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Triple negative breast cancer in South Africa

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Triple negative breast cancer in South Africa

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Declaration

I, Leanne May Prodehl, declare that this research is my own work. It is being submitted to the University of the Witwatersrand, in fulfillment of the requirements for the degree of Master in Medicine in the University of the Witwatersrand, Johannesburg. It has not been previously submitted for any degree or examination at this or any other university. This report does not utilise any previous or current work produced by another individual.

Signature: _____ Date: _____

Dedication

I would like to dedicate this dissertation to my parents for their unwavering support. I would also like to dedicate it to the young women with breast cancer who first piqued my interest in the topic.

Presentations arising out of this study

The preliminary results of this research were presented at the South African Surgical Research Society meeting in Pretoria, 2011.

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Abbreviations

BCS – breast conserving surgery

CPR – complete pathological response

DCIS – ductal carcinoma in situ

DFS – disease free survival

EGFR – epithelial growth factor receptor

ER – oestrogen receptor

FISH – fluorescent in situ hybridisation

IHC – immunohistochemistry

MDT - multidisciplinary team

mTOR – mammalian target of rapamycin

PARP – poly (ADP-ribose) polymerase

PR – progesterone receptor

SLNB – sentinel lymph node biopsy

USA - United States of America

VGFR – vascular endothelial growth factor receptor

Abstract

Introduction

Triple negative breast cancer is a breast cancer subtype associated with advanced stage at presentation, aggressive tumour biology and poor disease free and overall survivals. It is characterized by negative immunohistochemical staining for the oestrogen and progesterone receptors and Her 2 overexpression. Studies have shown an increased prevalence of triple negative breast cancer in young African-American women.

Young age at presentation is similarly associated with poor outcomes. It is not clear if young women with breast cancer do poorly because of increased representation of aggressive subtypes or genetic differences unique to young women within each subtype.

Methods

We undertook a retrospective file review to identify triple negative breast cancer cases at two breast care units in Johannesburg. Presentation, treatment and outcomes data were collected on the patients at Milpark Breast Care Unit. A prospective file review and telephonic interview were done for further follow up.

Results

There were 275 patients with triple negative breast cancer identified out of 1898 patients (14.4%). The prevalence at Milpark Breast Care Unit was 13.9% and 16.1% at Helen Joseph Hospital.

135 patients were further analysed. Stage at presentation was IIa and IIb in approximately half (47.23%) of the patients. Patients presented with large tumours - 57.78% greater than two centimeters, and lymph node positive disease (55.55%). The majority (72.73%) of patients had high-grade, poorly differentiated tumours. This is consistent with studies showing that triple negative breast cancers present with more advanced tumours. There was a non-significant trend for younger patients to present with more advanced tumours with more aggressive histology.

The triple negative breast cancer is challenging to manage due to its lack of response to steroid blockade and lack of biological therapy. This was reflected in the number of patients treated with multimodality therapy. 94.81% of patients had chemotherapy, 59.26% as neoadjuvant and 40.74% as adjuvant treatment. There were 93 (68.89%) patients treated with radiation therapy.

There was a recurrence in 22.96% of patients, with preponderance to lymph node and visceral metastases. Recurrences occurred early, the median was 23.1 months and all had occurred within eight years. Younger patients had more recurrences (32.35%) and all occurred within six years. Stage at presentation and lymph node involvement were significantly associated with recurrence.

Complete pathological response to neoadjuvant chemotherapy is associated with improved outcomes. The recurrence rate was 1.25% if there was a complete pathological response in both the breast and lymph nodes.

The mortality rate was 19.26% and was greater in younger patients, 23.53% for women 40 years old and younger and 31.82% for women 35 years old and younger. Mortality was significantly associated with stage at presentation and lack of surgery but not lymph node positivity.

Conclusion

The prevalence of triple negative breast cancer in two South African breast care units was similar to some studies but less than studies in West and East Africa. Patients in these units, similar to other units, presented at a more advanced stage and had poorer outcomes than luminal breast cancers. Younger patients showed a trend to more

advanced presentation and poorer outcomes than older patients within the triple negative subtype. This suggests than the outcomes of young patients cannot be explained by preponderance to aggressive subtypes alone.

Chapter 1. Introduction

Breast cancer in women is the second most common cancer in the world and the most common cancer among women globally. Breast cancer is the second highest cause of cancer death in women, after lung cancer (1) 411 093 women die of breast cancer a year (2). In sub-Saharan Africa breast cancer is the most prevalent cancer in women and since 2008 there has been a 20% increase in the incidence of breast cancer worldwide (3). Breast cancer in women 40 years or younger represents 7 % of all breast cancers (4).

1. 1 Breast cancer subtypes

In 2000 the Stanford Group described an intrinsic gene subset for breast cancers, describing four subtypes of breast cancer according to gene assay of molecular markers. The four subsets were ER positive or luminal, basal-like, Her2 positive and normal breast-like (5). Luminal tumours were characterised further and the resulting five subtypes used to describe breast cancers were:

- Luminal A (ER pos, PR pos, Her2 neg,)
- Luminal B (ER pos, PR pos, Her2 pos)
- Her2 overexpressing (ER neg, PR neg, Her2 pos)
- Basal-like (ER neg, PR neg, Her2 neg)
- Normal breast-like or unclassified (ER neg, PR neg, Her2 neg) (6)

The definition of breast cancer subtypes has been refined since the first publication in 2000. In clinical practice four subtypes are commonly used. Luminal A describes an oestrogen and progesterone receptor positive, low-grade tumour. Luminal B is used for oestrogen receptor positive tumours that may or may not be progesterone receptor positive or Her2 overexpressing and are high grade. Her2 tumours overexpress Her2 but are negative for oestrogen and progesterone receptors. Triple negative breast cancers do not express positivity for oestrogen, progesterone or Her2 receptors (7).

Luminal A is the predominant subtype (51.9%) followed by luminal B (27.5%). Triple negative breast cancer prevalence ranges from 10% to 55% depending on the population surveyed (8,9). The prevalence of Her2 overexpressing tumours is 7% (10).

These subtypes have been linked to clinical presentation and outcomes. The luminal tumours have the best outcomes and patients with luminal A breast cancer have the best prognosis. Patients with either Her2 overexpressing or basal-like subtypes have the worst overall survival. The outcomes have changed for Her2 overexpressing breast cancer with recent advances in targeted therapies for e.g. trastuzumab (6).

It has become more apparent that oestrogen receptor positive and receptor negative breast cancers differ at a molecular level (11). Kramangar et al (2), suggest that there should be a shift in breast cancer models to stratify breast cancer as either oestrogen receptor positive or negative. They describe two distinct types of breast cancer – oestrogen negative cancers, which have increasing prevalence in premenopausal women, are aggressive, have unusual morphology and an association with BRCA 1. These cancers have a higher incidence in low risk populations such as native Japanese women, and are associated with a higher mortality rate. The second type of breast

cancer is oestrogen receptor positive, which is more prevalent globally and predominantly affects postmenopausal women. It behaves in a less aggressive manner, with better differentiated histology and an association with BRCA 2 (2).

The recommended definition of breast cancer subtypes are based on immunohistochemical (IHC) tests that distinguish immunoreactive cells (12). The previously defined threshold was more than 10% immunoreactive cells, and this was used in a number of clinical trials and epidemiological studies, however nowadays oestrogen and progesterone receptor negative breast cancers are those that express less than 1% immunoreactive cells (1).

1. 2 Triple negative breast cancer

Triple negative breast cancer is a heterogeneous group of tumours. The defining features are a lack of expression of oestrogen and progesterone receptors, and lack of overexpression of the Her2 gene (13). Triple negative breast cancer is a clinical term that encompasses the basal-like and normal breast tissue-like cancers described by the Stanford group. The terms are sometimes used interchangeably but are not completely synonymous (6).

There is relatively uniform 20-30% discordance between triple negative breast cancer and basal-like breast cancer (14). Triple negative is often used as a surrogate for the prevalence of basal-like breast cancer in a population. Bauer et al (15), found that in a population-based study triple negative tumours can be used as a surrogate marker for

basal-like breast cancer as the proportion of basal-like to triple negative breast cancers corresponded with incidences reported in other studies.

It is important to note that the triple negative breast cancer subtype describes a heterogeneous group of breast cancers. Ongoing research has further defined triple negative breast cancer into six subgroups and used extensive immunohistochemical testing and cluster analysis to define their characteristics (16). These subgroups differ in presentation, tumour grade and survival outcomes (17). The basal-like phenotype comprises the majority of triple negative breast cancers, however other molecular subtypes exist: these include the Her2 overexpressing group, luminal A, luminal B, claudin-low, and a few normal-like tumours (18).

Other types of tumor classified as triple negative breast cancer include carcinomas morphologically designated as of no specific type (salivary gland-like carcinomas (particularly the adenoid cystic carcinomas and myoepithelial carcinomas), lobular, and/or mixed features. Some papillary and secretory-like carcinomas may also be regarded as triple negative disease (13).

There is also a link between triple negative breast cancer and the BRCA1 mutation with approximately 70% BRCA1 mutation breast cancers presenting as triple-negative. A number of histopathological features including genomic instability and DNA repair effects are shared by BRCA1- associated tumours and triple negative breast cancers (19).

There has been a surge of interest and research into triple negative breast cancer over the past few years. This is due to the unique presentation and management of these patients. Triple negative breast cancer is characterised by high cell proliferation, poor

cellular differentiation, many recurrent copy number imbalances, and, often mutations in the *TP53* tumour suppressor gene (14). Triple negative breast cancers present at an earlier age and have large tumour size, high histological and nuclear grade at presentation (20). Triple negative tumours are also more likely to present in the two years between screening mammograms (21).

Patients with triple negative breast cancer have lower disease free survival and higher mortality rates than other breast cancers. Studies have demonstrated 40.4% all cause mortality and 30.8% breast cancer specific mortality for triple negative breast cancer as opposed to 21.3% all cause mortality for luminal A tumours (22). The five year overall survival and five year disease free survival is 81-84% and 79-84% for triple negative tumours compared to 94-96% and 90-95% for luminal A breast cancer (20).

Studies have also shown that not only is the recurrence rate higher in triple negative breast cancer (30.8% vs. 14.8% in luminal A), the peak time of recurrence is earlier, typically within two to three years after treatment with poor post recurrence survival (23). Triple negative breast cancer is more prone to blood borne metastases and 13% of patients will have distant metastases two years after diagnosis with preponderance to visceral and brain metastases (24).

1. 2. 1 Basal-like breast cancer

Basal-like breast cancer is one of the genetic subtypes described by the Stanford group. It is characterised on immunohistochemistry by positive staining for cytokeratins of the basal layers of the duct epithelium and increased expression of proliferative markers.

Genetic studies of basal-like breast cancer show decreased expression of the hormone receptor cluster genes and increased expression of the proliferative cluster. This is in keeping with the poor differentiation of most basal-like tumours (14).

Basal-like breast cancer is a component of the triple negative breast cancers, and a more homogenous group. There is still, however no internationally accepted definition for basal-like breast cancers and how best to define these tumors is a matter of controversy and ongoing debate (21). A British group compared three methods of defining basal-like breast cancer – morphology, immunohistochemistry and transcriptional profiles. There were 116 basal-like breast cancers of which 13 were identified by all three methods (25).

The concept of a distinct aetiology is supported by a study looking at the risk factors associated with basal-like tumours. It was found that younger age at menarche and shorter duration of breast-feeding were strong risk factors, which is similar to the other breast cancer subtypes. In contrast to luminal A tumours, increased parity, younger age at first pregnancy, and suppression of lactation are also risk factors for basal-like tumours. Increased BMI and waist-hip ratio are associated with basal-like breast cancer, especially in pre-menopausal women. This is unlike luminal tumours where obesity is a risk factor in only post-menopausal women. Basal-like tumours were especially associated with an increase in adiposity since childhood (26).

1. 2. 2 Normal breast like breast cancer

The triple negative breast cancers that are not basal-like are a subtype described by Stanford Group as unclassified or normal breast like. These cancers do not stain positive for the basal cytokeratins but have high expression of the genes characteristic of basal epithelial and adipose cells and low expression of the genes characteristic of luminal epithelial cells (5).

Studies looking at the genetic profile of these cancers have described a non-homogeneous group with different presentations and outcomes (27). Groups have described six subgroups of triple negative breast cancer (16).

1. 3 Incidence and prevalence

Epidemiological studies have shown that the incidence of triple negative breast cancer is 10 to 20 % (15). There is, however, great variation amongst populations.

Numerous studies have shown an increased prevalence of triple negative breast cancer in premenopausal African American women. The Carolina Breast Cancer Study was one of the first published population based studies investigating the incidence of basal-like breast cancer, which they found to be 20-26% of all breast cancer. The incidence of basal-like tumours in African American women was 20-26% vs. 16% in non-African American women. Furthermore they demonstrated an increased prevalence in young women, with a 24% incidence in premenopausal women vs. 15% in postmenopausal (28).

The Carolina Breast Cancer Study showed an incidence of basal-like breast cancer of 16%, 56% luminal A, 10% luminal B, 8% Her2 overexpressing and 10% unclassified, thus triple negative cancers were 26% (28). In contrast a study amongst Japanese women showed an incidence of 10% of triple negative breast cancer (29), and in Sweden the incidence was 12% (20).

