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Clinical and Pathological Features of Triple Negative Breast Carcinoma

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the degree of

Masters of Medicine in Anatomical Pathology

Johannesburg, 2016

DEDICATION

To my family, who have always believed in me.

DECLARATION

I, Dr Jessica Charlotte Linden (0306328E) am a student registered for the degree of Masters of Medicine (Anatomical Pathology) in the academic years 2012 to 2016.

I hereby declare the following:

I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.

I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others.

I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature

Signed at the University of the Witwatersrand, Johannesburg

Date: 06/01/2016

ABSTRACT

BACKGROUND: Breast carcinoma is the most common carcinoma diagnosed in females worldwide. The mortality rate from breast carcinoma has declined over the last three decades. Receptor negative tumours, perhaps due to limited treatment options, have a higher mortality rate.

OBJECTIVES: The aim of this study is to determine patient demographics and tumour characteristics of triple negative breast carcinoma (TNBC) at the histopathology department of Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

METHOD: An observational, cross-sectional, retrospective study design was used. A search of our electronic database identified 1126 individuals with invasive breast carcinoma between 1 January 2011 and 31 December 2012. Tumour characteristics and patient demographics were subsequently assessed on those individuals with confirmed TNBC.

RESULTS: 13.0% of tumours had a triple negative phenotype. Individuals with TNBC ranged from 28.0 to 89.0 years with a median age of 44.0 years; the mean age was 55.4 years (SD ± 13.4 years). TNBCs were ~70 times more common in females than males. The tumours ranged in size from 6.0 mm to 160.0 mm, with a median size of 36.0 mm; the mean tumour size was 48.3 mm (SD ± 33.1 mm). The majority of tumours (90.8%) were invasive carcinomas of no special type. 67.9% of individuals presented with high grade tumours and the majority presented with stage II or III disease (43.8% and 45.6% respectively).

Concurrent in-situ malignancy was identified in 34.1% of cases. 69.6% of individuals presented with nodal involvement. Unifocal disease was considerably more common than multifocal disease (90.7% compared to 9.3%). The distribution of left and right sided disease was similar (54.5% and 43.2% respectively) and bilateral disease was uncommon (2.3%).

CONCLUSION: TNBCs comprise 13.0% of breast carcinomas, this is lower than the prevalence in Soweto and other African countries. Differences in racial and socioeconomic demographics may provide an explanation. Patient demographics and tumour characteristics of TNBCs in our population are largely similar to the data available in the literature. The substantial size and stage difference amongst studies suggests that factors other than the triple negative phenotype could contribute to large tumour size and advanced stage at presentation. In the South African context, barriers to accessing healthcare provide a plausible explanation.

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LIST OF ACRONYMS / ABBREVIATIONS

AR	Androgen receptor
ASCO	American Society of Clinical Oncology
BRCA	Breast cancer susceptibility gene
CAP	College of American Pathologists
CK	Cytokeratin
CHBAH	Chris Hani Baragwanath Academic Hospital
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
DCIS	Ductal carcinoma in-situ
ER	Oestrogen receptor
H&E	Haematoxylin and eosin
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
MRI	Magnetic resonance imaging
NHLS	National Health Laboratory Services
PR	Progesterone receptor
SANAS	South African National Accreditation System
SD	Standard deviation
TNBC(s)	Triple negative breast carcinoma(s)

CHAPTER 1: INTRODUCTION

Breast carcinoma is the most common malignancy diagnosed in females worldwide. Developing countries carry a large burden of this disease, with 50.0% of breast carcinoma diagnoses and 58.0% of breast carcinoma deaths occurring here (The World Health Organisation, 2015).

Breast carcinoma accounts for 16.0% of malignancy related deaths in South African females, making it the second most common cause of malignancy related death. It is second only to cervical carcinoma, which accounts for a marginally higher 16.9% of malignancy related deaths (The World Health Organisation, 2014). Breast carcinoma is the most common carcinoma diagnosis in South African females, with a 1 in 35 lifetime risk (Cancer Association of South Africa, 2014).

The mortality rate from breast carcinoma has declined over the last three decades as a result of improvements in prevention, early detection and the introduction of chemotherapy, including hormonally targeted therapies (Jemal et al., 2010). The improved survival outcome, attributed to the use of targeted treatment, has resulted in receptor analysis becoming standard procedure in the diagnosis of breast carcinomas (Sharma et al., 2014). Receptor negative tumours, perhaps due to their limited treatment options, have shorter disease free and overall survival rates (Bose, 2015 & Yadav et al., 2014).

To our knowledge, the demographics and tumour characteristics of triple negative breast carcinomas (TNBCs) have not been investigated in patients' biopsies evaluated at the histopathology laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). While a study of the receptor status of breast carcinoma in individuals attending Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto has been completed (McCormack et al., 2013), the population served by CMJAH encompasses a potentially different patient demographic and spans possibly a wider geographic area. No other data are available on the receptor status of breast carcinomas or triple negative tumours in South Africa.

A better knowledge of the epidemiology and tumour demographics of TNBC is essential to better understand the disease. This could influence preventative strategies and will have important prognostic implications. It may provide a platform for the development of improved treatment policies in the future. Furthermore, in a time where research is aimed at personalised medicine, understanding TNBC in the South African population is fundamental.

CHAPTER 2: AIMS AND OBJECTIVES

The aims of this study were to determine patient demographics and tumour characteristics of TNBCs at the histopathology department of CMJAH between 1 January 2011 and 31 December 2012.

The objectives of this study were as follows:

1. To assess patient demographics in terms of age, gender and race (Asian, Black, Coloured, White and other).
2. To assess the characteristics of TNBC in terms of size, modified Bloom and Richardson grade (Appendix B), histological subtype (Appendix C), nodal involvement, concurrent in-situ malignancy, focality and laterality.
3. To quantify the total number of breast carcinomas as well as the total number of TNBCs.
4. To compare these findings to the data available in the literature.

CHAPTER 3: LITERATURE REVIEW

3.1 Definitions

A triple negative breast carcinoma phenotype is defined as a tumour that does not overexpress human epidermal growth factor receptor 2 (HER2) and does not express oestrogen receptors (ER) and progesterone receptors (PR) immunohistochemically (Schmadeka et al., 2014).

Perou et al. (2000) proposed the first molecular classification of breast carcinoma based on gene expression analysis using microarray studies. Five distinct molecular subtypes were originally described in this innovative study. These are known as the intrinsic subtypes and include luminal A, luminal B, HER2-enriched, basal-like and the normal breast-like subtypes. More recently, the claudin-low, molecular apocrine and interferon-rich subtypes have been described (Alizart et al., 2012 & Prat et al., 2010). These different molecular subtypes have important clinical implications as they differ in terms of incidence, risk factors, prognosis, age at diagnosis and response to treatment (Prat et al., 2013).

Immunohistochemical markers have been used to identify these molecular subtypes. Luminal A tumours have a high level of ER expression and a relatively low proliferation index while luminal B tumours have a lower level of ER expression and a higher proliferation index. Luminal A tumours, by definition, are low grade tumours and luminal B tumours are designated as intermediate grade. HER2-enriched tumours are HER2 positive, ER negative and PR negative while basal-like tumours are ER, PR and HER2 negative. Both the HER2-

enriched and basal-like subtypes are high grade tumours (Cornejo et al., 2014 & Lakhani et al., 2012).

Basal-like breast carcinomas constitute approximately 15.0% of all breast carcinomas (Perou et al., 2000). The terms basal-like breast carcinoma and TNBC are often used interchangeably, however, these terms are not synonymous (Cadoo et al., 2013). Although the majority of TNBCs (56.0 to 85.0%) will belong to the basal-like molecular category, it is important to note that not all TNBCs are basal-like (Li et al., 2015, Peng, 2012 & Prat et al., 2013). Microarray studies have shown a discordance rate of 20.0 to 30.0% between these two tumour types based on patterns of gene expression (Prat et al., 2013). As gene microarray studies are used mainly in the research rather than the clinical setting, immunohistochemically determined TNBCs have been studied as a surrogate marker for the basal-like phenotype (Schmadeka et al., 2014 & Sharma et al., 2014).

3.2 Epidemiology

3.2.1 Prevalence

The reported prevalence of TNBC is variable (Table 1). In London, individuals with breast carcinoma have a lower occurrence of triple negative tumours, with a reported prevalence of 10.0% (Pal et al., 2014). This is in contrast to India, where 46.0% of breast carcinomas were triple negative tumours (Zubeda et al., 2013).

In the United States, the prevalence of TNBC ranged from 12.0 to 24.0% (Schmadeka et al., 2014). Hispanic females have a prevalence of 23.1% (Lara-Medina et al., 2011). This is comparable to the 21.0% prevalence reported in individuals from Soweto, South Africa

(McCormack et al., 2013). Other studies emanating from the African continent showed that 34.0% of Ugandan females (Galukande et al., 2014) and 20.2% of Kenyan females had triple negative tumours (Sayed et al., 2014). 31.9% of females from North East India had TNBC (Sharma et al., 2014) whilst 12.2% of females from Kuwait had TNBC (Fayaz et al., 2013).

Table 1. Table comparing the prevalence of TNBC amongst various studies.

Study	**Country**	**Sample size**	**Prevalence of TNBC (%)**
Bauer et al., 2007	California, USA	92 358	12.5
Fayez et al., 2014	Kuwait	2 986	12.2
Galukande et al., 2014	Uganda	226	34.0
Lara-Medina et al., 2011	Mexico	2 074	23.1
Li et al., 2013	China	21 749	12.2
McCormack et al., 2013	Soweto, South Africa	1 218	21.0
Pal et al., 2014	London, England	2 394	10.0
Sayed et al., 2014	Kenya	304	20.2
Sharma et al., 2014	North East India	972	31.9
Somali et al., 2013	Turkey	882	14.9
Zubeda et al., 2013	India	619	46.0

3.2.2 Demographics

3.2.2.1 Age

Individuals with TNBC span a wide age range. A single retrospective study showed an age range from 21.0 to 89.0 years (Li et al., 2013). The mean age at presentation varied between studies, from 40.0 years (Sharma et al., 2014) to 56.0 years of age (Ishikawa et al., 2011).

Overall, triple negative tumours tend to occur at a younger age than non-TNBC (Ray et al., 2010). Females from North East India with triple negative tumours presented at a mean age of 40.0 years compared to 49.0 years in those with non-TNBC (Sharma et al., 2014). In California it was demonstrated that the likelihood of being diagnosed with TNBC was higher in younger females. Females younger than 40.0 years of age were shown to be 1.5 times more likely than older females (those aged between 60.0 and 69.0 years) of being diagnosed with TNBC (Bauer et al., 2007). Hispanic females with TNBC presented at a mean age of 49.2 years compared to their non-TNBC counterparts who presented at a mean age of 52.2 years (Lara-Medina et al., 2011).

In contrast to the majority of available data, a single study showed no significant difference between the mean age at presentation of individuals with TNBC compared to those with non-TNBC (52.3 compared to 53.4 years) (Somali et al., 2013). It is noted that this was a relatively small study comprising 750 participants. Lara-Medina et al. (2011) and Bauer et al. (2007) had 2 074 and 51 074 participants respectively.

Breast carcinoma in young females is rare with only 5.0 to 6.0% of total breast carcinomas occurring in females younger than 40.0 years of age (Vogel et al., 2011). A retrospective

study showed that breast carcinoma in young females (≤35.0 years of age), when compared to elderly individuals (≥60.0 years of age), was associated with increased aggressiveness. Furthermore, breast carcinoma in young females were characterized by large tumour size, higher histological grade, more advanced stage and elevated lymph node metastasis rates when compared to tumours in older individuals (Zhang et al., 2015).

A study evaluating the molecular subtypes of breast carcinoma in 451 females ≤40.0 years at diagnosis revealed that there were a significantly higher proportion (34.3%) of basal-like tumours in this age group compared to females between 41.0 and 52.0 years of age (27.7%). A higher proportion of HER2-enriched carcinomas were also detected in young patients. This group of females were less likely to have luminal A tumours compared to other age groups (Azim et al., 2012).

Young females with breast carcinoma are faced with unique challenges requiring further consideration. This includes issues associated with premature menopause, loss of fertility and fertility preservation, family planning, sexual functioning, beauty and body image, establishing careers and raising children (Ribnikar et al., 2015 & Zhang et al., 2015).

3.2.2.2 Race and socioeconomic status

Although breast carcinoma is diagnosed more frequently in Caucasian females, mortality is higher amongst females of African ancestry. These racial trends have also been shown to vary with age. Black females younger than 50.0 years of age have a higher incidence of breast carcinoma than their Caucasian counterparts whilst later in life this trend reverses and the incidence is higher in older Caucasian females (Wheeler et al., 2013).

TNBCs are more common in Black individuals, especially African American females and females of West African ancestry (Cadoo et al., 2013, Schmadeka et al., 2014 & Wheeler et al., 2013). Additionally, breast carcinomas diagnosed in African American females are twice as likely to have a triple negative phenotype compared to those diagnosed in White females. Overall, Black females are diagnosed with more advanced stage disease than Caucasian females (Schmadeka et al., 2014). Survival differences persist between Black and White females diagnosed at similar stages of illness, suggesting that breast carcinoma in Black individuals is fundamentally a different disease (Wheeler et al., 2013).

Individuals with a lower socioeconomic status have a higher rate of TNBC compared to patients with a high or moderate socioeconomic status. Regardless of socioeconomic status, however, Black females were 1.8 times more likely than White females to be diagnosed with TNBC (Sineshaw et al., 2014).

The increasing rate of breast carcinoma in Africa is thought to be due to 'westernisation' of the developing world. The main factors implicated are socioeconomic improvements that increase life expectancy as well as those that allow females reproductive choices such as delayed childbearing, decreased parity and decreased breast feeding. Less desirable lifestyle changes and a sedentary way of life are also thought to contribute to this increase (Porter, 2008).

In South Africa, females have a 1 in 35 overall lifetime risk of developing breast carcinoma (Cancer Association of South Africa, 2014). This risk, however, varies between racial groups. Black females have an overall 1 in 53 lifetime risk of developing breast carcinoma,

Caucasian females have a 1 in 15 chance, Coloured females a 1 in 21 chance and Indian females, a 1 in 20 chance (National Institute for Occupational Health, 2006). In Soweto, 94.2% of females with TNBC were Black, 2.4% were White, 1.9% were Coloured and 1.4% were Asian (McCormack et al., 2013).

3.2.2.2 Gender

A medical papyrus from ancient Egypt, discovered by Egyptologist Edwin Smith and dating back to between 3 500 and 2 500 BC, is thought to be the first record of breast carcinoma. Nine patients were described in this papyrus and interestingly all nine individuals were male (Serarslan et al., 2015).

Male breast carcinoma is uncommon, accounting for approximately 1.0% of all breast carcinomas and less than 1.0% of all malignancies in males (Ge et al., 2009 & Kornegoor et al., 2012). As male breast carcinoma is rare, available data are limited and the majority of existing data refer to female individuals with TNBC.

Invasive carcinoma of no special type is the most common histological subtype of male breast carcinoma (Giordano et al., 2004 & Ge et al., 2009). The prevalence of TNBC in males ranges from 4.0% to 5.7% (Aschie et al., 2013 & Sayad et al., 2014).

Male breast carcinoma is biologically different to female breast carcinoma with higher rates of hormone receptor positivity and lower rates of HER2 overexpression (Kornegoor et al., 2014, Masci et al., 2015 & Serarslan et al., 2015). This was demonstrated in a relatively

large study conducted by Giordano et al. (2004) comprising 2 537 men with breast carcinoma, where it was shown that 90.0% of male breast carcinomas were ER positive and 81.0% were PR positive. These findings are similar to a study comprising 42 men which demonstrated that 100.0% of male breast carcinomas were ER positive (Ge et al., 2009). A smaller study of 35 males with breast carcinoma established that luminal A and luminal B tumours were the most common subtypes in men, while TNBC contributed to only 5.7% of the tumours (Aschie et al., 2013). These findings are congruent with those of Ge et al. (2009) and Kornegoor et al. (2012) who showed that luminal A and luminal B tumours were the most common subtypes of male breast carcinoma. Furthermore, Kornegoor et al. (2012) showed that only 4.0% of male breast carcinomas were classified as triple negative tumours.

In contrast to the above findings, a study of 22 Greek men with breast carcinoma revealed that the triple negative phenotype was the second most common subtype, after the luminal A phenotype (Tsoukalas et al., 2014). This difference may be attributed to the study's relatively small sample size.

Females and males present with breast carcinomas which are similar in size (Serarslan et al., 2015). As is the case in females, oestrogen is important in the development of male breast carcinoma. In males, however, circulating levels of oestrogen are low and most oestrogens are synthesised in the peripheral tissue and have local effects. In addition, male breast carcinoma shows high rates of androgen receptor (AR) positivity (Kornegoor et al., 2014). Male breast carcinomas progress more rapidly to advanced stage lesions with involvement of the chest wall or ulceration of the overlying skin, due to a lack of breast tissue (Lakhani et al.,

2012 & Serarslan et al., 2015). When male and female patients of similar age and tumour stage are compared, the prognosis is similar (Kornegoor et al., 2014).

3.2.3 Risk factors

Females with a high body mass index and a lack of recreational physical activity are at higher risk for developing triple negative tumours. Multiparity as well as early parity are generally considered to be protective against the development of breast carcinoma. These two factors, however, are reported risk factors for the development of TNBC (Kandil et al., 2012 & Wheeler et al., 2013). Increased parity as a risk factor for triple negative tumours is supported by the data available for TNBC in Hispanic females. Premenopausal status and hormonal contraceptive use have also been suggested as risk factors for the development of triple negative tumours (Lara-Medina et al., 2011).

Data pertaining to family history in individuals with TNBC are variable. Fayaz et al. (2013) demonstrated that 21.0% of individuals with triple negative tumours had a family history of breast carcinoma. There was a significantly higher prevalence of breast carcinoma in family members of TNBC patients compared to non-TNBC patients (65.2% compared to 32.9%).

