IMMUNOHISTOCHEMICAL SUBTYPES OF BREAST CANCER AND THEIR ASSOCIATION WITH DEMOGRAPHIC AND CLINICO-PATHOLOGICAL CHARACTERISTICS IN THE LIMPOPO PROVINCE

by

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DISSERTATION

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DEDICATION

This study is dedicated to the families of Peka, Mathosa, Maluleke and Masenya for their unwavering support, motivation and understanding. For all the time and financial sacrifices they made to see me through. This achievement is yours too. Thank you.

DECLARATION

I declare that the **IMMUNOHISTOCHEMICAL SUBTYPES OF BREAST CANCER AND THEIR ASSOCIATION WITH DEMOGRAPHIC AND CLINICOPATHOLOGIC AL CHARACTERISTICS IN THE LIMPOPO PROVINCE** submitted to the University of Limpopo for the degree of **Master of Science in Medical Sciences**, has not been previously submitted by me for a degree at this or any other university; that it is my work in design and execution and all material contained herein has dully been acknowledged

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Peka L.N.J (Ms) **Date**

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ABSTRACT

Background: This study was designed to determine the relationship of immunohistochemical subtypes of breast cancer and clinicopathological features and demographic information in Limpopo since there has been no data published on the association of immunohistochemical subtypes, clinicopathological features and demographic characteristics in recent years.

Methods: Data was obtained from records of patients diagnosed with breast cancer between 2015 and 2020. Chi-Square and ANOVA tests were performed, and results considered significant at $p \leq 0.05$.

Results: The mean age was 50.32 ± 11.40, estrogen receptor positive(ER+), progesterone receptor positive(PR+) and human epidermal growth receptor 2 positive(HER2+) prevalence were 73.5%, 62.3% and 26.5% respectively. More than half of the patients (54.3%) had a Ki-67 level >15%. Grade II tumors were the predominant type of tumors in this study (51.7%). 43.7% of the cases showed lymph node involvement. Luminal B subtype was the most predominant immunohistochemical subtype in the study (46.4%), followed by Luminal A (24.5 %), TNBC (19.9%) and HER2- enriched (6.6%). 2.6 % of the cases were classified as undetermined.

Conclusion: A significant association was observed between immunohistochemical subtypes and tumor grade.

Key words*:* Immunohistochemical subtypes, breast cancer, clinicopathological features, Limpopo Province.

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ABBREVIATIONS

ASCO	American Society of Clinical Oncology
ATAC	Arimidex, Tamoxifen, Alone or Combined
CANSA	Cancer Association of South Africa
CDC	Centre for Disease Control and Prevention
DCIS	Ductal Carcinoma *in Situ*
ER	Estrogen Receptor
EBCTCG	Early Breast Cancer Collaborative Group
H&E	Haematoxylin and Eosin
HER2	Human Epidermal Growth Factor Receptor 2
HIV	Human Immunodeficiency Virus
IDC	Infiltrating Ductal Carcinoma
IDC- NST	Infiltrating Ductal Carcinoma-No Special Type
IHC	Immunohistochemistry
ILC	Infiltrating Lobular Carcinoma
PR	Progesterone Receptor
SEM	Standard Error of the Mean

TNBC	Triple Negative Breast Cancer
TNM	Tumour, Node, Metastasis

TERMINOLOGY

Breast cancer	Neoplasm of the breast due to abnormal growth of cells, which tends to proliferate in an uncontrolled way and, in some cases, metastasises and invades the surrounding tissue and destroys it (Dai, Xiang, Li & Bai, 2016). In this study, the term was used as is.
Clinicopathological Characteristics	Signs and symptoms observed by a physician and laboratory findings pertaining to the course of a disease (Sepe, Piscuoglio, Quintavalle & Perrinal, 2015). In this study, the term refers to characteristics associa ted with breast cancer, which include, but not limited to age at diagnosis, histological type, tumor size, tumor grade and lymph node status.
Immunohistochemistry	Laboratory technique used for molecular evaluation of tissues through the localisation of antigens and light microscopy (Hirsch, McElhinny, Stanforth, Ranger-Moore, Jansson, *et al.*, 2017). In this study, the term was used as is.
Breast Cancer Subtypes	Groups of breast cancer classified through tumor molec ular typing techniques such as microarray, genotyping a nd immunohistochemistry into *Luminal* subtype, basal-like subtype, human epidermal growth subtype and normal breast-like subtype (Tao, Song, Du, Han, Zuo, *et al.*, 2019). In this study, breast cancer subtypes refer to a classification of breast tumors based on histochemical profiling of ER, PR, HER2 receptors and Ki-67 proliferation marker into *Luminal* A, *Luminal* B, HER2-enriched and Triple negative breast cancer subtypes.

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CHAPTER 1

BACKGROUND AND MOTIVATION

1.1. INTRODUCTION

Breast cancer is a patho-physiologically heterogeneous disease which presents with a variety of histopathological features and clinical characteristics (Doval, Sharma, Sinha, Kumar, Dewan *et al.*, 2015). It is a global health problem, and the most frequently diagnosed cancer in women worldwide, accounting for about a quarter of all cancers in women (Ferlay, Soerjomataram, Dikshit, Eser, Mathers *et al.*, 2015).

