A COMPARISON BETWEEN OESTROGEN AND HER-2 IMMUNOHISTOCHEMICAL STAINING OF CORE AND EXCISION BIOPSY SPECIMENS IN BREAST CANCER

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A research report submitted to the Faculty of Medicine, University of the Witwatersrand, in partial fulfilment of the requirements for the Degree of Master of Medicine in Surgery.

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DECLARATION

I, Jacobus Stephanus Vermaak, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in General 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.

.....

Jacobus Stephanus Vermaak

3rd of May 2013

DEDICATION

My family:

To my loving and supportive wife, Sonja.

To my boys: Leon and Sean.

CONGRESS PRESENTATIONS FROM THIS STUDY

1. **Bert Myburgh Research Forum**

   Vermaak JS, Cairns A, Hale M. Comparison between core and excision biopsy oestrogen receptor immunohistochemical staining for breast cancer.

   Presented on the 25th of October 2009 to the Bert Myburgh Research Forum, University of the Witwatersrand, Johannesburg, South Africa. (See appendix A)

2. **Surgical Research Society of Southern Africa**

   Vermaak JS, Cairns A, Hale M. Breast Cancer: Comparing Oestrogen and HER-2 Immunohistochemistry Staining of Core and Excision Biopsy Specimens.

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3. **University of the Witwatersrand Faculty Research Day**

   Vermaak, JS, Cairns A, Hale M. Oestrogen and Her-2 Immunohistochemical Staining compared in the core and excision biopsy specimens of Breast Cancer.

   Poster presentation on the 22nd of September 2010 at the University of the Witwatersrand Faculty Research Day, Johannesburg, South Africa. (See appendix D)

4. **Academic Surgical Congress, USA**

Vermaak JS. Surgical Research Society of Southern Africa – Breast Cancer: Comparing Estrogen and Her-2 Immunohistochemical Staining of Core and Excision Biopsy Specimens.

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ABSTRACT

Introduction

Immunohistochemical staining for oestrogen and human epidermal growth factor receptor (HER)-2 is essential in the management of breast cancer patients. Two histological specimens are usually obtained during a breast cancer patient's treatment: the core biopsy and the final excision specimen.

Aim

The aim of the study was to compare oestrogen receptor and HER2 immunohistochemical staining of the initial core biopsies to that of the final excision biopsies in breast cancer. This is the first study of its kind in South Africa. It will help determine whether immunohistochemistry should be repeated on the excision biopsy specimen when already obtained from the core biopsy, or whether this is mere duplication of effort in a resource-limited environment.

Methods

Following approval by the ethics committee of the University of the Witwatersrand, a retrospective review was conducted using records from Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) Breast Clinic as of the 1st of January 2004 until the 30th of June 2009 (5 ½ years). All patients with a histological diagnosis of breast cancer were reviewed (*n*=718).

Patients who had oestrogen receptor immunohistochemical staining done on both the core and excision specimens were analysed for agreement (*n*=132, 18.4%). Oestrogen receptor immunohistochemistry was analysed when greater than 5% staining was considered positive and reanalysed when greater than 10% staining was considered positive.

Patients who had HER2 immunohistochemical staining done on both core and excision specimens were also analysed for agreement between the two specimens (*n*=124, 17.3%). HER2 immunohistochemical staining was analysed when both HER2(2+) and HER2(3+) staining were considered 'positive'. A separate analysis was conducted when only HER2(3+) staining was considered 'positive'.

Results

For the oestrogen receptor immunohistochemical staining:

A total of 132 patients were included in our study of which only one was male. The mean age ± SD was 56.7 ± 13.3 years (range: 30.0 to 90.0 years). Analysis of the agreement between core and final excision for oestrogen receptor staining above 5% revealed an excellent statistical agreement (91.7% agreement, *Kappa* = 0.784). Analysis of the agreement between core and final excision specimen for oestrogen receptor staining above 10% was even better (93.2% agreement, *Kappa* = 0.823). The core biopsy results were analysed using excision specimens as the gold standard for comparison, which yielded a sensitivity of 93.5%, a specificity of 92.3%, a positive predictive value of 96.7% and a negative predictive value of 85.7%.

For the HER2 immunohistochemical staining:

A total of 124 patients were included in our study of which one was male. There was a poor agreement between core and excision biopsy specimens when HER2(2+) and HER2(3+) were considered 'positive' (57.3% agreement, *Kappa* = 0.103). This agreement was found to be significantly influenced by neoadjuvant chemotherapy between the core and excision biopsy (*P*=0.04) and the number of core biopsies that were obtained (*P*=0.03). When HER2(3+) immunohistochemical staining was analysed as 'truly positive', a tendency towards agreement was demonstrated between the core and excision biopsy specimens (81.5% agreement, *Kappa* = 0.398). The core biopsy results were analysed using excision specimens

as the gold standard for comparison, which yielded a sensitivity of 57.1%, a specificity of 86.4%, a positive predictive value of 46.2% and a negative predictive value of 90.8%. Neoadjuvant chemotherapy was the only factor found that strongly influenced the agreement between the core and excision biopsy immunohistochemical staining for HER2(3+) (P=0.03).

Conclusion

There is an excellent correlation between the oestrogen receptor immunohistochemical staining of the core and the excision biopsy specimens. However, in a resource-constrained environment, this might be considered a duplication of effort. In contrast, there is no agreement between the core and final excision biopsy specimens with regard immunohistochemical staining for HER2. This study showed that neoadjuvant chemotherapy and the number of core biopsies taken influenced this agreement.

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  We should be reminded that every patient that was allocated a mere number in this dissertation, was an actual person with breast cancer. A person with family, friends, emotions, aspirations and desires. I thank each one of them for allowing me to utilize their data to increase our understanding of this devastating disease.

- Dr SM Vermaak

  My wife, friend and emotional support

- Our Father in Heaven

  Through which all things are possible

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LIST OF ABBREVIATIONS

AI	aromatase inhibitors
avg	average
BRCA	breast cancer gene
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
ER	oestrogen receptor
ESR	oestrogen receptor gene
FISH	fluorescence in situ hybridization
HER2	human epidermal growth factor receptor-2
LHRH-A	luteinizing hormone-releasing hormone antagonists
mm	millimetres
n	sample size considered
No.	number
pt	patient
SERM	selective oestrogen receptor modulators
VEGF	vascular endothelial growth factor

CHAPTER 1 - INTRODUCTION & LITERATURE REVIEW

1.1 The incidence of breast cancer in South Africa

There is a paucity of up-to-date statistics regarding breast cancer in South Africa, with most of the data being older than a decade[1]. Accordingly, the lifetime risk of a woman developing breast cancer in South Africa varies from one in 13 among Caucasian woman to one in 81 among African women[2]. The global mortality from breast cancer is decreasing, but African women seem to be adversely affected with regard to late presentation, high mortality, inadequate health care systems, socioeconomic factors and a poor cultural understanding of disease.[2,3]

1.2 The diagnosis of breast cancer

The diagnosis of breast malignancy involves three components: 1) clinical evaluation, 2) radiological evaluation and 3) histological evaluation. After a thorough clinical evaluation, the radiological component should consist of ultrasound, mammography or, in selected cases, magnetic resonance imaging (MRI). Initial histological confirmation of malignancy is usually provided by the core biopsy, which is the preferred method of obtaining cytology. The core sample not only provides confirmation of the diagnosis, type and subtype of malignancy, but also offers the ability to test for aspects of tumour biology that may help with prognostication and planning of chemotherapy. Biological markers that predict of tumour behaviour include hormonal markers (oestrogen and progesterone receptor status), epidermal growth factors (*e.g.* HER2), angiogenic markers (*e.g.* VEGF), genetic markers (*e.g.* BRCA) and cellular proliferation markers (*e.g.* Ki-67). Direct pharmacological manipulation is possible when HER2 and oestrogen-progesterone receptor expression is known. In certain breast cancer patients, the core biopsy might be the only tissue sample obtained. Examples of such patients include advanced disease who receive palliation, patients who are not operative candidates and those patients who have complete pathological

responses after neoadjuvant treatment. The question in these patients remains whether the core biopsy was indeed representative of the entire tumour.

1.3 The oestrogen receptor

Sir George Thomas Beatson was the first to demonstrate the hormonal susceptibility of breast cancer by slowing the progression of metastatic breast malignancy with bilateral oophorectomy.[4] There are two types of oestrogen receptors currently identified in humans: oestrogen receptor (ER)-α encoded on chromosome 6q by a gene called the oestrogen receptor gene (ESR)1, and ER-β encoded on chromosome 14q by a gene called ESR2.[5] Apart from for the lack of the C-terminal domain, the clinical significance of the two different types of oestrogen receptors has not yet been established.[5] The ER-α is documented in breast cancer cells, while ER-β is expressed in prostate cells and colonic tissue.[6] Oestrogen exerts its affect by binding to oestrogen receptors and interacting via oestrogen response elements in the DNA as a DNA-binding transcription factor.[5] This action is susceptible to manipulation by, amongst others, selective oestrogen receptor modulators (SERMS), aromatase inhibitors (AI), pure anti-oestrogens (*e.g.* Fulvestrant) and luteinizing hormone-releasing hormone antagonists (LHRH-A). The progesterone receptor status is considered to be a barometer of functional oestrogen receptors, and thus correlates with the degree of response to hormone manipulation.[5] The literature agrees that hormone receptor-positive tumours respond less favourable to conventional chemotherapy in general,[7-10] but benefit substantially from endocrine manipulation.[11] Most clinicians consider an oestrogen nuclear staining of greater than 10% of tumour cells as an 'oestrogen positive' breast carcinoma.