3.2.4 Hereditary breast carcinoma

The breast cancer susceptibility genes (BRCA) are tumour suppressor genes which are involved in DNA repair of double strand breaks. Mutations in the BRCA1 gene are associated with early onset breast cancer (Kandil et al., 2012). The evidence shows that individuals harbouring the BRCA1 gene mutation have a higher incidence of triple negative tumours than sporadic cases (Cadoo et al., 2013) and that these are more likely to be high

grade tumours (Kandil et al., 2012). 80.0% of BRCA1 associated breast tumours will have a basal-like phenotype (Kandil et al., 2012).

BRCA mutations are noted in individuals with TNBC, with 9.6% patients harbouring a BRCA1 or BRCA2 mutation. The mean age at diagnosis of individuals with the BRCA1 mutation was significantly younger than individuals without this mutation (Wong-Brown et al., 2015).

3.3 Pathological features

3.3.1 Gross pathology

3.3.1.1 Tumour size

TNBCs present at a larger size than non-TNBCs (Sharma et al., 2014). A retrospective study from North East India demonstrated that TNBCs were larger than non-TNBC, with a mean tumour size at presentation of 52.0 mm in TNBC compared to 34.0 mm in non-TNBCs (Sharma et al., 2014). In Chinese females, 53.2% of TNBCs were greater than 50.0 mm in size compared to 43.1% of non-TNBC (Li et al., 2013). These findings were mirrored in a study by Somali et al. (2013). Bauer et al. (2007) demonstrated a median TNBC size of 22 mm contrasted with 17 mm in non-TNBC. A failing of these studies was that none defined whether macroscopic, clinical or imaging techniques were used to determine tumour size.

3.3.1.2 Tumour laterality

Distribution of right and left sided disease are similar and bilateral disease is uncommon (Sharma et al., 2014). 0.3% of TNBCs were bilateral, 46.0% left sided and 36.8% right sided

(Sayed et al., 2014). Similarly, Fayaz et al. (2013) showed that 1.0%, 53.0% and 46.0% of TNBC were bilateral, right and left sided respectively.

3.3.1.3 Tumour focality

In triple negative carcinomas, unifocal disease is more common than multifocal and diffuse disease (Peker et al., 2013). Unifocal disease has been reported in up to 69.0% of cases (Pekar et al., 2013) with rates as high as 90.0% (Fayaz et al., 2013).

3.3.2 Imaging

Mammographic imaging of triple negative tumours identifies hyperdense, oval or lobulated masses with indistinct or circumscribed margins (Schmadeka et al., 2014). Calcifications, along with other typical features of non-triple negative tumours, such as an irregular shape and spiculated margins, are often absent (Boisserie-Lacroix., et al 2013, Dogan et al., 2012 & Schmadeka et al., 2014). Up to 18.0% of TNBCs are occult on mammography (Schmadeka et al., 2014).

On ultrasound, triple negative tumours have circumscribed or microlobulated margins with no posterior acoustic features or posterior enhancement (Boisserie-Lacroix et al., 2013).

Magnetic resonance imaging (MRI) shows rim enhancement which is highly suggestive of malignancy and identifies suspicious features more commonly in TNBC than in non-triple negative tumours (Boisserie-Lacroix et al., 2013). Triple negative tumours are poorly

defined mammographically and the extent of the disease may be better assessed with the use of MRI (Cadoo et al., 2013 & Dogan et al., 2012).

3.3.3 Histopathology

3.3.3.1 Histological subtypes

Numerous histological breast carcinoma subtypes have been described (Appendix C). The majority of triple negative tumours, up to 95.8%, are invasive carcinomas of no special type. Medullary carcinomas are the next most common histological subtype (Sharma et al., 2014). Less frequent histological subtypes include adenoid cystic, apocrine and metaplastic carcinomas (Leidy J et al., 2014 & Bose, 2015).

3.3.3.2 Grade

Breast carcinoma grade is assessed using the modified Bloom and Richardson score (Elston et al., 2002) (Appendix B). Triple negative tumours are usually high grade or modified Bloom and Richardson grade 3 (Cadoo et al., 2013, Galukande et al., 2014, Sharma et al., 2014 & Qingli et al., 2014). Galukande et al. (2014) showed that 68.0% of TNBCs have a high histological grade. The tumours often have high proliferation/mitotic rates although the exact values were not defined (Lara-Medina et al 2011).

In the Soweto based study, only 4.3% of TNBCs presented as grade 1 tumours. The majority of TNBCs were grade 3 tumours (66.5%) and the remaining 29.3% were grade 2 tumours (McCormack et al., 2013).

3.3.3.3 Histomorphological features

Although the diagnosis of a TNBC is made on immunohistochemistry, there are histomorphological features that may alert the pathologist to the possibility of a triple negative tumour. In addition to the high histological grade, the tumours may show central necrosis and pushing borders. Perilobular and intratumoural lymphocytes, variably sized vessels and an increase in fibrous tissue may also be noted (Schmadeka et al., 2014).

3.3.3.4 In-situ malignancy

Carcinoma in-situ is usually absent and if identified, usually comprises only a small focus (Kandil et al., 2012). Fayaz et al. (2014) showed that concurrent ductal carcinoma in-situ (DCIS) was present in 26.0% of individuals with TNBC.

3.3.3.5 Nodal involvement

Individuals with TNBC are more likely to present with lymph node involvement than individuals with non-TNBC (55.8% compared to 51.0%) (Sharma et al., 2014). This is supported by other studies which have shown that TNBCs have a high rate of lymph node positivity (Galukande et al., 2014 & Peng, 2012). In contrast to these findings, Lin et al. (2012) demonstrated that individuals with triple negative tumours are less likely to have lymph node involvement.

3.3.3.6 Stage at presentation

Individuals with TNBC often present with advanced disease, defined as WHO Stage III and IV tumours (Galukande et al., 2014 & Lara-Medina et al., 2011). These findings were

confirmed in the study of Sowetan females, where 2.9% presented with stage I disease, 38.8% with stage II disease, 57.2% with stage III disease and the remaining 7.2% with stage IV disease (McCormack et al., 2013).

Dickens et al. (2014) identified a number of factors contributing to a late stage at presentation in sub-Saharan Africa. These included poor existing healthcare infrastructure, lack of early-detection programs as well as unavailability, inaccessibility and lack of adherence to treatment. In some developing areas, a relatively low incidence of breast carcinoma is coupled with a high mortality rate and this has largely been attributed to late presentation (Porter, 2008).

3.3.4 Immunohistochemistry

Knowledge of the ER, PR and HER2 status of breast carcinomas is fundamental to patient management and assessment of these prognostic biomarkers in routine histological evaluation is thus compulsory (Allred, 2010).

3.3.4.1 Oestrogen and progesterone immunohistochemistry

According to guidelines published in 2010, a tumour is ER or PR negative if less than one percent of the tumour cells are immunoreactive (Hammond et al., 2010) (Appendix D). Prior to 2010, the criteria for assessing the ER and PR status of breast carcinomas were poorly defined and many laboratories used an arbitrary cut-off of greater than 10.0% ER positive tumour cells to define an ER positive tumour (Allred, 2008). It was demonstrated that 20.0% of immunohistochemical assessments of ER and PR status may be inaccurate due to false positive and negative results as a result of variation in pre-analytic variables as well as

differing thresholds for positivity and interpretation criteria. Consequently, the new guidelines for ER and PR testing were developed. Due to the impact of Tamoxifen on mortality reduction together with its low toxicity profile, the recommended cut-off for receptor positivity was changed to 1% or more immunoreactive tumour cells (Hammond et al., 2010).

ER testing is highly sensitive to tissue fixation and processing procedures. If standardised protocols, as outlined by the "Consensus Recommendations on Oestrogen Receptor Testing in Breast Cancer by Immunohistochemistry" are not strictly adhered to, false negative results may be obtained. These guidelines detail the importance of appropriate fixation and processing, the use of adequate positive and negative controls as well as standardised reporting criteria to ensure validity of the results (Yaziji et al., 2008). It has been suggested that the high rates of receptor negativity in sub-Saharan Africa may be due to false negative results (McCormack et al., 2013).

3.3.4.2 HER2 immunohistochemistry

HER2 negativity is defined, according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 guidelines, as a HER2 immunohistochemical (IHC) score of 0 or 1+. According to the 2013 guidelines, an IHC score of 0 is defined as "no staining or incomplete membrane staining that is faint/barely perceptible within ≤10% of tumour cells". An IHC score of 1+ is defined as "membrane staining that is incomplete and faint/barely perceptible within >10% of the tumour cells". An IHC score of 2+ is considered equivocal and is defined as "circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of tumour cells or

complete and circumferential membrane staining that is intense and within ≤10% of tumour cells". It is recommended that in-situ hybridisation be performed on all cases where the HER2 immunohistochemical status is equivocal (Wolff et al., 2013) (Appendix E).

The study period was prior to implementation of the current guidelines. According to the previous ASCO/CAP HER2 2007 guidelines, an IHC score of 0 or 1+ was defined as "no staining or weak, incomplete membrane staining in any proportion of tumour cells" (Wolff et al., 2007) (Appendix F).

The implementation of these new guidelines is important in the era of molecularly targeted therapy and personalised medicine (Rakha et al., 2014). There has been a large focus on pre-analytical factors which need to be optimised in order to increase the accuracy of the results. This is similar to the ER and PR assessment guidelines. These factors include time to fixation, duration of fixation and time from removal from the patient to incision of the specimen (Wolff et al., 2013). There is also an increase in recommendations regarding repeat or reflex testing. This has been criticised as it is felt that this recommendation is not adequately evidence based and will result in an increase in resource utilisation for which the benefits have not yet been adequately justified (Bethune et al., 2015 & Rakha et al. 2014). Bethune et al. (2015) have shown that the use of these new updated 2013 guidelines will result in a 9.4% reclassification of breast carcinomas. The impact of this is uncertain at present.

3.3.4.3 Factors influencing prognostic biomarker assessment

The prognostic biomarker status of tumours varies significantly between primary and recurrent tumours (Soomro et al., 2014). Neoadjuvant chemotherapy decreases the hormone receptor immunoreactivity of tumour cells (Pachnicki et al., 2012). Chemotherapy, especially Anthracycline-based chemotherapy, has been associated with a switch in ER status. There is a high discordance in ER and PR receptor status between primary breast carcinomas and corresponding metastatic lesions. HER2 status, however, remains relatively constant (Curtit et al., 2013). Hormone receptor status is consequently most reliably assessed on primary tumours and prior to treatment with chemotherapy.

Studies of hormone receptor testing on core biopsies and subsequent excision specimens have shown comparable results (Wood et al., 2007). Tumour progression may be associated with a loss of ER expression (Macfarlane at al., 2012).

3.3.5 Molecular profile

In addition to ER, PR and HER2 negativity, triple negative tumours with a basal-like phenotype can be identified immunohistochemically by the expression of one or more of the following; Cytokeratin (CK) 5/6, CK14, CK17 and epidermal growth factor receptor (EGFR) (Ho-Yen et al., 2012 & Peng, 2012).

Triple negative tumours with a basal-like phenotype have a worse prognosis than those with other molecular subtypes (Leidy et al., 2014). Identification of this subset of tumours therefore has important prognostic implications. Other molecular subtypes making up non

basal-like triple negative tumours include the normal breast-like, claudin-low and interferon-rich categories (Ho-Yen et al., 2012).

Recently, Lehmann et al. (2011), through the analysis of gene expression profiles of TNBCs, identified 6 subtypes of TNBC exhibiting unique gene expression. These included basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like and luminal AR subtypes. Further gene expression analysis allowed identification of cell line models for each of these subtypes. By pharmacologically targeting the "driver" signalling pathways of these groups of tumours, they demonstrated different responsiveness to therapies, proving that gene expression signatures can guide therapy selection, providing a platform for the development of targeted therapy in TNBC. These subtypes have shown different rates of treatment response, including complete pathological response, highlighting that TNBC are heterogenous tumours (Bose, 2015 & Lehmann et al., 2011).

3.4 Treatment

3.4.1 Targeted treatments

ER positive cancers may respond to ER antagonists such as tamoxifen as well as aromatase inhibitors (Allizart et al., 2012). Tamoxifen binds to the oestrogen receptor and blocks oestrogen stimulated growth. Its use has been shown to reduce disease recurrence and prolong life in individuals with ER positive breast carcinomas (Allred, 2010). Tumours which overexpress HER2 may respond to trastuzumab treatment, a monoclonal antibody that inhibits the HER2 receptor. If these receptors are negative, the tumour is unlikely to respond to targeted treatment, offering no improvement in outcome (Allizart et al., 2012).

3.4.2 Surgery and chemotherapy

Surgery is the best treatment modality for the local control of triple negative tumours (Schmadeka et al., 2014). Triple negative carcinomas lack any specific targeted treatments. Combined chemotherapy with anthracycline, taxanes, ixabepitine and platinum agents remains the standard systemic treatment (Hudis et al., 2011 & Sharma et al., 2014). TNBC associated with BRCA1 mutations are particularly susceptible to platinum based agents and taxanes (Sharma et al., 2014). The experimental work by Lehmann et al. (2011) demonstrated that basal-like carcinoma, whether associated with BRCA mutations or not, showed a relatively higher sensitivity to cisplatin treatment compared with all other TNBC subtypes.

3.4.3 Molecular profiling and potential treatment options

Molecular profiling of breast carcinoma can accurately subtype tumours and provide important prognostic information. This assists in the selection of individuals who will benefit from a particular treatment, thus avoiding unnecessary treatment side effects in those who would not respond to a particular therapy. A number of molecular risk scores are commercially available for this purpose, including PAM50®, MammaType®, MammaPrint®, Oncotype DX®, Endopredict®, Genomic Grade Index® (Allizart et al., 2012, Sinn et al., 2013 & Schmadeka et al., 2014).

Immunohistochemical risk scores, including Mammostrat® and IHC4, have been designed as a more affordable alternative to the molecular risk scores. These require a standardised immunohistochemical procedure which uses immunohistochemistry as a surrogate for RNA-based gene signatures (Sinn et al., 2013).

A prospective study examining the utility of MammaPrint® concluded that its use can impact recommendations for adjuvant chemotherapy. In addition, it can decrease variability regarding treatment selection within and between institutions (Cusumano et al., 2014). These findings were mirrored in a study by Whitworth et al. (2014) who assessed both the BluePrint and MammaPrint® molecular assays.

Currently, therapeutic strategies targeting particular mutations in TNBC are in clinical trials. These include poly(ADP-ribose) polymerase inhibitors for BRCA-mutated TNBC, antiandrogens for AR positive TNBC, fibroblast growth factor receptor (FGFR) inhibitors for TNBC harbouring FGFR amplifications and gamma-secretase inhibitors for TNBC with mutations in the PEST domain of NOTCH proteins (Lehmann et al., 2015). ARs are expressed in 25.0 to 75.0% of all TNBCs, making them an important potential target in the treatment of these tumours (Safarpour et al., 2015).

Management of TNBC is challenging. To date, within the clinical setting, these tumours lack molecular targets and systemic chemotherapy remains the only available systemic treatment modality (Yadav et al., 2014 & Schmadeka et al., 2014).

3.5 Prognosis

Receptor negative tumours, perhaps due to their limited treatment options, have a poor prognosis and a higher overall mortality rate (Yadav et al., 2014). In addition, TNBCs have a recurrence rate of 33.9% compared to the reported recurrence rate of 20.4% in non-triple negative tumours (Lara-Medina et al., 2011 & Schmadeka et al., 2014). TNBCs recur more quickly than non-TNBCs and they have high relapse associated mortality (Foulkes et al.,

2010 & Schmadeka et al., 2014). 22.0% of triple negative tumours will recur within the first three years (Fayaz et al., 2013).

The brain and lung are common sites for metastases (Foulkes et al., 2010 & Schmadeka et al., 2014). Central nervous system and visceral metastasis are more common in TNBC than in other breast carcinoma subtypes (Bose, 2015).

Neoadjuvant chemotherapy in TNBC is associated with a higher pathologic complete response when compared to receptor positive tumours treated with neoadjuvant chemotherapy. Despite this initial response to treatment, triple negative tumours have a significantly worse three year progression free and three year overall survival (Schmadeka et al., 2014). The five year survival rate is increased with surgery and chemotherapy, however, the prognosis remains poor (Pal et al., 2014).

In triple negative tumours, AR positivity may be associated with better survival outcomes, thus AR expression may have prognostic and therapeutic value (Kim et al., 2015). Triple negative tumours with a lower proliferative index, as assessed using a proliferation marker such as a Ki67 immunohistochemical stain, have a better prognosis than those showing higher proliferation rates. Exact values were not defined (Schmadeka et al., 2014). Molecular profiling of breast carcinomas, in addition to assisting in the planning of treatment, can offer important prognostic information.

These aforementioned findings highlight that improved knowledge of TNBCs will allow for more accurate prognostication and potentially provide a platform for the development of more effective treatment strategies.

CHAPTER 4: MATERIALS AND METHODS

4.1 Clinical and laboratory setting

Approval of the title of for this research project was obtained from the University of the Witwatersrand (Appendix A). Research was conducted at the National Health Laboratory Service (NHLS) Laboratory of CMJAH. This laboratory is accredited by the South African National Accreditation System (SANAS). CMJAH is a referral hospital in Parktown, Gauteng with a 1088 bed capacity. It offers secondary, tertiary and specialised services to the population of Gauteng Province as well as neighbouring provinces (Wikipedia, 2015).

4.2 Study design

An observational, cross-sectional, retrospective study design was used.

4.3 Sample and sample size

All cases of breast carcinoma, including biopsy and excision specimens, diagnosed at the NHLS laboratory of CMJAH between 1 January 2011 and 31 December 2012 were identified by performing a search of our electronic database (DISA) using the 'Snomed' search program.

The following 'Snomed codes' were used in order to retrieve the cases:

- T 04000 Breast
- T 04020 Right breast

- T 04030 Left breast
- M 85003 Infiltrating duct carcinoma
- M 80003 Neoplasm malignant
- M 80103 Carcinoma
- M 85223 Infiltrating duct and lobular carcinoma
- M 81403 Adenocarcinoma
- M 85213 Infiltrating ductular carcinoma
- M 85000 Infiltrating ductal, lobular and medullary neoplasm

4.4 Inclusion and exclusion criteria

Once all the histopathology reports were retrieved, inclusion and exclusion criteria were applied.