The incidence rate of female breast cancer ranges from about 9 per 10,000 in Europe to about 2 per 10,000 people in Africa (Adeloye, Sowunmi, Jacobs, David, Adeosun *et al.*, 2018). According to the Cancer Association of South Africa (CANSA), breast cancer is the most prevalent cancer in South African women, accounting for 22% of all cancers diagnosed in 2013 alone (Maree & Schmollgruber, 2014). It is the most prevalent cancer in White, Coloured and Asian women in South Africa, and second only to cervical cancer in Black women. Compared to women of Caucasian and Asian heritage, it is widely reported that the prognosis of breast cancer in black women is poorer (Smigal, Jemal, Ward , Cokkinides, Smith *et al.*, 2006; van Bogaert, 2013b; Parada, Sun, Fleming, Williams-DeVane, Kirk *et al.*, 2017) with reported mortality rates of 37%, which is higher in African-American women than in other races (Smigal *et al.*, 2006; van Bogaert, 2013b).

Breast cancer is clinically classified using different schemata based on the purpose and type of data set available. The American Joint Committee of Cancer provides two principal schemes of classifying breast cancer, namely: Prognostic and Anatomic staging (Koh & Kim, 2019).

The prognostic classification of breast cancer is dependent on the immunohistochemical profile or subtype of the steroid hormone receptors, estrogen (ER) and progesterone receptors (PR) and the human epidermal growth factor receptor-2 (HER2), which have been shown to determine the prognosis of breast cancer patients and inform the choice of suitable therapies (Bennis , Abbass, Akasbi, Znati, Joutei , *et al.*, 2015; van Bogaert, 2013a; Elidrissi Errahhali, Ouarzane, El Harroudi, Afqir, Bellaoui *et al.*, 2017; Sengal, Haj-Mukhtar, Elhaj, Bedri, Kantelhardt, *et al.*, 2017). For instance, patients with ER-positive breast cancer who are treated

with tamoxifen (selective ER influencer) survive for longer, experience a lower recurrence rate and reduced mortality, and an enhanced tumor response compared to breast cancer patients with ER and PR negative tumors (Patil, Bhamre, Singhai, Tayade, Patil *et al*., 2011; Doval *et al.*, 2015). Also, breast cancer tumors lacking the immunohistochemical expression of all three receptors (ER, PR and HER2), popularly known as the Triple negative breast cancer (TNBC), largely correlates with poor prognosis (Patil *et al.*, 2011; van Bogaert, 2013b; Doval *et al.*, 2015).

Anatomically, breast cancer classes include Invasive Ductal Carcinoma-No Special Type (IDC-NST), and Invasive Lobular Carcinoma (ILC), which accounts for approximately 10% of breast cancer cases. The less common types include micro papillary, mucinous, papillary, tubular, medullary, metaplastic and apocrine (Tsang & Gary, 2020).

Clinicopathological characteristics are both signs and symptoms observed by a physician on examination as well as laboratory findings pertaining to the course of a disease (Sepe, Piscuoglio, Quintavalle & Perrinal, 2015). Research indicates that some of the clinicopathological characteristics associated with breast cancer include, but not limited to, lymph node status, age of diagnosis, breast cancer histological subtype, Ki 67 proliferation marker as well as the ER, PR and HER2 receptor status (Ding, Zhang, Xu & Zhang, 2017).

It is well established that breast cancer is a biologically diverse disease characterised by a variety of histopathological features (San, Fujisawa, Fushimi, Soe, Min *et al.*, 2017). However, little is known about the characteristics of this disease among women in Limpopo Province. To our knowledge, only one study (van Bogaert, 2013a) on breast cancer molecular subtypes among women in Limpopo has been published thus far. The study, however, did not correlate the subtypes with the clinicopathological characteristics of breast cancer. This study was therefore designed to determine the Immunohistochemical profile of breast cancer patients in the predominantly black female population of Limpopo Province, South Africa as well as to correlate the IHC expression with standard clinicopathological features and demographic information.

1.2. PROBLEM STATEMENT

Immunohistochemical staining for all diagnosed breast cancer is now routinely done in both public and private histopathology facilities in South Africa, but published data on these IHC markers (ER, PR and HER2) is scarce, especially in Limpopo Province. The only study published on the immunohistochemical profile of breast cancer among South African women in Limpopo Province was conducted by van Bogaert (2013). It excluded patients seen at private histopathology facilities and did not correlate the subtypes with any clinicopathological feature of breast cancer, or give demographic information such as age of diagnosis. This study will therefore analyse the IHC profile of breast cancer specimens processed at public and private histopathology facilities in Limpopo Province as well as collect demographic data on breast cancer, and associate this information with standard clinicopathological features.

1.3. PURPOSE OF STUDY

1.3.1. Aim

The aim of the study was to determine the immunohistochemical subtypes and demographic information of breast cancer cases seen at public and private hospitals in Limpopo Province and to correlate these with clinicopathological characteristics..

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1.3.2. Objectives

- To determine the estrogen, progesterone and human epidermal growth factor receptor status of breast cancer samples seen at Limpopo Province tertiary and private hospitals between January 2015 and December 2019 in order to classify the breast cancer sub-type as being *Luminal* A, *Luminal* B, HER2- enriched or Triple negative (immunohistochemical subtypes).

- To determine the clinicopathological characteristics of breast cancer samples seen at Limpopo tertiary and private hospitals between January 2015 and December 2019, and to associate them with immunohistochemical subtypes of breast cancer samples.

- To collect demographic information of breast cancer patients and to correlate them with the subtypes and clinicopathological characteristics of breast cancer.

- To correlate demographic characteristics with immunohistochemical subtypes and clinicopathological characteristics amongst breast cancer patients.

1.4. RESEARCH QUESTION

What are the prevalent immunohistochemical subtypes of breast cancer and their clinicopathological characteristics among patients seen at public and private hospitals in Limpopo Province, and how are these associated with their demographics?