1. 4 The role of ethnicity

Early breast cancer studies of prevalence in triple negative breast cancer demonstrated a disproportionately high prevalence in African-American women; studies such as Bowen et al (30), showed an increased prevalence of triple negative breast cancer in young black British women (22% black women and 15% white women). Huo et al (9), studied the prevalence of triple negative disease in Nigeria and Senegal and showed an incidence of 55%. This marked prevalence of triple negative breast cancer may be linked to the increased incidence in African American women as many African Americans are of West African descent (31).

This significantly high prevalence in African women led to a number of studies investigating the prevalence of breast cancer subtypes in African populations. Incidence rates ranged from 16.5% in Morocco (32), 27% in Kenya (33) to 46% in Mali (34). All studies demonstrate younger age and more advanced stage at presentation.

It has been postulated that an increased prevalence of an aggressive triple negative subtype partly accounts for the poorer outcomes in young African American women (35). A study looking at breast cancer survival and ethnicity found that African American

ethnicity was an independent predictor of adverse outcome with a 22% excess risk of death from all causes (36). In 2006 Smigal et al (37), researched trends in breast cancer in the USA from 1995 to 2002 and showed that for women under the age of 35 the incidence of breast cancer was higher in African American women compared to Caucasian women. The study also showed that despite the overall incidence of breast cancer being highest in Caucasian women, African American women had 37% higher death rates.

1. 5 What is the prevalence in South Africa?

Kramangar et al (2), looked at patterns of cancer incidence, mortality and prevalence globally and found a lack of data to describe temporal trends in Africa. A Pub Med search in 2010 resulted in a limited number of studies investigating basal-like or triple negative breast cancer in Africa. Subsequently there have been a number of publications documenting prevalence rates of triple negative breast cancer in Africa – but all are from North, East and West Africa. There is still a dearth of publications from southern Africa.

In 2004 Walker et al (38), reviewed breast cancer trends in South Africa and found a low incidence compared with developed nations – 15.1 vs. 40-89 per 100 000. They reported that South African black women presented an average of ten years earlier with more advanced disease. They note that changes in lifestyle have decreased the protective factors such as late menarche and high parity, which has led to increasing incidence in black South African women. Breast cancer has overtaken cervical cancer as the most common cancer in women in South Africa between 1993 and 1995,

however, the national cancer registry published in 2007 may underestimate the more recent incidence of breast cancer in South Africa (39).

Research into tumour biology and behaviour in a low to middle income countries such as South Africa, adds valuable information to our understanding of breast cancer in that country. Many of the recommendations guiding management of breast cancer are derived from research undertaken in high-income countries, which may not be applicable elsewhere. Furthermore there are differences in breast cancer subtypes in different populations but these may not explain the differences in outcomes (40).

1. 6 Management of triple negative breast cancer

The mainstay of the management of triple negative breast cancer involves the use of systemic chemotherapy agents as its unique biology and lack of conventional targets and receptors renders hormonal therapy ineffective. Thus chemotherapy is the mainstay of treatment, usually anthracycline and taxane based regimens (19). Poor disease free outcomes are seen especially if the patient has a poor response to chemotherapy due to the dearth of other treatment options (41). The lack of targeted therapy (both hormonal and biological) has driven research into alternative chemotherapy regimens such as carboplatins (42). These have shown improved response rates with insufficient evidence to support universal use.

Triple negative tumours have traits of DNA damage repair defects possibly due to a disruption in the BRCA 1 pathway. The genetic link between triple negative tumours and

BRCA 1 has, with further study, led to possible targeted therapy for both (23). The ideal would be to find a targeted therapy such as trastuzumab, which has reduced disease recurrence by 52% in Her2 overexpressing breast cancers (22).

PARP - poly (ADP-ribose) polymerase – is a nuclear enzyme which plays a critical role in the response to DNA damage. Inhibitors of PARP are used to enhance the effects of cytotoxic agents such as anthracyclins and radiotherapy. PARP inhibitors were originally developed for BRCA 1 and BRCA 2 tumours, and it was thought that the BRCAness of triple negative breast cancers meant that they would also benefit from PARP inhibition (43). Early reports of phase III trials; however, failed to show any significant benefit for PARPs in triple negative breast cancer (19).

The failure of PARPs and the persistently poor outcomes fuels interest in targeted therapy for triple negative breast cancer. New targets are endothelium growth factor receptor (EGFR), checkpoint kinase 1, mammalian target of rapamycin (mTOR) and a host of others. The search for effective target therapy has resulted in substantial research in the molecular pathways of triple negative breast cancer (44).

1. 7 Breast cancer in young women

Breast cancer in young women is becoming a significant health care concern since 7% of patients in the developed world and 25% in the developing world are diagnosed with breast cancer below the age of 40 (45). The majority of breast cancers still present in older, post menopausal women but there is great interest in breast cancer in women

younger than 40. These younger patients have a more advanced stage at presentation and poorer outcomes such as disease free survival and overall survival.

Young women have higher mortality and recurrence rates than older women, with up to four times increased local recurrence in women younger than 45. Younger patients are more likely to have large tumours and advanced stage at presentation. Tumours in younger women are more likely to be grade III, steroid receptor negative, have lymphovascular invasion and positive lymph node disease (46).

There are two schools of thought to explain the documented poor outcomes in younger women. The question is whether breast cancer diagnosed at a young age has a unique biology or is a representative of an increased incidence of aggressive subtypes (47). Studies have documented an increased representation of aggressive subtypes such as: basal-like, unclassified and Her2 overexpressing in young women (48). It has been postulated that it is a preponderance to aggressive subtypes that is responsible for poor prognosis in young women. Authors such as Jenkins et al have concluded that age is not an independent prognostic factor in breast cancer (49). Adherents of this theory state that treatment should be guided by subtype biology and performance status and not age (50).

There are, however, conflicting studies demonstrating, using multivariate analysis, age is an independent predictor of outcome in breast cancer. These include studies based on microarray data, which have shown increased proliferation-related prognostic gene signatures in young women (47,51). This data implies that there is unique biology of breast cancer in young women regardless of breast cancer subtype. This theory is

supported by studies that have shown a difference in outcome according to age within the triple negative subgroup of breast cancers (52).

It is important to define how age at diagnosis impacts breast cancer outcomes as this may guide treatment. In 2001 the St Gallen Consensus Conference included age at presentation as an indication for chemotherapy. Recent studies have highlighted the importance of the unique breast stroma in young women, showing that this microenvironment is very responsive to growth factor stimulation, which could promote aggressive growth. This stroma is a potential target for treatment (47).

The definition of a 'young woman' in breast cancer research varies, with most articles referring to women under either age 35 or 40 years as 'young', however under 45 has also been used (53). The lack of a standard definition of what is ‘young’ in breast cancer is challenging especially as there appears to be a crossover in racial and survival differences after the age of 40. A cutoff of age 40 may allow more focused and accurate assessment of the differences in young and old patients with breast cancer (54). A literature review in 2004 found that the group of patients in whom age was an independent risk factor for recurrence was women aged 35 to 40 and younger (55).

The MD Anderson Cancer Centre group analysed their patients according to age and found age younger than 40 was significantly associated with poor prognosis even on multivariate analysis (51,52).

1. 8 Hypotheses

1) In patients with triple negative breast cancer women 40 years old and younger have different presentation and outcomes to women older than 40 years.

2) The proportion of triple negative breast cancer is higher in a South African population than North American or European populations.

3) Disease free survival and mortality of triple negative breast cancer in a South African Breast Care Unit is similar to international trends.

1. 9 Aim

To document the frequency, presentation and outcomes of South African patients who present with triple negative breast cancer according to age.

1. 10 Objectives

1) To determine the prevalence of triple negative breast cancer at both Milpark and Helen Joseph Hospital Breast Care Units.

2) To determine the disease free survival and mortality of triple negative breast cancer at Milpark Hospital Breast Care Unit.

3) To compare presentation and outcomes of patients with triple negative breast cancer according to age.

Chapter 2. Methods

This study consisted of two components - a retrospective file review and prospective file review and telephonic interviews. The file reviews were conducted at the Netcare Milpark Breast Care Centre and the Helen Joseph Hospital Breast Unit. Both units are in Johannesburg and affiliated with the University of the Witwatersrand.

Ethics approval was obtained from the University of the Witwatersrand Ethics Committee as well as approval from both the Netcare Milpark and the Helen Joseph Hospitals.

At the Netcare Milpark Breast Care Centre all files of patients seen between 1 January 2000 and 31 June 2010 were reviewed. Where possible, missing data were collected from the pathologist or treating oncologist; this was done by file review, and telephonic and email request.

Once all the patients at the Netcare Milpark Breast Care Centre were identified, follow up was assessed until 31 December 2012. Reviewing the files of all identified patients and telephonic interviews collected the follow up information. The telephonic interviews were conducted according to a predetermined questionnaire, and verbal consent was obtained prior to the interview.

The files of patients seen at the Helen Joseph Hospital Breast Unit were reviewed for data regarding the number of patients with breast cancer and their histological subtype. Where the histology was not available from the file, the National Health Laboratory Service computer records were searched for missing data.

Patients were deemed to have triple negative breast cancer if the immunohistochemistry testing for oestrogen, progesterone receptors and Her2 were negative. The threshold limit for immunohistochemical testing was reviewed and revised in 2009 by ASCO. Prior to this a result of less than 10% reaction was negative. The new threshold for a negative test is less than 1% (56). In this study the threshold in place at the time of testing was used.

Immunohistochemical testing for Her2 status provides three possible results: 1+, 2+ and 3+. 1+ is considered a negative result, 3+ a positive result and 2+ is equivocal. If the Her2 status was equivocal, in-situ hybridisation testing was performed to confirm the presence or absence of Her2 over-expression (57). This is similar to a study done at MD Anderson Cancer Centre (51).

Her2 was first described in 1985 (58) and testing only became routine in the late 1990s. Testing was not, however, universally performed especially if trastuzumab was not available, thus 30 patients from Milpark Breast Care Centre and 135 from Helen Joseph were excluded because no Her2 result as available. A further 26 patients at Milpark Breast Care Centre and 23 from Helen Joseph were excluded from the study when the Her2 testing was equivocal and a confirmatory in situ hybridisation test had not been done.

In cases where the receptor testing of the core biopsy and the final surgical specimen differed, the surgical specimen was used if the patient had not had neoadjuvant chemotherapy. If the patient had neoadjuvant chemotherapy and there was minimal

tumour response the receptor status of the surgical specimen was used. If there had been a significant response the receptor status of the core biopsy specimen was used. These cases were reviewed at the Milpark Breast Care Unit multidisciplinary meeting, with the pathologist involved, or the treating oncologist.

In patients where the histology was unclear e.g. triple negative disease in a lobular breast cancer, a pathologist from the Milpark Hospital Breast care Unit multidisciplinary team reviewed the specimen. Alternatively the original pathologist was asked to review the specimen. A number of cases were found to be ductal adenocarcinoma on review.

The breast cancer specimens were classified according to the Nottingham modification of the Scarff-Bloom-Richardson score, which grades tumours from 3-9. A score of 3-5 is low grade, 6-7 is intermediate and 8-9 is high grade (59). It has been clearly shown that the histologic grading of invasive breast carcinoma, in particular the Nottingham modification of the Scarff-Bloom-Richardson (NSBR) grading scheme provides significant prognostic information (60).

Nuclear grading was also assessed and graded according to Fisher's modification of Black's nuclear grade. Nuclear grade is considered to be one of the most important prognostic factors in breast cancer (61). The NSBR and the nuclear grade were then used to assign patients in the study a score from 1 to 3. 1 was poorly differentiated, 2 moderately differentiated and 3 well differentiated.

The histologic specimens were assessed at a number of laboratories over a long period of time and the reporting of specimens was not uniform. Where possible any discrepancies or missing data was clarified with the laboratory. The patients where the

histology was not clear were discussed at the multidisciplinary team meeting and a consensus decision taken.

Stage at presentation and after neoadjuvant therapy was classified according to the seventh edition of the American Joint Committee on Cancer staging manual (62).

Response to neoadjuvant chemotherapy was assessed as present or absent. Three modalities were used to assess response – clinical, radiological and pathological. Response in the breast was present if the size of the tumour decreased from the pre-chemotherapy assessment or there was histological evidence of a response to chemotherapy. Axillary nodes had responded if the number of involved nodes had decreased or if there was evidence of a response to chemotherapy.

Disease recurrence was noted clinically or radiologically. Where possible tumour recurrence was confirmed with a biopsy for either cytology or histology. In the case of visceral metastases e.g. brain or liver, biopsies were often not done, and the diagnosis was made on imaging.

Disease free survival was defined as the time in months from date of diagnosis to the date of diagnosis of recurrence (local, regional or distant), date last seen or death. Overall survival was defined as the time in months from the date of diagnosis to the date of last follow up or death from any cause (51).

Patients in our study were analysed in two major age groups- 40 years or younger and older than 40 years old. To assess whether there were further differences, women 35 years old and younger were assessed separately.

2. 1 Inclusion Criteria

- Women older than 18 years old.
- Women with invasive breast carcinoma.
- Patients for whom the results of oestrogen receptor, progesterone receptor and Her2 testing were known.