4.4.1 Inclusion criterion

- Histologically confirmed breast carcinoma in which ER, PR and HER2 staining had been performed.

4.4.2 Exclusion criteria

- Breast tumours other than carcinomas.
- Cases where the hormone receptor status had not been established.

- Post chemotherapy specimens or where no information was provided regarding chemotherapy.
- Metastatic breast carcinomas (confirmed breast carcinomas at any site other than the breast, where the primary breast tumour was not available for assessment).
- Where slides for confirmation of receptor negativity could not be retrieved/located from storage.

4.5 Collection and recording of data

4.5.1 Retrieval and review of relevant data

As the criteria for determining hormone receptor status, as per the ASCO/CAP guidelines, had changed over the study period, the slides of all cases classified as ER or PR negative were retrieved from archives and reviewed to assess whether they still met current recommended ER/PR negativity criteria (Appendix D).

All immunohistochemical stains during the study period were performed on fully automated DakoAutostainer Link 45 machines. Monoclonal Mouse Anti-human Oestrogen Receptor α clone ID5 has been used for ER staining, Monoclonal Mouse Anti-human progesterone receptor clone PgR 636 has been used for PR staining and Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein has been used for HER2 staining.

All samples have been processed in the presence of adequate positive and negative controls. Quality control has been achieved by assessment of both internal and external controls. The pattern of staining in normal breast tissue present on the test provided an internal control.

External positive and negative controls were run concurrently on a separate slide and have been checked by a qualified pathologist within the department prior to issuing of the test. In addition, the CMJAH histopathology laboratory participates in an external quality assurance program run by the Royal College of Pathologists of Australasia (RCPA).

In order to establish reliability of the data, techniques to reduce inter- and intra-observer variability were employed. The slides were all initially assessed by the principal investigator and subsequently, every fifth slide was reviewed by the supervisor. No discrepancies arose and therefore review of slides by a third nominated pathologist was not required. In all cases, the supervisor and the principal investigator were blinded to previous findings.

Additional histology or cytology reports were reviewed and relevant information was included in the study to maximise the amount of available data. Where more than one histology/cytology result were available for a patient, the patient was given one unique study number and all data pertaining to the patient entered under this one study number.

As menopausal age status was not known patients in this for the present study, a surrogate age of 50.0 years was used to allow comparison with available data. This is in line with the findings from a large study of 18 997 females from multiple countries which demonstrated that the median age of menopause was 50.0 years (Gold et al., 2001). Similarly, a surrogate age of 50.0 years was applied in the study by Somali et al. (2013), with which we have compared our results.

Where possible, the tumour size was attained from the macroscopy section of the histology report. If this was not available, clinical details and imaging studies were used.

A tissue diagnosis (histology/cytology) was required to determine nodal status. Where only a clinical history/imaging studies were available, these cases were regarded as having absent/missing data with respect to lymph node status.

4.5.2 Analysis of data

Information from each case was entered onto a Microsoft Excel® data capture sheet (Appendix G). The patients' names and hospital numbers were initially recorded along with unique study numbers. Subsequent analyses utilised only the study number in order to achieve anonymity. Only the principal investigator had access to the original data capture sheet and this was kept separately from subsequent data capture sheets.

On completion of the data entry on an Excel spreadsheet, the results were analysed using *Statistica 12*. This was done in consultation with a statistician from the University of the Witwatersrand.

4.6 Statistical analysis

Descriptive analysis was performed. Each parameter was assessed in isolation and combination. Categorical data were assessed as frequencies and ratios and their relationships assessed using the Chi-squared test. Continuous data were assessed for normality. Where

appropriate, parametric tests (Student's t test) and non-parametric tests (Mann-Whitney U test) were applied. A significance level of 5% was used for this investigation.

4.7 Ethical considerations

The Department of Anatomical Pathology, University of the Witwatersrand has been granted blanket approval by the Committee for Research on Human Subjects of the University of the Witwatersrand to conduct retrospective studies (M10744). A formal project specific ethics application was submitted and ethical clearance obtained from the Human Research Ethics Committee of the University of the Witwatersrand (M150383) (Appendix H).

CHAPTER 5: RESULTS

5.1 Overview

A search of the electronic database (DISA) at CMJAH for the period 1 January 2011 to 31 December 2012 identified a total of 1126 individuals with invasive breast carcinoma. 182 of the 1126 individuals, according to the histology reports, had triple negative tumours.

As the criteria for determining hormone receptor status had changed over the study period, the slides of all cases classified as ER or PR negative were retrieved from archives and reviewed to assess if they still met recommended current ER/PR negativity criteria (Figures 1 to 7). Only four cases initially classified as ER or PR negative were re-classified as positive based on the new guidelines and this number was not statistically significant (P-value = 0.74). 37 cases (20.3%) could not be retrieved from storage for confirmation of the ER and PR status. A total of 141 tumours could be confirmed as triple negative and formed our final sample size on which subsequent analyses were performed.

182 (16.2%) of the 1126 individuals with breast carcinoma were reported as having triple negative tumours according to the initial review of the histology reports. 141 (13.0%) of the individuals had confirmed triple negative tumours after retrieval and review of the slides. This difference in prevalence is attributed mainly to an inability to retrieve cases from storage for confirmation of receptor status, rather than a change in the receptor status after reviewing the slides. The difference in prevalence (3.6%; 95% CI: 0.7%–6.5%) is statistically significant (P-value = 0.01)

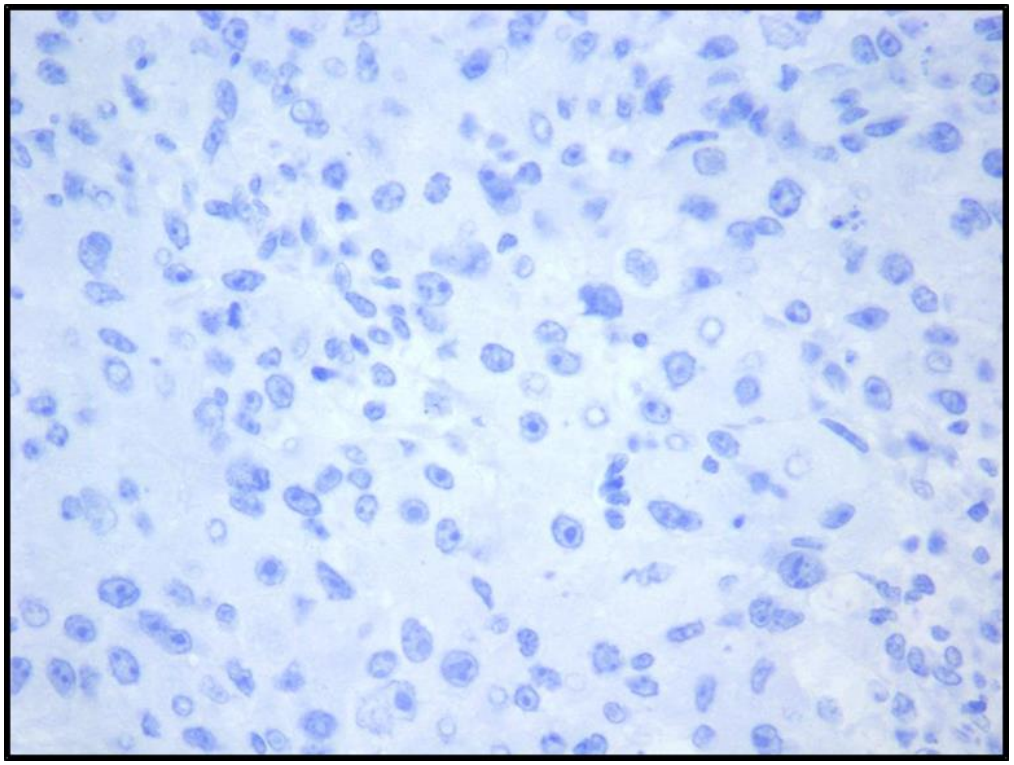


Figure 1. Micrograph (400x magnification) showing a negative ER immunohistochemical stain.

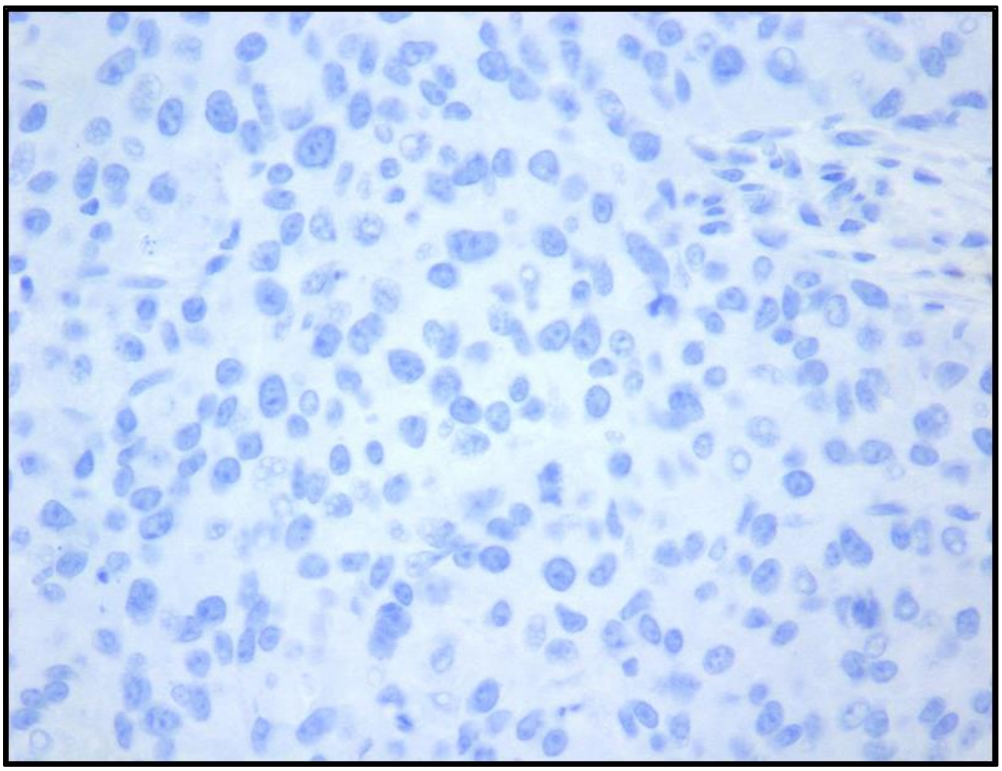


Figure 2. Micrograph (400x magnification) showing a negative PR immunohistochemical stain.

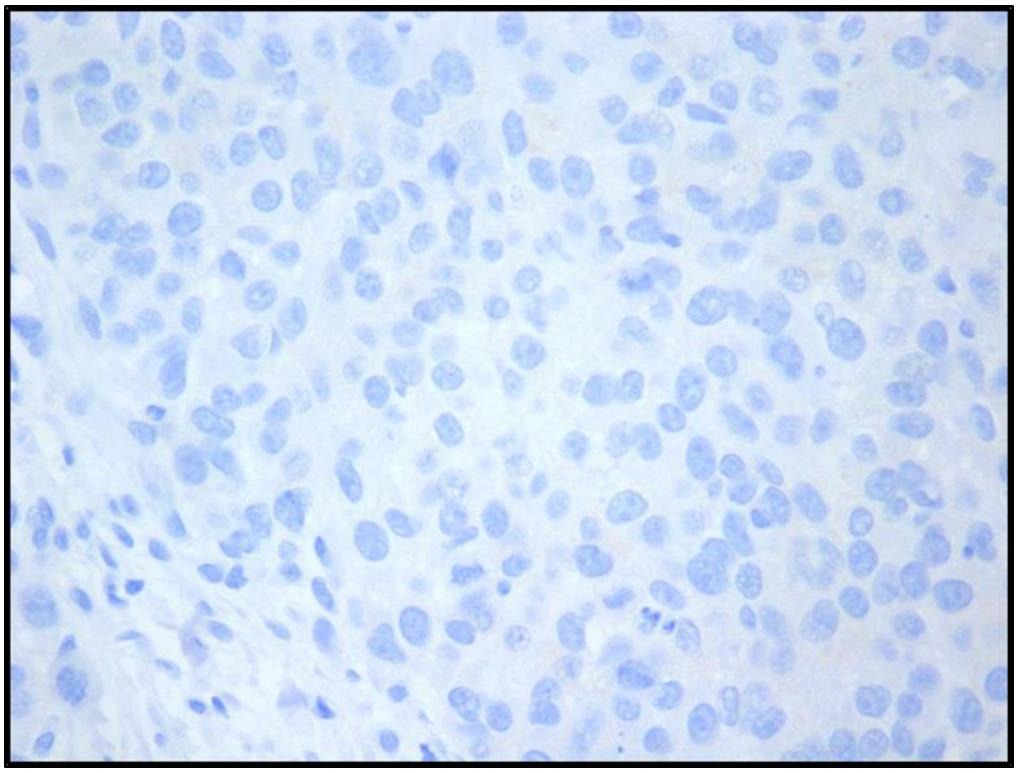


Figure 3. Micrograph (400x magnification) showing a negative HER2 immunohistochemical stain (IHC score 0).

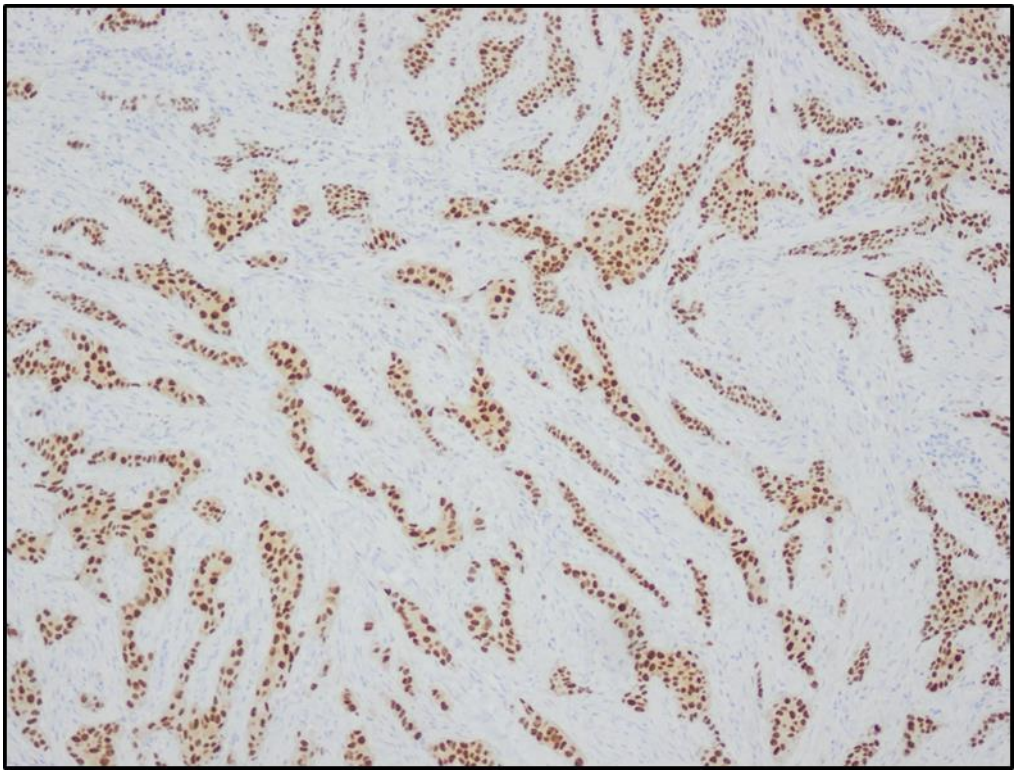


Figure 4. Micrograph (100x magnification) showing a positive ER immunohistochemical stain with strong nuclear staining in 66-100% of the tumour cells.

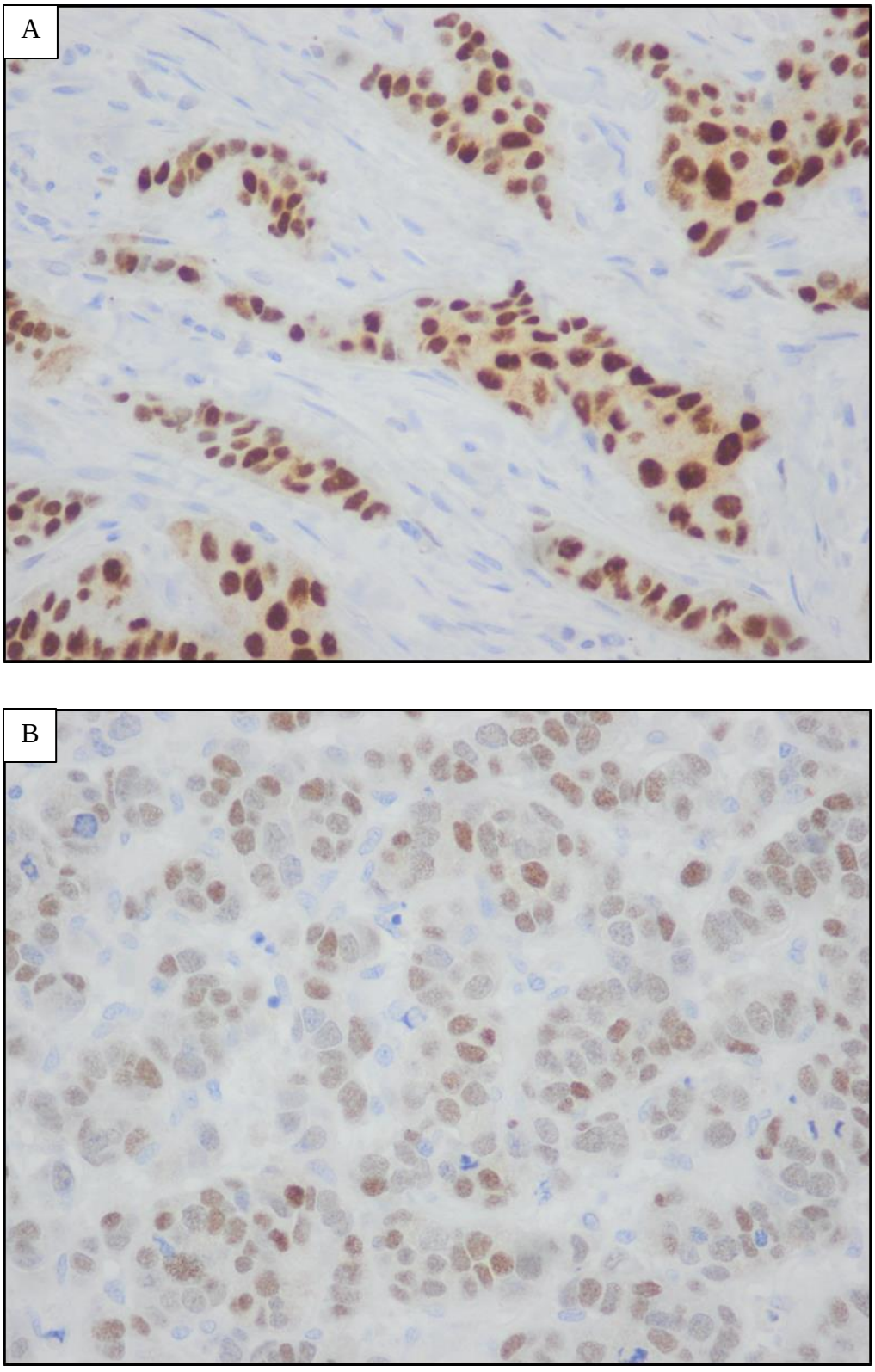


Figure 5. Micrographs (400x magnification) showing positive ER immunohistochemical stains with strong nuclear staining (A) and weak to moderate nuclear staining (B) within tumour cells.