CHAPTER 2

LITERATURE REVIEW

2.1 Breast Cancer

Breast cancer is a neoplasm of the breast caused by abnormal growth of cells, which tend to proliferate in an uncontrolled way, evading the body's regulatory mechanisms such as apoptosis and angiogenesis and, in some cases, metastasises and invades the surrounding tissues and destroy them (Gupta, Kim, Prasad & Aggarwal, 2010).

Globally, breast cancer represents about a quarter of total female cancers and is the second most common type of cancer diagnosed in developing countries, with an estimated 2 million new cases in 2019 alone (Donepudi, Kondapalli, Amos & Venkanteshan, 2014; Ghoncheh, Pournamdar & Salehiniya, 2016). The incidence rate of female breast cancer varies from 19.3 per 100,000 in Eastern Africa to 89.7 per 100,000 people in Western Europe, with approximately one in eight women and one in 1,000 men estimated to develop invasive breast cancer over the course of their lifetime (Ghoncheh *et al.*, 2016; Zaidi & Dib, 2019). According to the World Health Organization (WHO), an estimated 627 000 women died of breast cancer in 2019 alone, accounting for 15% of total cancer mortalities worldwide. Incidence rates are considerably lower in Africa than in most parts of the world; however, mortality rates in certain African countries (e.g., Nigeria, Egypt, Ethiopia) are among the highest worldwide (Adeloye, Sowunmi, Jacobs, David, Adeosun *et al.*, 2018).

In South Africa, breast cancer incidences have reportedly doubled in the past two decades, with the age-standardised mortality rate for breast cancer in South Africa estimated to be 16.5 per 100,000, compared with 14.1 per 100,000 in the United States of America (Coughlin & Ekwueme, 2009; Unger-Saldaña, 2014; Trupe, Rositch, Dickerson, Lucas & Harvey, 2017). South Africa has a higher incidence of breast cancer (13.1 %) in contrast with other Southern African countries such as Botswana (9.1 %) and Mozambique (5.3 %) (WHO, 2020). The Cancer Association of South Africa (CANSA) reports that 19.4 million South African women aged 15 and older are currently at risk of developing breast cancer over the course of their lives (CANSA, 2016).

General cancer statistics remain largely unreported across the nine South African provinces (Made, Wilson, Kerry, Jina, Ruxana *et al.*, 2017). A review of literature

indicates that the last report on cancer stats was released by the National Cancer Registry in 2014. This report outlines that breast cancer is the leading form of carcinoma amongst Asian and Coloured females, accounting for 39.3% and 28.57 % of all histologically confirmed cancers, respectively. Black and White females are less affected, with rates of 20.05 % and 20.51% of all diagnosed cancers, respectively.

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2.2 Risk Factors

Risk factors for breast cancer include social, demographic, and biomedical causes, which individually, or collectively, increase the risk of developing and dying from breast cancer. Identification of these factors is thus important clinically and in research. Common risk factors of breast cancer include age, which has been shown to have a positive direct relationship with cancer, that is, the risk of cancer increases with the progression of age. A history of breast cancer in the family, combined with the presence of genetic risk factors such as mutation of the BRCA1/BRCA2 breast cancer gene. Other factors include, but are not limited to, infertility, age of first fulltime pregnancy, age of menopause, post/pre-menopausal usage of hormones, short period of lactation and obesity (Donepudi *et al.*, 2014; Ghoncheh *et al.*, 2016).

A study conducted on 480 women aged 18-55 years of age in India by Prusty, Begum, Patil, Naik, Pimple *et al.* (2020) found that lack of knowledge on breast cancer and the importance of early testing is also a significant risk factor. This is because this factor perpetuates presentation of breast cancer at an advanced stage of the disease, resulting in overall poor prognosis (Prusty, Begum, Patir, Naik, Pimple *et al.*, 2020).

2.3 Breast Cancer Classification

Breast cancer is a heterogenous disease, encompassing a plethora of pathological, microscopic, clinical and genetic characteristics. As a result, classification systems have been developed for the purpose of treatment, management, and prognosis of the condition (Malhotra, Zhao, Band & Band, 2010). Based on the purpose and type of data set available, breast cancer can be classified using different schemata. A search of literature indicates that the schemes use different biological approaches and thus indicate different properties, which all combine to give a comprehensive breast cancer

profile (Taherian-Fard, Srihari & Ragan, 2014). The American Joint Committee of Cancer provides two principal schemes of classifying breast cancer, namely: anatomic and prognostic staging (Sparano, 2020).

2.3.1 Anatomical/histological Classification.

Breast cancer is classified based on microscopic assessment of tumor cells. According to the Centres for Disease Control and Prevention (CDC) in the United States of America, breast cancer is anatomically classified based on the TNM staging tool, which categorises breast cancer depending on the breast tissue that exhibit abnormality (T-tumor size), lymph node involvement (N- node status) and whether these cells invade surrounding tissues (M-distant metastasis) (CDC, 2019). This tool is known as the Bloom-Richardson classification. Based on this scheme, breast cancer can be broadly classified as being in-situ or invasive breast carcinoma with a given stage. *In situ*, breast cancer is observed within the tissues of the breast only, whilst invasive breast cancer metastasises and affects other normal tissues of the body, forming secondary tumors (CDC, 2019; Malhotra, Zhao, Band & Band, 2010).

