in a study comparing SLNB and completion ALND for breast cancer showed that compared to SLNB alone, completion ALND does not appear to improve outcomes for breast cancer patients with microscopic nodal metastasis. This study offered evidence that axillary recurrence and survival are comparable for SLNB alone versus SLNB with completion ALND for microscopic nodal disease and in selected patients for macroscopic nodal metastasis [(6)]. The median follow up in this study was 63 months. Longer surveillance may reveal a subtle increase in axillary recurrence.

Cyr et al. in a 2012 [7] retrospective study looked into the disease recurrence in sentinel lymph node positive breast cancer patients who had forgone axillary lymph node dissection. Their primary objective was to review if patents with a positive sentinel lymph node could have completion ALND omitted safely. Their conclusion was that ALND in women with a positive sentinel lymph node does not significantly impact nodal or distant recurrence or mortality [7]. The limitation with this study was the short follow up period. It is likely that a longer term follow up could have revealed higher recurrence rates. Although being a retrospective study, it showed the usefulness of SLNB as a procedure and its ability to offer similar outcomes to ALND in early node negative breast cancer consistent with the previous trials and studies.

Recently, sentinel lymph node biopsy has been extended to patients with multicentric breast cancer with clinically node negative axilla. Previously multiple foci of carcinoma especially when present in different quadrants of breast were considered a relative contraindication. Tousimis et al (2003) in a study conducted on 70 patients with multifocal or multicentric breast carcinoma underwent mastectomy and SLNB with a planned ALND [8]. The accuracy of SLNB was 96% and the sensitivity 92% with a false negative rate of 8%. Their results were comparable with outcomes of other trials for example during the validation phase of the ALMANAC trial [9], the false negative rate and the negative predictive value in patients with multifocal or multicentric breast cancer rate were 8.8% and 92.5 % respectively, results almost similar to the unifocal group , 7.2 and 96.5% respectively.

Moreover, in another study by Kumar et al. (2003) conducted on 48 patients with multifocal or multicentric breast cancer, success rate, sensitivity, negative predictive value and accuracy of SLNB were 93.5, 100, 100 and 100% respectively using radiocolloids and blue dye [10]. This

trial validated the use of SLNB in multifocal or multicentric, clinically node negative breast cancer.

Lymphoedema, an important clinical adverse event was also identified to occur with SLNB as well but at lower rates than those of patients undergoing ALND. In the ALMANAC trial[9], lymphoedema was measured by participant self-examination and reported as moderate or severe at 12 months. It was found to be 1% with SLNB versus 2% with ALND. The morbidity of ALND is described again in the NSABP B32 trial [3] as well as neurological or sensory deficit were found to be far worse in ALND and significantly lower in SLNB. In the ACOSOG Z0011 trial [4], although no objective measurement of lymphoedema was included in the protocol, it was subjectively reported that lymphoedema was higher with ALND than in those with SLNB alone especially from 12 months post operation.

In this era of minimally invasive procedures, SLNB offers excellent advantage to axillary staging of breast cancer whilst having reduced morbidity and acceptable locoregional clearance when compared to ALND in patients with early stage breast cancer. The American Society of Clinical Oncology (ASCO) published evidence based clinical practice guidelines on the use of sentinel lymph node biopsy for patients with early stage breast cancer in 2014[11]. The recommendations, which were based on randomized controlled trials, were as follows:

- Women without sentinel lymph node metastases should not receive ALND.
- Women with one or two metastatic SLNs planning to undergo breast conversing surgery with whole breast radiotherapy should not undergo ALND.
- Women with SLN metastases, who will undergo mastectomy, should be offered ALND.

As the significance of SLNB became obvious over the years through evidence from multiple controlled trials, attention turned to the need of a good method for intra-operative pathological

evaluation of sentinel lymph nodes. As previously mentioned, the benefits of intra-operative pathological evaluation are multiple, for example in cases of a positive sentinel lymph node, it allows one to proceed immediately to axillary dissection or sampling without having to return the patient to the operating room for a second procedure. Conventional intra-operative pathologic evaluation of sentinel lymph nodes consists of frozen section, touch imprint cytology and molecular assays such as polymerase chain reaction (PCR) [(12)] . These tests have been evaluated in different studies and found to be either inaccurate, expensive and some impractical.