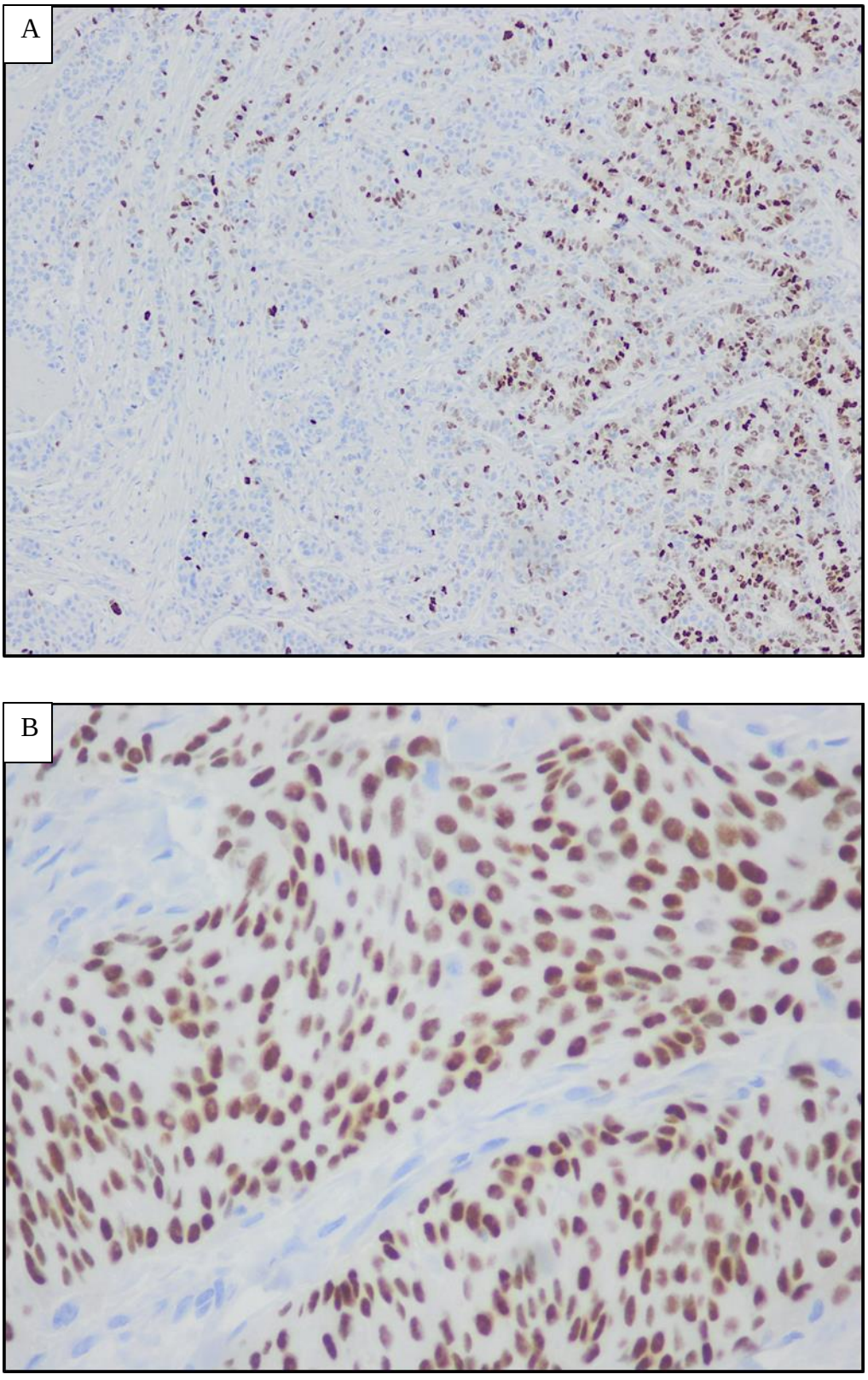


Figure 6. Micrographs showing positive PR immunohistochemical stains. Micrograph A (100x magnification) shows strong nuclear staining in 33-66% of tumour cells. Micrograph B (400x magnification) shows strong nuclear staining in 66-100% of tumour cells.

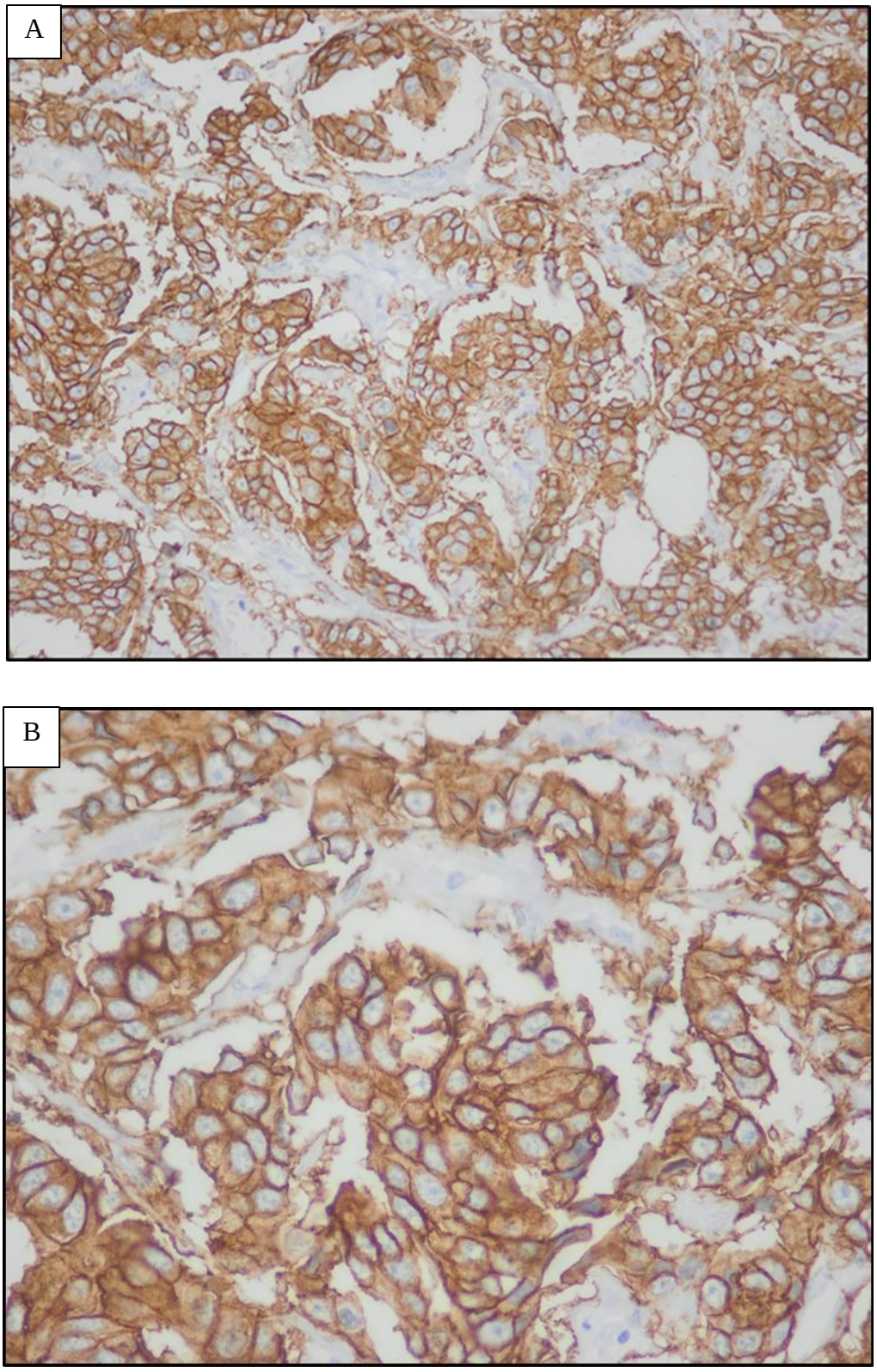


Figure 7. Micrograph showing a positive HER2 immunohistochemical stain/IHC score 3+ at 200x (A) and 400x (B) magnification.

Both core and excision biopsy results were assessed. In 36 cases (25.5%), both the core and excision biopsy results were available. For the majority of cases, 89 of the 141 (63.1%), only the core biopsy results were available while for the remaining 16 (11.3%), only excision biopsy results were available for assessment.

There was insufficient data available to assess concordance between the receptor statuses assessed on core and excision specimens from the same patients. This is as a result of departmental policy which dictates that receptor status is only repeated if the patient has undergone chemotherapy and if the time period lapsed between core biopsy and excision is greater than six months. Chemotherapy as well as disease progression are known to alter receptor status (Curtit et al., 2013 & Macfarlane et al., 2012).

Table 2. Characteristics of TNBC at CMJAH between 1 January 2011 and 31 December 2012.

Characteristic	**Category**	**Number (%)**
Total number of TNBCs		141
Gender	Male	2 (1.4)
	Female	139 (98.6)
Ethnicity	Black	3 (100.0)
	White	0

	Asian	0
	Coloured	0
	Missing data	138
Age at diagnosis (years)	<30	4 (2.9)
	31-40	12 (8.6)
	41-50	42 (30.0)
	51-60	38 (27.1)
	61-70	21 (15.0)
	71-80	15 (10.7)
	80-90	8 (5.7)
	>90	0 (0.0)
	Missing data	1
Tumour size (mm)	<20	12 (16.9)
	21-50	36 (50.7)
	>50	23 (32.4)
	Missing data	70

Histological subtype	Invasive carcinoma NST	128 (90.8)
	Lobular carcinoma	1 (0.7)
	Pleomorphic lobular carcinoma	1 (0.7)
	Metaplastic carcinoma	8 (5.7)
	Medullary carcinoma	2 (1.4)
	Apocrine Carcinoma	1 (0.7)
Tumour Grade	1	1 (0.7)
	2	42 (31.3)
	3	91 (67.9)
	Missing data	7
Tumour stage	I	5 (9.6)
	II	23 (44.2)
	III	23 (44.2)
	IV	1 (1.9)
	Missing data	89

In situ malignancy	Present	30 (34.1)
	Absent	58 (65.9)
	Missing data	53
Nodal involvement	Present	32 (69.6)
	Absent	14 (30.4)
	Missing data	95
Tumour focality	Unifocal	68 (90.7)
	Multifocal	7 (9.3)
	Missing data	66
Tumour laterality	Left	(54.5)
	Right	(43.2)
	Bilateral	(2.3)
	Missing data	9

5.2 Prevalence of triple negative tumours

182 (16.2%) of the 1126 individuals with breast carcinoma were reported as having triple negative tumours. After review of slides and confirmation of receptor negativity, 141 (13.0%) of the individuals had confirmed triple negative tumours.

5.3 Patient Demographics

5.3.1 Age

Age was available for 140 (99.3%) of the total 141 cases. Individuals with TNBC ranged from 28.0 to 89.0 years with a median age of 44.0 years. The mean age was 55.4 years with a standard deviation of 13.4 years. The majority of individuals (57.1%) were between 41.0 and 60.0 years of age. 2.9% of individuals were below 30.0 years of age (Figure 8).

Using 50.0 years of age as a surrogate for menopause, 41.4% of females with TNBC in the current study were premenopausal.

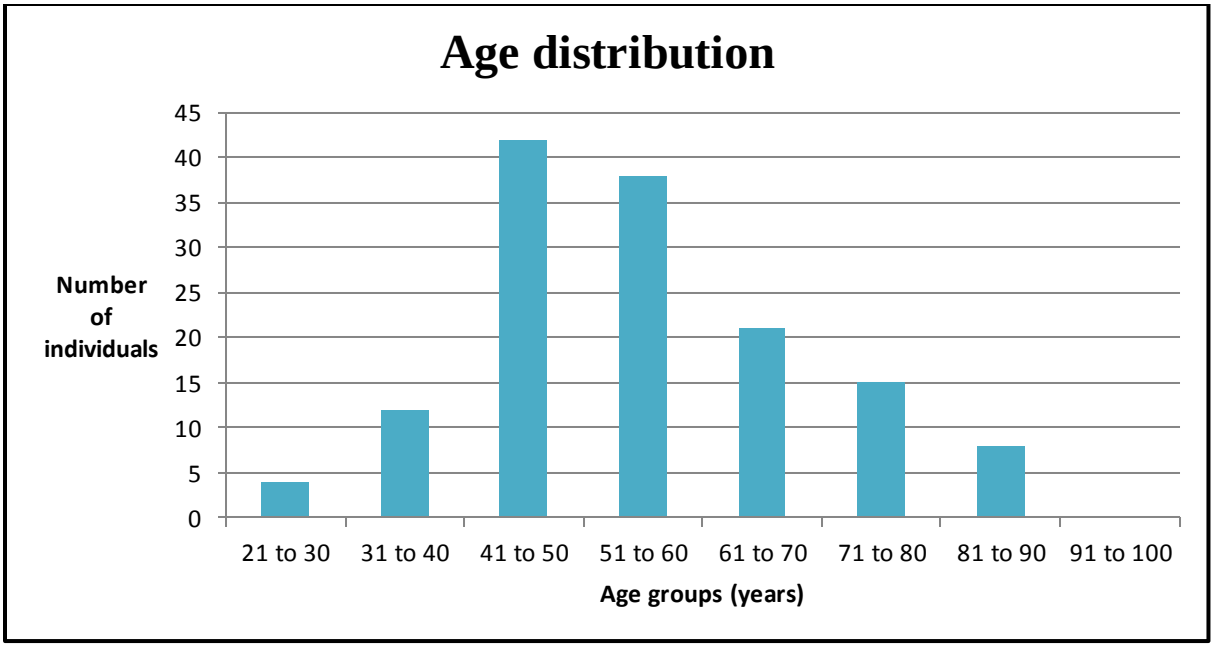


Figure 8. Graphical representation of the age distribution of individuals with TNBC.

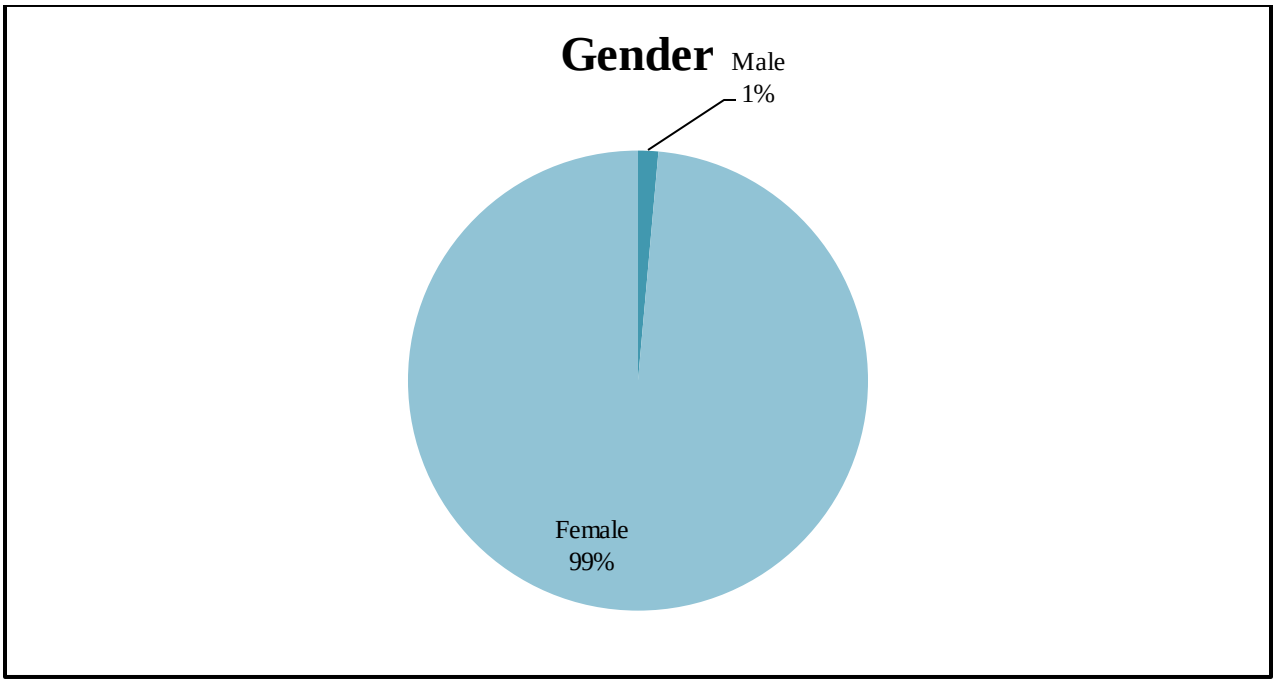


Figure 9. Graphical representation of the gender distribution of individuals with TNBC.