2. 2 Exclusion Criteria

- Patients for whom their receptor status could not be confirmed.
- Discrepancies in histology that could not be resolved.
- Missing data.

2.3 Ethics

The first component of the study was a retrospective review and there was no change to the management of patients. The second, prospective component extended the follow up of patients, and again no changes were made to management. Demographic data were collected but kept anonymous. Data were kept anonymous for analysis.

A submission made to the University of the Witwatersrand Human Research Ethics Committee in May 2010 was approved. The clearance certificate number is M10543. (Appendix 2)

A second application was made to the ethics committee in May 2013 for follow up data collected by file review and telephonic interview. The second clearance certificate is M130545. (Appendix 3)

2.4 Data analysis

All data were entered into Excel spreadsheets.
For the patients at Milpark Breast Care Centre, the following variables were collected: age, race, stage at presentation, size of tumour, axillary involvement, distant metastases, neoadjuvant and adjuvant treatment, surgery, response to treatment, recurrence and death. Where appropriate, variables such as age were categorised before analysis.

Statistical analysis was done with Strata. Significance of results was determined with either a Chi-squared or Fischer exact test. Patients were stratified by age: 40 years old and younger, and older than 40 years

2.5 Benefit

- To further knowledge about triple negative breast cancer and breast cancer in young women.
- To define a South African public health issue.
- To elucidate any differences between older and younger women with the same breast cancer subtype.
- To aid in outreach programmes to inform and educate the community.
- To be used as a tool to raise funds for awareness campaigns.

Chapter 3. Results

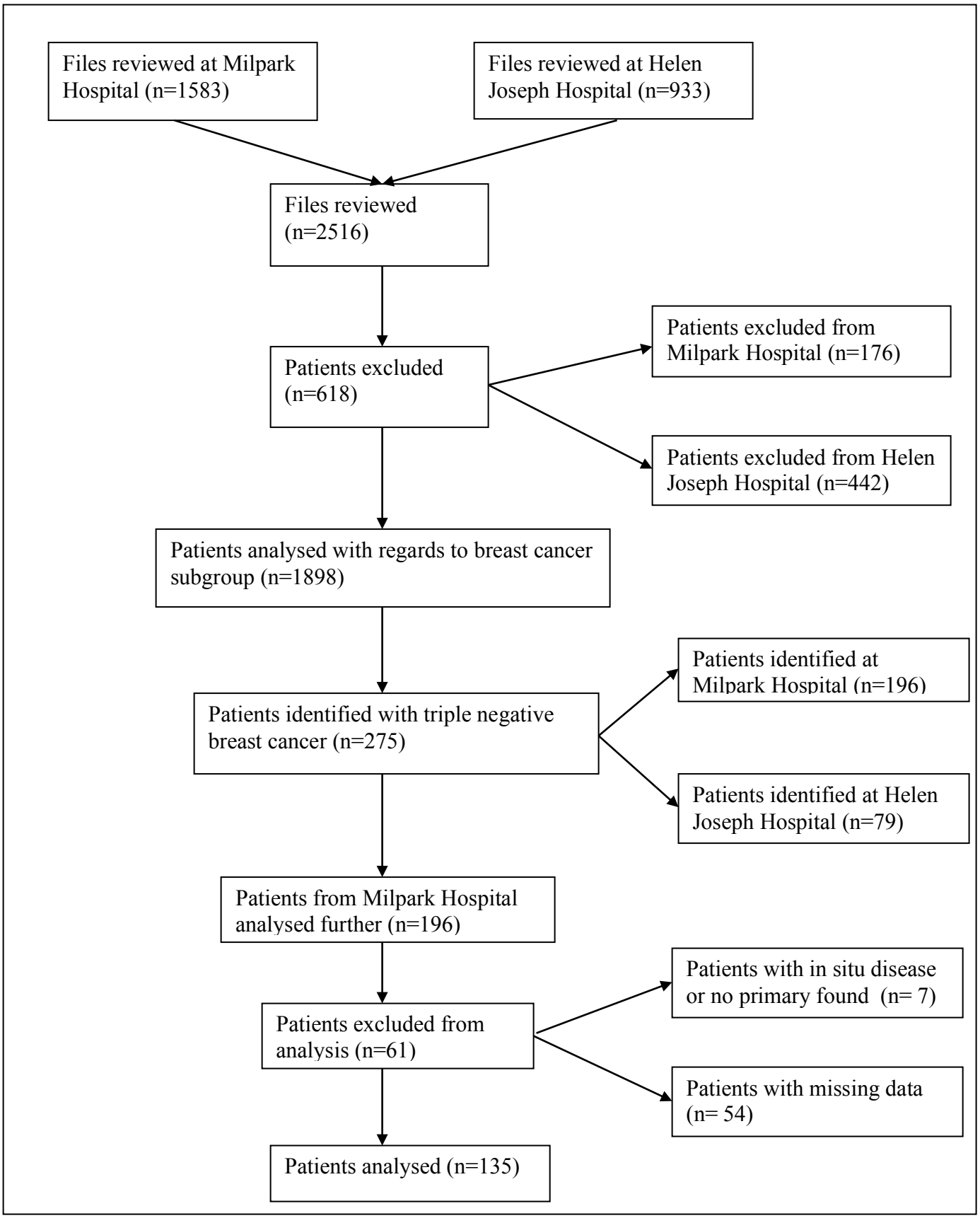
In this study 2516 files were reviewed, 1583 at Milpark Breast Care Centre and 933 at Helen Joseph Hospital. 618 patients were excluded, 176 from Milpark Breast Care Unit and 442 from Helen Joseph Hospital.

The patients from Milpark were excluded as histology was not available in 37 patients, receptor status was not clear or conflicting in 83 results, and in 56 patients the Her2 status could not be determined. The reason for 284 of the exclusions from Helen Joseph Hospital was that the receptor status of the breast tumour could not be determined. A further 158 patients were excluded as an immunohistochemistry result for Her 2 overexpression was not available or equivocal.

It was not possible to collect data regarding presentation, treatment and outcomes on the 79 patients identified with triple negative breast cancer at Helen Joseph. Of the 196 patients identified at Milpark Breast Care Centre, 61 were excluded because of in situ disease or no primary (7) and missing data (54).

Follow up of patients was determined from date of diagnosis to date of death or date last seen. The range is 1 to 121 months with a median of 45 months.

Figure 1: Consort diagram



3. 1 Incidence of breast cancer subtypes

A total of 275 patients with triple negative breast cancer were identified, 196 at Milpark Breast Care Centre and 79 at Helen Joseph Hospitals. Triple negative breast cancers comprised a greater percentage of the total number of breast cancers at Helen Joseph Hospital (16.1%) than at Milpark Breast Care Centre (13.9%). Luminal B and Her2 over expressing tumours were similarly present in greater proportion at Helen Joseph Hospital than Milpark Breast Care Centre.

Table 1. Patients at Milpark and Helen Joseph Hospital Breast Care Units according to breast cancer subtype.

	Milpark (percentage Milpark total) n=1407	Helen Joseph (percentage Helen Joseph total) n=491	Total n=1898
Total	1407	491	1898
Luminal A	909 (64.6%)	260 (52.9%)	1169 (61.6%)
Luminal B	150 (10.6%)	80 (16.3%)	220 (11.6%)
Her 2 over expressing	152 (10.8%)	72 (14.7%)	224 (11.8%)
Triple negative	196 (13.9%)	79 (16.1%)	275 (14.4%)

3. 2 Age and menopausal status at presentation

The patient's age at presentation could be analysed in 135 patients. Women 40 years or younger comprised 25.19% of the sample and women older than 40 years were 74.81%. In the cohort over half (57.8%) were postmenopausal.

Table 2. Age at presentation

Total	135	
Very young (≤35)	22	16.30%
Young (≤40)	34	25.19%
Older (>40)	101	74.81%

Figure 1: Age distribution of the patients

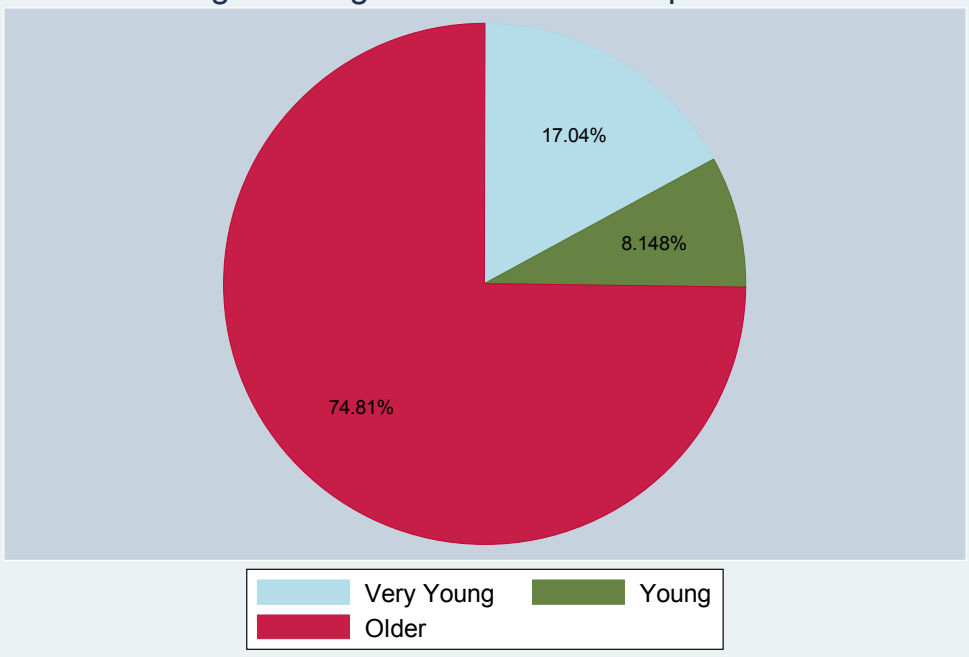


Table 3. Age ranges of patients

		Standard deviation
Very young (≤35)		
Mean	30.4	4.1
Median	31	
Range	18 - 35	
Young (≤40)		
Mean	34.1	5.1
Median	33	
Range	18 – 40	
Older (>40)		
Mean	57.7	11.4
Median	57	
Range	41 – 108	
Total		
Mean	51.3	14.6
Median	54	
Range	18 - 108	

Table 4. Menopausal status at presentation.

Total	135	
Premenopausal	49	36.30%
Perimenopausal	8	5.93%
Postmenopausal	78	57.78%

3. 3 Stage and tumour characteristics at presentation

The 135 patients were analysed for stage at presentation. Stages IIA and IIB accounted for almost half (47.23%) of stage at presentation.

Table 5. Tumour characteristics and stage at presentation.

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Stage					0.518
IA	3 (13.6%)	6 (17.7%)	21 (20.8%)	27 (20.0%)	
IB	0	0	2 (2.0%)	2 (1.5%)	
IIA	5 (22.7%)	8 (22.8%)	23 (23.0%)	31 (23.5%)	
IIB	9 (40.9%)	12 (35.3%)	20 (19.8%)	32 (23.7%)	
IIIA	3 (13.6%)	4 (11.8%)	16 (15.8%)	20 (14.8%)	
IIIB	2 (9.1%)	4 (11.8%)	14 (13.9%)	18 (13.3%)	
IV	0	0	5 (5.0%)	5 (3.7%)	
Early breast cancer	20 (91.0%)	30 (88.2%)	82 (81.2%)	112 (83.0%)	

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Tumour size					0.352
T1 – (<2cm)	5 (22.7%)	7 (20.6%)	31 (30.7%)	38 (28.1%)	
T2 – (2-5 cm)	10 (45.5%)	17 (50.0%)	45 (44.6%)	62 (45.9%)	
T3 – (>5cm)	5 (22.7%)	6 (17.7%)	10 (9.9%)	16 (11.9%)	
T4 (skin/chest wall/both)	2 (9.1%)	4 (11.8%)	15 (14.9%)	19 (14.1%)	
Lymph node involvement					0.276
N 0	9 (40.9%)	15 (44.1%)	45 (44.6%)	60 (44.4%)	
N 1	12 (54.6%)	17 (50.0%)	40 (39.6%)	57 (42.2%)	
N 2 and N 3	1 (4.6%)	1 (2.9%)	16 (15.8%)	18 (13.3%)	
Total positive	14 (63.6%)	19 (52.9%)	56 (55.4%)	75 (55.6%)	
Grade					
1	1 (4.6%)	2 (5.9%)	5 (5.8%)	7 (5.8%)	
2	4 (18.2%)	8 (23.5%)	18 (20.7%)	26 (21.5%)	
3	17 (77.3%)	24 (70.6%)	64 (73.6%)	88 (72.7%)	