1.4 HER2 expression

The epidermal growth factor receptors function as tyrosine kinases and include a family of receptors conveniently numbered HER1 to HER4.[75] HER2 is of particular interest in the management of breast cancer. Nomenclature for HER2 includes: Her2/neu, Neu, ErbB-2, CD340 and p185.[75] HER2

receptor expression is found in approximately 30% of breast cancers[12-14] and correlates with a worse prognosis, notably an increased recurrence and a decreased overall survival.[15,16] This prognosis could be improved with HER2 manipulation in the form of treatment with Trastuzumab[17] Trastuzumab is a monoclonal antibody that targets HER2 and is given for one year together with standard chemotherapy in patients with HER2 positive tumours. In such cases the relative risk of breast cancer recurrence is potentially decreased by as much as 50%.[18-20] In practice, HER2 status is determined by using immunohistochemical staining and confirmed by fluorescent *in situ* hybridization when equivocal.[75] Immunohistochemistry detects the protein expression of the HER2 proto-oncogene amplification,[75] while the actual HER2 amplification is detected by fluorescent *in situ* hybridization.[75] Two standardized techniques are available of which the Dako Hercept Test or the Ventana Pathway is used.[21] Immunohistochemical staining of HER2 is standardized: A score of 0 denotes no staining or membrane staining in less than 10% of the tumour cells. A staining score of 1+ indicates faint membrane staining in more than 10% of the tumour cells. Complete membrane staining of more than 10% of the tumour cells, but of a weak to moderate quality, was recorded as 2+. A score of 3+ was allocated to strong staining of more than 10% of the tumour cell membranes. Treatment with Trastuzumab is indicated when HER2 staining is recorded as "3+" by the pathologist.[75] An equivocal score of 2+ is referred for confirmation by fluorescent *in situ* hybridization (FISH), which is considered the 'gold standard' technique of establishing HER2 positivity. It is for this reason that the agreement of both HER2(2+) and HER2(3+) immunohistochemical staining was analysed. During the period of my study, routine FISH was not done for HER2(2+) immunohistochemical staining, because the treatment with Trastuzumab was not available at the particular institution. The amplification rate of the HER2(2+) group was observed to be up to 25% in certain published series.[20,22-24] The price tag attached to a year's course of Trastuzumab was estimated to be more than US $ 70 000 in 2006.[25]

1.5 Oestrogen receptor immunohistochemical staining on core and excision biopsies

Our study is the first of its kind performed on South African patients. Numerous international studies have been done in an attempt to compare the immunohistochemical staining of the core biopsy with the final excision biopsy specimen (Table 1.1). Of the 28 studies listed in Table 1.1, the mean percentage agreement between core and excision biopsies was 88%. However, there was considerable heterogeneity in the methodologies applied. Most studies did not state whether they were prospective or retrospective. Only Ozedemir *et al.* clearly stated that the data was collected prospectively for 61 patients.[26] Gödzinger *et al.* included patients who had ductal carcinoma *in situ* and invasive malignancy,[27] with ductal carcinoma *in situ* having a higher rate of oestrogen receptor positivity than invasive malignancy. [14] Furthermore, only Burge *et al.*[28] and Ozedemir *et al.*[26] stated that oestrogen receptor staining of more than 10% was considered 'positive'.

The percentage agreement between core and excision biopsy with regard oestrogen receptor immunohistochemical staining varied from 62% to 100% (Table 1.1). The analysis of percentage agreement can be deceiving as it is not a strong statistical measure of overall agreement and does not quantify the agreement due to chance. For this, a *Kappa* statistic has to be utilized. However, only four studies calculated the *Kappa* statistic for agreement.[29-32] Usami *et al.* conducted the only study to identify a *Kappa* statistic of more than 0.75, which implies that there was an excellent agreement between core and excision biopsies.[31] Sutela *et al.* achieved a *Kappa* statistic of 0.39 despite the seemingly acceptable 83% agreement between core and excision.[32] This implies that the agreement observed could be merely by chance and emphasises the importance of calculating the *Kappa* statistic. In an attempt to make sense of the heterogeneity involved, Li *et al.* recently conducted a meta-analysis comparing the core and excision immunohistochemical staining in invasive breast carcinoma of 2450 patients and found an overall agreement of 92.8% (*Kappa* = 0.78).[33]

Table 1.1. Overview of the literature comparing immunohistochemical staining of the oestrogen receptor between core and excision biopsies in breast cancer

Author	Year	No. of Pts	Neoadjuvant Chemotherapy	DCIS or Invasive Carcinoma	Percentage (%) Agreement Core vs. Excision	*Kappa* Value
Zidan A, et al[34]	1997	30			83	
Jacobs TW, et al[35]	1998	56	Excluded	Invasive	100	
Gödzinger P, et al[27]	1998	150		Both	97	
Connor CS, et al[36]	2002	44	Excluded	Invasive	98	
Taucher S, et al[29]	2003	180	Excluded	Invasive	91	0.69
Taucher S, et al[30]	2003	191	Included	Invasive	86	0.64
Harris K, et al[37]	2004	95			95	
Cavaliere A, et al[38]	2005	68			62	
Varge Z, et al[39]	2005	23	Included	Invasive	91	
Arens N, et al[40]	2005	25	Included	Invasive	80	
Arens N, et al[40]	2005	30	Excluded	Invasive	63	
Al Sarakbi W, et al[41]	2005	95			95	
Badoual C, et al[42]	2005	110			90	
Mann GB, et al[43]	2005	100		Invasive	86	
Burge CN, et al[28]	2006	87	Excluded	Invasive	95	
Cahill RA, et al[44]	2006	95			70	
Usami S, et al[31]	2007	112	Excluded	Both	95	0.84
Ozedemir A, et al[26]	2007	61	Included		90	
Hodi Z, et al[45]	2007	338			99	
Wood B, et al[46]	2007	100			96	
Kasami M, et al[47]	2008	173	Included	Invasive	89	
Kasami M, et al[47]	2008	117	Excluded	Invasive	93	
Sutela A, et al[32]	2008	41		Invasive	83	0.39
Arnedos M , et al[48]	2009	336		Invasive	98	
Hanley KZ, et al[49]	2009	41	Excluded	Invasive	95	
Park SY, et al[50]	2009	104		Invasive	99	
Uy GB, et al[51]	2010	160			82	
Lorgis V, et al[52]	2011	175	Excluded	Invasive	84	

1.6 HER2 immunohistochemical staining on core and excision biopsies

Studies comparing the agreement of HER2 immunohistochemical staining of core and final excision biopsy specimen are even more difficult to interpret than those done for the oestrogen receptor immunohistochemistry. The lack of standardization is remarkable with Table 1.2 outlining the fundamental conclusions, while concealing the heterogeneity among the different studies. The percentage agreement between the core and excision biopsy specimens for HER2 immunohistochemical staining varies from 64% to 98% in the 14 studies listed in Table 1.2, with a mean percentage agreement of 86%. The two studies done by Kasami *et al.* were not included in Table 1.2 as they compared the agreement of HER2(1+).[47,48] The study by Lorgis *et al.* was not included as they did not state what immunohistochemical staining level was compared.[52]

All of the studies reviewed in Table 1.2 were retrospective except for the studies done by Varge *et al.*[39] and Ozedemir *et al.*[26] Some of the studies compared the agreement between HER2(2+) (Connor *et al.*,[36] Burge *et al.*[28], Usami *et al.*[31]), while others compared the agreement between HER2(3+)(Ozedemir *et al.*)[26]. Again, in line with the discussion regarding comparison of the oestrogen receptor immunohistochemical staining under section 1.5, a *Kappa* statistic was only done in three of the studies listed in Table 1.2: Mueller-Holzner *et al.*,[14] Taucher *et al.*[53] and Osami *et al.*[31]. Five of the studies mentioned in Table 1.2 expanded the comparison by confirmation with FISH: Taucher *et al.*,[53] Varge *et al.*,[39] Burge *et al.*,[28] Apple *et al.*[53] and Hanley *et al.*[49] Interestingly, Hanley *et al.* found that the addition of FISH did not change any of the HER2(2+) equivocal staining to a negative result in a sample of 41 patients.[49]

Table 1.2. Overview of the literature comparing the immunohistochemical staining of HER2 between core and excision biopsies in breast cancer

Author	Year	No. of Pts	Neoadjuvant Chemotherapy	Percentage (%) Agreement Core vs. Excision	*Kappa* Value
Mueller-Holzner E et al[14]	2001	64		92	0.80
Connor CS et al[36]	2002	44	Excluded	91	
Taucher S et al[53]	2003	325		92	0.86
Cavaliere A et al[38]	2005	68		90	
Varge Z et al[39]	2005	23	Included	65	
Mann GB et al[43]	2005	100		80	
Burge CN et al[28]	2006	81	Excluded	96	
Cahill RA et al[44]	2006	95		64	
Usami S et al[31]	2007	60	Excluded	88	0.65
Ozedemir A et al[26]	2007	61		79	
Wood B et al[46]	2007	100		87	
Apple SK et al[53]	2009	125		98	
Hanley KZ et al[49]	2009	41	Excluded	93	
Park SY et al[50]	2009	104		87	

1.7 Factors that may influence the result of immunohistochemical staining on core and excision biopsies

From the literature it is evident that certain factors may influence the result of immunohistochemical staining on the core and excision biopsy specimens. Specifically, these factors include whether core or excision biopsy should be used as the gold standard for comparison, the size and number of the core biopsies taken, the tumour size in question, the effect of neoadjuvant chemotherapy and the effect of delay in sample processing between the core biopsy and final excision specimen. With reference to the literature, these factors are discussed below.