5.3.2 Race

Race was available for only three (2.1%) individuals, all of whom were Black.

5.3.3 Gender

Information regarding gender of the individuals was available for 100% of the cases. 139 (98.6%) of the individuals were female while 2 (1.4%) were male (Figure 9). The female to male ratio was 69.5:1.

5.3.4 Family history

There was no clinical information available regarding family history.

5.4 Tumour characteristics

5.4.1 Tumour size

Information regarding tumour size was available in 71 cases (50.3%). The tumours ranged in size from 6.0 mm to 160.0 mm, with a median size of 36.0 mm. The mean size was 48.3 mm with a standard deviation of 33.1 mm (Figure 10).

Macroscopic size, as assessed in the histology laboratory at time of grossing, was available for 52 (73.2%) tumours. Information regarding tumour size for the additional 19 cases was obtained from the clinical details section of the histology report; this included 14 (19.7%) cases where size was estimated on imaging studies and 5 cases (7.0%) where tumour size was estimated clinically.

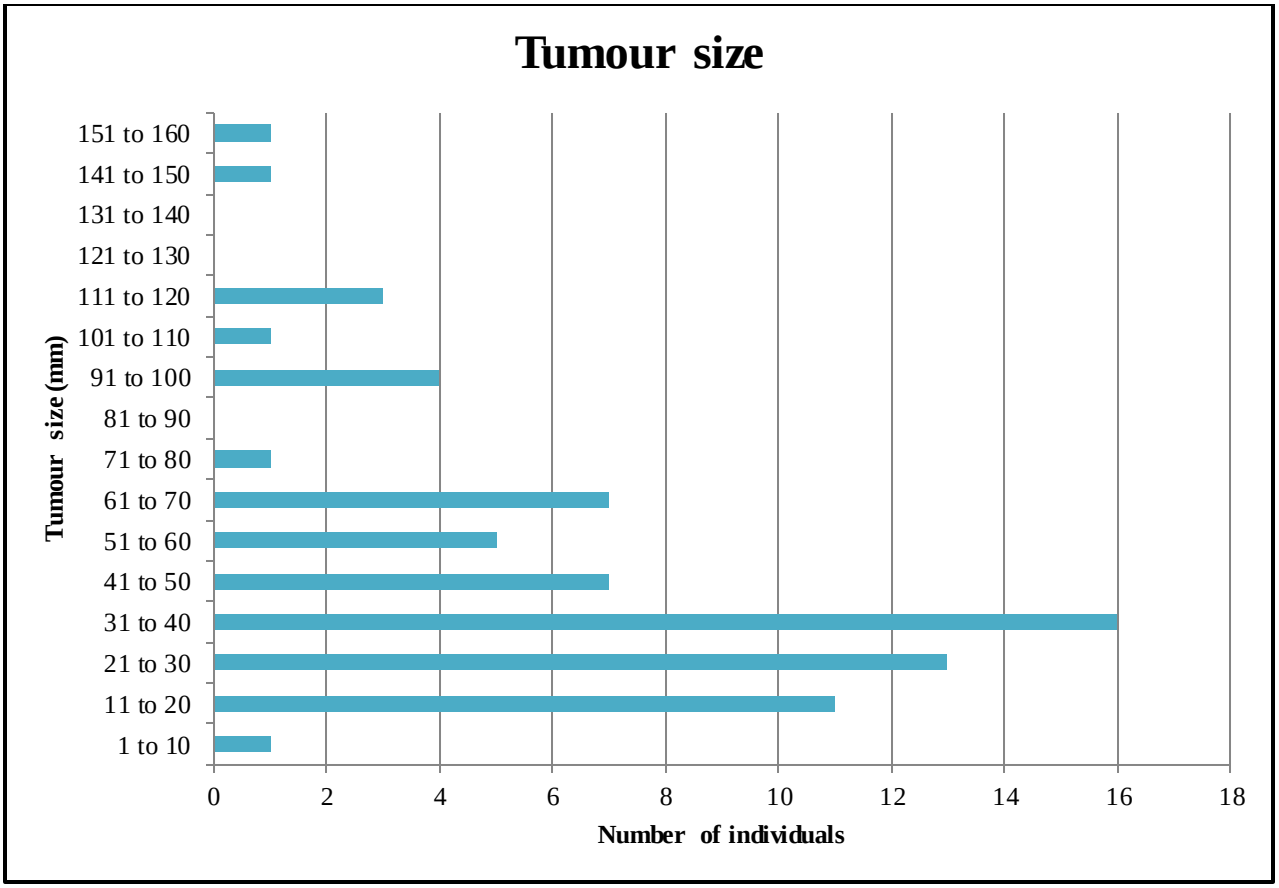


Figure 10. Graphical representation of the size of TNBCs.

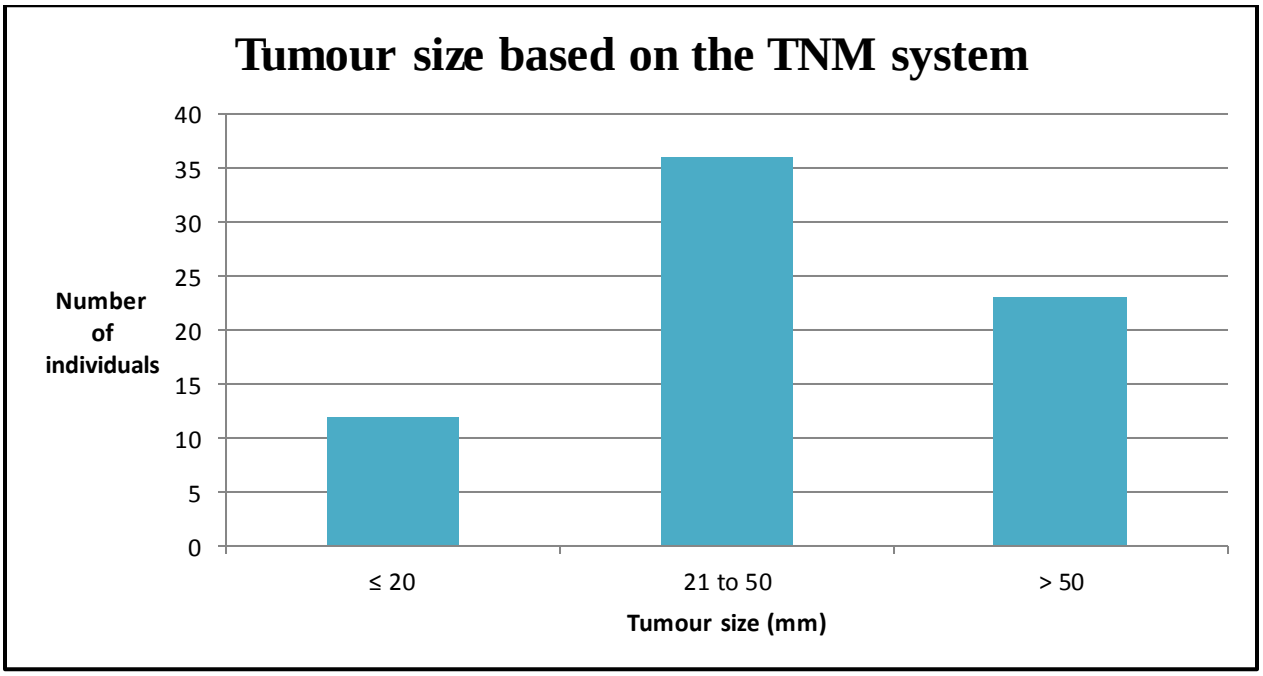


Figure 11. Graphical representation of TNBC size based on the TNM size groupings.

Evaluation of the size distribution of the tumours, using values which correspond to the TNM size grouping, revealed that the majority of tumours (50.7%) corresponded to T2 tumours (21 to 50 mm). T1 tumours (≤20 mm) comprised 16.9%, while T3 tumours (>50 mm) comprised 32.4% of the remaining TNBC tumours (Figure 11).

5.4.2 Histological subtypes

Information regarding the histological subtype of the tumours was available for 141 cases (100%). 128 cases (90.8%) were invasive carcinomas of no special type and 2 (1.4%) were lobular carcinomas. One lobular carcinoma was classified as a pleomorphic lobular carcinoma. The remaining histological subtypes included 8 (5.7%) metaplastic, 2 (1.4%) medullary and 1 (0.7%) apocrine carcinomas (Figure 12).

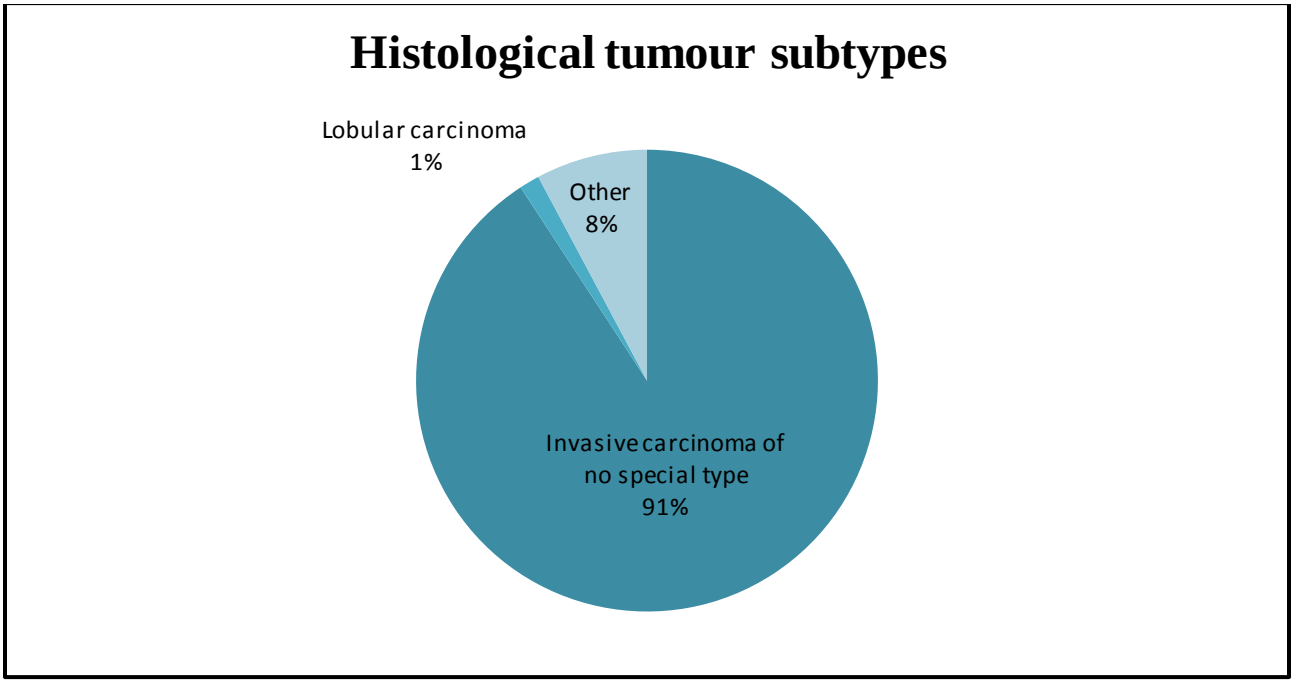


Figure 12. Graphical representation of the histological subtypes of TNBCs.

5.4.3 Tumour grade

Information regarding the tumour grade, as per the modified Bloom and Richardson grading system, were available for 134 (95.0%) of the 141 cases. A single tumour (0.7%) was grade 1, 42 tumours (31.3%) were grade 2 and 91 tumours (67.9%) were grade 3 (Figures 13 and 14).

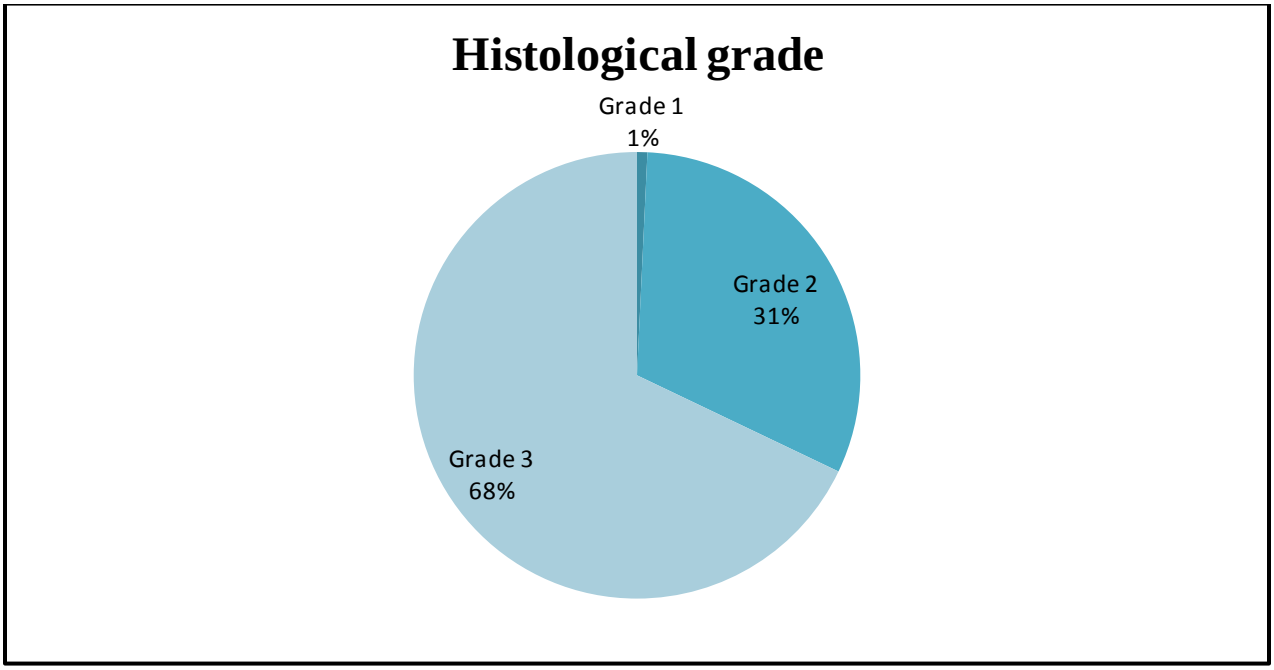


Figure 13. Graphical representation of the histological grade (based on the modified Bloom and Richardson score) of TNBCs.

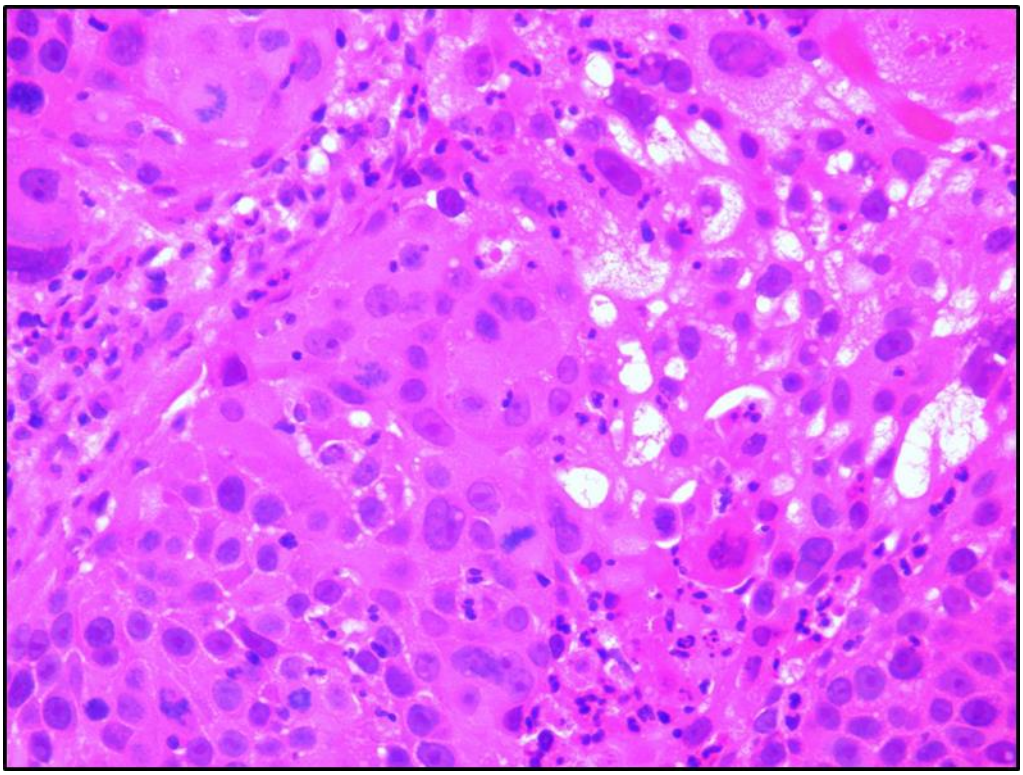


Figure 14. Haematoxylin and eosin stain (H&E) at 400x magnification demonstrating high grade nuclear features, including mitotic activity, lack of tubule formation and marked nuclear pleomorphism.