Francissen et al. (2013) published a retrospective study on the benefits of intra-operative frozen section analysis of the sentinel lymph node with regard to false negative rate and influence on operation time [(13)]. Their results showed that frozen section accurately predicted axillary status in 83.6% of the patients and a false negative rate of 56.4%. In this study, of the 628 patients who underwent sentinel lymph node biopsy, only 78 patients (12.4%) benefited from intra-operative frozen section analysis [(13)]. In 101 cases frozen section was also false negative and these patients had to undergo axillary lymph node dissection as a separate procedure. The authors attributed the unacceptable high false negative rates and low sensitivity due to increasing recognition of micro metastases and publication bias. They also evaluated the benefits of frozen section by comparing operation times of the different procedures to see if frozen section of the sentinel lymph node was either time saving or time consuming. The authors showed that additional operation time of a secondary ALND after wide local excision and SLNB is 17 minutes and in case of ablative surgery 35 minutes [(13)]. Therefore in patients with a positive sentinel lymph node identified by frozen, section, extra operation time was scheduled due to the need for ALND. Although frozen section as a procedure to detect intra-operative positive nodes causes an uncertainty in the operation room schedule and takes up additional operation time which is factored in for every patient undergoing SLNB, patients

with a true positive frozen section sentinel node benefit by undergoing immediate ALND, thus avoiding a second procedure[13]. Disadvantages of a second procedure are increased morbidity, costs and the emotional impact on the patient.

The value of an accurate intra-operative method to determine positive sentinel lymph node allows a decision to skip ALND during primary surgery in cases of a negative sentinel lymph node. Touch imprint cytology (TIC) has been evaluated in different studies and found to have sensitivity equivalent to or even better than frozen section. The major advantages of TIC are that there's no need for special equipment, there is minimal tissue loss and it provides good cytological details. However, of concern are the false negative rates of TIC which may lead to unnecessary secondary ALND. In 2011, Chen et al. looked into the factors associated with the misdiagnosis of sentinel lymph nodes using touch imprint cytology for early stage breast cancer [14]. Their primary aim was to evaluate the clinical value of TIC with a normative procedure of the pathological evaluation of sentinel lymph nodes. A total of 366 patients with T1 – T2 breast cancer were included after undergoing successful SLNB. TIC was routinely performed intra-operatively and results were compared to standard histological analysis. Compared to final histopathology results, the sensitivity, specificity and overall accuracy of TIC was 76.6, 98.9 and 92.3% respectively on a patient basis. It was also noted that TIC was more sensitive for macrometastases than micrometastases [13]. The problem with this trial was the low accrual and the documented number of false negatives of 15%.

In another publication on using anti-cytokeratin immunostaining of imprint cytology as an intra-operative diagnostic tool for the assessment of sentinel lymph node by Lambah et al, they studied 238 SLNs from 53 patients with early T1 – T2 breast cancer [15]. In their study, imprints were stained for Immunohistochemistry (IHC) and they reported sensitivity of 36.4% for IHC imprints. Such a low sensitivity was due to the IHC method used and the use of formalin fixed

paraffin sections which resulted in imprint cell loss because of absence of a supportive tissue frame [15].

Salem et al. (2006) performed a study to determine the feasibility and accuracy of immunohistochemistry stained touch imprints in detecting metastatic focus of axillary lymph nodes intra-operatively [16]. The authors evaluated 432 axillary nodes from 52 patients (23 ALND, 15 axillary node sampling and 14 SLNB), all were bisected, imprinted and stained with anti-cytokerin 19 IHC. They compared their results with standard histopathology. In their results, compared to histopathology, IHC imprint cytology had sensitivity of 91.4 and a negative predictive value of 99.2 on a node basis. Standard histopathology had sensitivity of 88.5 and negative predictive value of 99.0% [16]. Overall accuracies were 96.1 and 94.2% for IHC imprints and Histopathology sections respectively [16]. The challenge with this prospective study was the small study population and the ability of a non – histopathology trained surgeon to correctly interpret the results is questionable although the method itself is feasible.

Currently in the public health sector in South Africa, all sentinel lymph node biopsy specimens are sent for histopathological analysis. This process of specimen travel, analysis and reporting may take time. The faxitron biovison x-ray machine is a self-contained unit with four times the resolution of standard mammography[17]. It is capable of developing a digital image within 15 seconds per exposure. Multiple views can be obtained within one or two minutes if required. There's no need to squash the specimen with the faxitron biovision x-ray machine, its portable and the image is immediately developed for the surgeon to decide if margins are clear or if a sentinel lymph node has disease present in it [17]

In the quest to find an accurate and rapid intra-operative method to evaluate sentinel lymph nodes with a view to perform a definitive operation during primary surgery, we aim to show

that the faxitron biovison x-ray machine can be used as an intra-operative tool to identify positive sentinel lymph nodes.

The features or parameters to assess or measure and hence determine disease presence in the SLNs when imaging on FBXM in our study include assessing the presence of:

1. Asymmetrical lesion density/intensity and distortion
2. Mass and micro calcifications or
3. No abnormality
4. The intensity units of the SLN measured on the FBXM reflecting metastasis.

Although the presence of calcifications and a mass as visualized on the faxitron biovison x-ray machine are sensitive indicators of disease presence or axillary sentinel lymph node metastasis, they are subjective parameters. A more objective method we will employ is to measure the intensity units generated by imaging the sentinel lymph nodes on the faxitron biovison x-ray machine. To measure the X-ray attenuation of images on faxitron biovison x-ray machine, the intensity generated is measured to give a value. This value was then used to determine the likelihood of axillary sentinel lymph node metastasis. An intensity unit is the equivalent of the density generated by the sentinel lymph node on the faxitron biovison x-ray machine [17]. Therefore, the faxitron biovison x-ray machine study aims to evaluate sentinel lymph node metastasis against Intensity Units (IU) to determine optimal cut off values. All lymph nodes were evaluated without any prior information on Histopathological results.