Histologic classification is based on the pathological development of cancer cells. This classification provides for over 20 different histological types of breast cancer. Over 70% of breast cancer cases are classified as being Invasive Ductal Carcinoma-No Special Type (IDC-NST), followed by Invasive Lobular Carcinoma (ILC), which accounts for approximately 10% of breast cancer cases. The less common types include micro papillary, mucinous, papillary, tubular, medullary, metaplastic and apocrine (Tsang & Gary, 2020)

Histological assessments provide for classification of breast cancer based on a modification of the TNM staging. This mechanism uses the Nottingham modification of Bloom-Richardson classification. In this classification,thetumor cells are compared with normal breast cells, and a numerical grade is provided for the tumor cells based on how abnormal they are compared to normal cells. Three criteria are used for this assessment. The first criterion directs assessment of glandular or tubular formation in the tumor cells, which reflects differences of tumor cells from normal ductal or lobular cells. The second criterion calls for assessment of nuclear polymorphism, which indicates cytomorphology of the cells. The last criterion looks at the number of mitotic figures, which reflects the proliferation index of the tumor cells (Pradhan, Paudyal,

Sinha & Agrawal, 2017). Grade I tumor cells show little abnormality when compared to normal breast cells. This serves as an indication of the slow spread and growth of the tumor. Grade II and III tumors exhibit great difference to normal cells and are seen to spread and grow more rapidly (Taherian-Fard, Srihari &Ragan, 2014).

2.3.2 Prognostic Classification

Prognostic classification encompasses the TNM classification and histological grading as well as evaluation of biomarkers such as ER, PR, HER2 and Ki-67, which are evaluated through immunohistochemical subtyping (Sparano, 2020).

Immunohistochemical subtyping of breast cancer has become routine in laboratory investigations across many laboratories in the world (Zaha, 2014). This technique is based on the ability of antibodies to hybridise with specific antigens on surfaces of cells (Ivell, Teerds & Hoffman, 2014). Immunohistochemistry is used to detect molecular markers that are important in guiding treatment decisions, classifying breast cancer into subtypes that are distinct in morphology and behaviour, and are used as prognostic tools (Zaha, 2014).

Molecular markers such as ER, PR, HER2 and Ki-67 are key in the classification of breast cancer into immunohistochemical subtypes (Dai, Xiang, Li & Bai, 2016). These markers reflect the conditional gene expression of tumor cells and can individually or collectively indicate the best treatment options for patients as well as indicate mammary origin of tumor cells in metastasised cancer (Dai, Xiang, Li & Bai, 2016).

2.4 Molecular Markers used in Immunohistochemical Subtyping

2.4.1 Estrogen receptor.

The estrogen receptor is a member of the nuclear receptor superfamily of transcription regulators. These receptors bind the four types of estrogen hormones, namely estrone, estradiol, estriol and estetrol (Hua, Zhang, Kong & Jiang, 2018). The hormones have several physiologic functions, including regulation of reproduction as well as breast and sexual organs development (Hua, Zhang, Kong & Jiang, 2018). Estrogen receptors are important in the regulation of breast cancer. Three different types of the estrogen receptors are currently known, ERα, ERβ and the G-couple

protein receptor 1(GPER) (Hua, Zhang, Kong & Jiang, 2018). Research indicates that current therapies target specifically ERα for endocrine therapy (Hua, Zhang, Kong & Jiang, 2018). The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines of 2010 recommend that a breast cancer specimen is ER-positive (ER+) if ≥1 % of the tumor cells have the estrogen receptor (Groenendijk, Treece, Yoder, Baron, Beitsch, *et al.* 2019). ER+ malignancies usually have a superior prognosis and are generally a lot more responsive to hormonal treatment with drugs such as Tamoxifen and Arimidex (Anderson Society, 2019). ER+ breast cancers are more predominant in Caucasian women, with 79% of breast tumors in US-born white women being ER+ (calculated amongst women with known ER-status) (Jemal & Fedewa, 2012). The percentage of ER+ cancers is reportedly lower among US-born African American women (61%, all ages combined) (Jemal & Fedewa, 2012). Some reports (Bird, Hill & Houssami, 2008; Huo, Ikpatt, Khramtsov, Dangou-M, Nanda, Dignam, *et al.*, 2009) indicate a significantly higher percentage of ER-negative cancers in indigenous populations in Africa, which may contribute to the poor survival from this malignancy.

2.4.2 Progesterone Receptor

The progesterone receptor is an intracellular receptor that belongs to the nuclear receptor superfamily of transcription regulators. It has two isoforms PR-A and B (Patel, Elguero, Thakore, Dahoud, Bedaiwy, *et al.*, 2015). They bind and therefore aid in the signal transduction of the hormone progesterone, which functions in the development of breasts, lactation and the establishment of pregnancy (Patel *et al.*, 2015). The role of PR in breast is poorly understood because of the difficulty to isolate progesterone and study independent of other hormones such as the growth factor (Yao, Song, Wang, Yang & Song, 2017). Furthermore, PR isoforms are expressed in response to ER transcriptional events. However, the significance of PR status in breast cancer prognosis is supported by worldwide clinical trials of ATAC *(*Arimidex, Tamoxifen, Alone or Combined), which indicates that patients who are ER+/PR+ respond better to endocrine therapy and show a lower recurrence rate than those who are ER+/PR- (Yao, Song, Wang, Yang & Song, 2017). Like with ER status, breast cancer is considered PR+ when ≥1 % of the tumor cells express PR.