1.7.1 Considering core biopsy as a gold standard for comparison

I used the surgical excision biopsy as the 'gold standard' for comparison with the core biopsy samples. My initial logic was that the entire specimen should be more accurate than a mere sample taken by core and that large tumours might be heterogeneous in nature with regard to the receptor status distribution. The same logic was applied in other comparative studies,[52] for example, Sutela *et al.* evaluated 41 invasive breast cancers with core biopsies and compared the results with excision specimens.[32] They found that the core biopsy was more likely to be positive for oestrogen receptor immunohistochemical staining and HER2 assessment by FISH. [32] The difference was not, however, statistically significant. Wood *et al.* suggested that a small number of additional hormone receptor positive cases could be detected by performing immunohistochemical staining on a previously received core biopsy in the case of a negative result on the excision specimen. [46] Douglas-Jones *et al.* found that the oestrogen receptor immunohistochemical staining was significantly higher in core biopsies compared to that of the excised specimen.[54] Importantly, Mann *et al.* suggested that

13% of oestrogen receptor positive patients and 1% of HER2 positive patients will be 'missed' with reliance on excision biopsy immunohistochemical staining alone.[43]

In contrast, Khoury *et al.* found that excision biopsy specimens were more likely to have a positive receptor status (81.3%) for oestrogen receptor than the core biopsy (80.1%).[55] This was not statistically significant. The study by Arnedos *et al.*[48], with a sample size of 336 patients, also failed to show a higher tendency of positive staining for oestrogen receptor in core samples.

Chen X *et al.* suggested in their meta-analysis that the correlation between core and excision biopsy was better when the population tested had a higher oestrogen receptor positive rate (>78%) in general ($P < 0.05$).[56] Even though the meta-analysis by Li *et al.* found excellent agreement between core and excision biopsies for oestrogen receptor staining (92.8%, *Kappa* = 0.78), they still maintained that a negative hormonal receptor status should be interpreted with caution or repeated on excision biopsy.[33]

It seems as though a positive immunohistochemical staining result for oestrogen receptor could be missed on both the core biopsy and the excision specimen. If either of these tests are negative, one can repeat the immunohistochemical staining on the other to confirm. The number of extra patients who would stain positive seems small: 1.2% for oestrogen receptor in Khoury *et al.*'s series, for example.[55] It is not clear if this translates in a cost-effective search for effective benefit in clinical outcome or indicate an adequate number needed to treat. This research has not been done as yet.

1.7.2 The size of the core biopsies

Earlier studies suggested that large core biopsies are required for the analysis of invasive breast carcinomas.[57] The thought was that the quality of analysis correlates with the quantity of tissue examined.[58] However, high accuracy with regard to histological diagnosis and

immunohistochemistry has been obtained even with the limited tissue quantity acquired using a thin needle (16 Guage).[31] Currently, with the advent of tissue microarray technology, the size of the cores does not seem to influence the biomarker results significantly, provided that the core biopsies are still larger than 0.6 mm in diameter.[59]

1.7.3 The number of the core biopsies taken

Regarding the histological diagnosis: O'Leary *et al.* concluded in their review of a 113 patients, that the number of cores collected and the total amount of material reviewed by the pathologist did not influence the agreement between core and excision specimens.[58]

Concerning immunohistochemical staining for oestrogen receptor and HER2, the literature is ambiguous. For example, Sutela *et al.* suggested that 3 core samples are needed for reliable assessment of HER2 and progesterone receptor status, whilst the oestrogen receptor sensitivity remained low even after multiple core biopsies.[32] Al Sarakbi *et al.* did not find a significant difference in the number of core samples obtained between concordant and discordant cases regarding oestrogen and progesterone receptor immunohistochemical staining.[41]

Furthermore, Tamaki *et al.* concluded from their sample size of 353 patients that the concordance in diagnosis between core biopsy and final surgical excision specimen approached 100% for both oestrogen receptor and HER2 detection when four or more core biopsies were taken.[60] They concluded that the optimal number of core biopsies to be taken should be four in the pre-operative setting.[60]

1.7.4 Tumour size and the results from core biopsies

There is a concern that the core biopsy could misrepresent large tumours where heterogeneity might exist throughout the tumour. Howell *et al.* suggested that oestrogen and progesterone receptor status could be altered in advanced carcinoma of the breast due to tumour heterogeneity.[61] However, Mueller-Holzner *et al.* suggested that the HER2 status seems to be stable throughout the tumour.[14] Importantly, few studies reported on the intra-tumoural distribution of the oestrogen receptor, progesterone receptor or HER2 using immunohistochemical staining. This may become even more relevant in the evaluation of large tumours, as reported on in our study. Moreover, Douglas-Jones *et al.* found that there was a significant decrease in oestrogen receptor positivity from the periphery of the tumour toward the centre.[54]

1.7.5 Effect of neoadjuvant chemotherapy on core biopsies

Although survival rates for neoadjuvant and adjuvant chemotherapy have been found to be equal, the response to neoadjuvant chemotherapy is an important predictor of outcome. [62,63] Neoadjuvant chemotherapy has become standard treatment in locally advanced-, metastatic- and inflammatory breast cancers and allows *in vivo* assessment of the tumour's response while permitting an increasing proportion of patients the option of breast-conservation therapy. [62,63] Neoadjuvant chemotherapy continues to evolve and the decision making often depend on the results obtained from a core biopsy specimen alone. [62,63]

The study by Taucher *et al.* implied that neoadjuvant chemotherapy significantly decreased the expression of oestrogen receptor status from a positive core biopsy to a negative final excision specimen.[29] Rody *et al.* observed a change in receptor expression of either oestrogen, progesterone or HER2 from positive to negative in 16/35 cases (45.7%) and from negative to positive in 5/22 cases (22.7%) following neoadjuvant Docetaxel, Adriamycin and

Cyclophosphamide treatment.[64] Lee *et al.* found that hormone receptor status changed in 34/56 patients (61%) treated with neoadjuvant chemotherapy and 27/56 patients (48%) who were not treated with neoadjuvant chemotherapy.[68] These changes influenced the decision regarding endocrine manipulation in only 3 patients (5%) in the neoadjuvant and non-neoadjuvant arms of the study, for example: patients staining positive for both oestrogen and progesterone receptors were treated the same manner to a change where oestrogen receptor stained positive and progesterone receptor stained negative.[68]

Arens *et al.* found that the HER2 receptor remained stable in their small cohort of 25 patients, all of whom received neoadjuvant chemotherapy with Adriamycin and either Docetaxel or Cyclophospahmide.[40] This conclusion is in agreement with that of Taucher *et al.*[53], Vincent-Salomon *et al.*[67] and numerous other studies[39,40,47,69] in which stable HER2 expression was found after neoadjuvant chemotherapy. The stability in HER2 over-expression was often attributed to the fact that HER2 amplification occurs late in tumour genesis and is thus a robust marker.[67,69]

Conversely, Zheng *et al.* found that the expression of HER2 was significantly different before and after neoadjuvant chemotherapy (P=0.049), while the oestrogen receptor rates were not as severely affected.[65] Nonetheless, they still suggested re-evaluating a patient's receptor status following neoadjuvant chemotherapy.[65]

Thirty two studies were reviewed by van de Ven *et al.* that included neoadjuvant chemotherapy (with or without Trastuzumab) given between the core and final excision specimen.[66] They found a significant range of discordance between the core and final excision ranging from 2.5% to 17.0%.[66] There was a good agreement between the core biopsy and excision specimens when HER2 amplification was tested with FISH, but poor when HER2 was assessed using immunohistochemistry.[66] Van de Ven *et al.* found that a switch to a negative HER2 receptor status occurred in up to 43% of patients when

neoadjuvant chemotherapy was combined with Trastuzumab.[66] In conclusion, this review stated that the receptor status of the residual tumour should be re-determined after neoadjuvant chemotherapy and before prescribing further adjuvant treatment.[66]

For obvious reasons, patients who had a complete pathological response were not included in our study. This would also have been the case if data were collected prospectively.