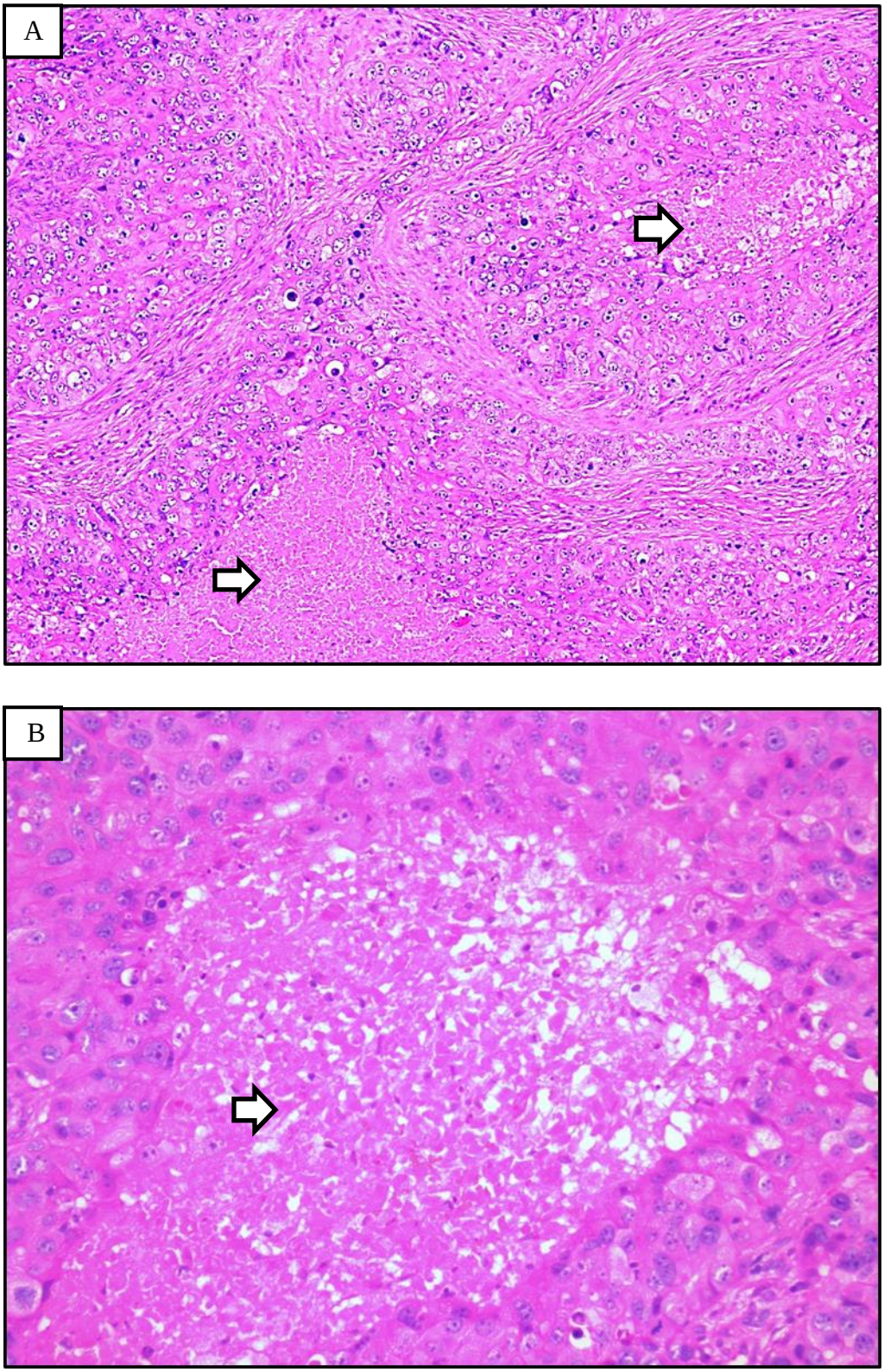


Figure 15. H&E micrograph showing areas of central necrosis (arrows) at 100x (A) and 400x (B) magnification.

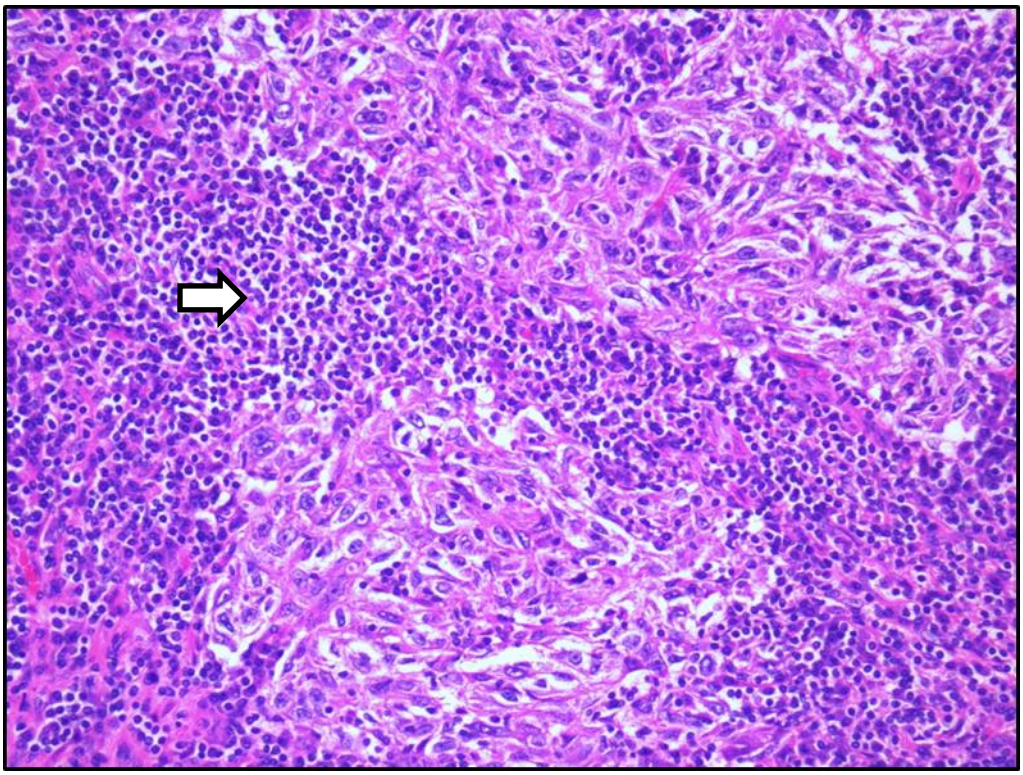


Figure 16. H&E (200x magnification) showing peritumoural lymphocytes (arrow).

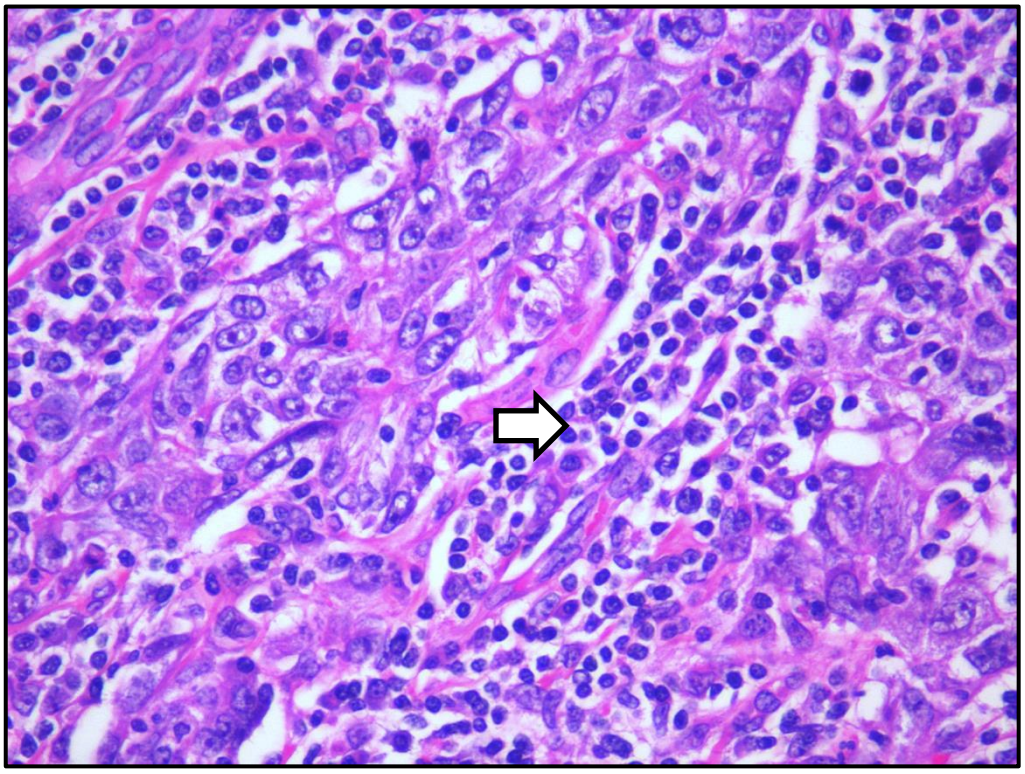


Figure 17. H&E (400x magnification) showing intratumoural lymphocytes (arrow).

5.4.4 Tumour Stage

Information regarding pathological tumour stage was available for 52 (36.9%) of the 141 cases (Figure 18).

- 5 individuals (9.6%) had stage I disease.
- 23 individuals had stage II disease. Of the 23 individuals with stage II disease, 47.8% (n=11) had stage IIA and 52.2% (n=12) had stage IIB disease.
- 23 individuals had stage III disease. Of the 23 individuals with stage III disease, 43.5% (n=10) had stage IIIA, 26.0% (n=6) had stage IIIB and 30.4% (n=7), stage IIIC disease.
- A single individual (1.9%) had stage IV disease.

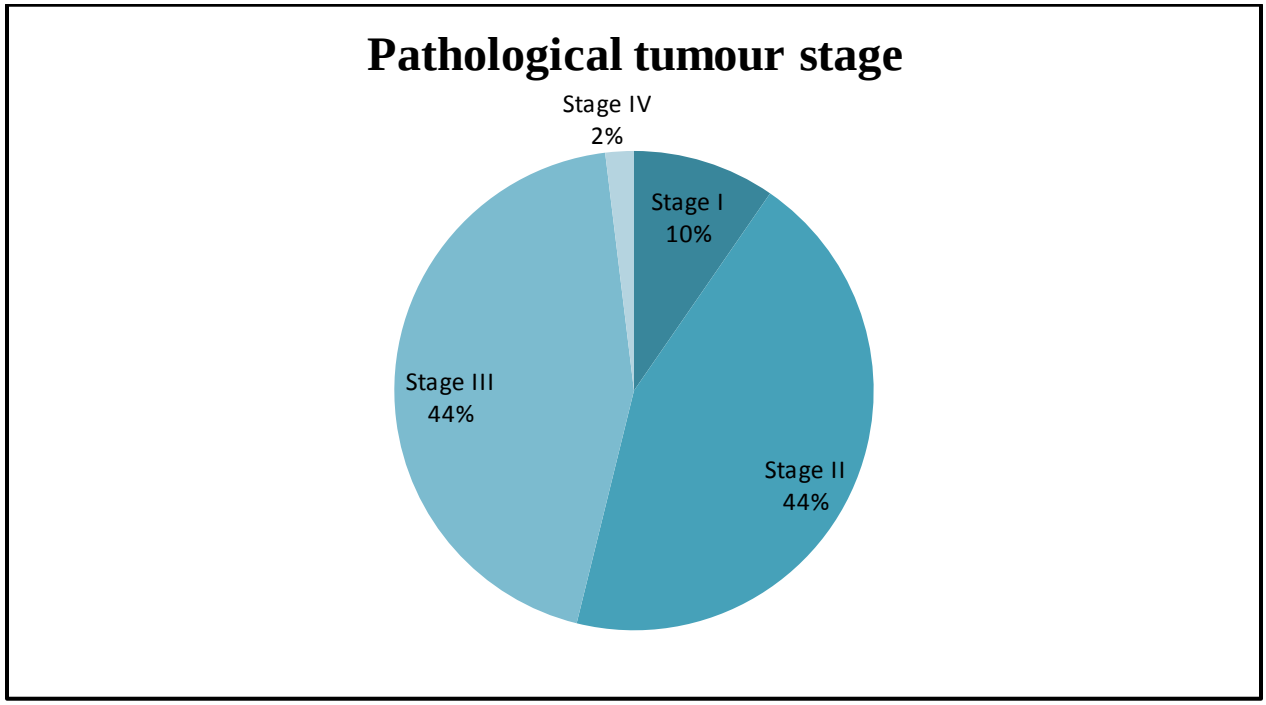


Figure 18. Graphical representation of the pathological tumour stage in individuals with TNBC.

5.4.5 In situ malignancy

The presence or absence of concurrent in situ malignancy was known in 88 cases (61.1%). 30 cases (34.1%) had concurrent in situ malignancy, the remaining 58 (65.9%) did not (Figures 19 and 20).

5.4.6 Nodal involvement

The presence or absence of nodal involvement was known in 46 of the 141 cases (32.6%). 32 cases (69.6%) showed nodal involvement, the remaining 14 cases (30.4%) were node negative (Figure 21). In all but one case, nodal involvement was determined by histological evaluation of the lymph nodes (Figure 22). The remaining lymph node was assessed as positive on cytological examination of material obtained by fine needle aspiration.

5.4.7 Tumour focality

Information regarding tumour focality was available in 75 (53.2%) of the 144 cases. In 68 individuals (90.7%), a single tumour was identified while the remaining 7 individuals (9.3%) had multifocal tumours (Figure 23).

5.4.8 Tumour laterality

Information with respect to tumour laterality was present in 132 (91.7%) of the 144 cases. 72 tumours (54.5%) were left sided, 57 tumours (43.2%) were right sided and 3 individuals (2.3%) had bilateral tumours (Figure 24). The proportion of individuals presenting with right and left sided tumours was not significantly different (P-value = 0.065).

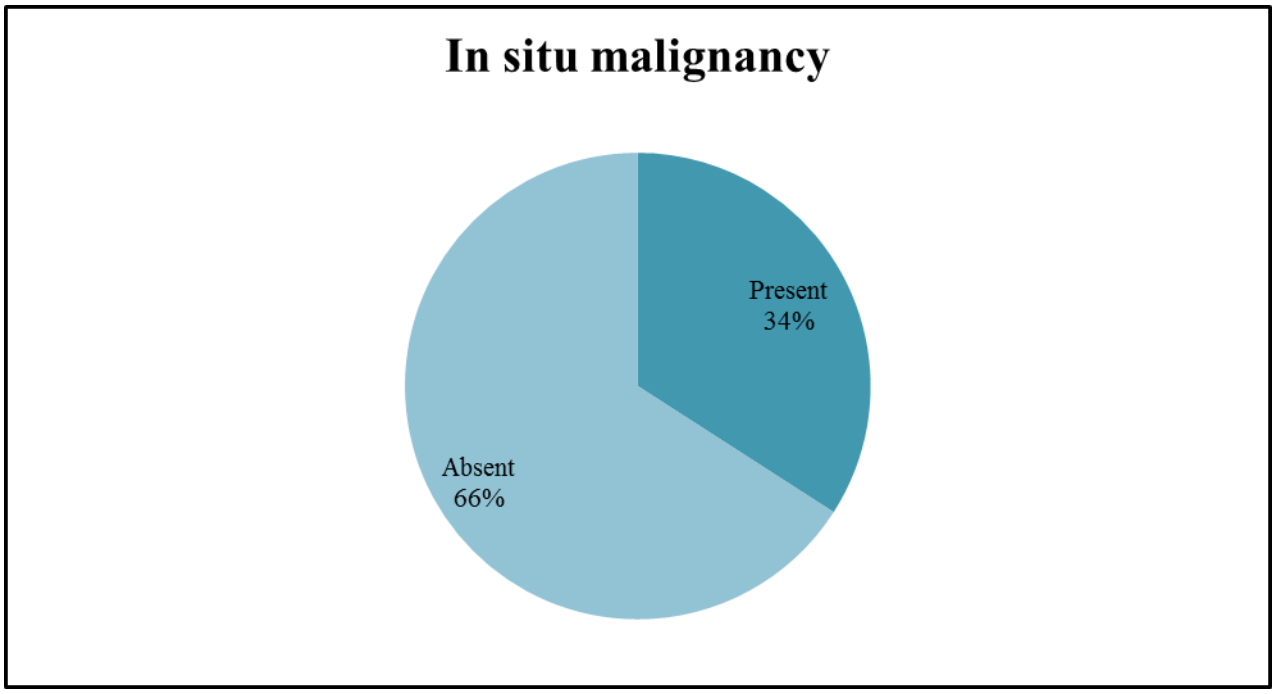


Figure 19. Graphical representation of concurrent in-situ malignancy in individuals with TNBC.

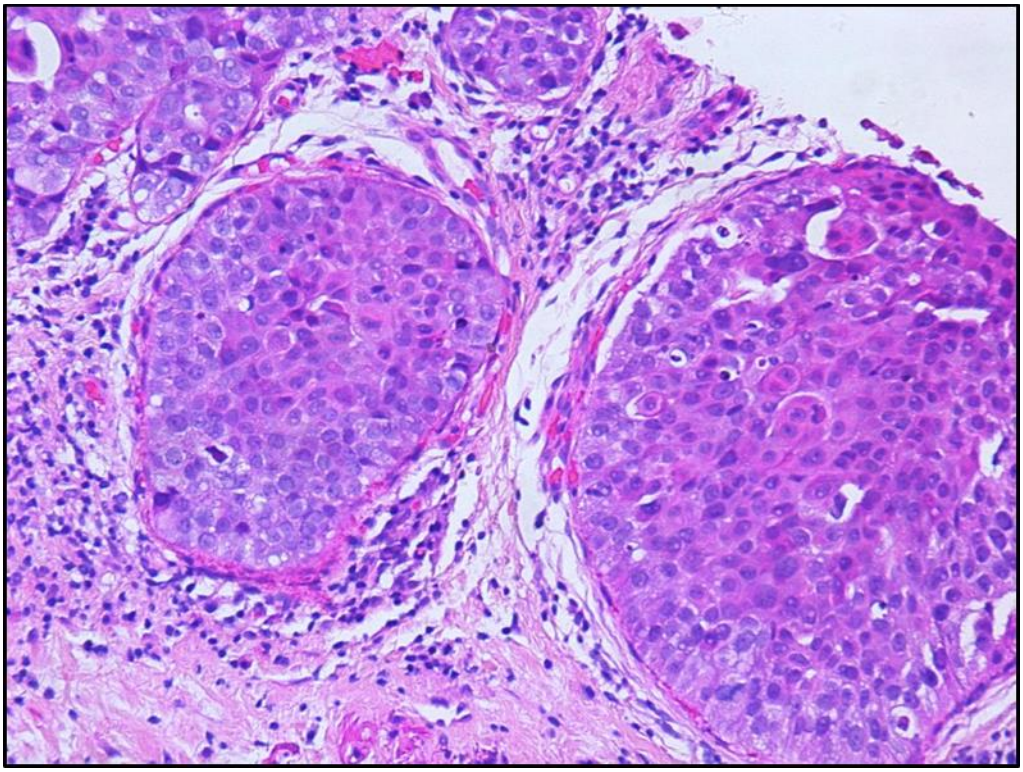


Figure 20. H&E (200x magnification) showing ductal carcinoma in-situ of the breast.