2.4.3 HER2

HER2 or human epidermal growth factor 2 is a transmembrane receptor protein, a member of the epidermal growth receptor family, which also includes HER1, HER3, and HER4 (Viale, 2015). In normal cells, its activation stimulates downstream pathways that regulate cell proliferation and subsistence (Viale, 2015). In breast cancer, HER2 acts as an oncogene, its overexpression resulting in uncontrolled cell proliferation, evasion of apoptosis and promotion of angiogenesis and evasion of other tissues. Studies show that this receptor is seen in 20-25% of breast cancer cases (Viale, 2015). HER2 overexpression has been associated with aggressive phenotypes of breast cancer, as well as poor prognosis and greater chances of recurrence of diseases. Breast cancer cases that test positive for the marker are ruled to be more responsive to treatment with the monoclonal antibody-based treatment Herceptin (Trastuzumab), a drug used to treat tumor cells by restricting spread through the altering of the subunits of the receptor (Viale, 2015). Breast cancer cases that are positive for the receptor respond poorly to endocrine treatments with drugs such Tamoxifen. HER2 testing is routinely done in laboratories using IHC or in situ hybridisation. Following testing, a sample is considered HER2+ if it has IHC score of 3+ in ≥10% of the tumor cells (Viale, 2015). A score of 2+ in ≥10 of tumor cells indicates a borderline reactivity of the cells and intensity, and normally requires further investigation with other mechanisms such as the Polymerase Chain Reaction (PCR). A sample with a score of 1+ in ≥ 10% of tumor cell or a score of 0 in ≤ 10 % of the tumor cells is considered HER2 negative (Viale, 2015).

2.4.4 Ki-67

Ki-67 is a non-histone protein encoded by the *MKI-67* gene and is involved in ribosomal RNA synthesis. It is strictly associated with cell proliferation (Sobecki, Mrouj, Camasses, Parisis, NicolasF *et al.*, 2016). According to the St. Gallen guidelines of 2011, Ki-67 labeling index is used to classify breast cancer as being of low proliferation (labeling index <15%), intermediate proliferation (labeling index ranges from 15-30%) or high proliferation (labeling index >30%) (Bustreo, Osella-Abate, Cassoni, Donadio, Airoldi *et al.*, 2016). Clearly, high levels of the marker are used to indicate if a patient needs chemotherapy in addition to endocrine/hormonal therapy. Furthermore, it is

used to determine the effect of different doses of Tamoxifen on tumor proliferation. Breast cancer patients that are found to be Ki-67 positive are classified as high-risk patients (Bustreo *et al*., 2016).

2.4 Immunohistochemical Subtypes of Breast Cancer

Breast cancer is classified into four distinct subtypes based on the above-mentioned molecular receptors, namely: *Luminal* A, *Luminal* B, HER2-enriched and Triple negative (Sohn, Han & Seo, 2016).

2.5.1 Luminal A.

This subtype exhibits the following characteristics: ER+, PR+ (>20%), HER2- and Ki-67 < 14% (Goldhirsch, Winer, Coates, Gelber, Piccart-Gebhart *et al.*, 2013). Studies show that Luminal A has a better prognosis than the other subtypes, and that patients with this subtype benefit more from endocrine therapy as opposed to chemotherapy (Nielsen, Jensen, Burugu, Gao, Jorgensen, *et al.*, 2017). This is supported by the theory that chemotherapy targets replicating cells while Luminal A tumor cells show low expression of proliferation genes and markers (Ki-67<14%). In a study conducted in Morocco, Bennis *et al.* (2012) found that the Luminal A subtype was more prevalent (53.6%) and is associated with favourable clinicopathological characteristics.

2.5.2 Luminal B

Luminal B breast cancer is characterized as being ER+, HER2-, and at least one of: Ki-67 ≥ 15%, PR < 20% or ER+, HER2+, Any Ki-67, Any PR (Note:once a breast cancer case is ER positive and also HER2 positive, it does not matter the status of PR and the level of Ki-67, it is classifiedas Luminal A) (Goldhirsch, *et* al.,2013). Research indicates that this subtype is associated with aggressive clinical behaviour and a prognosis like that of basal-like and HER2- enriched subtypes of breast cancer. A study conducted on 1951 patients at the Liaquat National Hospital in Karachi found that Luminal B breast cancer was significantly associated with a younger age group as compared to *Luminal* A (Hashmi, Aijaz, Khan, Mahboob, Irfan *et al.*, 2018).

2.5.3 HER2-enriched breast cancer

HER2-enriched breast cancer is histochemicaly characterised by being HER2+, ER-, PR- (Goldhirsch, *et al.*, 2013). This subtype accounts for 20% of all diagnosed breast

cancers (Mendes, Alves, Afonso, Cardoso, Passos-Coelho *et al.*, 2015), and has been known to have a poor prognosis and an aggressive phenotype than *Luminal* subtypes. However, the development of HER2 targeted therapies has improved the disease outcome and patient survival (Mendes *et al.*, 2015). Studies show that this form of breast cancer responds well to treatments such as trastuzumab and pertuzumab (Mendes *et al.*, 2015).

2.5.4 Triple negative breast cancer

TNBC (Triple negative breast cancer) is characterised by testing negative for all the hormonal receptors ER, PR and HER2 (Goldhirsch *et al.*, 2013). This breast cancer subtype accounts for 15-20% of all breast cancer cases and has been associated with a poor prognosis, aggressive phenotype, and limited treatment options than the other subtypes (Shimelis, LaDuca, Hu, Hart, Na *et al.*, 2018). Studies also indicate that TNBC is associated with hereditary conditions, namely; mutations in the *BRACA1* and BRACA*2* genes. A case of TNBC in a patient of 60 or less years of age is an important criterion for testing *BRACA1* and BRACA*2* mutations (Shimelis *et al.*, 2018).