1.7.6 Effects of sample processing delay on biopsy results

Delay in the processing of samples allow for proteolytic degradation of the antigen. This has been demonstrated for oestrogen and progesterone receptor staining.[70] Meyer *et al.* showed only a small reduction in the proportion of positive oestrogen receptor assay results in fresh mastectomy specimens compared to biopsy specimens, and concluded that no significant losses occurred.[71] However, Khoury *et al.* observed significant alterations in the membranous markers (epidermal growth factor receptor and E-cadherin) when specimens were stored overnight without fixation.[72] The effect of a delay in sample processing was not incorporated into my study.

1.7.7 Effects of delay between the core biopsy and the final excision

Hodi *et al.* mentioned that tumours analysed in their study were all excised within 60 days of core.[45] Arnedos *et al.* achieved a median time of 27 days between core biopsy and final excision of the tumour.[48] Since most institutions strive to expedite the care of patients suffering from malignancy, the effects of delays between the core and excision biopsy specimen immunohistochemical staining is not known.

1.8 Aim of the study

The aim of this study was to compare the oestrogen receptor and HER2 immunohistochemical staining results from the core biopsy to that of the final surgical excision specimen, in sequential patients with confirmed breast cancer, who had both specimens analyzed at the Charlotte Maxeke Johannesburg Academic Hospital Breast Unit. This study, being the first study of its kind in South Africa, allows us to examine the agreement in a South African context with regard to the unique challenges present in our patient population: tumour biology, advance presentation of disease and delays between core and excision specimens. Comparison of results obtained from this study with international studies will determine whether there are differences in our population group and will help determine future policies in our unit.

CHAPTER 2 - PATIENTS AND METHODS

2.1 Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand before conducting the study (clearance certificate number: M090523, Appendix F). Patients were allocated study numbers to protect their identity.

2.2 Patient selection and data collection

A retrospective analysis was conducted from the 1st of January 2004 to the 30th of June 2009 (5 ½ years). Data was obtained using the records from the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) Breast Clinic and the National Health Laboratory Service (NHLS) pathology database. All patient records with a histological diagnosis of breast cancer made during this period were reviewed. Patients who had immunohistochemical staining for oestrogen receptors done on both the core and excision specimens were identified. Furthermore, patients who had immunohistochemical staining for HER2 on both the core biopsy and the excision specimen were also identified for comparison between the core and excision specimens.

Immunohistochemical staining was performed by the Department of Histopathology at the University of the Witwatersrand, Johannesburg, South Africa. Staining methods were not altered during the study period. The final excision biopsy (from mastectomy or wide local excision) was used as the 'gold standard' to compare to the core biopsy specimen.

2.3 Analysis of oestrogen receptor immunohistochemical staining

For the purpose of this study, oestrogen receptor positivity was defined in the report by the pathologist as both 1+ positive staining in 5% or more of the tissue sample, and 1+ positive

staining in 10% or more of the tissue sample. Most specimens were immunostained using the DAKO Autostainer®. Due to the number of specimens received by the laboratory, manual staining was also performed. Unfortunately it was not possible to obtain the proportions of staining performed manually compared to auto-stained samples.

The *Kappa* statistic was employed to compare the agreement between core and excision biopsy specimens. A *Kappa* statistic of less than 0.40 signifies no agreement, a *Kappa* statistic between 0.40 and 0.75 indicates poor agreement and a *Kappa* statistic higher than 0.75 implied excellent agreement between the core and excision biopsy results.

Subgroup analysis was conducted to assess whether chemotherapy, tumour size, the number of core biopsies performed, patient age or the time delay from the core biopsy to the final excision biopsy had any impact on the agreement between the core biopsy and excision specimen immunohistochemical staining. Fisher's exact and Student's *t*-tests were used to calculate a two tailed *P*-value. Statistical significance was defined as $P \leq 0.05$.

2.4 Analysis of HER2 immunohistochemical staining

For the purpose of this study, HER2 positivity was examined at two levels. In the first instance, I considered HER2 immunohistochemical staining as positive when HER2 staining was classified by the histopathologist as '2+' or '3+'. This was referred to as the "HER2(2+) positive group". This particular denotation is used as the threshold by clinicians for further confirmation using FISH. In the second instance, I considered HER2 immunohistochemical staining as positive when HER2 staining was classified by the histopathologist as '3+'. This was referred to as the "HER2(3+) positive group". This denotation is used by clinicians to resemble the 'true' HER2-positive patients.

Similarly, the *Kappa* statistic was employed to quantify agreement between the core and excision biopsy specimens, and subgroup analysis was conducted to assess whether

chemotherapy, tumour size, the number of core biopsies performed, patient age or the time delay from the core biopsy to the final excision biopsy had any impact on the agreement or non-agreement between the core and excision immunohistochemical staining. Fisher's exact and Student's t-tests were used to calculate a two tailed *P*-value. Statistical significance was defined as $P \leq 0.05$.

CHAPTER 3 - RESULTS

3.1 Patients identified for analysis

A total of 718 patients (2 males) had a histological diagnosis of breast cancer made at CMJAH during the five and a half year period that extended from the 1st of January 2004 until the 30th of June 2009. Immunohistochemical staining for oestrogen receptor status was done on core alone in 360 (50.1%) patients, and on excision biopsy specimen only in 226 (31.5%) of patients. Immunohistochemical staining for oestrogen receptor status was done on both the core and excision biopsy specimens on 132 patients (18.4%). HER2 immunohistochemical staining was done on both the core and excision biopsy specimens of 124 patients (17.3%). An overlap existed in that all patients who had HER2 staining in core and excision, had oestrogen receptor immunohistochemical staining as well, but not *vice versa*.

3.2 The oestrogen receptor group

3.2.1. General characteristics

132 patients (one male) had oestrogen immunohistochemical staining status of both core and excision specimens analysed (see Table 3.1). The mean age of these patients with one standard deviation was 56.7 ± 13.3 years (range, 30.0 to 90.0 years). Breast cancer was diagnosed in the left breast of 71 patients (54.2%, n=131) and in the right breast of 60 patients (45.8%, n=131) (P=0.33). The average number of core biopsies done before excision of the final specimen was 3.5 (range, 1 to 11 core biopsies; n = 131).

Table 3.1. Demographics, general characteristics and immunohistochemistry of patients with positive oestrogen receptor staining on both the core and excision biopsies

Variable	Value	No. of patients in analysis
Patients with oestrogen receptor staining on both core and excision biopsies	132 (18.4%)	718
Gender		132
Female	131 (99.2%)	
Male	1 (0.8%)	
Mean age in years (range)	56.7 (30.0 – 90.0)	132
Tumour laterality		131
Left breast	71 (54.2%)	
Right breast	60 (45.8%)	
Number of cores (range)	3.5 (1.0 – 11.0)	131
Method of excision		132
Mastectomy	126 (95.5%)	
Wide local excision	6 (4.5%)	
Tumour size of excision specimen in mm (range)	37.1 (2.5 – 230.0)	132
Histology		132
Core = Excision	131 (99.2%)	
Core = Excision = Ductal	121 (91.7%)	
Core = Excision = Lobular	9 (6.8%)	
Core = Excision = Ductal & Lobular	2 (1.5%)	
Core = Ductal ; Excision = Lobular & Ductal	1 (0.8%)	
Days between core and excision		
Mean no. of days for all patients (range)	136 (8 - 449)	130
No. of patients with delay of ≤60 days		41
No. of patients with delay for 61 to 179 days		41
No. of patients with days for ≥180 days		48
Patients receiving chemotherapy between core and excision	58 (43.9%)	132
*Effect of chemotherapy on the delay between core and excision**		
Mean no. days for chemotherapy pts (range)	202 (26 – 449)	57
Mean no. days for pts without chemotherapy(range)	74 (8 – 273)	73

**P < 0.001*

3.2.2 Histology of the final excision specimen

The histology of the final excision biopsy revealed ductal carcinoma in 121 patients (91.7%, n = 132), lobular carcinoma in 9 patients (6.8%, n = 132) and both lobular and ductal carcinoma in 2 patients (1.5%, n = 132). The core biopsy agreed with the final excision biopsy histology in all except one patient (99.2% agreement, n = 132). The core biopsy for the latter patient revealed ductal carcinoma only, whereas the excision specimen had elements of both ductal and lobular carcinoma (Table 3.1).

3.2.3 Time from core to excision and neoadjuvant chemotherapy

The average time delay from core biopsy to final excision was 136 days (range: 8 - 449, n = 130). Fifty eight patients (43.9%, n = 132) received chemotherapy in the time interval between core and excision biopsies. Patients who received neoadjuvant chemotherapy had a mean delay of 202 days (range: 26 - 449) between core biopsy and excision specimen compared with only 78 days (range: 8 - 273) in patients who did not have neoadjuvant chemotherapy ($P < 0.001$) (Table 3.1).

3.2.4 Tumour size and method of obtaining the final excision specimen

The average tumour size as determined by the final excision specimen was 37.1 mm (range: 2.5 - 230.0 mm; n = 132). The final excision biopsy specimen was obtained by mastectomy in 126 patients (95.5%; n = 132) and by wide local excision in 6 patients (4.5%; n = 132).