1.3 Problem Identification

The long turn -around time (between harvesting a SLN and getting histopathological results) and the need for a second procedure in the staging process leads to delays in managing breast cancer. The economic burden associated with a second procedure and the difficulties of re-operating the axilla necessitates the need for an intra-operative test that can reliably and accurately identify positive lymph nodes. If this intra-operative method is accurate, it would allow a one stage procedure were a SLN is harvested, decision made intra-operatively and definitive surgery performed in the same setting.

1.4 Aim of the study

1. To show that the FBXM can be used to identify positive sentinel lymph nodes.
2. To compare the sensitivity and specificity of the FBXM to determine positive lymph nodes metastasis intra-operatively as compared to standard histopathology.

CHAPTER 2

2.1 Study Method

Equipment: The faxitron biovison x-ray machine provides immediate verification of excised breast tissue margin in patients undergoing surgical excision or biopsy procedures. It can improve efficiency, save theatre time and overall procedure cost when specimens are imaged intra-operatively as per manufacturer's claim. It allows simultaneous analysis of images via a PACS system. Images are sent directly to mammography unit and the specimen directly to pathology. The faxitron biovison x-ray machine used for this study is shown in Figure 1 (Bioptics model: pixarray, USA company) [17]

Figure 1: The Faxitron Biovison X-ray machine (FBXM) [(17)]

After a SLNB, the specimen was immediately placed in the radiation compartment of the FXBM and images taken in an antero-posterior orientation. The images were analyzed followed by retrieval of specimen and submission for histological evaluation. The X-ray attenuation of images on FBXM called the intensity unit, generated was measured to give a value and displayed on a screen. This value was then used to determine the likelihood of axillary SLN metastasis. An intensity unit is the equivalent of the density generated by the sentinel lymph node on the FBXM. The histogram below (Figure 2) is an example of intensity units generated by the SLN density on measurement.

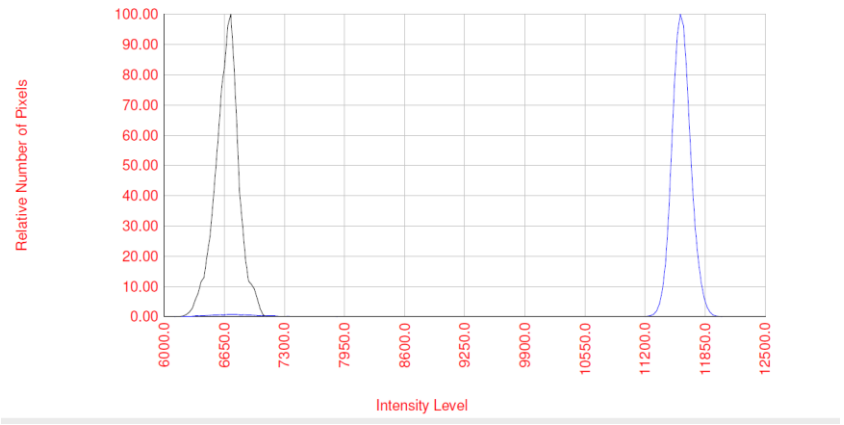

Fig 2: Histogram showing mean and maximum intensity level of a SLN as measure of density to determine SLN metastasis.

2.2 Study design – This study was carried out at the Netcare Breast Care Centre of Excellence, Milpark Hospital, Johannesburg, South Africa. Ethics approval was granted by the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg. An initial pilot study of 30 specimens was assessed and the results analyzed to determine a statistically ideal sample size and congruency between intra-operative imaging with FBXM vs. histopathology. Eventually, 104 specimens were analyzed. All patients who underwent sentinel lymph node biopsy at Milpark hospital with T1 – T2 clinically and radiologically node negative

breast cancer who were admitted for a SLNB at Milpark hospital were included. Any patient with T3- T4 breast cancer and post neoadjuvant treatment was excluded.

In this prospective, blinded study, the first sentinel harvested was imaged as a whole and the data automatically stored on the FBXM for evaluation. If more than one sentinel lymph node was positive, the second node was imaged separately from the first and evaluated separately. The SLN was imaged in an antero- posterior or posterior – anterior orientation for this study. The time taken for an image to be captured and stored on the FBXM was 40 seconds. To measure the X-ray attenuation of the images on the faxitron biovison x-ray machine, the intensity generated was measured to give a value and displayed on the screen. This value was then used to determine the likelihood of axillary sentinel lymph node metastasis. An intensity unit is the equivalent of the density generated by the sentinel lymph node on the faxitron biovison x-ray machine. Therefore, we evaluated sentinel lymph node metastasis against IU to determine optimal cut off values. All lymph nodes were evaluated independently and without any prior information on histopathological results to avoid potential bias.