2.6 Prevalence of the Immunohistochemical subtypes and molecular markers in studies.

In a study conducted in Morocco, Bennis *et al.* (2015) found that the Luminal A subtype was more prevalent (53.6%), and is associated with favourable clinicopathological characteristics, followed by Luminal B (16.4%), HER2-overexpressing (12.6%), basal-like (12.6%) and unclassified subtype (4.9%) (Bennis *et al.*, 2015). In another Moroccan study, Elidrissi, *et al.* (2017) reported that most tumors were hormone receptor positive (73%), and 28.6% were HER2 positive (Elidrissi *et al.*, 2017). In a tertiary care hospital in India, breast cancer hormone receptor positivity (ER and/or PR) was seen in 63.4% of patients, while 23.8% of malignancies were triple negative. Only 23.0% were HER2 positive, and around 10.0% of malignancies were both ER and HER2 positive (Doval *et al.*, 2015).

In a large study evaluating breast cancer receptor status in more than 1,200 public hospital patients in Soweto, South Africa, 35% of the cancers were ER-, 47% PR-,

74% HER2-, and 21% were triple negative status (McCormack, Joffe, van den Berg, Broeze, dos Santos Silva *et al.*, 2013). The authors reported that advanced stage malignancies had a tendency to be ER- and PR-, and were not really associated with HER2 status (McCormack *et al.*, 2013) . They also noted that age was not strongly associated with ER or PR status, but older women had a greater tendency to have HER2- tumors, and concluded that age-specific ER- percentages in black South African women were comparable to American women of African ancestry, mainly for postmenopausal women.

In the only published study regarding breast cancer subtypes in Limpopo Province, van Bogaert (2013) found that HER2 was overexpressed in 26.0% of cases, while the Triple negative subtype constituted 27.9%. He also reported that there was a high level of ER negativity (42.7%) in Limpopo Province (van Bogaert, 2013), contrary to the report by Adebamowo *et al.* (2008) that there is no difference between black Africans and Caucasians.

2.5 Clinicopathological Characteristics

Clinicopathological characteristics are signs and symptoms observed by a physician on examination as well as laboratory findings pertaining to the course of a disease (Sepe, Piscuoglio, Quintavalle & Perrinal, 2015). Research indicates that some of the clinicopathological characteristics associated with breast cancer include, but not limited to, tumor size, tumor grade, lymph node status, histological type and age at diagnosis (He, Wu, Yang, Sun, Li *et al*., 2015). Existing literature only outlines statistics on the histochemical subtypes of breast cancer in Limpopo Province. However, it does not correlate these immunohistochemical subtypes to clinicopathological characteristics of breast cancer and demographic information on breast cancer in Limpopo Province, hence this study.

2.6 Demographic Characteristics

In the latest cancer report by the National Cancer Registry in 2014, breast cancer is the leading form of carcinoma in women in South Africa, accounting for 22% of all cancers diagnosed in 2013 alone. Literature search indicates that the last

characterisation of Limpopo demographics about cancer was done in the year 2000. This outcome adds to the significance of this study as it will look at the current demographic, immunohistologic and clinicopathologic statuses of breast cancer in Limpopo Province.

CHAPTER 3
METHODOLOGY

3.1 Research Design

This study was a quantitative and retrospective study. A quantitative study is a research approach that places emphasis on numbers and figures that can be manipulated to give statistical significance (National Council for Osteopathic Research, 2014). This study was quantitative as it quantified and statistically analysed data relating to immunohistochemical profiling of breast cancer samples over a five-year period. A retrospective study is one that looks at the data that already exists and data that was initially collected for other purposes than research (Ranganathan & Aggarwal, 2019). This study was based on all female breast cancer case records that were registered at Mankweng Hospital and Polokwane Lancet Laboratories from 2015 to 2020.

3.2 Sampling

3.2.1 Study site

The study was conducted at Polokwane Lancet Laboratories and the breast cancer unit of Mankweng Hospital. Polokwane Lancet Laboratories are private institutions that offer medical science services to the public. Its histopathology unit diagnoses and histologically confirms many of the breast cancer cases in the private sector of the province (exact statistics currently not available). It is in the city of Polokwane in the Capricorn district. Mankweng Hospital is one of the two provincial tertiary hospitals, alongside Pietersburg Hospital. It is located in Mankweng township, also in Capricorn District. The breast cancer unit of Mankweng Hospital receives and processes most of the breast cancer samples from its breast cancer care unit and other public hospital breast cancer care units in Limpopo Province. The map below reflects the region of Limpopo in which the two institutions where the study was conducted are located (circled in red).

Figure 1: Map of Limpopo Province reflecting the five districts of the province and the study site (circled in red). Source: Limpopo Tourism Agency, 2017, Limpopo regions. Accessed: 08 January February 2020, Accessed from: http://www.golimpopo.com/regions

3.2.2 Study Population

The population used in this study included female patients diagnosed with breast cancer during the period of 2015 to 2020. Breast cancer patients seeking treatment in the public sector are all referred to the oncology unit at Mankweng tertiary hospital, while patients in the private sector are mostly referred to Polokwane Lancet Laboratories for their histopathology report by private oncologists (Private communication – Dr Mahlakwane – the only private histopathologist in Limpopo Province).

Histopathology reports for the public sector patients were accessed from the patients' files at Mankweng Hospital while the histopathological report for private sector patients were accessed at Polokwane Lancet Laboratories in Polokwane.

3.2.3 Sampling Method

Consecutive sampling of the histopathological records of female patients diagnosed with breast cancer and referred to Polokwane Lancet histopathology laboratory and the breast cancer unit of Mankweng Hospital during the period from January 2015 to December 2019 was used in the study. Consecutive sampling is defined as a sampling technique in which every consecutive patient who presents with the condition of interest is approached for enrolment until a minimum sample size is reached (Mathieson, 2014). This was done to ensure that the minimum sample size is reached (considering records that will be excluded due to incomplete data) and to prevent

sampling bias which may result from choosing records with only specific characteristics, such as a particular geographical location. In this study, all patient records that fit the selection criteria were enrolled.