3.2.5 Analysis of oestrogen receptor positive staining above 5%

Immunohistochemical staining above 5% was recorded as “positive” for oestrogen receptor and analysed. The agreement between the oestrogen receptor status of core and excision biopsy specimens was 91.7% (*Kappa* = 0.784). When the excision biopsy specimen was

considered the 'gold standard' for comparison, the core biopsy had a sensitivity of 92.9%, a specificity of 87.9%, a positive- predictive value of 95.8% and a negative predictive value of 80.6% (Table 3.2). Patients who had agreement between core and excision biopsies were compared with patients who had no agreement between them. As seen in Table 3.3, none of the following factors influenced the agreement between core and excision biopsy results: chemotherapy ($P = 0.75$), tumour size ($P = 0.33$), number of core biopsies ($P = 0.76$), patient age ($P = 0.71$) or the delay between the core and excision biopsy ($P = 0.83$).

Table 3.2. Comparison of oestrogen receptor positive staining ≥5% for the core biopsy using the excision biopsy as the 'gold standard'.

	Excision (+)	Excision (-)	TOTAL
Core (+)	92	4	96
Core (-)	7	29	36
TOTAL	99	33	132

Table 3.3. Factors that might affect oestrogen receptor staining when ≥5% staining is considered positive

Factors	Agreement between core and excision	No agreement between core and excision	Significance
Total patients (n = 132)	121 (91.7%)	11 (8.3%)	κ = 0.784
Chemotherapy (n = 58)	54/121 (44.6%)	4/11 (36.4%)	*P* = 0.75
Average tumour size in mm (range)	35.5 (2.5 – 135.0) *n* = 121	54.7 (16.0 – 230.0) n = 11	*P* = 0.33
Average number of cores (range)	3.6 (1 – 11) *n* = 120	3.5 (2 – 6) n = 11	*P* = 0.76
Average patient age in years (range)	56.8 (30 – 90) *n* = 121	55.6 (35 – 67) *n* = 11	*P* = 0.71
Average days of delay between core and excision (range)	136.2 (8 – 449) *n* = 119	128.5 (25 – 401) *n* = 11	*P* = 0.83

Abbreviations: κ = *Kappa* value

3.2.6 Analysis of oestrogen receptor positive staining above 10%

When immunohistochemical staining above 10% for oestrogen receptors was considered "positive", the agreement between core and excision biopsy specimens was 93.2% (*Kappa* = 0.823). When the excision biopsy specimen was considered the 'gold standard' for comparison, the core biopsy had a sensitivity of 93.5%, a specificity of 92.3%, a positive predictive value of 96.7% and a negative predictive value of 85.7% (see Table 3.4). We compared patients who had agreement between core and excision biopsy results with patients who had no agreement. None of the following factors influenced the agreement between core and excision biopsy results: chemotherapy (*P* = 0.11), tumour size (*P* = 0.70), number of core

biopsies ($P = 0.80$), patient age ($P = 0.63$) or the delay between the core and excision biopsy ($P = 0.75$) (Table 3.5).

Table 3.4. Comparison of oestrogen receptor positive staining ≥10% for the core biopsy using the excision biopsy as the 'gold standard'.

	Excision (+)	Excision (-)	TOTAL
Core (+)	87	3	90
Core (-)	6	36	42
TOTAL	93	39	132

Table 3.5. Factors that might affect oestrogen receptor staining when ≥10% staining is considered positive.

Factors	Agreement between core and excision	No agreement between core and excision	Significance
Total patients (n = 132)	123 (93.2%)	9 (6.8%)	κ = 0.823
Chemotherapy (n = 58)	56/123 (45.5%)	2/9 (22.2%)	$P = 0.11$
Average tumour size in mm (range)	37.3 (2.5 – 230.0) $n = 123$	33.9 (16.0 – 95.0) $n = 9$	$P = 0.70$
Average number of cores (range)	3.6 (1 – 11) $n = 122$	3.4 (2 – 6) $n = 9$	$P = 0.80$
Average patient age in years (range)	56.8 (30 – 90) $n = 123$	55.1 (35 – 67) $n = 9$	$P = 0.63$
Average days of delay between core and excision (range)	136.5 (8 – 449) $n = 121$	122.8 (33 – 401) $n = 9$	$P = 0.75$

Abbreviations: κ = *Kappa* value

3.3 The HER2 group

3.3.1 General characteristics

Eight patients from the oestrogen receptor group had to be excluded in this analysis, since HER2 immunohistochemistry was not performed on both the core and final excision specimens (n = 124). The average age ± SD was 56.4 ± 13.4 years (range: 30.0 - 90.0 years). There was one male patient (0.8%). Breast cancer was diagnosed in the left breast of 63 patients (51.2%, n = 123) and in the right breast of 60 patients (48.8%, n = 123) (P = 0.34). The average number of core biopsies done before excision of the final specimen was 3.6 (range: 1 - 11; n = 124). (Table 3.6)

3.3.2 Histology of the final excision specimen

Histology of the final excision biopsy revealed ductal carcinoma in 116 patients (93.5%, n = 124), lobular carcinoma in 6 patients (4.8%, n = 124) and both lobular and ductal carcinoma in 2 patients (1.6%, n = 124). The core biopsy agreed with the final excision biopsy histology in all except one patient (99.2% agreement, n = 124), where the core biopsy in the latter patient revealed ductal carcinoma only and the excision specimen had elements of both ductal and lobular carcinoma. (Table 3.6)

3.3.3 Time from core to excision and neoadjuvant chemotherapy

The average time delay from core biopsy to final excision was 141 days (range, 8 days to 449 days, n = 122). Fifty five patients (44.4%, n = 124) had chemotherapy in the time interval between the core and excision biopsy. Patients who had neoadjuvant chemotherapy had a mean delay of 212 days (range: 42 - 449 days) between core biopsy and excision specimen compared with only 75 days (range: 8 - 273 days) in patients who did not have neoadjuvant chemotherapy ($P < 0.001$) (Table 3.6).

Table 3.6. Demographics, general characteristics and immunohistochemistry of patients with HER2 staining on both the core and excision biopsies

Variable	Value	No. of patients in analysis
No. of patients with HER2 staining on both core and excision biopsies	124 (17.3%)	718
Gender		124
Female	123 (99.2%)	
Male	1 (0.8%)	
Mean age in years (range)	56.4 (30.0 – 90.0)	124
Tumour laterality		123
Left breast	63 (51.2%)	
Right breast	60 (48.8%)	
Number of cores (range)	3.6 (1.0 – 11.0)	124
Method of excision		124
Mastectomy	118 (95.2%)	
Wide local excision	6 (4.8%)	
Tumour size of excision specimen in mm (range)	37.8 (2.5 – 230.0)	124
Histology		124
Core = Excision	123 (99.2%)	
Core = Excision = Ductal	116 (93.5%)	
Core = Excision = Lobular	6 (4.8%)	
Core = Excision = Ductal & Lobular	2 (1.6%)	
Core = Ductal ; Excision = Lobular & Ductal	1 (0.8%)	
Days between core and excision		
Mean no. of days for all patients (range)	141 (8 - 449)	122
No. of patients with delay for ≤60 days		35
No. of patients with delay for 61 to 179 days		39
No. of patients with delay for ≥180 days		48
Patients receiving chemotherapy between core and excision	55 (44.4%)	124
*Effect of chemotherapy on the delay between core and excision***		
Mean no. days for chemotherapy pts (range)	212 (42 – 449)	53
Mean no. days for pts without chemotherapy(range)	75 (8 – 273)	69

**P < 0.001*

3.3.4 Tumour size and method of obtaining the final excision specimen

The average tumour size as determined by the final excision specimen was 37.8 mm (range: 2.5 - 230.0 mm, *n* = 124). The final excision biopsy specimen was obtained by mastectomy in 118 patients (95.2%, *n* = 124) and by wide local excision in 6 patients (4.8%. *n* = 124).

3.3.5 Analysis of HER2(2+) and HER2(3+) as 'positive'

Immunohistochemical staining for HER2 was analysed where HER2(2+) and HER2(3+) staining were both taken as 'positive', and HER2(1+) and HER2(0) staining were both considered 'negative'. The agreement between core and excision biopsy specimens was 57.3% (*Kappa* = 0.103). When the excision biopsy specimen was considered the 'gold standard' for comparison, core biopsy had a sensitivity of 39.6%, a specificity of 70.4%, a positive predictive value of 50.0% and a negative predictive value of 61.0% (Table 3.7) Patients that had agreement between core and excision biopsies were compared to patients who had no agreement. None of the following factors influenced the agreement between core and excision: tumour size (*P* = 0.25), patient age (*P* = 0.52) or the delay between the core and excision biopsy (*P* = 0.45). (Table 19) Neoadjuvant chemotherapy (*P* = 0.04) and the number of core biopsies done (*P* = 0.03) significantly influenced the agreement between the core biopsy and excision specimen (Table 3.8).

Table 3.7. Comparison using HER2(2+) and HER2(3+) as positive staining from the core biopsy against the excision biopsy as the 'gold standard'.

	Excision (+)	Excision (-)	TOTAL
Core (+)	21	21	42
Core (-)	32	50	82
TOTAL	53	71	124

Table 3.8. Factors that might affect HER2 staining when HER2(2+) and HER2(3+) is considered positive.