Two values were determined from imaging the sentinel lymph nodes namely the mean intensity and maximum intensity units. Considering that surrounding fat tissue on the harvested lymph nodes could contribute towards the tissue intensity/ density, measures were taken to circumvent this. In the case were the IU was measured with or without internal fat at normal lymph node hilum, tracing outlines of sentinel lymph nodes by hand to highlight a Range of Interest (ROI) was done. To eliminate artificial contamination these outlines were excluded when mean IU values were evaluated but included to determine maximum IU values.

In circumstances were two SLN's were harvested, they were both evaluated with the same method and the highest IU value obtained was used as the value for the patient in question. If other non-objective parameters were used to detect the presence of disease such as

calcifications or obvious presence of a mass and were such was the case, it was correctly highlighted on the data capturing sheet under others.

The SLN was then retrieved and taken to the laboratory for standard histopathological assessment. The operation was completed and immediate evaluation of the images on FBXM was done. An example of the datasheet used for capturing data is appended as Appendix I.

2.3 Data analysis

In the present study, the agreement between the faxitron biovison x-ray machine and standard histopathology was compared using a Chi-squared test. An independent sample T-test was used to assess whether the minimum, maximum and average intensity differed significantly. The SPSS Statistical software version 20 was used for analysis.

CHAPTER 3: RESULTS

A total of 104 patient samples were analyzed and three cases without FBXM data were excluded. Thus, 101 patient samples were analyzed. The FBXM and Gold standard results are summarized below (Table 1 and 2). A total of 29.7% (30/101) and 23.8% (24/101) positivity and 70.3 (71/101) and 76.2% (77/101) negativity was reported respectively.

Table 1: Faxitron Biovision X-ray Machine Results (A)

A		Frequency	Percent
faxitron biovison x-ray machine SLNB Results	Positive	30	29.7
	Negative	71	70.3
	Total	101	100.0

Table 2: Gold standard (histopathology) results (B)

B		Frequency	Percent
SLNB Histology Results (Gold standard)	Positive	24	23.8
	Negative	77	76.2
	Total	101	100.0

Table 3: Summarised data of table 1 and 2 *(showing a direct comparison of the FBXM results with gold standard)*

		faxitron biovison x-ray machine results			P-value
		Positive	Negative	Total	
Gold Standard (Histology)	Positive	17 (56.7%)	7 (9.9%)	24 (23.8%)	.000
	Negative	13 (43.3%)	64 (90.1%)	77 (76.2%)	

Summarized table showing a direct comparison of the FBXM results with gold standard. There was a significant difference in the sensitivity and specificity of the FBXM results compared to the gold standard. FBXM demonstrated poor sensitivity when compared to histology.

3.1 Was there an association between the faxitron biovison x-ray machine results and the Gold Standard (Histology) results?

3.1.1 Using the chi-square test

The chi-square test of association was used to assess whether there was an association between results obtained using the Gold Standard (Histology) and those from the faxitron biovision x-ray machine. The results are shown in Table 3 below.

Table 4: FBXM Results vs. the Gold Standard (Histology)

SLNB Histology Results (Gold Standard) Vs. faxitron biovison x-ray machine **SLNB Results Cross tabulation**					
			Faxitron Biovision Machine Results		Total
			Positive	Negative	
Gold Standard (Histology)	Positive	Count	17	7	24
		% within Faxitron Biovision Machine Results	56.7%	9.9%	23.8%
	Negative	Count	13	64	77
		% within Faxitron Biovision Machine Results	43.3%	90.1%	76.2%
Total		Count	30	71	101
		% within Faxitron Biovision Machine Results	100.0%	100.0%	100.0%

Gold Standard (Histology) * faxitron biovison x-ray machine **Results Crosstabulation**					
			faxitron biovison x-ray machine results		Total
			Positive	Negative	
Gold Standard (Histology)	Positive	Count	17	7	24
		% within Gold Standard (Histology)	70.8%	29.2%	100.0%
	Negative	Count	13	64	77
		% within Gold Standard (Histology)	16.9%	83.1%	100.0%
Total		Count	30	71	101

	% within Gold Standard (Histology)	29.7%	70.3%	100.0%

Therefore, the sensitivity of the FBXM was (17/24) = 70.8% and the Specificity of the FBXM was (64/ 77) = 83.1%. The FBXM had aPositive predictive value of (17/30) = 56.7% and Negative predictive value of = (64/71) = 90.1%

3.1.2 Using the receiver operating characteristic (ROC) curve

To further determine the sensitivity and specificity of the two methods, a ROC curve was generated using SPSS statistical software program. This is a plot of true positive rate (sensitivity) against the false positive rate (100-specificity) in what was referred to as the Youden's Index. A Youden's Index was used to assess the performance of a diagnostic test with dichotomous results.