3.2.4 Inclusion Criteria

Records of female patients diagnosed with breast cancer during the period of 01 January 2015 to 31 December 2019 and referred to Lancet histopathology laboratory and the breast cancer unit of Mankweng Hospital were included in the study. All breast carcinomas that were histologically confirmed and processed for hormone receptor status were included in the study.

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3.2.5 Exclusion Criteria

All records which had no data on hormonal receptors were excluded. Cases which had no Ki-67 proliferation marker data and were also ER+/PR+ and HER2- were not included in the study (this is because an ER+/PR+ and HER2- profile require a Ki-67 level to distinguish whether it is a Luminal A or Luminal B subtype). Records that had hormonal receptor data but did not have any clinicopathological data records were excluded.

3.2.6 Sample Size

All records that met the inclusion and exclusion criteria were included in the study to meet the minimum sample size. The research aimed to determine the different molecular subtypes of breast cancer in Limpopo Province and their association with clinicopathological characteristics. Therefore, the sample size was calculated as follows:

- For a confidence level of 95 % (alpha level of 0.05), Zα/2 = 1.96
- For a power of 80%, Z1-β =0.84
- Effect Size (a measure of the strength of the relationship between two variables on a numerical scale) = $ES = \frac{p_1 - p_0}{\sqrt{p_1(1-p_1)}}$,

Where P0 is the population proportion from previous studies and P1 is the level of significance of the study (5%). According to a report on breast cancer by the Cape Town etc. in 2018, breast cancer affects 27 in 100 000 women, which translates into a prevalence of 0.027 %. The prevalence value here is reflected because it is needed to calculate the appropriate effect size, an index is needed in the formula to calculate a sample size for a correlation study which is going to use inferential statistics for analysis (Anova and the chi-square).

$$ES = \frac{p_1 - p_0}{\sqrt{p_1(1-p_1)}}$$

ES= $\frac{0.05-0.00027}{\sqrt{0.05-(1-0.05)}}$

=0.228

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{ES} \right)^2$$

N= $(1.96+0.84/0.228)^2$
;
=151

Therefore, a minimum of 151 records were required in the study.

3.3 Data Collection

Reports of female patients diagnosed with breast cancer that have been histologically confirmed and processed for hormone receptor status were used to obtain relevant data. The data was collected using a tool developed from histopathology reports (Appendix 1).

3.3.1 Age at Diagnosis

The age at diagnosis was obtained from histological records of patients in respective institutions. The age was expressed as a mean ± standard deviation and categorised into age groups.

3.3.2 Molecular Markers

Immunohistochemistry, a technique that is used for molecular evaluation of tissues through the localisation of antigens and light microscopy is used to screen for the hormonal receptors. The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines of 2010 direct that a breast cancer specimen is ER+/PR+ if ≥1 % of the tumor cells stain for these markers (Groenendijk *et al.*, 2019). The testing of hormonal receptors has been routine in histological laboratories of South Africa since the early 2000. The Ki- 67 marker was adopted as routine in 2014. Data on these markers was obtained from patient records as testing of the hormonal receptors has been routine in histological laboratories of South Africa since the early 2000 Ki- 67 marker was adopted as routine in 2014.

3.3.3 Histological type, tumor grade, tumor size, lymph node status and histological type

These indices were also collected from histology records using the data collection tool in the appendix 1.

3.3.4 Immunohistochemical Subtypes

Cases of breast cancer were classified into immunohistochemical subtypes based on the St. Gallen guidelines of 2013 as follows:

Table 1: Criteria and conditions of classification of immunohistochemical subtypes based on molecular markers. Adopted from: Goldhirsch, Winer, Coates, Gelber, Piccart-Gebhart (2013).

Subtype	**Marker conditions**
Luminal A	ER+, PR+, HER2-, Ki-67 < 14%.
Luminal B	ER+, HER2-, and at least one of: Ki-67 ≥ 15%, PR < 20%.
	ER+, HER2+, Any Ki-67, Any PR.
HER2- Enriched	HER2+, ER-, PR-.
Triple Negative	HER2-, ER-, PR-.

3.4 Data Analysis

The data was tabulated and cleaned for errors in Microsoft Excel 2010. Analysis was done using the Statistical Software Package for the Social Sciences (SPSS) version 26 from IBM(released in 2019). Frequencies were determined for all the indices and the clinicopathological characteristics per each immunohistochemical sub-type. The mean age and range as well as immunohistochemical subtypes per age group were also determined. A chi-square test was used to analyse associations between immunohistochemical subtypes and histological type, tumor grade, tumor size range and lymph node status. An ANOVA test was used to determine the relationship between age and immunohistochemical subtypes. Results were considered significant at $p<0.05$ for the chi-square and ANOVA tests.

3.5 Reliability and Validity

Reliability is the extent to which a data collection tool captures and produces stable and consistent results (Noble & Smith, 2015). This speaks to the ability of the research tool to produce repeatable results. Data obtained from the cancer facilities has been subjected to quality assurance procedures, as required by the Breast Cancer Control Policy of South Africa published in 2017. Immunohistochemical profiling was done using tested standard immunohistochemical staining procedures and the scoring of the immunohistochemical stains repeated on the 10X and 100X magnification on the microscope. The results were then assessed by pathologists, clinicians and external quality assurance bodies in accordance with the policy. The data was captured by the researcher and further checked by the supervisor to ensure correctness.

In data collection, validity refers to research methods measuring the data that is intended to be measured (Noble & Smith, 2015). In this study, to ensure validity, the data collection was validated through past literature and a mini pilot on the first 30 records obtained at Polokwane Lancet Laboratories. Furthermore, validation is strengthened by the adherence of the histological laboratories to daily quality assurance measures on an immunohistochemical apparatus. The laboratories use external control staining, presence of benign breast tissue as an internal positive control and non-epithelial elements as negative controls as required by the above-mentioned policy to ensure that the correct data is being collected.