Factors	Agreement between core and excision	No agreement between core and excision	Significance
Total patients (n = 124)	71 (81.5%)	53 (18.5%)	κ = 0.103
Chemotherapy (n = 55)	29/71 (39.6%)	26/53 (65.2%)	***P* = 0.04**
Average tumour size in mm (range)	40.1 (2.5 – 230.0) *n* = 71	34.6 (3.5 – 110.0) *n* = 53	*P* = 0.25
Average number of cores (range)	3.8 (1 – 11) *n* = 71	3.2 (2 – 5) *n* = 53	***P* = 0.03**
Average patient age in years (range)	57.0 (31 – 79) *n* = 71	55.4 (30 – 90) *n* = 53	*P* = 0.52
Average days of delay between core and excision (range)	134.7 (8 – 444) *n* = 69	148.6 (23 – 449) *n* = 53	*P* = 0.45

Abbreviations: κ = *Kappa* value

3.3.6 Analysis of HER2(3+) as 'positive'

When the immunohistochemical staining was analysed considering HER2(3+) staining as positive and HER2(2+), HER2(1+) and HER2(0) staining as negative, the agreement between core and excision biopsy specimens was 81.5% (*Kappa* = 0.398). When the excision biopsy specimen was considered the 'gold standard' for comparison, the core biopsy had a sensitivity of 57.1%, a specificity of 86.4%, a positive predictive value of 46.2% and a negative predictive value of 90.8% (Table 3.9). Patients who had agreement between core biopsy and excision specimens were compared to patients who had no agreement. None of the following factors influenced the agreement between core and excision: tumour size (*P* = 0.93), number of cores (*P* = 0.52), patient age (*P* = 0.17) or the delay between the core and excision biopsy (*P* = 0.12) (Table 3.10). Neoadjuvant chemotherapy before excision did, however, significantly influence the agreement between core and excision biopsy results (*P* = 0.04).

Table 3.9. Comparison using HER2(3+) as positive staining from the core biopsy against the excision biopsy as the 'gold standard'.

	Excision (+)	Excision (-)	TOTAL
Core (+)	12	14	26
Core (-)	9	89	98
TOTAL	21	103	124

Table 3.10. Factors that might affect HER2 staining when HER2(3+) is considered positive.

Factors	Agreement between core and excision	No agreement between core and excision	Significance
Total patients (n = 124)	101 (81.5%)	23 (18.5%)	κ = 0.398
Chemotherapy (n = 55)	40/101 (39.6%)	15/23 (65.2%)	***P* = 0.04**
Average tumour size in mm (range)	37.8 (2.5 – 230.0) *n* = 101	37.3 (15 – 110.0) *n* = 23	*P* = 0.93
Average number of cores (range)	3.6 (1 – 11) *n* = 101	3.4 (2 – 8) *n* = 23	*P* = 0.52
Average patient age in years (range)	57.1 (30 – 90) *n* = 101	52.7 (32 – 84) *n* = 23	*P* = 0.17
Average days of delay between core and excision (range)	134.8 (8 – 449) *n* = 99	166.6 (32 – 304) *n* = 23	*P* = 0.12

Abbreviations: κ = *Kappa* value

CHAPTER 4 – DISCUSSION AND CONCLUSION

4.1 General observations regarding the study population

A considerable number of new breast cancer cases are diagnosed at the CMJAH each year. My analysis only included patients who had their diagnosis made on both core and final surgical excision results at CMJAH. This analysis only included patients who had their diagnosis made on both the core and final surgical excision at CMJAH. All specimens were analysed at the NHLS

There is currently no policy in place at CMJAH to guide the decision with regard to which specimens undergo immunohistochemical staining. While some clinicians treat patients solely based on either core biopsy or the excision biopsy specimen, others repeat immunohistochemical staining. Clinician bias as to which patient had their immunohistochemical staining for the oestrogen receptor and/or HER2 repeated would have influenced the results of this retrospective analysis.

A very high proportion of patients had the final excision biopsy specimen obtained by mastectomy (95.5%), rather than by breast conservation. In contrast, investigators such as Arnedos *et al.* obtained 70% of surgical excision samples from breast conservation specimens and only 30% from mastectomy.[48] The high proportion of mastectomies done in our population is most likely a reflection of our larger than reported average tumour size (37.1 mm). The mean tumour sizes reported in other studies were much smaller: 14.0 mm (Mann *et al.*[43]), 18.6 mm (Ozdemir *et al.*) and 25.8 mm (Arens *et al.*[40]). These tumour size discrepancies might be a reflection of the effectiveness of breast screening programs available in other countries which diagnose breast tumours at a much earlier stage.

Importantly, this study confirms that there is good agreement between the core and excision biopsy in identifying the histological type of carcinoma.. Core biopsy correlated with excision biopsy in all except one patient (99.2% agreement, n = 132). This is similar in other

studies, depending on what detail is considered with regard to the histological diagnosis. In support of my findings, Richter-Ehrenstein *et al.* showed that 99.6% of core biopsies had the identical histological type as the final excision in 488 patients.[73] In contrast, Sharifi *et al.* found the correlation between histologic type on core needle biopsy correlated with that on excision biopsy in only 64 of their 79 cancers (81%). However, the specific detail assessed for agreement in this particular study included the histological grade, lymphatic vessel invasion and the presence of an extensive intraductal component, which was not analysed to such detail in this study.[74]

An area of concern in this study is the time delay between the core and excision biopsy specimens, which averaged 135.6 days (19.4 weeks) for the group of patients analysed regarding oestrogen receptor staining. The time delay is long, especially in patients who received chemotherapy ($P < 0.001$). Four patients had a delay of longer than a year between the core and excision specimens. The delay between the core and final excision biopsy did not influence the agreement between these biopsies for either the oestrogen receptor or HER2. Investigators such as Hodi *et al.* mentioned specifically that all the tumours were excised within 60 days of the core.[45] The delay demonstrated in the current study raises concerns regarding overall patient care and communication between medical and surgical oncologists to expedite care after neoadjuvant chemotherapy.

4.2 Results for immunohistochemical staining for the oestrogen receptor

The agreement between the core and the final excision specimen was analysed considering a ≥ 10% oestrogen receptor immunohistochemical staining as a "positive" result. Although the agreement for ≥ 5% positive staining was excellent (91.7%, *Kappa* = 0.784), the ≥ 10% immunohistochemical staining group revealed even better agreement between the core biopsy and excision specimens (93.2%, *Kappa* = 0.823). Indeed, all the parameters of sensitivity,

specificity, positive predictive value and negative predictive value improved when considering the cut-off level at 10% as a minimum for a positive oestrogen receptor immunohistochemical staining.

Factors such as chemotherapy, tumour size, number of core biopsies, patient age or the time delay between the core and final excision biopsy did not influence the agreement between these biopsy results for oestrogen receptor staining. Importantly, because of the excellent agreement between the core and final excision biopsy specimens for oestrogen receptor immunohistochemical staining in general, the number of patients who did not have congruent results were small (n=9 of 132; 6.8%)

4.3 Results for immunohistochemical staining for HER2

For the purpose of this analysis, I compared two separate groups: the first group of patients (described under section 4.3.1) were labelled HER2 'positive' when their immunohistochemical staining was denoted as HER2(2+) or HER2(3+). All patients that stained HER2(0) and HER2(1+) were considered 'negative'. This analysis was done because the HER2(2+) group would be the trigger for further investigation with FISH, should the treatment with Trastuzumab become the standard of care at CMJAH. In the second group of patients (described under section 4.3.2) our definition of what was considered HER2 'positive' was more stringent. Patients were labelled HER2 'positive' when their immunohistochemical staining was denoted as HER2(3+). All patients that stained HER2(0), HER2(1+), HER2(2+) were thus considered 'negative'. The reason for this change in defining the positive result was that the HER2(3+) group could be considered as the 'truly positive' HER2 patients in which FISH analysis is not required.

4.3.1 HER2(2+) and HER2(3+) defined as a positive result

There was no agreement observed between the HER2-positive and HER2-negative categories in the core and final excision biopsy specimens. Indeed, the agreement result of 57.3% with a *Kappa* statistic of 0.103 indicates that this observation of agreement was mere chance. This was one of the lowest congruencies demonstrated in any study to date, with Varga *et al.* obtaining 65% agreement[39] and Cahill *et al.* obtaining a 64% agreement[44], as listed in Table 1.2. Notably, these two studies did not report the *Kappa* statistic.

Although tumour size, patient age or the time delay between the core and excision biopsies did not have a statistical influence on the agreement between HER2-positive and HER2-negative patients, neoadjuvant chemotherapy ($P = 0.04$) and the amount of core biopsies taken ($P = 0.03$) significantly influenced the agreement between core and excision biopsy.

4.3.2 HER2(3+) defined as a positive result

In this group, the agreement between core and excision biopsy specimens improved to 81.5% compared to the 57.3% agreement found when the positively-stained group included the HER2(2+) patients. The *Kappa* statistic of 0.398 falls just short of the minimum of 0.400 which is required for this not to be due to chance, resulting in what seems to be a tendency towards agreement between the core and excision specimens.