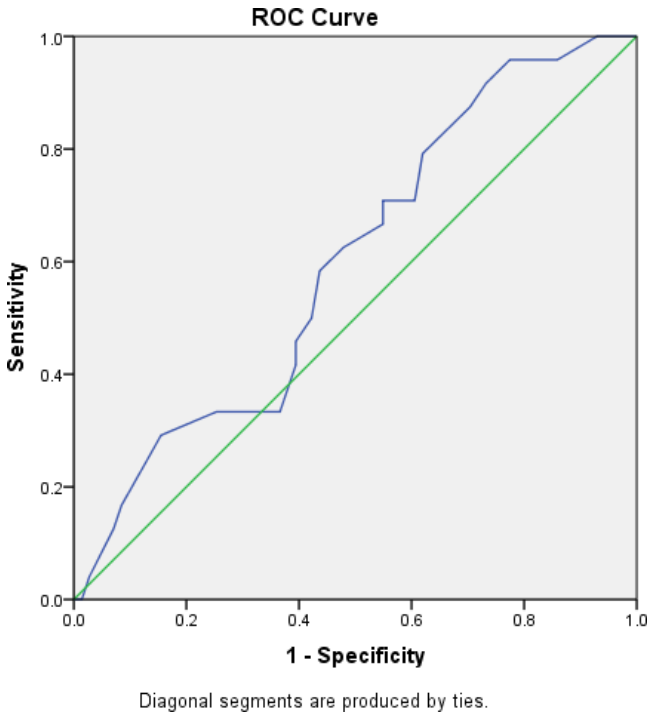

Figure 3: ROC curve of mean IU of a SLN. The AUC of this case was 0.599. We made a ROC curve and found the cut-off value using the Youden index.

Table 5: Area under the Curve

Area Under the Curve				
Test Result Variable(s): Maximum Intensity				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.599	0.064	0.148	0.475	0.724
The test result variable(s): Maximum Intensity has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.				
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

From the curve, the area under the curve (AUC) of 0.599 was obtained. This means that the FBXM results differed significantly with the gold standard since the AUC was < 0.6. The p-value was also greater than 0.05 (p-value = 0.148). This means that there was no significant relationship between the faxitron biovison x-ray machine and the Gold Standard (Histology).

Area under the curve shows that a good predictive value should be 0.8 and above. A value of less than 0.6 is poor

The Sensitivity, Specificity & Youden's Index values to determine the cut off values are shown in Table 4. The Youden index which is the difference between the true positive rate and the false positive rate was calculated using SPSS statistical software program (IBM Corporation in, New York, USA). The Youden's Index indicates the probability of a positive test result in subjects who have the condition and a negative result for patients without the condition. It is calculated using the formulae, Youden’s index = (Sensitivity + Specificity - 1). Youden’s index values ranges from 0 to 1, where a value of 1 indicates a perfect situation where all positive results are correctly classified as positive and negative values are all classified as negative. On

the other hand, a Youden's Index value of 0 indicates a situation where all values are incorrectly classified. In a perfect test, Youden's index equals 1.

Table 6: Sensitivity, Specificity & Youden's Index

Coordinates of the Curve			
Test Result Variable(s):	Maximum Intensity		
Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity	Youden's Index
7487.000	1.000	1.000	.000
7493.500	1.000	.986	.014
7649.500	1.000	.972	.028
9300.000	1.000	.958	.042
10850.000	1.000	.944	.056
11100.000	1.000	.930	.070
11325.000	.958	.859	.099
11400.000	.958	.845	.113
11475.000	.958	.831	.127
11600.000	.958	.775	.184
11725.000	.917	.732	.184
11775.000	.875	.704	.171
11825.000	.792	.620	.172
11875.000	.708	.606	.103
11950.000	.708	.563	.145
12075.000	.708	.549	.159
12175.000	.667	.549	.117
12250.000	.625	.479	.146
12350.000	.583	.437	.147
12450.000	.500	.423	.077
12525.000	.458	.394	.064
12575.000	.417	.394	.022
12650.000	.333	.366	-.033
12750.000	.333	.352	-.019
12850.000	.333	.254	.080
12950.000	.292	.155	.137
13050.000	.167	.085	.082
13150.000	.125	.070	.055
13250.000	.042	.028	.013
13350.000	0.000	.014	-.014
13401.000	0.000	0.000	.000

The results in the Sensitivity, Specificity & Youden's Index shows that the best Youden's Index would be 1.84. This shows that a maximum cut-off of 11600 will have a sensitivity of 0.958 and a 1- specificity of.775. This means that 95.8% of the cases with a positive outcome will be

correctly classified by the faxitron biovison x-ray machine as positive and 77.5% of the negative outcomes will be incorrectly classified as positive outcomes.

3.2 **Comparison of histology results between positive and negative samples**

Independent sample t-test was used to assess whether the minimum, maximum and average intensity differed significantly between positive or negative samples on the Gold Standard (Histology). The results are shown below (Table 6).