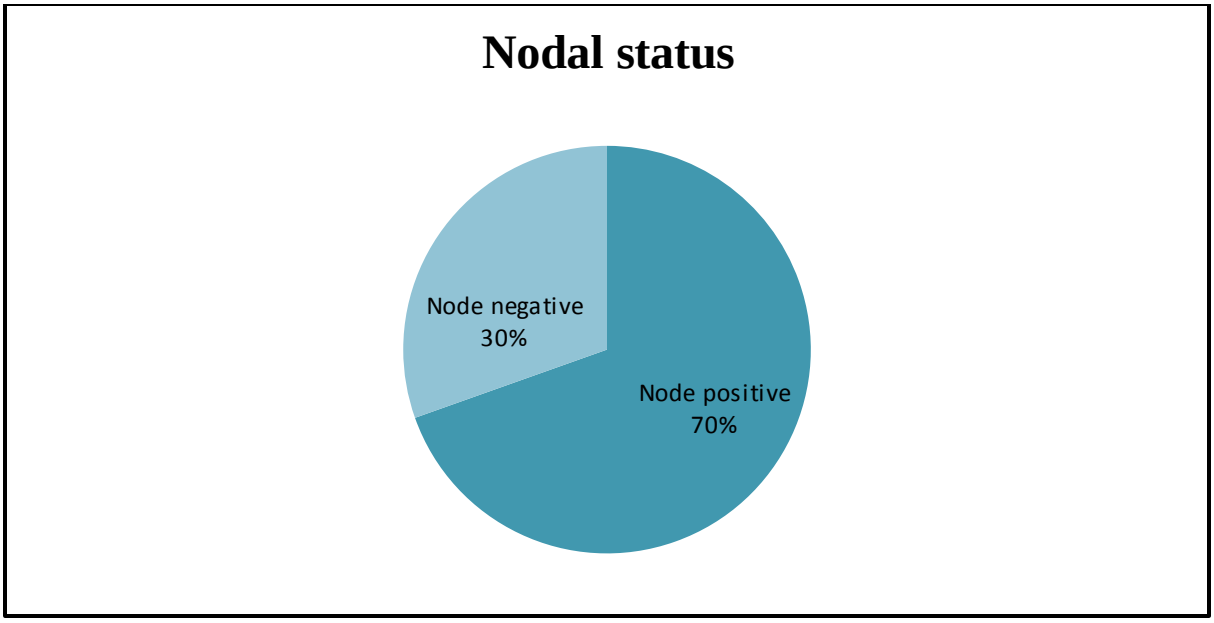


Figure 21. Graphical representation of nodal involvement in individuals with TNBC.

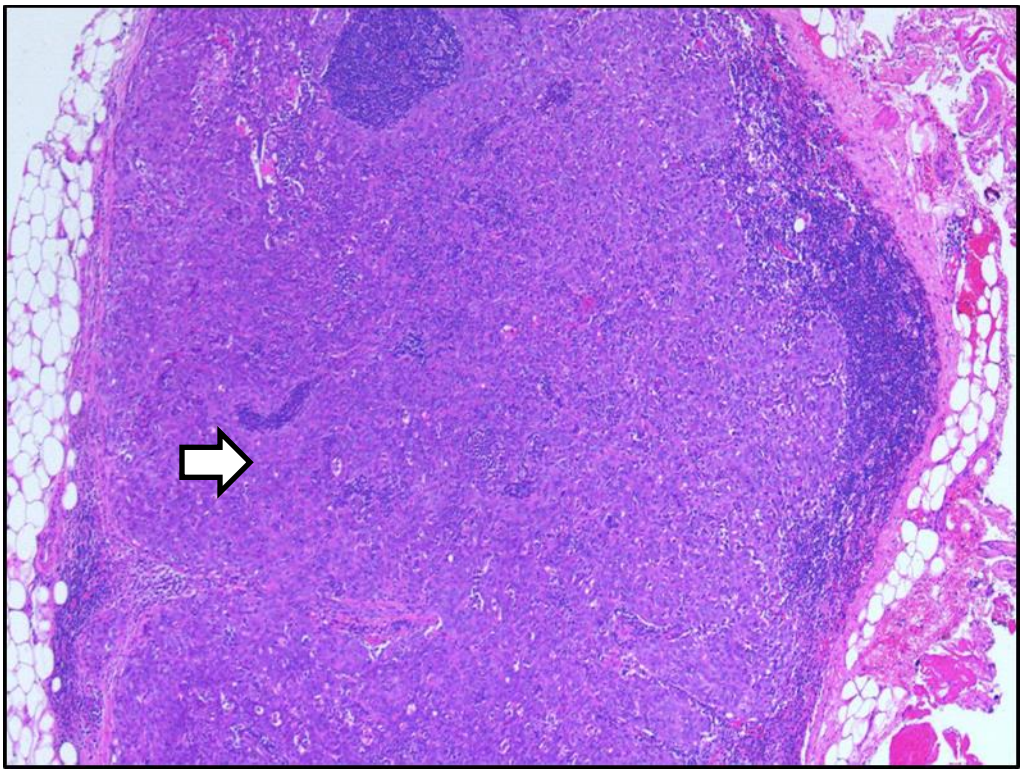


Figure 22. H&E (40x magnification) showing lymph node involvement by a metastatic TNBC (arrow) with a rim of lymphocytes seen at the periphery.

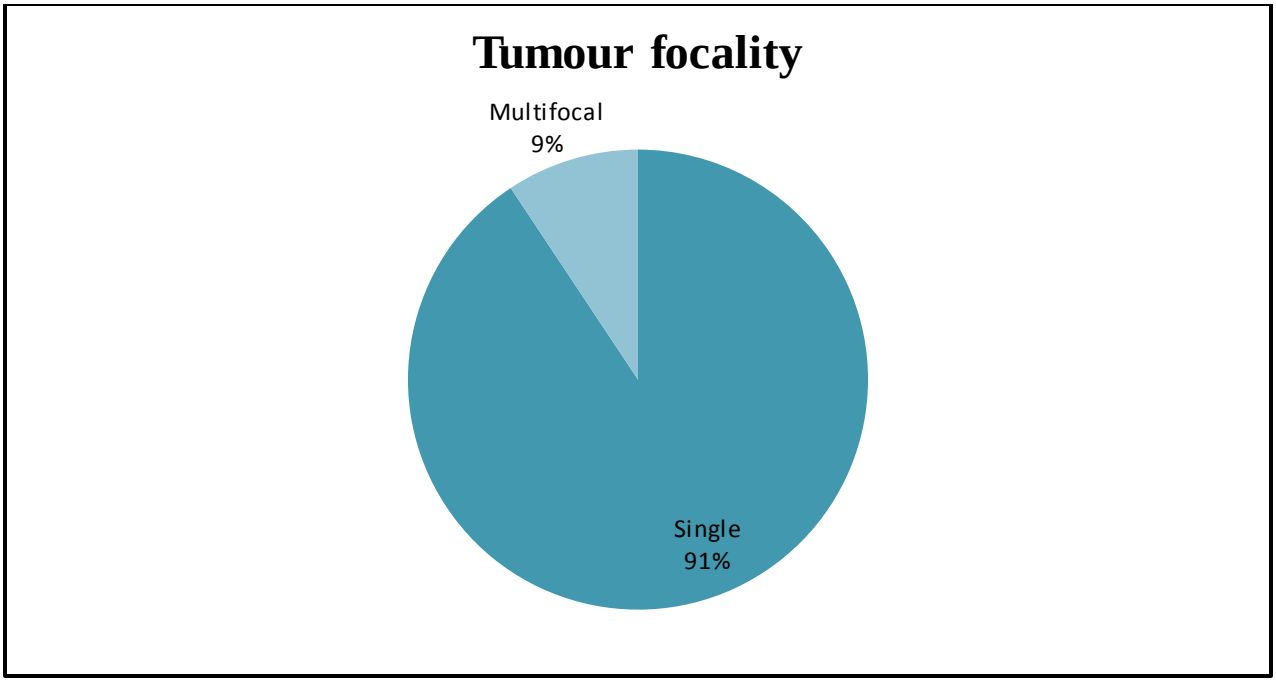


Figure 23. Graphical representation of tumour focality in TNBC.

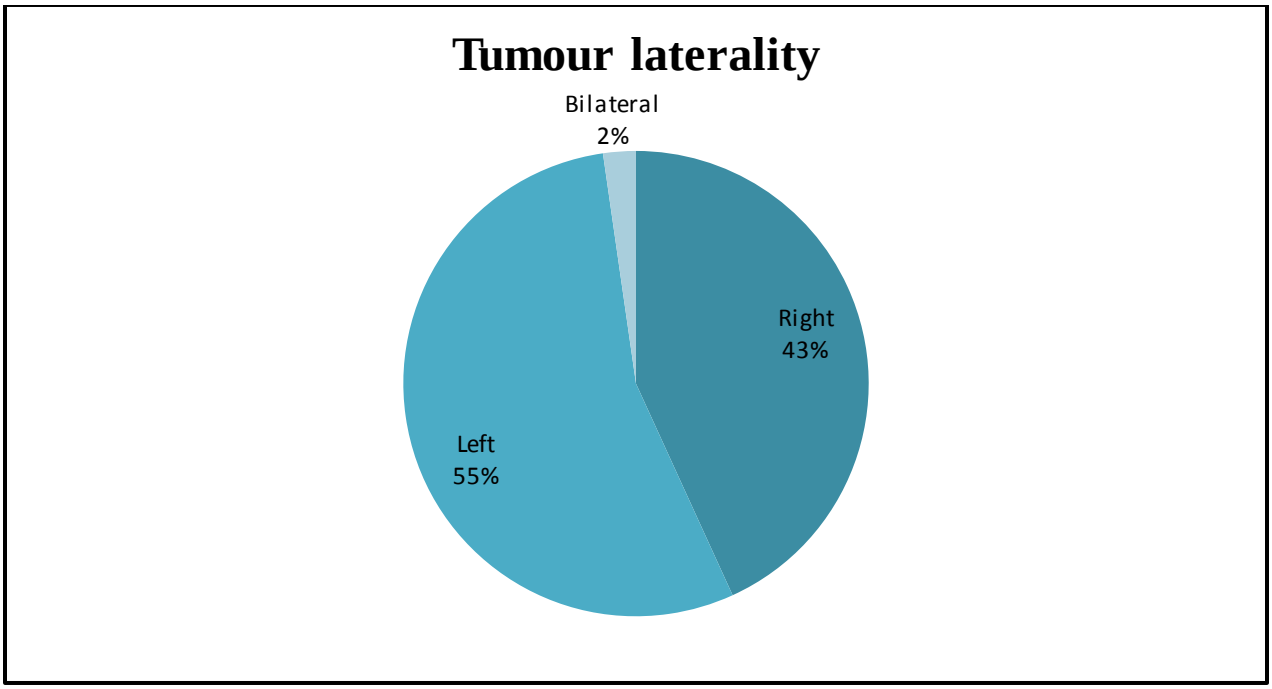


Figure 24. Graphical representation of tumour laterality in TNBC.

CHAPTER 6: DISCUSSION

In this observational, cross-sectional, retrospective study at CMJAH, a South African public sector hospital, we observed a 13.0% prevalence of triple negative tumours. This prevalence is lower than the 21.0% reported prevalence at CHBAH, Soweto (McCormack et al., 2013). One possible explanation for this difference in prevalence is a difference in racial demographics between the two study populations. TNBCs are more common in Black individuals (Cadoo et al., 2013, Schmadeka et al., 2014 & Wheeler et al., 2013) and the majority of the study population (91.0%) from Soweto were Black (McCormack et al., 2013). TNBCs are also more common in individuals of a lower socioeconomic status (Sineshaw et al., 2014). It may be that the population attending CMJAH is of a higher socioeconomic status or that the population included less Black individuals.

Unfortunately, information with respect to race and socioeconomic status was not available for the present study in order to confirm or exclude this hypothesis. Statistics regarding the racial demographics of individuals attending CMJAH were not available from the hospital itself or from online government resources. Subsequent studies on the receptor status in individuals from CMJAH should strive to ensure that the demographic profile, inclusive of race and socioeconomic status, is available.

Our 13.0% prevalence is also lower than that reported in other African studies. Although our sample size was similar to the Soweto based study (McCormack et al. 2013), the studies

emanating from the other African countries were small, with a total size of 226 from Uganda (Galukande et al., 2014) and 304 from Kenya (Sayad et al., 2014).

It has been suggested that the high rates of receptor negativity in sub-Saharan Africa may be due to false negative results (McCormack et al., 2013). Taking this into consideration, part of our relatively low prevalence could be attributed to our laboratory being subjected to stringent quality assurance, including accreditation by SANAS as well as various internal and external quality control programmes.

Our prevalence is similar to that seen in China (12.2%), California (12.5%), Kuwait (12.2%), England (10.0%) and Turkey (14.9%) (Table 1). The studies from China and California were robust, comprising 21 749 and 92 358 participants respectively (Bauer et al., 2007 & Li et al., 2013).

In 2010, ASCO/CAP published new guideline recommendations for immunohistochemical testing of ER and PR in breast carcinoma (Hammond et al., 2010) and these updated guidelines where used in the present study. The studies with which we compared our findings were published between 2007 and 2014 and it is therefore likely that the criteria for determining ER and PR status varied between these studies. Within the present study, only four cases initially classified as ER or PR negative were re-classified as positive based on the new guidelines and this number was not statistically significant (P-value = 0.74). It is thus unlikely that the different guidelines used in assessment of ER and PR status amongst the various studies would alter the validity of our comparisons.

Data pertaining to age are well represented in our study with age being available for 99.3% of cases. Individuals in the study population had a median age of 44.0 years and a mean age of 55.4 years (SD ± 13.4 years). The majority of individuals in this study were between 41 and 60 years of age and 2.9% of individuals were below 30 years of age. These findings are similar to those available in the literature (Ishikawa et al., 2011, Li et al., 2013 & Sharma et al., 2014). The similarity of our results with those of other studies, including international studies, allows for comparability between these populations and validates our data.

Table 3. Table comparing the percentage of premenopausal females with TNBC amongst various studies.

Study	**Country**	**Sample size**	**Premenopausal (%)**
Fayez et al., 2014	Kuwait	2,986	61.0
Li et al., 2013	China	21,749	54.9
Sayed et al., 2014	Kenya	304	62.4
Somali et al., 2013	Turkey	882	42.2
Present study, 2015	Johannesburg, South Africa	1,126	41.4

TNBCs are more common in premenopausal than postmenopausal females (Fayez et al., 2014). As menopausal age was not known for the present study, a surrogate age of 50 years was used to allow comparison with available data. 41.4% of females with TNBC in the current study were premenopausal. This percentage of premenopausal females is lower than that of the studies from China, Kuwait and Kenya. This difference is statistically significant

in all three cases (P-value <0.001) (Table 3). Different racial and socioeconomic factors, with differences in contraceptive methods, hormone replacement therapy use, obesity and genetic risk factors may provide a possible explanation.

The percentage of premenopausal females in our study is almost identical to the 42.2% identified in the study from Turkey (Somali et al., 2013). Further investigation into this similarity may draw valuable comparisons. The study from Turkey used a surrogate age of 50 years to define menopausal status whilst in the remaining studies menopausal status was known. Additional studies with accurate determination of menopausal status are required to validate our finding of higher prevalence in postmenopausal females and to identify the possible causes for variability of prevalence/incidence of TNBC among postmenopausal and premenopausal females.

Race was only available for three individuals in the present study, all of whom were Black. This has formed a major limitation of the current study. Drawing conclusions based on race with respect to prevalence would have been very informative, especially when comparing the present study with the study from our neighbouring hospital in Soweto.

Likewise, there was no clinical information available with respect to family history. Information regarding family history may provide valuable insight into the risk factors for TNBCs. The presence of breast carcinoma at a young age, especially TNBC, coupled with a positive family history could alert the pathologist to the possibility of a BRCA mutation. This information would be of assistance in terms of risk factor stratification and determining the pathogenesis of these tumours. Furthermore, a comment in the histology report regarding

the possible need for BRCA mutational analysis could assist the treating clinician with further management. For a single case of a 34 year old female, there was mention of previous ovarian carcinoma by the clinician on the requisition slip. Despite a search of the NHLS electronic database, the histology report for this ovarian tumour could not be located. Females with BRCA mutations have an increased risk of developing ovarian carcinomas (George et al., 2014).

The lack of data is attributed to the retrospective nature of the study which relies on appropriate completion of requisition slips by the attending clinician. Clinicians should be aware of the benefits of including all relevant clinical information, including pertinent negatives, on the histology requisition slip.

Information regarding gender was available for 100% of the cases and in the present study TNBCs are approximately 70 times more common in females than in men. This is in keeping with available data which shows that the prevalence of TNBC is lower in males than in females, ranging from 4% (Sayad et al., 2014) to 5.7% (Aschie et al., 2013). The study by Sayad et al. (2014) had a sample size of 304 individuals and included 12 men while the study by Aschie et al. (2013) was relatively large comprising 35 males with breast carcinoma. Further large studies are required for the accurate evaluation of receptor status in men.

Exposure to oestrogen is central to the development of breast carcinoma, providing an explanation for the lower incidence of breast carcinoma in men compared to females (Abdulkareem, 2013). Androgen deficiency and conditions associated with excess oestrogen are risk factors for the development of breast carcinoma in men (Thomas et al., 1992).

France et al. (2000) outlined the social aspects of male breast carcinoma. Men showed a greater failure to report symptoms than their female counterparts, in most cases due to a lack of awareness of what the symptoms could indicate. In addition, most breast masses were not painful and were therefore ignored. Men were shown to visit the doctor less frequently than females. This finding is substantiated by a study from the United Kingdom which showed that the consultation rate of men was 32% lower than that of females and that this difference was only partially accounted for by reproductive associated consultations. Non-consultation of men was greatest in individuals between the ages of 16 to 60 years (Wang et al., 2013) and most breast carcinomas in our population occurred within this age group. McLaren et al. (2014) identified distance to a healthcare facility as a barrier to accessing healthcare. Males and females have different patterns of health care utilisation, with decreased utilisation associated with distance being larger for men than it is for females. There is a low degree of suspicion for male breast carcinoma amongst doctors and both male and female patients typically believe that breast carcinoma is a female disease (France et al., 2000). These findings highlight the need for education pertaining to male breast carcinoma, aimed at both healthcare professionals as well as at the public.