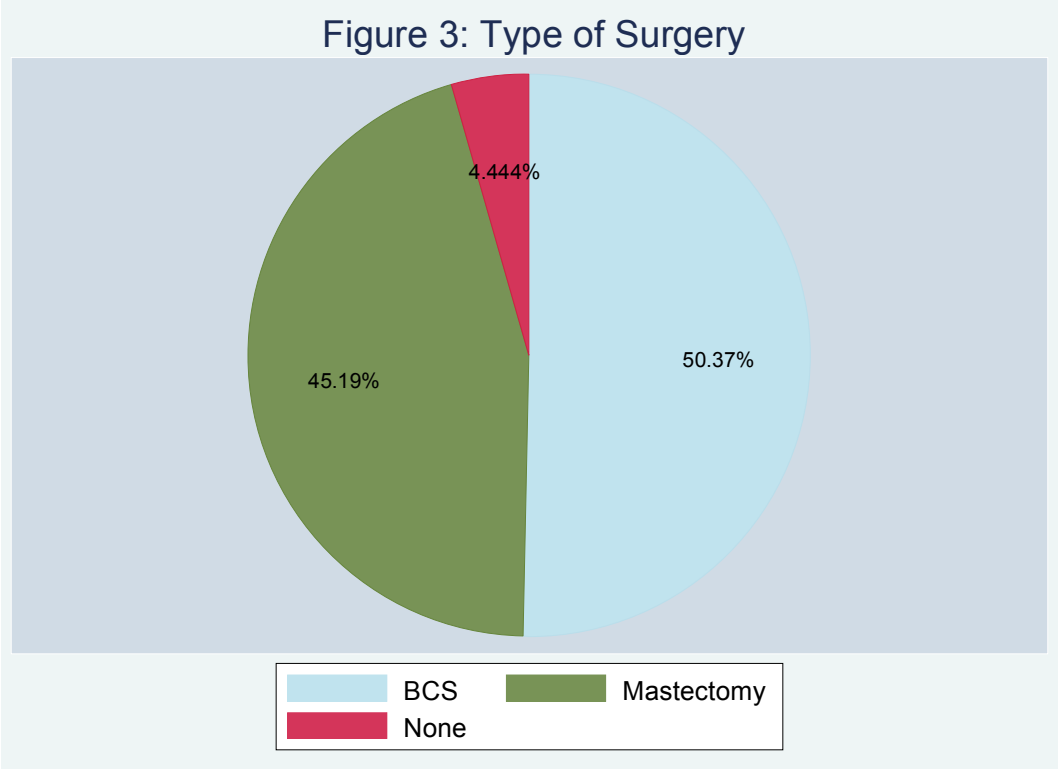
3. 4 Surgery for the breast primary

Surgery remains a key component in the management of breast cancer and the majority of patients in this study underwent surgery. The reasons why patients did not have surgery were varied and included patient choice, the presence of metastatic disease and the patient's medical fitness for surgery.

These results demonstrate the greater proportion of younger women who required more extensive surgery.

Table 6. Surgical management

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Surgery					0.172
BCS	8	15	53	68	
Mastectomy	14	19	42	61	
None	0	0	6	6	


Figure 3: Type of Surgery
4.444%
50.37%
45.19%
BCS
Mastectomy
None

3. 5 Neoadjuvant and adjuvant therapy

The majority of patients in this study had chemotherapy either as neo-adjuvant or adjuvant treatment and seven patients had both.

Table 7. Neoadjuvant and adjuvant treatment

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Neoadjuvant chemotherapy					0.120
Yes	16 (72.7%)	24 (70.6%)	56 (55.5%)	80 (59.3%)	
No	6 (27.3%)	10 (29.4%)	45 (44.5%)	55 (40.7%)	
Adjuvant therapy					0.371
Yes	20 (90.9%)	31 (91.2%)	86 (85.2%)	117 (86.7%)	
No	2 (9.1%)	3 (8.8%)	15 (14.8%)	18 (13.3%)	
Adjuvant chemotherapy					
yes	6 (27.3%)	10 (29.4%)	45 (44.6%)	55 (40.7%)	
Adjuvant radiation therapy					
yes	16 (72.7%)	24 (70.6%)	69 (68.3%)	93 (68.9%)	

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Neoadjuvant and adjuvant chemotherapy					
Yes	2 (9.1%)	3 (8.8%)	4 (4.0%)	7 (5.2%)	
All patients chemotherapy					
Yes	20 (90.9%)	31 (91.2%)	97 (96.1%)	128 (94.8%)	

In the group of patients who had adjuvant radiation therapy we assessed the type of surgery those patients had had. This was to determine whether the major indication for adjuvant radiation therapy was breast-conserving surgery.

Table 8. Radiation therapy by type of surgery

	Very young (<35) n=16	Young (≤40) n=24	Older (>40) n=69	All n=93
Radiation therapy				
BCS	6 (37.5%)	11 (45.9%)	45 (65.2%)	56 (60.2%)
Mastectomy	10 (62.5%)	13 (54.1%)	24 (34.8%)	37 (39.8%)

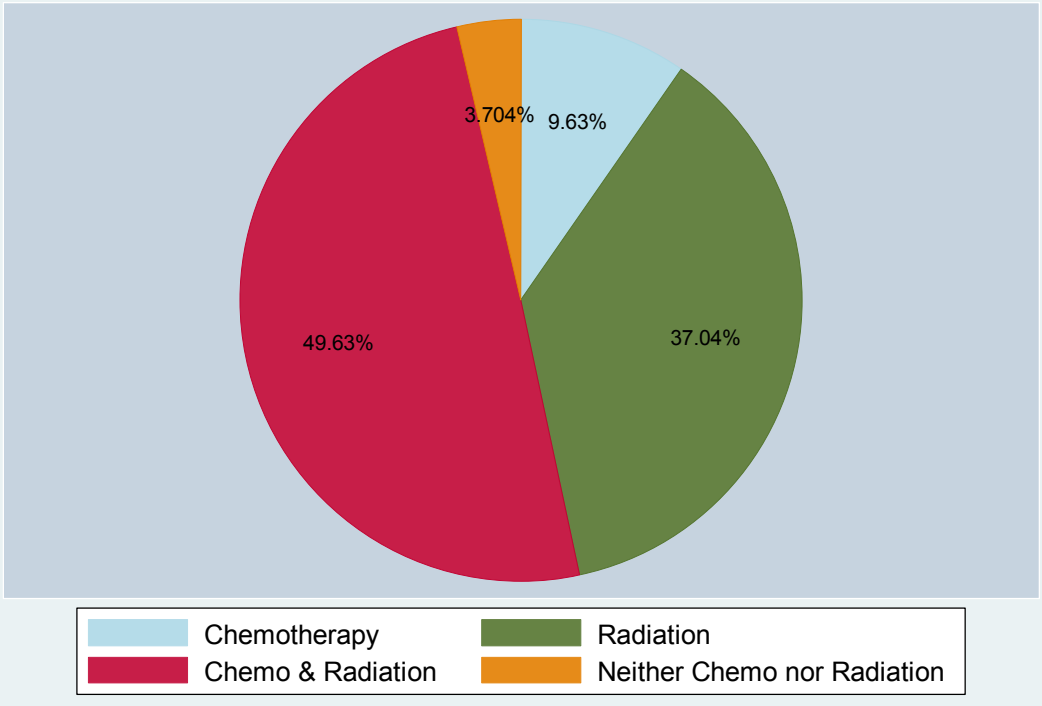
3. 6 Multimodality treatment

Table 9. Modalities of treatment

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135
Surgery	22 (100%)	34 (100%)	95 (94.1%)	129 (95.6%)
Surgery alone	2 (9.1%)	2 (5.9%)	3 (3.0%)	5 (3.7%)
Chemotherapy alone	0	0	3 (3.0%)	3 (2.2%)
Radiation therapy alone	0	0	0	0
Surgery and chemotherapy	20 (90.9%)	31 (91.2%)	91 (90.1%)	122 (90.4%)
Surgery and radiation therapy	16 (72.7%)	24 (70.6%)	70 (69.3%)	93 (68.9%)
Chemotherapy and radiation therapy	0	0	3 (3.0%)	3 (2.2%)
Three modalities	16 (72.7%)	23 (67.7%)	68 (67.3%)	91 (67.4%)

Breast cancer is a complex disease with many factors that determine management and outcome. It is recognized that breast cancer should be treated in a multidisciplinary environment with multiple modalities of management (63). It is a reflection of this that the majority of patients in this study (67.41%) had management that involved surgery, chemotherapy and radiation treatment. The patients 35 or younger were especially likely to have multimodality treatment (72.73%) because of a number of poor prognostic features.

Figure 3: Chemotherapy/Radiation



3.7 Response to neoadjuvant chemotherapy

The 80 patients who had neoadjuvant chemotherapy were assessed for their response to chemotherapy and the rates of complete pathological response.

Table 10. Response to neoadjuvant chemotherapy

	Very young (<35) n=16	Young (≤40) n=24	Older (>40) n=56	All n=80	p value
Response to neoadjuvant treatment					0.254
Yes	15 (93.75%)	22 (91.67%)	51 (91.07%)	73 (91.25%)	
CPR breast	9 (56.25%)	11 (45.83%)	18 (32.14%)	29 (36.25%)	
CPR nodes	3 (18.75%)	4 (16.67%)	9 (16.07%)	13 (16.25%)	
CPR breast and nodes	3 (18.75%)	3 (12.75%)	8 (14.29%)	11 (13.75%)	

3.8 Recurrence of triple negative breast cancer

Triple negative breast cancer is recognized as a risk factor for recurrence of breast cancer and recurrence within the first two years after diagnosis (64).

Table 11. Breast cancer recurrence

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Recurrence					0.215
Total	8	11	20	31	
Breast	3	4	5	9	
Lymph nodes	0	1	4	5	
Distant metastases	3	4	7	11	
Breast and lymph nodes	1	1	2	3	
Breast and distant metastases	0	0	1	1	
Lymph nodes and distant metastases	1	1	1	2	

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Recurrence outside the breast	5	7	15	22	

Data on time from diagnosis to recurrence data was available for 30 of the 48 recurrences. The range was wide, from 4.4 to 92.8 months, but patients 40 years or younger had all recurred within 75 months.

Table 12. Time to recurrence in months

	Very young (<35) n=8	Young (≤40) n=10	Older (>40) n=20	All n=30
Time to recurrence				
Range	4.5 – 74.6	4.5 – 74.6	4.8 – 92.5	4.5 – 92.5
Mean	26.8	28.5	23.9	24.8
Median	19.5	24.3	21.3	23.1
Standard deviation	25.6	23.1	19.4	20.4

Table 13. Breast cancer recurrence according to stage at presentation

	IA n=27	IB n=2	IIA n=31	IIB n=32	IIIA n=20	IIIB n=18	IV n=5	p value
								0.12
Breast cancer relapse	2	2	7	7	8	7	0	
No breast cancer relapse	25	0	24	25	12	11	5	

Table 14. Breast cancer recurrence according to lymph node positivity at presentation

	Lymph node negative n=60	Lymph node N1 n=57	Lymph node N2 and N3 n=18	All n=135	p value
					0.008
Breast cancer relapse	7	20	6	33	
No breast cancer relapse	53	37	12	102	

3. 9 Response to neoadjuvant chemotherapy and recurrence

Response to chemotherapy is been shown to have an impact on outcomes in triple negative breast cancer. Data is available for 80 patients who received neo-adjuvant chemotherapy.

Table 15. Recurrence in patients treated with neoadjuvant chemotherapy

	Very young (<35) n=16	Young (≤40) n=24	Older (>40) n=56	All n=80
Recurrence in patients treated with neoadjuvant chemotherapy	5	7	19	26
Median time to recurrence in months	11	14.9	18.2	18.2
Recurrence in patients with CPR in breast	3	3	4	7
Median time to recurrence in months	24.5	24.5	19	27.2

	Very young (<35) n=16	Young (≤40) n=24	Older (>40) n=56	All n=80
Recurrence in patients with CPR in the breast and nodes	0	0	1	1
Median time to recurrence in months	0	0	4.8	4.8

3. 9 Mortality from triple negative breast cancer

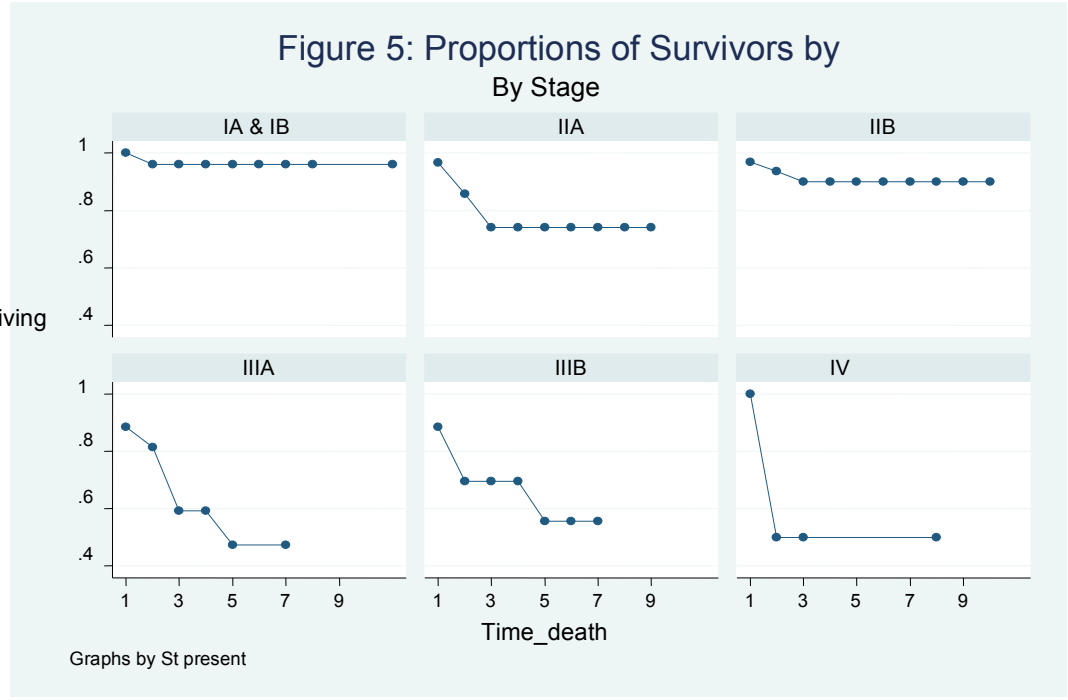
The mortality rate was 19.26% and was highest in the youngest group of patients (31.82%). The time to death from time of diagnosis for all patients ranged from 5.1 to 87.4 months.