Table 7: Comparison of histology results between positive and negative samples

	Gold Standard (Histology)		
	Positive (n= 24)	Negative (n=71)	P-value
Maximum Intensity	12435.42 ± 573.03	12059.68 ± 1147.59	0.128
Minimum Intensity	5652.08 ± 993.51	5926.77 ± 850.54	0.193
Average Intensity	9043.75 ± 489.52	8993.23 ± 609	0.684

Mean ± Standard Deviation

To better understand if the maximum, minimum and average intensity differed significantly between positive or negative samples on the Gold Standard (Histology), T-test subgroup analysis was performed as above. Significant statistical difference was not shown when comparing the maximum, minimum and average intensity units for positive and negative SLNB's specimens compared to standard histology. On the independent sample t- test, it can be noted that there was no significant difference in the maximum, minimum and average intensity units from the final outcome of the Gold Standard results since all the p-values were greater than 0.05. This means that the mean Maximum Intensity, Minimum Intensity, and Average Intensity differed less significantly for both the positive and negative patients.

CHAPTER 4: DISCUSSION

4.1 Study findings

The ability to accurately determine axillary lymph node status is important in estimating the 5 to 10 year survival rate in breast cancer patients. In patients with ALN metastasis, the 10-year survival rate depends on the number of involved nodes, and ranges from 30% for those with > 10 metastases to 90% for those with no metastasis[(2)]. Staging the axilla is not only important for estimating prognoses, but also for selecting individual treatment regimens [(19)]

The American College of Surgeons Oncology Group Z0011 trial showed that patients randomized for sentinel lymph node biopsy (SLNB) alone or for SLNB + ALN dissection (ALND), did not significantly differ in local or regional recurrence. The ACOSOGZ0011 enrolled patients who were diagnosed as N0 before randomization, which supports the importance of preoperative ALN evaluation.
In this study, we assessed the ability of the faxitron biovison x-ray machine in identifying positive sentinel lymph nodes intra-operatively in a clinically node negative axilla in breast cancer patients.

The sensitivity of the faxitron biovison x-ray machine compared to standard histopathology was 70.8% (17/24) while the specificity was 83.1% (64/ 77). That gives the faxitron biovison x-ray machine a positive predictive value of 56.7% (17/30) and a negative predictive value of 90.1% 64/71. Considering that these results were inferior to the findings of standard histopathology, the clinical use of FBXM at these cut-off values is not feasible. At such low sensitivity, the FBXM cannot be used reliably as an intra-operative tool to confidently and accurately determine positive SLNs. Although the FBXM has high magnification and resolution, that generated good intensity units and theoretically translated to equivalent of Hounsfield units generated by Computer Tomography (CT), it did not reflect this in practice. Why this did not happen is unclear. The sensitivity, specificity and Youden's Index values determined cut off values 11600 – 11725 maximum IU and Mean IU of 8993.23 - 9043.75 at

which a sentinel lymph node is likely to be positive. Mean IU can be influenced by the ROI selection, whereas maximal IU does not vary with ROI placement. Although selecting an ROI cannot be automated or standardized on the FBXM, it is an easy manual skill that requires no special technique. However, surrounding structures such as fatty hilum and subcutaneous tissue could potentially cause errors or bias if accidentally selected when selecting a ROI, as maximum IU markedly changes when structures other than the lymph node itself are included. Our results showed that the faxitron biovison x-ray machine did not effectively detect positive sentinel lymph nodes intra-operatively and is clearly inferior to standard histopathology, even from the viewpoint of reduced turnaround time and cost.

In figure 3 above, on the Youden index value, Area under the Receiver Operating Curve (ROC) used to determine cut-off scores based on the Specificity and sensitivity (Figure 3, Table 4, and Table 5). The test result variable(s): Maximum Intensity had at least one tie between the positive actual state group and the negative actual state group. The test variable of 0.599 shows a poor result or insignificancy of the findings. The FBXM had a specificity of 83.1%, this isn't accurate enough to be used in clinical practice.
An independent sample t-test assessed whether the minimum, maximum and average intensities differed significantly when a patient was positive or negative on the gold standard. The results showed no significant differences in the histopathology intensity findings and the final outcome since all the p-values were greater than 0.05. Although the results of IU when analyzed by the independent T-test showed no significant difference with histopathology, this in actual fact highlights the inaccuracy of the FBXM. Perhaps suggesting that using IU generated by imaging a SLN do not directly reflect its density and using it as a standard of measurement may be inaccurate.

4.2 Limitations of the study

Usually, when performing a sentinel lymph node biopsy, some surrounding tissues is incorporated and a range of interest has to be selected. Selecting a range of interest in a sentinel lymph node is a subjective process and surrounding fat and other tissues if not eliminated could significantly reduce the sensitivity. However, measuring both maximum and minimum intensity should reduce the error rate.

This study relied on measuring the density generated by a sentinel lymph node when imaged on the faxitron biovision x-ray machine which is quantified as intensity units. However, if a sentinel lymph node was not well skeletonized, this could lead to an overestimation of its true or actual density which could result in overestimation of its intensity and subsequently resulting in a false positive diagnosis.