Importantly, patients who received neoadjuvant chemotherapy after the core biopsy had a significantly lower agreement between the core and final excision specimens ($P = 0.04$). The number of cores taken, tumour size, patient age or the excessive time delay between the core and excision biopsies did not have a statistical influence on the agreement between HER2-positive and HER2-negative patients.

4.4 Criticism of the study

The retrospective nature of the study reflects the selection bias of clinicians as to whether or not the immunohistochemical testing of the core specimen was repeated. By the nature of this study, a complete pathological response to neoadjuvant chemotherapy would have been difficult to interpret, even if the study was to be conducted prospectively.

It would have been interesting to analyse the results of the progesterone receptors as well, since the progesterone receptor status influences the decision of hormonal manipulation, even if the oestrogen receptor status is negative.

The pathology results were based on the final written report provided to the clinician. A possible problem related to this may include a long delay before specimens are processed and analysed (anecdotal). This delay, and the exact staining methods, were not scrutinized due to the retrospective nature of this investigation. The exact number of specimens stained by hand *versus* those stained by machine could not be verified. Hand and machine staining generated their own controls for comparison, but were not compared against each other. Moreover, FISH analysis would have added interesting information to the results obtained from the HER2 immunohistochemical staining, but was unfortunately not done routinely.

Although the sample size was adequate to analyze statistic agreement, the individual sample sizes in the sub-analysis regarding the impact of patient age, number of core biopsies, time delay to excision, tumour size and chemotherapy were too small, especially in the analysis of the oestrogen receptor immunohistochemical staining.

4.5 Conclusions and recommendations

4.5.1 General observations

This study highlights some of the challenges that South Africa faces with regard to the management of breast cancer. Striking elements included the large tumour sizes and the time delay between the core biopsy and excision specimen.

4.5.2 Oestrogen receptor immunohistochemistry

There was good agreement for oestrogen receptor immunohistochemistry between the initial core and the final excision biopsy specimens. As would be expected, this agreement improved when considering a $\geq$ 10% oestrogen receptor staining as a positive result as opposed to a $\geq$ 5% positive oestrogen receptor stain. This raises the question to clinicians as to whether the 93.2% agreement with a 10% minimal positive stain for the oestrogen receptor, as observed in this study, is acceptable for diagnosis rather than considering a repeat immunohistochemical staining on the excision biopsy as a duplication of effort. In the 10% minimum positive stained oestrogen receptor group, this decision would translate in denying 4.5% of patients (n = 6/132) hormonal manipulation and 'over-treating' 2.3% of patients (n = 3/132) based on core biopsy alone. That is, if the core biopsy is considered as the 'gold standard' as per discussion in section 1.7.1. I would recommend a cost and risk analysis before deciding whether the agreement for oestrogen receptor staining is good enough to avoid repetition on the excision specimen.

In this regard, authors such as Lee *et al.* considered a 5% change in the status enough to warrant re-analysis of the oestrogen and progesterone receptor status in the final surgical specimens,[68] while Kasami *et al.* suggested that the immunohistochemical staining be repeated if the initial core result was negative for the oestrogen receptor.[47]

4.5.3 HER2 immunohistochemistry

There was no agreement between the core biopsy and final excision specimen with regard HER2(2+) in this analysis. Based on these results, I would definitely suggest a repeat of the core HER2 immunohistochemical stain on the excision biopsy specimen if a HER2(2+) stain or less was obtained from the core biopsy. Analysis of agreement between the core biopsy and the excision specimen with regard HER2(3+) was also very disappointing. Interestingly, these results were influenced by neoadjuvant chemotherapy. A prospective study is required to fully analyze the impact of neoadjuvant chemotherapy on HER2 immunohistochemical staining in our patient population.

Chivukula *et al.* suggested that FISH on core needle biopsies be performed as this would almost completely resolve the issue of heterogeneous expression of HER2.[16,20] However, this idealistic opinion would be difficult to execute in our financially restrained environment in South Africa.

In conclusion, this is the first study of its kind from South Africa comparing the results of core and final excision biopsies regarding oestrogen receptor and HER2 immunohistochemical staining in breast cancer patients. While the oestrogen receptor staining showed a good agreement between the core and excision biopsy specimens, this is not true for the HER2 staining. Notably, neoadjuvant chemotherapy had a significant impact on the agreement of these results for HER2 staining and future prospective work is necessary to investigate this further.

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APPENDIX A: BERT MYBURG RESEARCH FORUM PRESENTATION

University of the Witwatersrand

25 October 2009

Comparison between core and excision biopsy oestrogen receptor immunohistochemical staining for breast cancer

Authors: JS Vermaak, A Cairns, Hale M.

Aim: To compare the oestrogen receptor status of core biopsies and excision biopsies in breast cancer.

Methods: A retrospective review of records from Charlotte Maxeke Johannesburg General Hospital Breast Clinic from the 1st of January 2004 till the 30th of June 2009 (5 ½ years). All patients with a histological diagnosis of breast cancer were reviewed (n = 718). Patients who had oestrogen receptor immunohistochemical stains done on both core and excision specimens were included in the study for comparison (n = 132, 18.4%). Any receptor staining, even if weakly positive, was considered for our review.

Results: 132 patients were included in our study. There were 131 female and 1 male patient with a mean age of 56.7yrs (range 30 to 90 years). From the core biopsy, 96 patients stained +ve for the oestrogen receptor and 36 stained negative; from the excision biopsy, 99 patients stained +ve and 33 stained negative. Using the excision biopsy as gold standard for comparison, there were 4 false positive and 7 false negative results. There was no statistical difference between the core and excision biopsy staining (P=0.366) and excellent agreement between the two tests (91.6% agreement, *Kappa* = 0.784). The core biopsy had a sensitivity of 92.9%, a specificity of 87.9%, a positive predictive value of 95.8% and a negative predictive value of 80.6%.

Conclusion: There is an excellent correlation between the core and excision biopsy with oestrogen immunohistochemical staining. In a resource constraint environment, this might be considered a duplication of effort.

APPENDIX B: SURGICAL RESEARCH SOCIETY OF SOUTHERN AFRICA PRESENTATION

38th Annual Meeting, East London, South Africa

15 July 2010

Breast Cancer: Comparing Oestrogen and HER-2 Immunohistochemical Staining of Core and Excision Biopsy Specimens

Authors: JS Vermaak, A Cairns, Hale M.

Aim: To compare the oestrogen and HER-2 immunohistochemical staining of the initial core and final excision biopsies in breast cancer.

Methods: A retrospective review of records from Charlotte Maxeke Johannesburg General Hospital Breast Clinic from the 1st of January 2004 till the 30th of June 2009 (5 ½ years). All patients with a histological diagnosis of breast cancer were reviewed (n = 718). Patients who had oestrogen and HER-2 immunohistochemical staining done on both the core and excision specimens were included in the study for comparison (n = 132, 18.4%). Oestrogen staining was considered positive even if there was only weak staining (5% positivity) and the HER-2 receptor was considered positive when the staining was designated 2+ by the pathologist.

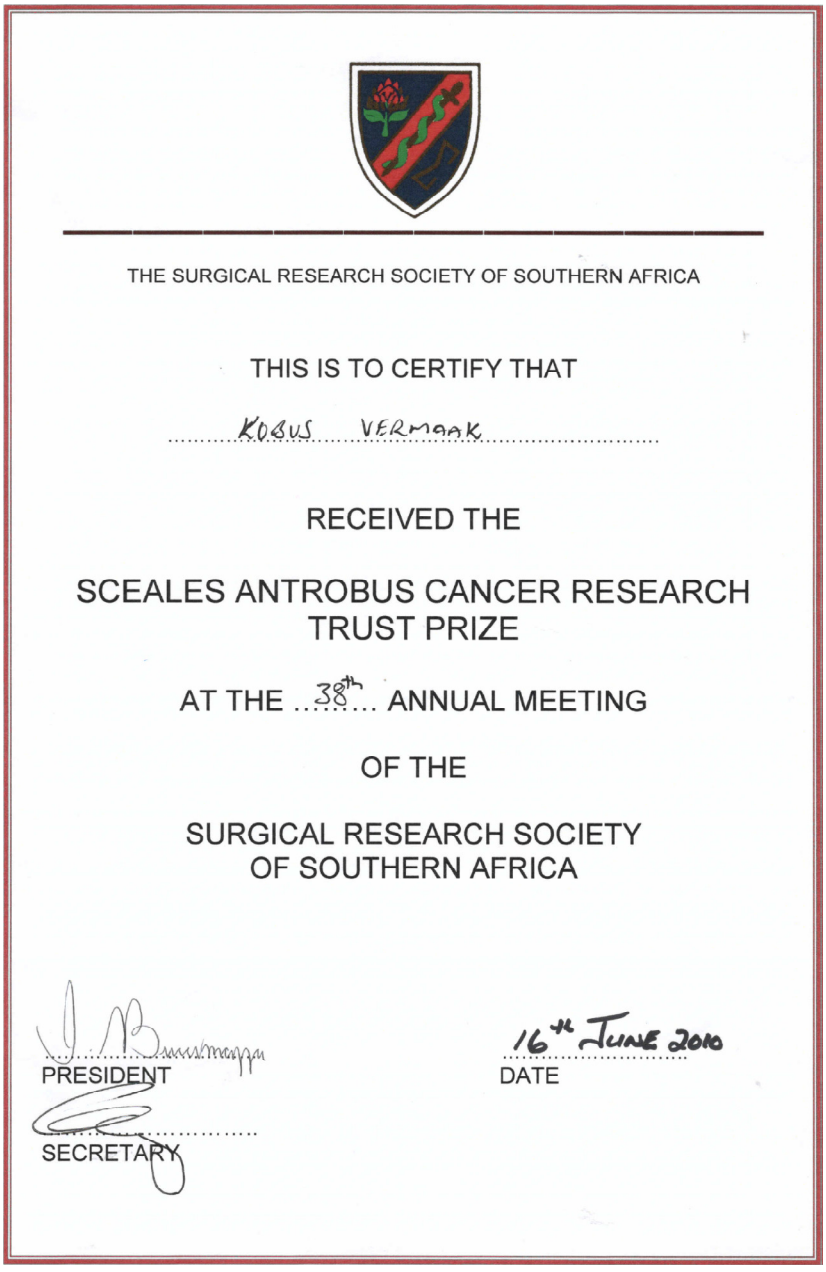
Results: 132 patients were included in our study of which only one was male. The mean age of 56.7yrs (range 30 to 90 years). Immunohistochemical staining of the oestrogen receptor revealed excellent statistical agreement between the core and excision biopsies (91.6% agreement, *Kappa* = 0.784). There was a poor correlation when the immunohistochemical staining for the HER-2 receptor was compared between the core and excision biopsies (57.3% agreement, *Kappa* = 0.103)