Table 16. Mortality rates

	Very young (<35) n=22	Young (≤40) n=34	Older (>40) n=101	All n=135	p value
Mortality					0.465
All cause	7 (31.8%)	8 (23.5%)	18 (17.8%)	26 (19.3%)	
Time to death in months					
Range	9.5 – 56.0	9.5 – 73.7	5.1 – 87.4	5.1 – 87.4	
Mean	30.6	40.4	35.1	36.5	
Median	30.4	30.9	25.53	30.6	
Disease progression prior to death	5 (71.43%)	5 (62.5%)	10 (55.56%)	15 (57.69%)	

Table 17. Mortality according to stage at presentation

	IA n=27	IB n=2	IIA n=31	IIB n=32	IIIA n=20	IIIB n=18	IV n=5	p value
								0.010
Deaths	0	1	7	3	7	6	2	
Alive	27	1	24	29	13	12	3	



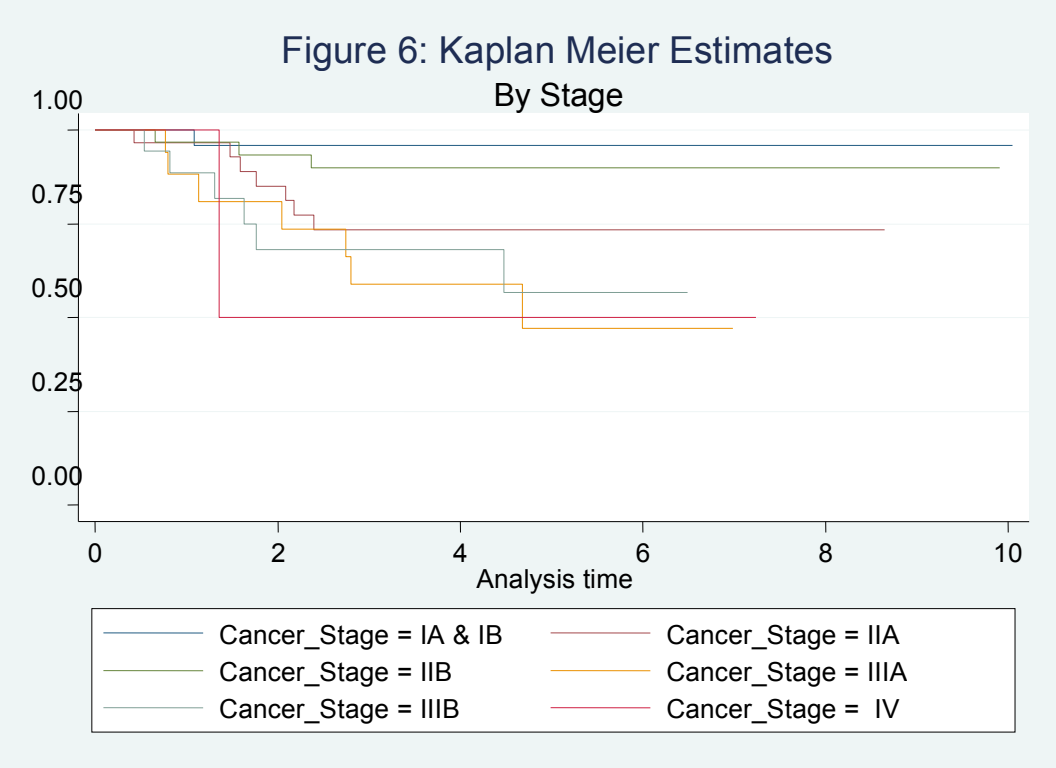


Table 18 . Mortality according to lymph node positivity at presentation

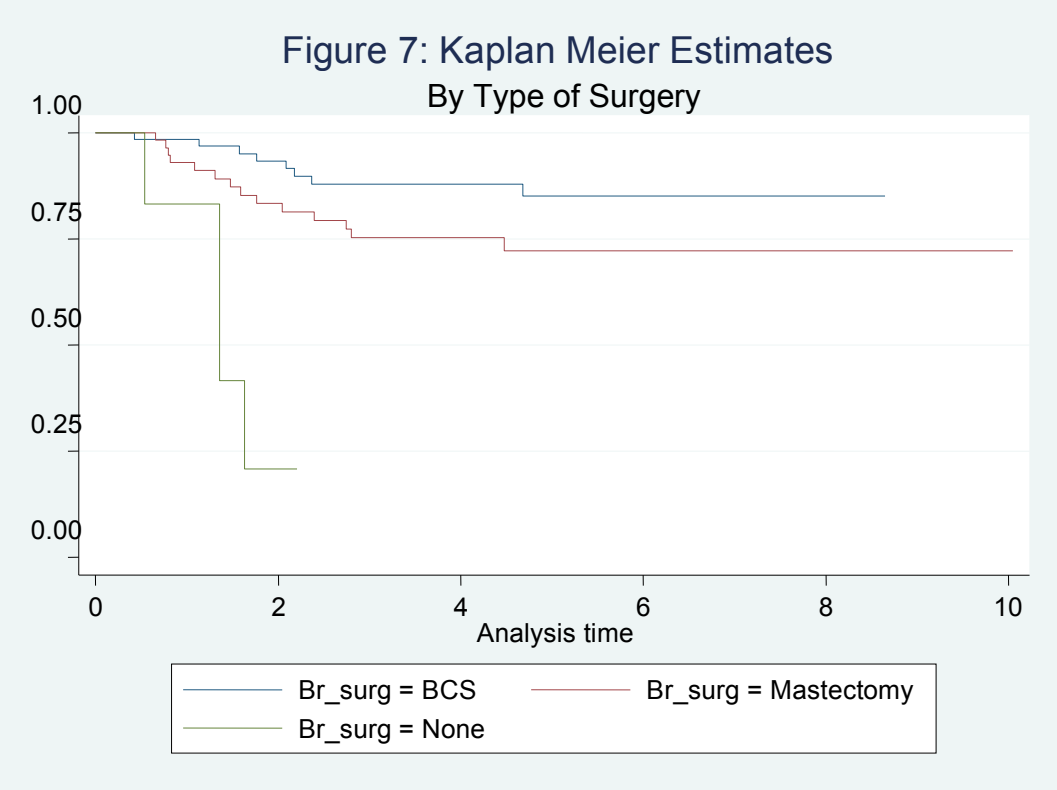
	Lymph node negative n=60	Lymph node N1 n=57	Lymph node N2 and N3 n=18	All n=135	p value
					0.265
Deaths	10	10	6	26	
Alive	50	47	12	109	

Table 19. Mortality according to response to neoadjuvant chemotherapy

	Deaths	Alive	p value
Response to neoadjuvant chemotherapy n=62	11 (18%)	51 (82%)	0.680
No response to neoadjuvant chemotherapy n=73	15 (21%)	58 (79%)	
CPR in the breast alone after neoadjuvant chemotherapy n=29	4 (14%)	25 (86%)	0.400
No CPR in the breast alone after neoadjuvant chemotherapy n=106	22 (21%)	84 (79%)	
CPR after neoadjuvant chemotherapy n=11	1 (9%)	10 (91%)	0.372
No CPR after neoadjuvant chemotherapy n=124	25 (20%)	99 (80%)	

Table 20. Mortality according to surgical procedure

	Breast conserving surgery n=68	Mastectomy n=61	No surgery n=6	p value
				0.003
Deaths	8 (12%)	14 (23%)	4 (67%)	
Alive	60 (88%)	47 (77%)	2 (33%)	



Chapter 4. Discussion

Triple negative breast cancer is an aggressive subtype of breast cancer, associated with advanced stage at presentation, early recurrence and increased mortality. It is a breast cancer subtype of interest to clinicians as there is still incomplete understanding of the cancer biology, no optimal treatment and poor outcomes. The challenge remains to improve the outcomes in this complex and heterogeneous breast cancer subtype.

Triple negative and Her2 over expressing breast cancers are the two most aggressive subtypes of breast cancer. The five year disease free and overall survivals of patients with Her2 over-expressing breast cancer has improved dramatically with the use of monoclonal antibodies targeted against Her2 (65). At present there is no targeted therapy to achieve similar improvements in outcome for triple negative breast cancer. Disease free and overall survival is poorer for any patient with triple negative breast cancer than the other breast cancer subtypes, regardless of age, stage at presentation and management.

Triple negative breast cancers are a heterogeneous group of cancers and many units throughout the world are reassessing the patients seen, with special reference to patient demographics, and outcomes both loco-regional and metastatic.

Early studies demonstrated triple negative breast cancer to be more common in North American and European Black African women (26,30). The number of publications regarding triple negative breast cancers in East, West and North Africa has increased noticeably. These describe high incidences, late stage at presentation and poor

outcomes (9,33). Studies have investigated genetic links between African-American and Sub-Saharan African breast cancer patients and postulate that there is a genetic link (66). To date there is no published data regarding triple negative breast cancers in Southern Africa.

Young women with breast cancer tend to present with an advanced stage of disease and a poor prognosis despite intensive treatment. It is postulated that the poor outcomes in young women with breast cancer are due to overrepresentation of aggressive subtypes. An alternate theory is that there are genetic differences with increased expression of aggressive cancer genes resulting in breast cancer with unique biology. It is not clear if young women with triple negative breast cancer have poor outcomes because of their age or because of the subtype of breast cancer (50).

This study aimed to determine the incidence of triple negative breast cancer at two specialist breast units and to assess presentation and outcomes as well as any differences related to the age of patients at presentation.

Milpark Hospital is a private facility that caters to insured and private paying patients. The Helen Joseph Hospital is a public sector hospital that treats an uninsured population. A study at both units found that black women comprised only 8% of all patients at Milpark Breast Care Unit, but 48% of all patients at Helen Joseph. There were 53% of patients at Milpark who had a tertiary education versus 13% at Helen Joseph (67).

The two units are multidisciplinary units involving the same surgeons although the rest of the multidisciplinary team varies between the two units.

Limitations

This was primarily a retrospective file review subject to certain limitations.

It was not possible to assess the patients from Helen Joseph Hospital comprehensively as the data regarding staging and management were not readily available. This resulted in a large number of patients being excluded.

The study reviewed patients seen over a ten-year period, during this time the criteria for receptor status and classification and staging systems have changed. There is, therefore, potential for bias due to subtype misclassification and stage migration.

The majority of data collected was from the retrospective file review. Reasons for incomplete data collection included:

- Patients who were referred after surgery and their pre-operative information were not available.
- Patients who did not follow up at the practice and it was not possible to contact them, this included patients referred from overseas for whom travel to South Africa was not feasible and patients who followed up with their oncologist.
- Patients who were seen for a second opinion and then followed up with their initial healthcare provider.
- Patients who chose not to have traditional medical management.

The final sample size for analysis was 135 patients; hence some variables were unsuitable for significance analysis as the sample was too small.

A further limitation of this study is that certain data are not part of routine history and examination, for instance, ethnicity. It was thus unfortunately not possible to determine the ethnicity of the patients in this study, which is regrettable as ethnicity has been proposed as a major risk factor for triple negative breast cancer (29).

4. 1 Incidence of triple negative breast cancer

Incidence rates of triple negative breast cancer vary throughout the world, and even within a country the rates may vary. The incidence of triple negative breast cancer ranges from 10 to 20%, and as high as 55% in one study (9,29).

The present study established that 14.4% of the women diagnosed with breast cancer had the triple negative subtype. The incidence was 16.1% at Helen Joseph Hospital and 13.9% at Milpark Hospital. The total number of patients with breast cancer seen at Helen Joseph Hospital during the study period was 491 of which 79 patients had the triple negative subtype. In the cohort at Milpark Breast Care Centre 1407 patients were evaluated, of which 196 had triple negative breast cancer.

Previous studies indicated that the highest incidence of triple negative breast cancer is in premenopausal African-American and African women. Population based studies demonstrated that women with triple-negative breast cancers were significantly more likely to be black or Hispanic and under the age of 40 years (15,28). These findings were replicated in a study done in British women (30). Certain countries have a lower incidence of triple negative disease, such as Sweden where the incidence is 12% (20), and in Japanese women the incidence is as low as 10% (29).

The first study in Africa to be published by Huo et al (9), examined triple negative disease in Nigeria and Senegal and showed an incidence of 55%. The incidence of triple negative breast cancer in subsequent studies varies was 16.5% in Morocco (32), 27.9%

in Kenya (33), and 46% in Mali (34). No studies in South Africa have been published as yet.