Information regarding the histological subtype of the tumours was well represented, being available for 100% of individuals in our study. The majority of cases (90.8%) were invasive carcinomas of no special type. This is in keeping with the findings in the literature (Schmadeka et al., 2014, Sharma et al., 2014 & Qingli et al., 2014). The remaining histological subtypes included lobular, pleomorphic lobular, metaplastic, medullary and apocrine carcinomas. Although metaplastic, medullary and apocrine carcinomas are well recognised histological subtypes of triple negative tumours (Bose, 2015) invasive lobular

carcinomas are a less common subtype. Somali et al. (2013) demonstrated that although invasive lobular carcinomas were overall the second most common histological breast carcinoma subtype, they were uncommon in TNBCs, accounting for only 1.5% of the tumours. This is identical to the 1.5% incidence of invasive lobular carcinoma in the present study. The similarity of our results with those of international studies allows for comparability between these populations and corroborates our data.

Information regarding tumour grade was well represented in the current study, being available for 95% of the cases. Grade 3 tumours were most common and accounted for 67.9% of cases. Our findings are in keeping with those in the literature (Table 4). In all but two studies, grade 3 tumours were the most common. In these two studies from Turkey and China, the majority of tumours were grade 2. Lara-Medina et al. (2011) showed that in Hispanic females, 78.5% of TNBC were grade 3 tumours. Our findings are comparable to the study on receptor status conducted in Soweto, where only 4.3% of TNBCs presented as grade 1 tumours. The majority of the tumours in this study comprised grade 2 (29.3%) and grade 3 tumours (66.5%) (McCormack et al., 2013). Individuals with TNBCs, whether from developed or developing countries i.e. irrespective of geographic location, present most commonly with a high histological grade. This may indicate that high tumour grade in TNBC is unaffected by factors such as race and socioeconomic status. This is in contrast to non-TNBCs where high grade breast carcinomas are associated with lower socioeconomic status.

Table 4. Table comparing the tumour grade of TNBCs amongst various studies.

Study	**Country**	**Sample size**	**Tumour grade (%)**		
			1	2	3
Bauer et al., 2007	California, USA	92358	3.1	15.7	71.1
Fayez et al., 2014	Kuwait	2986	10	33	57
Lara-Medina et al., 2011	Mexico	2074	4.1	17.5	78.5
Li et al., 2013	China	21749	23.3	42.2	34.5
McCormack et al., 2013	Soweto, South Africa	1218	4.3	29.3	66.5
Sayed et al., 2014	Kenya	304	7	39.3	53.7
Sharma et al., 2014	North East India	972	4	28.3	67.7
Somali et al., 2013	Turkey	882	6.2	53.5	40.3
Present study	Johannesburg, South Africa	1126	0.7	31.3	67.9

Information regarding tumour size was available for 50.3% of cases. The tumours ranged in size from 6 mm to 160 mm, with a median size of 36 mm. The mean tumour size of 48.3 mm is comparable to the study from North east India where TNBCs had a mean size of 52.0 mm (Sharma et al., 2014). Bauer et al. (2007) demonstrated a median size of 22 mm in females from California. Although this difference in size is large, adequate information was not available to conduct formal tests of significance (Table 5).

In the majority of cases (73.2%), tumour size was determined on macroscopic evaluation of the excision specimen. Having obtained this information is valuable as other studies reviewed in the literature, to the best of our knowledge, did not stipulate the method with which tumour size was determined. This information is important as there is discordance in the estimation of tumour size by various methods. High tumour grade and the presence of DCIS contribute to inaccuracies in the estimation of tumour size using MRI. Tumour size, to a lesser extent, can also influence the accuracy of MRI measurements (Jethava et al., 2015). Menella et al. (2015) showed that 44.3% of cases showed concordance between pathological tumour size and estimation of tumour size using MRI. In 36.7% of cases, MRI overestimated the tumour size. Similarly, a separate study showed a 55.8% concordance, within 5 mm, of MRI and pathologic tumour size. MRI overestimated tumour size in 32.0% of cases and underestimated size in 12.2% of cases (Jethava et al., 2015). This has bridged a gap in knowledge and will allow more precise comparisons with future studies.

Table 5. Table comparing the mean and median tumour size of TNBCs amongst various studies.

Study	**Country**	**Sample size**	**Mean tumour size (mm)**	**Median tumour size (mm)**
Bauer et al., 2007	California, USA	92,358	-	22.0
Sharma et al., 2014	North east India	972	52.0	-
Present study	Johannesburg, South Africa	1,126	48.3 (SD 33.1)	36.0

Information regarding pathological tumour stage was available for only 52 (36.9%) of the 141 cases. This is attributed to the fact that our study included both core and excision specimens. Excision specimens were available for 52 cases and it was only on these that pathological tumour stage could be assessed. The majority of individuals presented with stage II and stage III disease (44.2% and 44.2% respectively). The findings within the present study were similar to those of the study from Soweto as well as from other developing counties (Table 6). This is in sharp contrast to the study from California, USA, where the majority of individuals (81.4%) presented with stage I and II disease. In the present study, 54.0% of individuals presented with I and II disease. This difference is statistically significant (P-value <0.001).

Table 6. Table comparing the stage of TNBCs amongst various studies.

Study	**Country**	**Sample size**	**Stage (%)**			
			I	II	III	IV
Bauer et al., 2007	California, USA	92,358	32.8	48.6	10.8	3.9
Fayez et al., 2014	Kuwait	2,986	14.0	42.0	37.0	7.0
Lara-Medina et al., 2011	Mexico	2,074	3.7	31.4	52.7	12.2
Li et al., 2013*	China	21,749	19.6	25.2	21.2	20.4
McCormack et al., 2013	Soweto, RSA	1,218	2.9	38.8	51.2	7.2
Sayed et al., 2014	Kenya	304	12.1	26.3	43.4	18.2
Present study	Johannesburg, South Africa	1,126	10.0	44.0	44.0	2.0

* clinical stage

This substantial size and stage difference amongst studies may highlight the possibility that factors other than the triple negative phenotype could contribute to large tumour size and advanced stage at presentation. Within the present study population, these reasons could be similar to those identified in a study conducted in Soweto by Dickens et al. (2014) where a number of factors contributing to a late stage at presentation in sub-Saharan Africa were identified. These included poor existing healthcare infrastructure, lack of early-detection programs and unavailability, inaccessibility and lack of adherence to treatment.

The Cancer association of South Africa (CANSA) 2015 recommends monthly breast self-examination from 20 years of age and mammographic screening of non-symptomatic females yearly from the age of 40. In spite of free public sector healthcare in South Africa, barriers to accessing healthcare exist and this influences the number of females who present for screening. These barriers include monetary costs of transport, time costs and distance to a health care facility (McLaren et al., 2014). Dickens et al. (2014) showed that living just beyond 20 km from a health care facility increases the probability of late stage at diagnosis. Additional barriers to accessing these facilities include long queues, disrespectful treatment by staff at the facility, medication that is out of stock and perceived ineffective care (Harris et al., 2011).

The presence or absence of concurrent in situ malignancy was known for 61.1% of the cases and 34.1% of individuals had concurrent in situ malignancy. Available data for comparison is limited. Our findings, however, were similar to those in a study by Fayaz et al. (2014) who showed that concurrent DCIS was present in 26.0% of individuals with TNBC.

The presence or absence of nodal involvement was available for only 32.7% of individuals as this information was derived from excision specimens. Nodal involvement was present in 69.6% of cases and confirmed absent in 30.4%. A positive nodal status was more common in all the studies which were reviewed (Table 7). Our findings were most similar to those of a study emanating from China, where 71.4% of individuals had positive lymph nodes.

Table 7. Table comparing the lymph node status of individuals with TNBC amongst various studies.

Study	**Country**	**Sample size**	**Lymph node status (%)**	
			Positive	Negative
Li et al., 2013	China	21749	71.4	28.6
Sharma et al., 2014	North East India	972	55.8	39
Somali et al., 2013	Turkey	882	50.8	49.2
Present study	Johannesburg, RSA	1126	69.6	30.4

Information regarding tumour focality was available for 53.2% of the cases. The majority of individuals (90.7%) had single tumours. Information with respect to tumour laterality was present for 91.7% of the cases. The proportion of individuals presenting with right and left sided tumours was not significantly different (P-value = 0.065). This is in keeping with the available literature which shows that distribution of right and left sided disease are similar, bilateral disease is uncommon and unifocal disease is more common than multifocal, diffuse disease (Fayaz et al., 2013, Pekar et al. 2013 & Sharma et al., 2014).

We acknowledge the following limitations in our study. Firstly, missing data has been identified as a limitation. This is attributed to the absence of clinical information on the histology requisition slips as well as the retrospective nature of this study. As previously mentioned, information regarding race, family history and socioeconomic status would be valuable in comparing and drawing conclusions with available data. The lack of clinical information could be overcome by communication between the pathologist and clinician. Clinicians should be aware of the benefits of including all relevant clinical information, including important negatives, on the histology requisition slip. This could potentially increase the amount of information available for future retrospective studies.

The second limitation is the inability to retrieve a significant proportion of cases from storage. 182 (16.2%) of the 1126 individuals with breast carcinoma were reported as having triple negative tumours according to the initial review of the histology reports. 141 (13.0%) of the individuals had confirmed triple negative tumours after retrieval and review of the slides. This difference in prevalence is attributed mainly to an inability to retrieve cases from storage for confirmation of receptor status, rather than a change in the receptor status after reviewing the slides. The difference in prevalence (3.6%; 95% CI: 0.7%–6.5%) is statistically significant (P-value = 0.01). It is essential to address this within the department, to ensure improved filing and retrieval of archived material. This could improve the accuracy of future studies within the department. The use of prospective studies could overcome the difficulties attributed to the retrospective study design, including slide retrieval and a paucity of information provided by the clinicians.

Another potential limitation is that HER2 status was assessed on immunohistochemistry alone. The current guidelines suggest that in-situ hybridisation be performed on all cases where HER2 is equivocal on immunohistochemistry. HER2 reporting guidelines on FISH have also changed and the inclusion of these would necessitate costly review of these cases. In a study conducted at CHBAH, 50.0% of cases that were classified as HER2 equivocal using immunohistochemistry were positive on subsequent FISH analysis (McCormack et al., 2013). This implies that 50.0% of these cases could have been HER2 negative. If these HER2 negative cases confirmed on FISH were included, it may have been possible to include additional cases within our study.

Lastly, it is important to recognise that hospital based studies may not be fully representative of the population and will only include individuals who have accessed formal, westernised healthcare. People who do not report symptoms and those consulting traditional healers would not be represented. It should be acknowledged that individuals using traditional medicine may only account for a small percentage of the South African population. A large systematic review showed a decrease in utilisation of traditional medicines to levels as low as 0.1% of the population (Peltzer, 2009). Similarly, in a national household survey, only 1.2% of South Africans reported the use of traditional medication (Nxumalo et al., 2011).

This is the first study to investigate TNBCs within the population of CMJAH and is only the second study from South Africa to assess TNBCs, thus providing valuable new information from a South African perspective. A relatively large sample size has been used and this is comparable with that of the international studies with which we compared our data. To the

best of our knowledge, this is the first study to stipulate the method in which tumour size was determined, providing a baseline for further comparisons.

CHAPTER 7: CONCLUSION

Breast carcinoma is a leading cause of malignancy associated death amongst South African females (Cancer Association of South Africa, 2014) and its reported incidence has doubled in Africa over the last 40 years (Porter, 2008). Health status influences capital acquisition, economic status as well as transmission of socioeconomic status from one generation to the next (McLaren et al., 2014). A reduction in breast carcinoma associated mortality would have important immediate and long term socioeconomic benefits, making this disease a priority in South Africa.

Although screening programmes are in place, barriers preventing access to healthcare need to be addressed. Earlier detection would result in an earlier stage at diagnosis and overall reduced mortality. This is essential in the South African context, as many elderly females are the primary caregivers for their grandchildren; providing financial, emotional and physical support. This need arises as a result of mortality of their children, migration and re-arrangement of family structures (Schatz, 2007).

An improved knowledge of the demographics and histological characteristics of TNBC is essential to better understand the disease. This is the first study to investigate TNBCs within the population of CMJAH and is only the second study from South Africa to assess TNBCs, thus providing valuable new information from a South African perspective.

TNBCs comprise 13.0% of breast carcinomas, this is lower than the prevalence in Soweto and other African countries. Differences in racial and socioeconomic demographics may provide an explanation. Patient demographics and tumour characteristics of TNBCs in our population are largely similar to the data available in the literature. The substantial size and stage difference amongst various studies suggests that factors other than the triple negative phenotype could contribute to large tumour size and advanced stage at presentation. In the South African context, existing barriers to accessing healthcare provide a plausible explanation.

The need for further research on TNBCs has been highlighted. Information with respect to race, menopausal status and socioeconomic status would be particularly valuable.
Furthermore, molecular analysis of TNBCs using microarray studies would allow comparison with international data.

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APPENDICES

Appendix A: Approval of title letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
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Reference: Ms Thokozile Nhlapo
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31 October 2014
Person No: 0306328E
PAG

Dr JC Linden
Postnet Suite 113
Private Bag X10010
Edenvale
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South Africa

Dear Dr Linden

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Clinical and pathological features of triple negative breast carcinomas* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix B: The Modified Bloom and Richardson Score

The modified Bloom and Richardson score is used in tumour grading.

Three tumour characteristics are evaluated; tubule formation, nuclear pleomorphism and mitotic counts. A numerical score, from one to three, is assigned to each characteristic. The scores are subsequently combined to determine the tumour grade (Elston et al., 2002).

Tubule formation:

- 1 point Tubule formation in more than 75% of the tumour.
- 2 points Tubule formation in 10 to 75% of the tumour.
- 3 points Tubule formation in less than 10% of the tumour.

Nuclear pleomorphism:

- 1 point Small, regular, uniform cells.
- 2 points Moderate increase in size and variability.
- 3 points Nuclei with marked variation in size and shape.

Mitotic count:

- 1 point 0-9 mitoses per 10 high power fields.
- 2 points 10-19 mitoses per 10 high power fields.
- 3 points 20 or more mitosesper10 high power fields.

Total scores and grade:

- Score 3-5 Grade 1 tumour (well differentiated).
- Score 6-7 Grade 2 tumour (moderately differentiated).
- Score 8-9 Grade 3 tumour (poorly differentiated).

Appendix C: Histological subtypes of breast carcinoma

The following is a list of the histological subtypes of breast carcinomas according to the current WHO classification (Lakhani et al., 2012).

- Acinic cell carcinoma
- Adenoid cystic carcinoma
- Carcinoma with apocrine differentiation
- Carcinoma with medullary features
- Carcinoma with neuroendocrine features
- Carcinoma with signet ring differentiation
- Cribriform carcinoma
- Glycogen-rich clear cell carcinoma
- Invasive carcinoma of no special type (previously invasive ductal carcinoma)
- Invasive lobular carcinoma
- Invasive micropapillary carcinoma
- Invasive papillary carcinoma
- Lipid-rich carcinoma
- Metaplastic carcinoma
- Mucinous carcinoma
- Mucoepidermoid carcinoma
- Oncocytic carcinoma

- Polymorphous carcinoma
- Salivary gland/skin adnexal type tumours
- Sebaceous carcinoma
- Secretory carcinoma
- Tubular carcinoma

Appendix D: ER and PR Receptor status

The following criteria are from the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) Guideline recommendations for immunohistochemical Testing of ER and PR status in breast cancer (Hammond et al., 2010).

ER or PR positive: ≥ 1% of tumour cell nuclei are immunoreactive.

ER or PR negative: < 1% of tumour cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PR (positive intrinsic controls are seen).

Uninterpretable for ER or PR if no tumour nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining.

Appendix E: HER 2 receptor status (2013)

The following criteria are from the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 updated guidelines (Wolff et al., 2013).

IHC 0 (negative) is defined as "no staining or membrane staining that is incomplete and is faint/barely perceptible within ≤10% of tumour cells".

IHC 1+ (negative) is defined as "membrane staining that is incomplete and faint/barely perceptible within > 10% of the tumour cells".

IHC 2+ (equivocal) is defined as "circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the tumour cells or complete and circumferential membrane staining that is intense and is within ≤10% of the tumour cells".

IHC 3+ (positive) is defined as "circumferential staining that is complete, intense and within >10% of tumour cells".

Appendix F: HER 2 receptor status (2007)

The following criteria are from the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 updated guidelines (Wolff et al., 2007).

IHC 0 (negative) is defined as "no staining or weak, incomplete membrane staining in any proportion of tumour cells".

IHC 1+ (negative) is defined as "weak, incomplete membrane staining in any proportion of tumour cells".

IHC 2+ (equivocal) is defined "complete membrane staining that is either non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells" or "intense, complete membrane staining of 30% or fewer tumour cells".

IHC 3+ (positive) is defined as "uniform intense membrane staining of > 30% of invasive tumour cells".

Appendix G: Data capture sheet

Study number	**Age**	**Gender** m/f*	**Race** b/w/a/c/o**	**Specimen type** c/e/b***

*male/female
**Black/White/Asian/Coloured/Other
***core/excision/both

Size				**Histological subtype**		**Grade** 1/2/3
mm	macro	imaging	clinical	Duct	Other	

In-situ ca y/n	**Nodal involvement**			**Number of tumours** u/m*	**Laterality** l/r/b**	**Family history** y/n
	y/n	histo	cyto			

*unifocal/multifocal
**left/right/bilateral

Imaging findings		**Other clinical information**
mammogram	sonar	

Appendix H: Ethics clearance certificate

R14/49 Dr Jessica Linden

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150383

NAME: (Principal Investigator)	Dr Jessica Linden
DEPARTMENT:	Pathology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Clinical and Pathological Features of Triple Negative Breast Carcinoma
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Reena Mohanlal
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	

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 28/03/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix I: Turnitin report