Conclusion: There is an excellent correlation for the oestrogen receptor immunohistochemical staining of the core and excision biopsy specimens. In a resource constrained environment, this might be considered a duplication of effort. In contrast, immunohistochemical staining for the HER-2 receptor had no correlation. The reasons for s require further investigation.

APPENDIX C: THE SCEALES ANTROBUS CANCER RESEARCH TRUST PRIZE

The 38th annual meeting, East London, South Africa

The Sceales Antrhobus Cancer Trust Prize



THE SURGICAL RESEARCH SOCIETY OF SOUTHERN AFRICA

THIS IS TO CERTIFY THAT

KOBUS VERMAAK

RECEIVED THE

SCEALES ANTROBUS CANCER RESEARCH TRUST PRIZE

AT THE 38th ANNUAL MEETING

OF THE

SURGICAL RESEARCH SOCIETY OF SOUTHERN AFRICA

PRESIDENT

SECRETARY

16th JUNE 2010

DATE

APPENDIX D: UNIVERSITY OF THE WITWATERSRAND FACULTY RESEACH DAY PRESENTATION

OESTROGEN and HER-2

Immunohistochemical Staining compared in the core and excision biopsy specimens of

BREAST CANCER

[*] JS Vermaak, [*]A Cairns, [#] M Hale

[*]Department of General Surgery, [#]Department of Histological Pathology, University of the Witwatersrand

INTRODUCTION

The management of breast cancer requires histological confirmation which is facilitated by an initial core biopsy and then confirmed by the final excision specimen.
Sometimes immunohistochemical staining for oestrogen and HER2 is done on both the core and the final excision specimen after surgery.
We want to investigate if this is a duplication of effort and what the agreement between the core and final excision is.

AIM

To compare the oestrogen and HER-2 immunohistochemical staining of the initial core and final excision biopsies in breast cancer.

METHOD

A retrospective review of records from Charlotte Maxeke Johannesburg General Hospital Breast Clinic from the 1st of January 2004 till the 30th of June 2009 (5 ½ years).

n = 718 Total number of patients during this period with a histological diagnosis of breast cancer were reviewed.

n = 132 (18.4%) Patients who had oestrogen immunohistochemical staining done on both core and excision specimens
Oestrogen staining was considered positive even if there was only weak staining (5% positivity)

n = 124 (17.3%) Patients who had HER-2 immunohistochemical staining done on both the core and excision specimens.
HER-2 receptor was considered positive when the staining was designated "2+" by the pathologist.

The Kappa statistic was utilized to assess agreement between the core and excision specimens

Results: Oestrogen Receptor Immunohistochemistry

n = 132 patients (1 male)

Mean age = 56.7 years (range 30 to 90 years).

Excellent Agreement between the core and excision
(91.6% agreement, Kappa=0.784).

When using the excision biopsy as the 'gold standard' for comparison:

	Excision +ve	Excision -ve
Core +ve	92 patients	4 patients
Core -ve	7 patients	29 patients

Sensitivity of core biopsy	Sens = 92.9%
Specificity of core biopsy	Spec = 87.9%
Positive Predictive Value of Core biopsy	PPV = 95.8%
Negative Predictive Value of Core biopsy	NPV = 80.6%

Comparison of parameters:

	FALSE NEGATIVE	"OTHER"
Patient age (yrs)	n=7 35 – 65 (avg=53.7)	n=125 30 – 90 (avg=56.9)
Tumour size (mm)	n=7 16 – 230 (avg=63.9)	n=125 2.5 – 135 (avg=35.6)
Amount of cores	n=7 3 – 6 (3.7)	n=124 1 – 11 (3.5)

Results: HER2 Receptor Immunohistochemistry

n = 124 patients (1 male)

Poor Agreement between core and excision
(57.3% agreement, Kappa = 0.103)

When using the excision biopsy as the 'gold standard' for comparison:

	Excision +ve	Excision -ve
Core +ve	21 patients	21 patients
Core -ve	32 patients	50 patients

Sensitivity of core biopsy	Sens = 39.6%
Specificity of core biopsy	Spec = 70.4%
Positive Predictive Value of Core biopsy	PPV = 50.0%
Negative Predictive Value of Core biopsy	NPV = 61.0%

Comparison of parameters:

	FALSE NEGATIVE	"OTHER"
Patient age (yrs)	n=32 30 – 90 (avg=53.7)	n=92 31 – 84 (avg=57.4)
Tumour size (mm)	n=32 6 – 70 (avg=32.6)	n=92 2.5 – 230 (avg=39.6)
Amount of cores	n=32 3 – 5 (3.3)	n=92 1 – 11 (3.7)

Apologia to the statistical illiterate:

Kappa < 0.4	*no agreement*
Kappa 0.4 to 0.7	*agreement possible*
Kappa >0.7	*strong agreement not ascribed to chance*

CONCLUSION

1. There is an excellent agreement with regard receptor immunohistochemical staining of oestrogen in the core and excision biopsy specimens. In a resource constrained environment, this might be considered duplication of effort.

2. In contrast, immunohistochemical staining for the HER-2 receptor had no agreement. This is in contrast to what has been described in the breast oncology literature. The reasons for this is unclear and requires further investigation.

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Department General Surgery and Histological Pathology
Staff and Patients of Charlotte Maxeke Johannesburg General Hospital

APPENDIX E: ACADEMIC SURGICAL CONGRESS, USA PRESENTATION

6th Annual Academic Surgical Congress, Huntington Beach, California, USA

1 February 2011

Breast Cancer: Comparing Estrogen and HER-2 Immunohistochemical Staining of Core and Excision Biopsy Specimens

Authors: JS Vermaak, A Cairns, Hale M.

Aim: To compare the oestrogen and HER-2 immunohistochemical staining of the initial core and final excision biopsies in breast cancer.

Methods: A retrospective review of records from Charlotte Maxeke Johannesburg General Hospital Breast Clinic from the 1st of January 2004 till the 30th of June 2009 (5 ½ years). All patients with a histological diagnosis of breast cancer were reviewed (n = 718). Patients who had oestrogen and HER-2 immunohistochemical staining done on both the core and excision specimens were included in the study for comparison (n = 132, 18.4%). Oestrogen staining was considered positive even if there was only weak staining (5% positivity) and the HER-2 receptor was considered positive when the staining was designated 2+ by the pathologist.

Results: 132 patients were included in our study of which only one was male. The mean age of 56.7yrs (range 30 to 90 years). Immunohistochemical staining of the oestrogen receptor revealed excellent statistical agreement between the core and excision biopsies (91.6% agreement, *Kappa* = 0.784). There was a poor correlation when the immunohistochemical staining for the HER-2 receptor was compared between the core and excision biopsies (57.3% agreement, *Kappa* = 0.103)

Conclusion: There is an excellent correlation for the oestrogen receptor immunohistochemical staining of the core and excision biopsy specimens. In a resource constrained environment, this might be considered a duplication of effort. In contrast, immunohistochemical staining for the HER-2 receptor had no correlation. The reasons for this require further investigation.

APPENDIX F: ETHICAL APPROVAL FOR CONDUCTING THIS STUDY

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Jacobus S Vermaak

CLEARANCE CERTIFICATE	**M090523**
PROJECT	A Comparison of Core Biopsy Receptor Status to Post Excision Receptor Status in Breast Cancer
INVESTIGATORS	Dr Jacobus S Vermaak.
DEPARTMENT	Department of General Surgery
DATE CONSIDERED	09.05.29
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 2009/10/01 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr A Cairns

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...