The incidence of 14.4 % is less than the findings in the Carolina Breast Cancer Study – which had a prevalence of 20% basal like breast cancer (28). It is, however, more than the study from California where the incidence was 12.5% triple negative breast cancer (15) and the study by Dent et al (68), where the incidence was 11.2%. The incidence in this study is similar to that in a study from Morocco, where the incidence of triple negative breast cancer was 16.5% (32).

This is differs from other studies showing an increased incidence in African populations which may reflect that the study was done in an urban centre in a population with access to private healthcare. The results may be different if the study were conducted in a rural area or hospitals serving low income areas (69). It has been postulated that the increased incidence in young African-American women may be related to socio-economic factors and different access to screening and treatment (28).

4. 2 Age and menopausal status at presentation

There was a wide range of age at presentation, from 18 to 108. Patients over the age of 40 years compromised 101 of the 135 patients (74.81%) and 34 patients were 40 years old or younger (25.19%), 22 were 35 years old or younger (16.30%). The median age of the patients in the study was 54. This is very similar to the California study of triple negative breast cancer where the median age at presentation was 54 (15) and a Slovenian population where the median age was 55 (24). The median age at presentation of patients in our study was older than two studies in African patients, a Moroccan study where the median age at presentation was 46 years (32), and a Nigerian cohort, where the median age at presentation was 47 years old (70).

Dent et al (68), looked at the differences in presentation between triple negative breast cancers and cancers positive for any of ER, PR or Her2. They showed that patients with triple negative breast cancer were younger at presentation – 53 vs. 57.7 years with a p value of ≤ 0.0001. In this study 25% of women were 40 years old or younger, which is similar to the study by Liedtke et al (51) where 21.1% of the patients with triple negative breast cancer were younger than 40.

The 135 patients were assessed for menopausal status at presentation and 58% were postmenopausal. Premenopausal patients formed 36% and perimenopausal 6% of the cohort. Breast cancer is often a disease of postmenopausal women, confirmed by Kwan et al (71), where 76% of patients with luminal breast cancers were postmenopausal, but in triple negative breast cancers only 64% of patients postmenopausal, although they did not include perimenopausal women.

4. 3 Stage and tumour characteristics at presentation

In this study stage Ia tumours were more frequent in patients over the age of 40 years (21%). In patients 40 years old and younger 18% of patients had stage Ia tumours, a difference more pronounced in women 35 years old or younger, where 13.65% had stage Ia tumours. This difference was not, however, significant with a p value of 0.518.

In the women older than 40 years, as the stage at presentation advanced, the number of patients affected decreased. In the younger patients the greatest proportion of patients had stage IIB tumours at presentation. This was again more pronounced in women 35 years old or younger, 41% compared to 35% in women 40 years old and younger. In stage IIIa and IIIb the number of patients was lower in all age groups. It is interesting to note that in this cohort only women older than 40 years of age presented with stage IV disease.

There are well-described prognostic and predictive factors in breast cancer to estimate a patient's outcome, and guide management. Prognostic factors in breast cancer include time-dependent prognostic variables, biological prognostic variables and patient-related variables such as age and menopausal status.

Time-dependent prognostic variables include tumour size, lymph node stage, and extent of distant tumour spread. Biological prognostic variables are intrinsic tumour biological characteristics that determine tumour behavior and response to therapy. They are assessed using morphologic surrogates such as tumour differentiation, proliferation status and growth rate. Biologically aggressive tumours are more likely to present at an

advanced stage and the majority of advanced tumours show poor biological prognostic features. Advanced stage at presentation and aggressive biology are major factors associated with the poor outcomes of triple negative breast cancer patients.

The association of triple negative disease with advanced stage at presentation is well documented (8). Studies have shown that triple negative breast cancers are more likely to be larger, node positive (54.4% vs. 45.6%), and have grade III nuclear grade (66% vs. 28%) (68). This is similar to the findings of Rais et al (32), from in Morocco and one of the most recent studies published (24).

The poor prognosis of breast cancer in young women is linked with a number of factors that include: large tumour size at diagnosis, higher tumour grade, mitotic rate and lymph vascular invasion (47). It is unclear if advanced stage at presentation is intrinsic to breast cancers in young women or related to other factors such as breast cancer subtype (72).

The Helen Joseph Hospital and Milpark Hospitals both have a multimodality approach to the management of breast cancer. Patients are initially diagnosed and staged with a triple assessment, starting with a clinical examination. Patients are sent for both a digital mammogram and ultrasound, although young patients and those with dense breasts may only have ultrasound assessment. If a suspicious lesion is seen on imaging, a core biopsy is done. Magnetic resonance imaging may be used in certain patients, especially young patients.

An important aspect of the initial staging is the assessment of axillary nodes. If clinically or radiologically there is nodal involvement this is confirmed with a fine needle aspiration

or core biopsy. If there is no nodal involvement after initial assessment the patient has a dye and gamma probe guided sentinel lymph node biopsy.

In this study most patients had tumours between zero and five centimeters (86%), and 45% of all patients had tumours of two to five centimetres. Younger patients tended to present with larger tumours, the most marked difference was in tumours larger than five centimetres. Only 10% of patients older than 40 years old had tumours greater than five centimetres and 18% of patients 40 years old or younger and 23% of patients 35 years old or younger. Lesions that were T4 (involving skin, chest wall or both), were more common in women older than 40 years (15%) than women 40 years or younger (12%). This difference was not significant with $p = 0.352$.

If T4 tumours are excluded 58% of all patients had primary tumours larger than two centimetres. In the group of patients 40 years old and younger 68% had tumours larger than two centimetres, with little difference in women 35 years old and younger (68%). This corresponds with studies showing that younger women present with bigger tumours.

Tumour size is an independent predictor of recurrence, especially in patients without lymph node involvement. There is an increase in recurrence rates with increasing size, tumours greater than two centimeters are associated with 20% recurrence rates *(73)*. Studies of triple negative breast cancer describe that patients presented with significantly larger tumours – 22 vs. 17 mm in the California Study (15).

Early breast cancer is defined by the National Cancer Institute as stage I, II and IIIa cancer. If this definition is used, then the majority of women 35 years old and younger

(91%) and women 40 years old and younger (88%) presented with early breast cancer. This suggests that younger women present with less advanced disease, which is contrary to most current evidence.

The behaviour of breast cancer, however, is based on a number of factors of which lymph node involvement is a significant prognostic indicator *(73)*. Lymph node involvement is associated with increased local recurrence rates (5.5% vs. 24.7%) and poorer disease-free and 5-year survival (75). The association with poorer disease-free and overall survival rates holds true even for lymph node metastases of 2 mm or less, and lymph node positivity does not correlate with tumour size *(68,76)*.

Axillary lymph nodes were positive at presentation in 56% of all patients assessed; 13% had more than three nodes positive. This is much higher than the 10.7% lymph node positivity in luminal A breast cancer (77). The overall rate of positive lymph nodes was almost identical between patients 40 years and younger (56%) and those older than 40 years (55%) (p=0.276). The incidence, however, was higher in women 35 years and younger (64%). This is consistent with studies that have shown significant associations between lymph node involvement and age at presentation (78).

Tumour grade assesses gland formation, nuclear features and mitotic activity, and is also a significant prognostic factor in breast cancer outcomes; it strongly correlates with both progression free and overall survival (60). There is a correlation between grade and 5 year disease free survival with a relative risk of recurrence of 4.4 in grade 3 compared to grade 1 (79).

It was not possible to determine nuclear grade on all patients so only 121 of the 135 were analysed. It is a reflection of the aggressive biology of triple negative breast cancer that 73% of all patients assessed in the study had grade 3 tumours. There was no clear pattern: in patients 35 years old and younger 77% had grade 3 tumours and in patients 40 years old and younger 71% had grade 3 tumours. This proportion was similar to patients over 40 years old, where 74% of patients had grade 3 tumours.

This cohort of patients has demonstrated a trend to an association between presentation at a more advanced stage, larger tumours, and more lymph node involvement with younger age at presentation especially in in patients 35 years old and younger. In the study by Liedtke et al in 2013 (78), they showed that younger patients with triple negative breast cancer were more likely to have grade 3 tumours, as well as lymph node positive disease.

Seven patients with ductal carcinoma in situ or no tumour found in the breasts were excluded from analysis. Two patients with no tumour found in the breast were older than 40 years and presented with axillary masses that were lymph nodes on imaging and breast cancer on biopsy. The significance of the triple negative subtype in in situ tumours is not clear as the study of biomarkers are in their relative infancy in DCIS and key prognostic and predictive markers associated with invasive breast cancer have not been adequately studied in DCIS (74). Five patients with metastatic disease were also excluded; they were all over the age of 40 years, ranging in age from 53 to 79 years old.

The other predictors of recurrence such as lymphovascular invasion and proliferative markers are not reported, as the data were not available on sufficient patients for meaningful analysis.

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This study assessed a population with some uniformity in patient education level, socioeconomic status and equivalent access to healthcare. Despite this there was a trend to differences in stage at presentation and tumour characteristics between women 40 years and younger and women older than 40 years.

It is interesting to note that the trend to locally advanced disease at presentation persists in a more privileged cohort at Milpark Hospital. This may be due to the aggressive nature of triple negative breast cancer rather than delayed presentation due to poor access to healthcare.

4. 4 Surgery for the breast primary

Almost all of the patients had surgical resection of their breast cancer (96%) and half of all patients had breast conserving surgery (50%). There is, however a difference in the type of surgery that a patient had according to age at presentation as younger patients tended to have more aggressive surgery, although the difference was not significant. In the group of patients 35 years old or younger 64% had a mastectomy, in contrast to 56% of women 40 years old and younger, and 43% had a mastectomy in the group of patients older than 40, the p value was 0.172.

The difference in surgical approach according to age is a reflection of the concerns about recurrence after breast conserving surgery in young patients (80). There is no indication for more aggressive surgery in triple negative breast cancer on its own. This is supported by several studies which have demonstrated no significant differences in the local control rates between triple negative breast cancer and the other subtypes when breast conserving surgery was carried out (81). A study from 2011 showed increased local recurrence in patients with triple negative breast cancer treated with mastectomy than breast conserving surgery. They related this to an increased benefit on recurrence rates from adjuvant radiation post breast conserving surgey (82).

Younger patients with breast cancer especially patients 35 years and younger have an increased risk of locoregional recurrence after breast conserving surgery than older patients (83). The increased locoregional recurrence is probably multifactorial but the morphologic characteristics of breast cancer in young patients in a significant factor (84).

4. 5 Neoadjuvant and adjuvant therapy

The main modalities of treatment for breast cancer are: surgery, chemotherapy, hormonal therapy, targeted therapy and radiation therapy. The indications for each modality are based on tumour characteristics, patient characteristics, surgeon and oncologist approach, and patients' concerns and choices.

Prognostic factors such as lymph node involvement and tumour size are commonly used for decision-making regarding neo-adjuvant and adjuvant therapy. It is a reflection of the number of adverse prognostic factors in this group of patients that almost two-thirds had neo-adjuvant chemotherapy (60%). Age is another important factor in the decision to give neo-adjuvant chemotherapy, and 71% of patients 40 years and under had neo-adjuvant chemotherapy versus 55% of women older than 40 years. This was not a significant difference, p=0.120.

Neoadjuvant chemotherapy is also indicated for downstaging of tumours for breast conserving surgery. In this cohort there was equivalent number of patients having mastectomies to those having breast conserving surgery, thus downsizing to offer breast conserving surgery does not appear to have been a major indication for neoadjuvant chemotherapy. In the group of younger patients who were more likely to have a mastectomy, they were also more likely to have neoadjuvant chemotherapy. This decision was based on tumour characteristics and poor prognostic factors.

The number of patients having neoadjuvant chemotherapy is an indicator of the proportion presenting with locally advanced disease or adverse prognostic features such as stage, age and tumour grade.

Neoadjuvant chemotherapy is not routine in all units managing breast cancer. Protocols may differ according to factors such as limited access to adjuvant therapy or sentinel lymph node biopsies; and the preferences of the multidisciplinary teams. A number of patients in the cohort were referred from other units and were offered adjuvant treatment if indicated.

There were 55 patients who did not receive neoadjuvant chemotherapy, of whom 49 (89%) received adjuvant chemotherapy. The indications for adjuvant chemotherapy were based on the findings of the surgical specimen histology, patient factors and discussion in the multi-disciplinary meeting.

Adjuvant therapy consisted of chemotherapy and radiation therapy. Hormonal therapy was not offered to patients, as they were negative for oestrogen and progesterone receptors. The number of patients who had adjuvant treatment reflects the aggressive biology of the tumours reviewed in this study. In the group of all patients 117 (87%) had adjuvant therapy, of these 55 patients (41%) had chemotherapy and 93 (69%) had radiation therapy. There was no significant difference according to age at presentation, p=0.371.

Adjuvant chemotherapy was given to 41% of all patients, but there was a difference according to age at presentation. Patients who were 40 years and younger had adjuvant chemotherapy in 29%, and 27% of patients 35 years and younger. This is contrast to

45% of patients over 40 years old; in this study older patients were more likely to receive chemotherapy after surgery.

The number of patients who had either neoadjuvant or adjuvant chemotherapy was 128 of 135 patients (95%). When analysed in this way patients older than 40 years old were slightly more likely to have chemotherapy (96%) than women 40 years old and younger (91%).