4.3 Recommendations

This study revealed that the Biovision X-ray machine has a very low sensitivity for positive lymph nodes intra-operatively and thus, histopathological analysis remains the standard of care. The primary objective of having a simple tool or test that is capable of identifying positive lymph nodes intra-operatively was partly considered in view of the turnaround time of histopathology results and the need for avoiding a second procedure after histopathological evaluation. If the FBXM was sensitive in identifying positive SLNs intra-operatively it could have gone a long way in expediting and reducing economic cost especially in low income countries. Whether sensitivity could be improved by sectioning, staining and fixing the sentinel lymph nodes before intra-operative imaging was beyond the scope of this study but could be explored in a future, similar version of the same study. The FBXM has decent specificity and could be used for confirmation of margins in wide local excisions for breast cancer.

CONCLUSION

Determination of metastatic foci in a sentinel lymph node intra-operatively remains a challenge. Findings from this study showed a very poor sensitivity of the faxitron biovision x-ray machine in identifying positive sentinel lymph nodes intra-operatively in patients with early breast cancer with clinically node negative axilla. This means the FBXM cannot be used confidently and reliably as a method of choice for intraoperative testing of sentinel lymph nodes. Therefore, histopathology tests remain the standard and better test in evaluating sentinel lymph nodes.

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APPENDICES

Appendix I: Example of data Capturing sheet

Study name. Efficacy of the faxitron biovision x-ray machine in intra - operative determination of metastatic foci in a sentinel lymph node.

Study number.

Date of operation.

Demographics.

Age ……

Gender …….

BMI ……..

Pathological staging

T1a ……

T1b ……

T1c …….

T2 ……..

T3 ……..

T4 …….

Clinical staging ……

Radiological staging. ……..

FBXM sentinel lymph node metastasis.

Mean Intensity unit ……

Maximum intensity unit …….

Status positive/negative.

Others

Mass YES/NO

Microcalcifications YES/NO

Histopathology result. (Negative/ positive)

Agreement between the tests. (Yes/No

Appendix 2: Consent Form

Dear patient,

You are currently admitted to Netcare Breast Care Centre for treatment of problems you are currently experiencing. The Netcare Breast Care Centre not only renders treatment but is also actively involved in conducting research aimed at improving the quality of care that we deliver. Our research involves imaging of lymph nodes (glands) harvested from the armpit for tasting the presence of cancer before the lymph nodes are taken to the laboratory for analysis. Usually the glands are taken directly to the laboratory for histological evaluation and the results usually coming out within a week.

In our study in which you are enrolled, we aim to find a faster method to determine presence of disease in the glands by imaging them in a special machine. The picture generated by the machine will be able to tell us if the glands have disease in them and a decision taken immediately with regards to surgical treatment. If our study is a success, it could be used in the future to make rapid intra-operative decisions, avoiding a second operation and reducing overall cost of operation to the patient.
Our study will not compromise your treatment in any manner and no delays whatsoever will be linked to the study. Partaking in the study is completely voluntary and no incentives will be offered for participation.

The use of your information is subject to the following,

1. Approval from the Human Research Ethics Committee (medical) of the University of the Witwatersrand.
2. Identity of a patient from whose file information is extracted is never revealed to anyone but the researcher unless specific consent is obtained to do so. The information gathered does not contain the name of the patient but only a coded number so as to maintain anonymity.

We would like to obtain your consent to use information from your file for the purpose of research, subject to the aforementioned conditions. If you choose not give consent, this will not compromise your treatment it anyway. If at any time you choose to withdraw consent you are free to do so and will not be prejudiced in any way. Should you wish to contact us at any stage regarding the study and consent, contact us at 0114805779/0726377644.

A. Consent given

I .....hereby give consent for my records to be used as per the above mentioned conditions for the purpose of research:
Signature: …… Date: ……

B. Consent not given

I .....do not give consent for my records to be used:
Signature: …… Date: ……

Appendix 3: Ethics Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 George Manyangadze et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170975

NAME: (Principal Investigator)	George Manyangadze et al
DEPARTMENT:	Surgery Netcare Milpark Hospital Breast Care Centre of Excellence
PROJECT TITLE:	Efficacy of the Faxitron Biovision X-Ray Machine in Intra-Operative determination of Metastatic Foci in a Sentinel Lymph Node
DATE CONSIDERED:	29/09/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Carol Benn
APPROVED BY:	Prof C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/02/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 4: Permission letter to conduct research at Milpark Netcare

Tel: +27 (0) 11 480 5600
Fax: +27 (0) 11 482 3317
9 Guild Road, Parktown West, Johannesburg, South Africa
PO Box 91155, Auckland Park, 2006, South Africa
www.netcare.co.za