There was seven patients who had both neoadjuvant and adjuvant chemotherapy. This suggests particularly aggressive disease. The seven patients comprised three patients 40 years or younger, of whom two were 35 years old and younger, and four patients older than 40 years.

In the Milpark breast cancer unit multidisciplinary team adjuvant radiation is prescribed for: breast conserving surgery, involvement of more than three nodes, extra nodal spread, tumour size greater than five centimeters, insufficient margins and T4 tumours. The team accepts 10 millimetres as a clear margin.

Adjuvant radiation therapy was given to 69% of all patients and the proportions were similar despite different age at presentation. Women older than 40 years old had adjuvant radiation in 68%, women 40 years and younger in 71% and women 35 years and younger 73%.

It may be argued that one of the primary reasons for adjuvant radiation therapy is breast conserving surgery. In this cohort of 135 patients 93 (69%) had adjuvant radiation therapy, 56 (60%) of these patients had breast conserving surgery. The ratio for

younger women is, however, different to the entire cohort. Patients who were 35 years old and younger had radiation therapy after mastectomy in 63% and patients 40 years and younger in 54%. Patients older than 40 years old had adjuvant radiation therapy after mastectomy in 35%. This suggests that the indication for radiation therapy was other factors such as: tumour size, grade and lymph node status.

4. 6 Multimodality treatment

It can be seen that most of the cohort had multimodality treatment of their breast cancer. 4% of all patients had surgery alone and some of these declined adjuvant chemotherapy or radiation therapy for personal reasons. When all patients were assessed, 90% had surgery and chemotherapy, 69% surgery and radiation therapy and 67% had all three modalities. This illustrates two features of triple negative disease: the aggressive tumour biology, and the lack of targeted therapy.

Patients older than 40 years old (68%) and those 40 years or younger (67%) were equally likely to have three modalities of treatment. Patients who were 35 years old or younger, however, were more likely to have multimodality treatment – 73%. These numbers reflect the increased rate of radiation therapy in patients 35 years or younger. The trend to multimodality treatment in young patients reflects a more aggressive approach to treating breast cancer in these women because of the known poorer outcomes and the benefit of adjuvant radiation therapy in triple negative breast cancer.

In the current literature triple negative breast cancers have the poorest outcomes of all the subtypes. The progression-free and breast cancer specific survivals of the luminal A and B subtypes can be significantly improved by the addition of hormonal blockade such as tamoxifen (85). The Her2 overexpressing subtype had the poorest outcomes before the use of trastuzumab and more recently lapatinib and pertuzumab (86). The introduction of targeted therapy has improved the disease-free progression and overall survival of Her2 overexpressing tumours from 75% and 87% to 81% and 92% respectively (65).

There is as yet no targeted therapy available for triple negative breast cancers. The poly (ADP-ribose) polymerases (PARPs) were investigated as targeted therapy for triple negative breast cancers but did not show efficacy in phase III trials. There has been an explosion of research into potential targets and agents for the management of this subtype. A number of potential targets have been identified such as: epidermal growth factor receptors (EGFR); vascular endothelial growth factor and its receptors (VEGF, VEGFR); DNA repair capacity - PARPs, epigenetic regulation, androgen receptor (AR) and folate receptor (FR) signaling; and mammalian target of rapamycin (mTOR) inhibitors (44).

The number of patients requiring more than one modality of treatment and the persistently poor outcomes highlights the need for better and more treatment options.

4. 7 Tumour response to neoadjuvant chemotherapy

In this cohort 80 patients (59%) had neoadjuvant chemotherapy. The majority (91%) of these patients responded in some manner – clinically, radiologically, pathologically or a combination thereof. This may reflect the nature of the chemotherapy given as most patients were given anthracycline and taxane based regimens, which are associated with optimal outcomes (87). The patients 35 years old and younger had a marginally better response (94%).

The triple negative subtype, grade and age of patients in this study may account for the complete pathological response in the breast in 29 patients (36%) of the 80 patients who had neo-adjuvant chemotherapy. It is important to note that not all patients had a response in both the breast and lymph nodes; only 11 patients (14%) had a complete pathological response in both breast and nodes. In the data on outcomes it can be seen that patients who still had viable cancer cells in their lymph nodes after neoadjuvant chemotherapy had worse outcomes than the patients who did not.

There was a non-significant difference in complete pathological response according to age at presentation, p=0.254. Patients who were 35 years old and younger had higher rates of complete pathological response with 56% in the breast, 19% in the lymph nodes and 19% in both the breast and lymph nodes. In the group of patients 40 years old and younger the rates of complete pathological response were 46% in the breast, 17% in the lymph nodes and a poorer rate of 13% for both breast and lymph nodes. The patients who were older than 40 years old had the worst complete pathological response in the breast at 32%, but a better response in the lymph nodes at 16%. In these patients the

response rate of 14% in both the breast and lymph nodes was better than the patients 40 years old and younger.

Complete pathological response to neoadjuvant chemotherapy has been associated with improvement in disease-free and overall survival, which translates to long-term clinical benefit. Complete pathological response translates to improved survival with a 0.36 risk of death and 5 year overall survival rates of 83.9% and 5 year disease-free survival rates of 83.4% compared to 67.4% and 50% respectively (91). The definition of complete pathological response, however, is not standardized. Some studies define complete pathological response as no tumour found in the breast or lymph nodes, some studies only include the absence of tumour in the breast and not in the nodes, and some studies include in situ disease as complete pathological response. This has led to variations in the estimation of benefit of complete pathological response on clinical outcomes (88). The greatest benefit is shown when there is complete response in the breast and the lymph nodes (92). Some studies have shown that complete pathological response in the primary breast tumour predicts response in the axillary nodes (93).

In a study of Turkish patients, factors that predicted complete pathological response were grade, menopausal status, triple negativity, percentage of ER positivity, and Her2 expression (89). The benefit of complete pathological response is not seen in all subtypes of breast cancer. The three subtypes predictive of benefit from complete pathological response are: luminal B Her2 negative patients, Her2 overexpressing patients and triple negative breast cancer patients (88). Triple negative breast cancer treated with chemotherapy and Her2 overexpressing breast cancer in patients treated with trastuzumab demonstrate the greatest benefit on long term clinical outcomes after complete pathological response (90). In the triple negative breast cancer subtype there

may be further variations in rates of complete pathological response according to which of the subtypes of triple negative breast cancer is present.

4. 8 Recurrence of triple negative breast cancer

In the present study 23% of all patients had disease progression after initial management. There was a greater recurrence rate in patients 40 years old and younger (32%) than patients older than 40 years old (20%), p =0.215. This difference was more pronounced in women 35 years and younger (36%). In the study by Dent et al (68), the recurrence rate for triple negative breast cancer was 34% vs. 20% for other breast cancer subtypes. The recurrence rate for all patients in this study was lower, this may be due to a smaller cohort as Dent et al reviewed 180 patients; or shorter follow up time. This cohort may also be different in terms of population group.

The triple negative breast cancer subtype is associated with shorter relapse free survival times and a trend to visceral metastases (5,94). Many other studies have confirmed these poor outcomes; they have also shown that recurrences in triple negative breast cancer occur earlier than the other breast cancer subtypes. Dent et al (68), demonstrated high rates of recurrence in the first four years after diagnosis and no recurrences after eight years from diagnosis. This was confirmed in the present study (tables 11 and 12).

Breast cancer in young women is a recognized risk factor for recurrence and poor outcomes regardless of subtype. Women 40 years old and younger have a 1.53 (95% CI 1.37-1.74) increased risk of recurrence than women older than 40 years (46). A key question in breast cancer management is whether young patients do poorly because they have preponderance to aggressive subtypes such as triple negative breast cancer or whether breast cancer in the young has unique biology (50). This study determined

outcomes according to age in the subtype of triple negative breast cancer to assess if the poor outcomes of younger women with breast cancer persisted when compared to older women with the same aggressive subtype. This is a similar question to the study done by Liedtke et al at the MD Anderson Cancer Center (78), and it appears that age at presentation may be an independent predictor of outcome regardless of subtype.

Although the difference in recurrence rates according to patient age was not significant (p=0.215), it was one of the most marked differences in this cohort. Some studies have attributed the poorer disease free survival rates in younger women to increased local recurrence in young women treated with breast conserving surgery (95). In this study, however, most patients 40 years old and younger had a mastectomy, and there was still a high rate of local recurrence despite more aggressive surgery.

Distant recurrences accounted for almost half of all recurrences (14 out of 31). Recurrences outside the breast occurred in 22 patients (16%). This corresponds with data showing that there is a defined pattern of locoregional recurrence in triple negative breast cancer. Dent et al (68), found that locoregional recurrences occurred in 25% of triple negative breast cancers as opposed to 44% of the other subtypes. A difference was again seen according to age with women 35 years and younger having 23% of recurrences outside the breast. The rate was 21% in women 40 years old and younger and 15% in women older than 40 years old.

Six patients had recurrences at more than one site and two had recurrences in both regional lymph nodes and distant sites. This suggests that triple negative breast cancer is a systemic disease with early micrometastases. This is consistent with other studies

that have postulated that the predominant mode of spread in triple negative breast cancer is haematogenous with a tendency to develop visceral metastases early (24).

Early recurrence is a recognized feature of triple negative breast cancer and in the whole cohort the median time to recurrence was less than two years (23 months). The range was 4.5 – 75 months in women 40 years and younger and 5 – 92 months in women older than 40 years. Recurrences in younger women occurred within 6 years with a median of 23 months, and while the median in older women was 19 months there were still recurrences after seven years. It is similar to the findings of Dent et al that no recurrences occurred after eight years (68).

The two patients with the longest time to recurrence in women 40 years old and younger were 54 and 74 months. They both presented with early breast cancer – stage I and IIb, had breast conserving surgery, chemotherapy and radiation therapy. The patient with the longest time to recurrence in the group of women 40 years and older had stage IIa cancer treated with breast conserving surgery, chemotherapy and no radiation therapy. This patient had a better outcome than younger women despite less aggressive treatment.

The results of this study are similar to other studies, which have shown that the peak risk of recurrence of triple negative breast cancer is within three years (24). Studies have also shown a significant correlation between age at diagnosis and both disease free survival and distant disease free survival. The MD Anderson Cancer Centre data show a 30% greater risk for recurrence in younger patients, comparing women younger than 40 to women older than 40 (78).

The recurrence rates, site and time to recurrence are in contrast to the luminal A subtype where the proportion of patients with disease progression is both less and the progression is later. Patients with luminal subtypes have a 0.38 (CI 0.23-0.61) relative risk of developing locoregional recurrence after surgery compared to triple negative breast cancers (96).

Recurrence was analysed according to stage at presentation, this showed a significant difference (p=0.010) showing increased recurrence rates with more advanced stage at presentation. There was almost a doubling of the recurrence rate as the stages progressed from I to II and then to III. The exception is the recurrence in both the patients with stage IB cancer at presentation. Both of these patients were older than 40 years, had neoadjuvant treatment and mastectomies. One patient developed metastatic disease within a year of diagnosis and died 13 months after diagnosis – this may reflect particularly aggressive disease and the poor prognosis associated with lymph node positivity.

The impact of lymph node positivity on recurrence was assessed. It is a significant finding (p=0.008) that lymph node positive disease was associated with a three times increased rate of recurrence.

4. 9 Response to neo-adjuvant chemotherapy and recurrence

There were 80 patients treated with neoadjuvant chemotherapy and 24 (30%) had a recurrence. This proportion was similar in all ages at presentation. The median time to recurrence in all patients treated with neoadjuvant chemotherapy was 18 months. There was a difference in time to recurrence according to age at presentation with younger patients presenting with recurrences earlier. Women 35 years old and younger had a median time to recurrence of 11 months, in women 40 years old and younger time to recurrence was 15 months and 19 months in women older than 40 years.

The recurrence rates in patients who had neoadjuvant therapy differed with the degree of pathological response. The recurrence rate was 10% in all patients if there was no viable tumour found in the breast and 1.25% if there was no viable tumour found in the breast and lymph nodes. Patients who were 40 years old and younger had a 29% recurrence if treated with neoadjuvant chemotherapy, if there was a complete pathological response in the breast alone the recurrence rate was 13% at a median time to recurrence of 25 months. When these patients had a complete pathological response in both breast and lymph nodes there were no recurrences. These data were similar for women 35 years old and younger. The only recurrence after complete pathological response in both breast and lymph nodes was in a patient older than 40 years who had breast conserving surgery and no adjuvant radiation therapy. Radiation therapy reduces the ten year risk of recurrence after breast conserving surgery from 35.0% to 19.3% (97).

The difference in recurrence rate according to the degree of pathological response is in agreement with international trends.

4 .10 Mortality

The overall mortality rate for the 135 patients was 19%. Age at presentation did affect the mortality rate. Women who were 40 years and younger had a mortality of 23%, and women older than 40 years had a mortality of 17%. This was not a significant difference – p value 0.465.

Studies of triple negative breast cancer have showed poor 3 year progression free and overall survival rates (41). A study in London comparing triple negative breast cancer to all other subtypes showed a 5 year survival of 62% for triple negative breast cancer and 75% for all other patients. Survival in the triple negative group was better for those patients who had both surgery and chemotherapy (8). A similar study in Ljubljana found a 5 year disease free survival of 68.2% and 5 year overall survival of 74.5% (24).

A large study from the MD Anderson Cancer Centre on triple negative breast cancers describes median survival according to age groups. The worst overall survival was in the 31-40 age group, followed by the younger than 30, 41-50, older than 60 and 51-60 age groups in that order (78).

The effect of stage at presentation on mortality was assessed as it was for recurrence. The association was also significant (p=0.010) with an approximate 10% increase in mortality with increase in stage from I to II, III and IV. The outlier is again the patient with stage IB cancer who died 13 months after diagnosis. Stage IIB is also remarkable for a lower than expected mortality rate (4%), especially as the mortality rate for stage IIA cancer was 23%.

It is contrast to the significant association of lymph node positivity with recurrence, there was not a significant association with mortality (p=0.265). The difference in mortality rate between lymph node negative and up to three lymph nodes positive was less than one percent. There was, however, a doubling of mortality rate if more than three lymph nodes were positive.

Neoadjuvant chemotherapy did not affect mortality rates in a significant manner (p=0.680), as mortality was 18% if there was some response to chemotherapy and 21% if there was none. There was an impact on mortality, however, if there had been a complete pathological response. The benefit was greater if there was complete pathological response in the breast and lymph nodes (9%) than just the breast (14%).

It is interesting to note that the mortality of patients who had had a mastectomy was double that of patients having breast conserving surgery. This is especially interesting as a number of the patients having a mastectomy were younger.

One of the most significant findings of this study was the significant impact of not having surgery on mortality (p=0.003). Patients who did not have surgery had a mortality rate three times those having a mastectomy and six times those having breast conserving surgery. This may not reflect the impact of surgery on survival alone. The women who did not have surgery were likely to have advanced disease such as metastatic spread. Their mortality was thus determined by the extent of their disease not the surgery or lack thereof. Evidence indicates that triple negative breast cancer has earlier haemotogenous spread, thus the significant impact of a local intervention is noteworthy, however the numbers are small and definitive conclusions cannot be made.

Chapter 5: Conclusion

This study looked at breast care units at two hospitals in South Africa to assess the prevalence and outcomes of triple negative breast cancer and the impact of age at presentation.

This study of South African women did not demonstrate the higher incidences described in other studies with a large cohort of black women especially in West Africa. The incidence was similar to a number of other studies in Africa, and higher than studies in North America, Europe and Asia.

It has been postulated that the presentation and outcomes in triple negative breast cancer are related to socioeconomic issues. Patients in this study tended to present with advanced disease despite the majority coming from an insured population with good access to health care. This suggests that factors other than socio-economics contribute to the more advanced stage at presentation.

Triple negative breast cancer is marked by poor outcomes and lack of effective treatment. The majority of patients in this study required multimodality treatment. Radiation therapy was often indicated for reasons other than breast-conserving surgery. This not only indicates the aggressive nature of triple negative breast cancer but also that a mastectomy does not mean that a patient does not require radiation.

The patients with triple negative breast cancer in our study showed a variable response to neoadjuvant treatment. Those patients with a complete response to neoadjuvant

treatment had better progression free and overall survival. The different response of the breast and the lymph nodes to neo-adjuvant treatment has a prognostic implication. Patients who did not have a complete pathological response in the lymph nodes as well as the breast had poorer outcomes. This is consistent with international opinion that complete pathological response to neoadjuvant treatment is only significant if there is no viable tumour in the breast or lymph nodes.

One of the debates in breast cancer surgery is whether young patients with breast cancer do poorly because of preponderance to aggressive subtypes or if breast cancer in young patients has a unique biology. This study looked at one subtype and assessed whether the differences according to age persisted within one subtype and found that they do. The findings were in accord with other studies investigating triple negative breast cancer and age at presentation.

In our cohort presentation and outcomes differed with age at presentation. Younger patients presented with larger, more poorly differentiated tumours, and were more likely to have lymph node involvement at presentation. This was associated with shorter recurrence free survival and higher mortality rates despite more aggressive treatment. Younger patients were more likely to require neoadjuvant chemotherapy and multimodality treatment.

In the analysis of the data the patients were categorised into two groups – those 40 years old and younger and those older than 40 years. A subgroup of the younger patients was also analysed, those patients 35 years old and younger. Tests of significance were done on the groups 40 years and younger and older than 40 years. This was based on previous studies showing that age at presentation became an

independent predictor of outcome at the age of 40. Studies investigating the significance of age at presentation do, however, use other age cutoffs. In our study the differences in presentation, management and outcomes were greater when the group of patients 35 years old and younger were analysed.

Future studies to assess age and stage at presentation in the other breast cancer subtypes would demonstrate if this phenomenon is limited to triple negative breast cancers only. It would also be interesting to look at a triple negative cohort and use immunohistochemistry markers for basal breast cancer and proliferation. It may then be determined that the differences in presentation, management and disease progression between the young and the older patients are related to differences in the subtype of triple negative breast cancer.

This cohort of patients demonstrates that triple negative breast cancer is a disease with aggressive tumour biology and poor outcomes. Age at presentation appears to affect presentation and outcomes independent of breast cancer subtype.

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Appendices

Appendix 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Leaane M Prodehl

CLEARANCE CERTIFICATE	**M10543**
PROJECT	Triple Negative Breast Cancer in South African Women-A Cause for Conver?
INVESTIGATORS	Dr Leaane M Prodehl.
DEPARTMENT	Department of Surgery
DATE CONSIDERED	25/08/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 31/05/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

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...

Appendix 2

M130545

R14/49 Dr Leanne Prodehl

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130545

NAME: (Principal Investigator)	Dr Leanne Prodehl
DEPARTMENT:	Department of Surgery Medical School
PROJECT TITLE:	Triple Negative Breast Cancer in South African Women-a Cause for Concern?
DATE CONSIDERED:	31/05/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr CA Benn
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 31/07/2013

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 Secretary in Room 10004, 10th floor, Senate House, University.

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**.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3

TRIPLE NEGATIVE BREAST CANCER STUDY

DATABASE

2010

PATIENT CHARACTERISTICS

Name:_____

Date of birth:_____ Age:_____

Sex: Female ☐ Male ☐

Ethnicity: White ☐ Black ☐ Coloured ☐ Indian ☐

European ☐ Chinese ☐ Other ☐

Menopause: Pre ☐ Peri ☐ Post ☐

Diagnosis:_____

Date of diagnosis:_____

Age at diagnosis:_____

Personal history of breast cancer: _____

Family history of breast cancer: _____

Family history of ovarian cancer: _____

Family history of other cancers: _____

TUMOUR CHARACTERISTICS

Date mass first noticed:

_____

Histology: DCIS ☐ Infiltrating ductal ca ☐ Infiltrating lobular ca ☐Other ☐

Differentiation: Poorly ☐ Moderately ☐ Well ☐ Cannot be assessed ☐

Lymphovascular invasion: Yes☐ No☐

Markers: ER pos ☐ neg ☐ %positivity _____

PR pos ☐ neg ☐

Her2 pos ☐ neg ☐ FISH ☐

Grading:_____

Laterality: Left ☐ Right ☐ Bilateral ☐

Axillary node status:

Method: SLNB ☐ ALND ☐ USS ☐ Biopsy ☐

Date:_____

Number of glands involved:_____

Number of glands examined:_____

Staging: Clinical:

T_____ N_____ M_____ Tumour size_____

Pathological:

T_____ N_____ M_____ Tumour size_____

NEOADJUVANT MANAGEMENT

Neoadjuvant treatment given: Yes ☐ No ☐ Unknown ☐

Chemotherapy: Yes ☐ No ☐

Regimen:_____

Number of cycles:_____

Number of cycles before surgery:_____

Hormonal: Tamoxifen ☐ AI ☐ None ☐

Date start:_____ Date end:_____

Ongoing: ☐

Radiation: Yes ☐ No ☐

Target: Herceptin ☐ None ☐

Response to neoadjuvant treatment:

Clinical ☐ Radiological ☐ Pathological ☐ None ☐

SURGICAL MANAGEMENT

BCS ☐ Mastectomy ☐ Other ☐

Date of surgery:_____

SLNB +/- sampling ☐ ALND ☐ None ☐

Date of surgery:_____

ADJUVANT MANAGEMENT

Adjuvant treatment given: Yes □ No: □ Unknown □

Chemotherapy: Yes □ No □

Regimen:_____

Number of cycles:_____

Hormonal: Tamoxifen □ AI □ None □

Date start:_____ Date end:_____

Ongoing: □

Radiation: Yes □ No □

Target: Herceptin □ None □

BREAST CANCER RELAPSE

Relapse: Yes□ No: □

Date of relapse:_____

Local: Yes □ No □

Clinical diagnosis: Yes □ No □

Pathological proof obtained: Yes □ No □

Date:_____

Regional: Yes □ No □

Clinical diagnosis: Yes □ No □

Pathological proof obtained: Yes □ No □ Date:_____

Distant: Contralateral breast cancer □

Liver □ Lung □ CNS □

Skin □ Other □ Bone □

Pathological proof obtained: Yes □ No □ Date:_____

SECONDARY PRIMARY CANCER

Contralateral breast cancer □ Endometrial cancer □ Ovarian cancer □

Leukamia □ Other □

SURVIVAL DATA

Alive: Yes □ No □

Date of

death:_____

Cause of

death:_____

DISEASE FREE SURVIVAL

Disease progression: Yes □ No □

Date of

progression:_____

Date last

seen:_____

Appendix 4

TRIPLE NEGATIVE BREAST CANCER STUDY
TELEPHONIC FOLLOW UP
VERBAL CONSENT 2013

Hello Ms_____, I am _____. I am calling you from the Milpark Breast Care Centre regarding your diagnosis of breast cancer.

We are doing a study on the prevalence and outcomes of women with breast cancer that does not respond to hormones or Herceptin, or triple negative breast cancer. The aim of the study is to understand how many women in South Africa have triple negative breast cancer and how it affects them. The study is part of a Masters of Medicine in the Department of Surgery at the University of the Witwatersrand. It is anonymous and has ethics approval. The first part of the study looked at the files of all the breast cancer patients seen at the Milpark Breast Care Centre.

We would like to find out what has happened to you since your last follow up, by asking you a short questionnaire. Your management will not change whether you choose to answer or not and regardless of your answers.

Do you understand the purpose and implications of the questionnaire and do you wish to continue with the questionnaire?
Yes □ No □

Appendix 5

TRIPLE NEGATIVE BREAST CANCER STUDY TELEPHONIC FOLLOW UP QUESTIONNAIRE 2013

Date:_____

Time:_____

Verbal consent obtained: Yes ☐ No ☐

Patient requests email of information sheet: Yes ☐ No ☐

Patient email address:_____

Patient identity confirmed: DOB ☐ Address ☐ Diagnosis ☐

Questions:

Are you well? Yes ☐ No ☐

Have you had a recurrence of your breast cancer? Yes ☐ No ☐

If yes

when?_____

Where?_____

Have you been diagnosed with a cancer other than breast cancer?

Yes ☐ No ☐

If yes – where?_____

Appendix 6

List of corrections

1. *The results would benefit from regression analysis especially of the results are going to be published (as the study should be). This would determine important predictors of survival/recurrence.*

Thank you for your valuable comments, the regression analysis will indeed help determine predictors of outcome. This will be performed in phase two of the study as part of the preparation for publication.

2. *The discussion has unnecessary duplication of the introduction.*

This has been duly noted and the discussion amended accordingly.
For example: page 64, paragraph 2- the results of three studies have been consolidated and summarised.

Page 66, paragraph 1 – the results of three studies have also been consolidated.

Page 69, paragraph 2 - The results of various studies have been summarised into one sentence.

3. *In the discussion moving the main study findings to the beginning of each section to provide context should be considered.*

The comments are appreciated and the text has been revised accordingly, thank you for the constructive criticism.
For example: page 65, paragraph 2 has been moved to page 64, paragraph 2.

Page 69, paragraph 4 to page 70 paragraph 1 has been moved to page 68, paragraph 1.

Page 82, paragraph 3 to page 83, paragraph 1 has been moved to page 81 paragraph 2.

page 84,paragraph 3 has been moved to paragraph 1 on the same page.

page 85, paragraph 4 has been moved to page 84 paragraph 2.

4. *Study limitations are normally described toward the end of the discussion. The candidate has outlined these early in the discussion to give context. Positioning early in the discussion detracts from the study findings.*

Thank you for your constructive input, the value of the comments has been noted and reviewed with the supervisor. After much discussion it was felt that the results may difficult to interpret without the context of the limitations being described, and the order has been left unchanged.

5. *Avoid repeating results in the discussion.*

The text has been amended to clarify the implication and impact of results as opposed to repeating them without interpretation.
For example: page 74, paragraph 1 – context has been given to the rate of mastectomy in women 35 years old and younger by comparing it to the lower rates in older women.

Page 75, paragraph 2 – the rate of neo-adjuvant chemotherapy has been compared between the different age groups.

6. *For the purpose of the Research report, a one page Turnitin plagiarism report summary should suffice, especially when the percentage similarity is low.*

This revision has been noted and the full Turnitin plagiarism report amended to a one page summary as suggested.

7. *Minor errors*

Thank you for your constructive criticism and assistance with editing, all comments have been accepted and changes made to correct errors.