LETTER CONFIRMING KNOWLEDGE RESEARCH TO BE CONDUCTED IN THIS NETCARE FACILITY

Dear Dr George Manyangadze

Efficacy of the Faxitron Biovision X-ray machine in intra - operative determination of metastatic foci in a sentinel lymph node

We hereby confirm knowledge of the above named research, application to be made to the Netcare Research Operations Committee and in principle agree to the research application for Netcare Milpark Hospital, subject to the following :

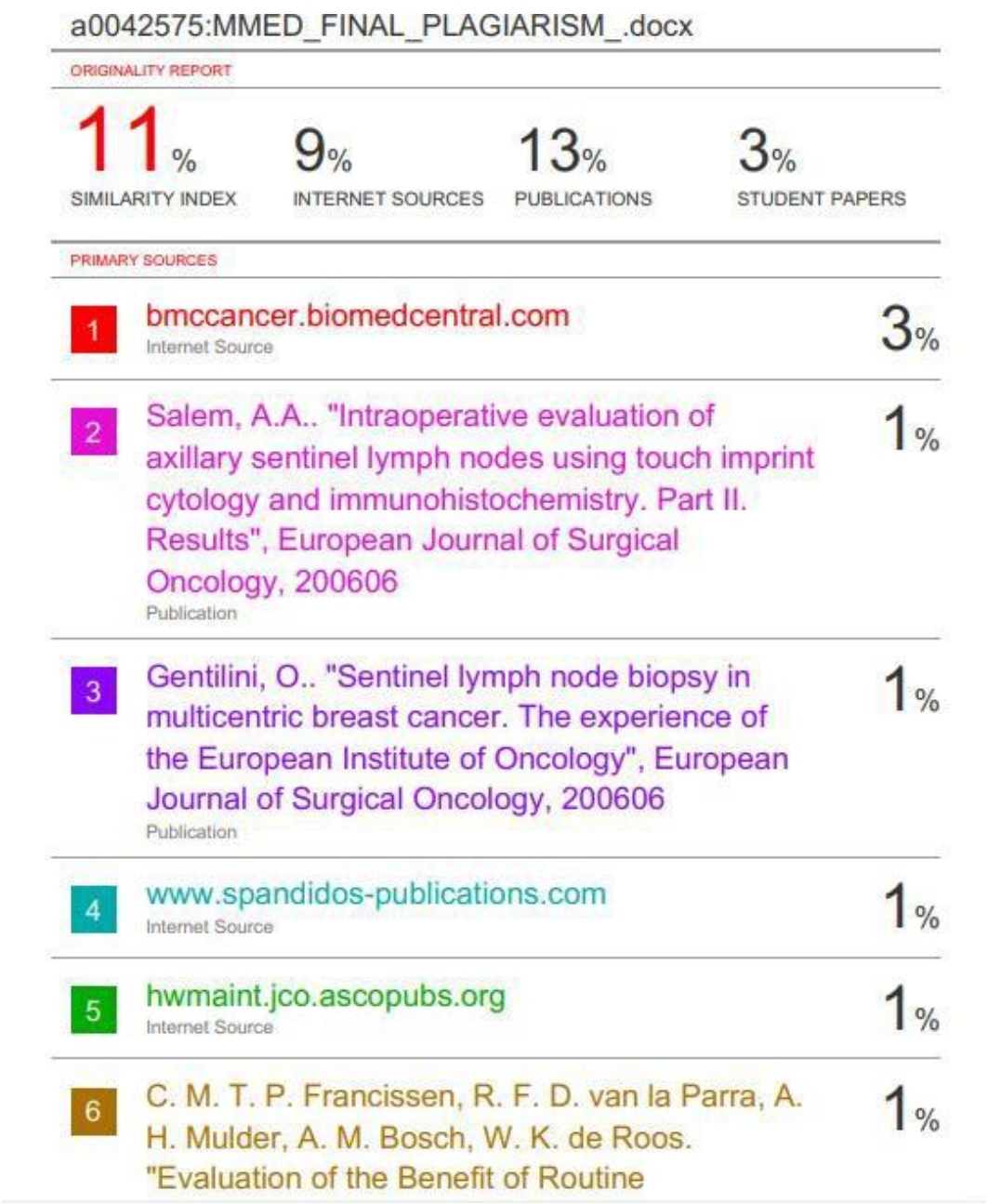
1. That the research (data collection) may not commence prior to receipt of FINAL APPROVAL from the Netcare Research Operations Committee.
2. A copy of the research report will be provided to Netcare Research Operations Committee once it is finally approved, or once complete.
3. Netcare has the right to implement any recommendations from the research.
4. That the Hospital/Site/Division Management reserves the right to withdraw the approval for research at any time during the process, should the research prove to be detrimental to the subjects / Netcare or should the research prove to be detrimental to the subjects / Netcare or should the researcher not comply with the conditions of approval.

We wish you success with your research.

Yours faithfully

Dr Justin Gavanescu
Hospital General Manager

Appendix 5: Turnitin Report



a0042575:MMED_FINAL_PLAGIARISM_.docx

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