3.6 Ethical Considerations

3.6.1 Ethical Clearance and Approval

Ethical approval to carry out this research was granted by Turfloop Research Ethics Committee (TREC) of the University of Limpopo (TREC/91/2020: PG), Polokwane-Mankweng Research Ethics Committee (PMREC 29 July 2020 UL 2020/B), Limpopo Department of Health and Lancet management. Further permission was requested from Lancet Polokwane director, Dr Mahlakwane and Mankweng clinical director, Dr Moila.

3.6.2 Anonymity and Confidentiality

No patient names or identifying information were used in the recording of data or in the dissemination of results as reflected in the data collection tool (Appendix 1). Data collected was assigned an identity code that ensures the above, and was stored electronically in Microsoft Excel® and the file was password encrypted.

3.6.3 Informed Consent and Voluntary Participation

This study made use of secondary data from hospital records which is data that has been previously collected for other purposes to aid in diagnosis and treatment/management. Based on Section 3.3.7 of the Department of Health's Ethics in Health Research guideline, research of this nature does not require consent from participants. The section also states that if the data collected are anonymous and no harm is envisaged on any individual, family or community, then new consent is not required. Therefore, in this research, new informed consent was not required as records that were used have signed consent from patients for broad and future use of their information in research. Furthermore, no interaction or collection of new samples from patients was done in this research.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter outlines the results obtained from the study. Singular indices, that is, the age, molecular markers, histological type, tumor size, tumor grade, lymph node status and immunohistochemical subtypes are reported first in frequencies and other measures of central tendency. This is then followed by the results on the association relationships between each index and immunohistochemical subtypes. Where there are variations and/or omissions, a reason is provided.

4.2 Presentation of Results

This study was conducted retrospectively on 151 patient records generated between 2015 and 2020 from Polokwane Lancet Laboratories and Mankweng Hospital.

4.2.1 Age

The mean age of the population was 50.32 ± 11.40 standard deviation, with a range of 28 to 90 years of age. Participants were grouped according to specific age ranges. Just over one percent (1.3%) of the population was less than 30 years of age, 15% were 31-40, 42.4% were 41-50, 23.2% were 51-60, 12.6% were 61-70, 4.6% were 71-80 and 0.7% were 80-90 years of age. Figure 2 expresses the age distribution of breast cancer patients in the study.

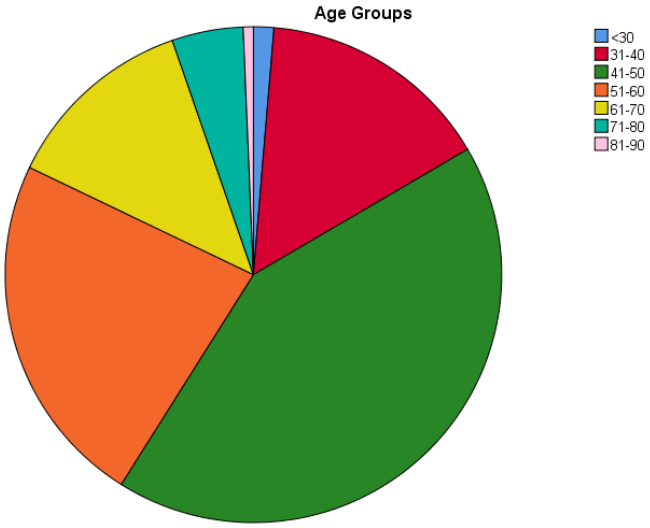


Figure 2: Age distribution of 151 breast cancer patients at Polokwane Lancet Laboratories and Mankweng Hospital in Limpopo Province.

4.2.2 Molecular Marker Frequencies

Over 100 cases (111, 73.5%) had ER+ tumors, 94 cases (62.3%) had PR+ tumors and 40 cases had HER2 positive tumors. One case showed an equivocal level of HER2 (2+), and SISH(Silver in situ hybridization-a technique used to further classify HER2 levels, especially if the level obtained in immunohistochemical tests is equivocal) was not performed to further classify the case as being positive (3+). Over 50 cases (57, 37.7%) had a Ki-67 level of <15% and 82 (54.3%) had a level >15%. 12 cases (7.9) % did not have a Ki-67 level on record. Table 2 below shows the frequency levels of positive and negative cases of each molecular marker.

Table 2: Molecular marker frequencies and percentages for 151 cases of breast cancer at Lancet Laboratories and Mankweng Hospital, Limpopo Province

Molecular Marker	**Status**	**Frequency**	**Percent**
ER	Negative	40	26.5
	Positive	111	73.5
PR	Negative	57	37.7
	Positive	94	62.3
HER2	Equivocal (SISH not performed)	1	0.7
	Negative	110	72.8
	Positive	40	26.5
Ki-67 proliferation marker	< 14%	57	37.7
	> 15%	82	54.30
	Not determine	12	7.9

4.2.3 Histological Type

Infiltrating ductal carcinoma (IDC) of no special type was the most predominant histological type in the study population, with a frequency of 142 cases (94.0%). This was followed by IDC of the mucinous/colloid type, with a frequency of 5 cases (3.3 %). The less frequent histological types of breast cancer included IDC of the papillary type, infiltrating lobular carcinoma, ductal carcinoma *in-situ* and secretory breast cancer, each at a frequency of 1 case (0.7%). Figure 3 shows the frequency distribution of each breast cancer histological type observed in the study.

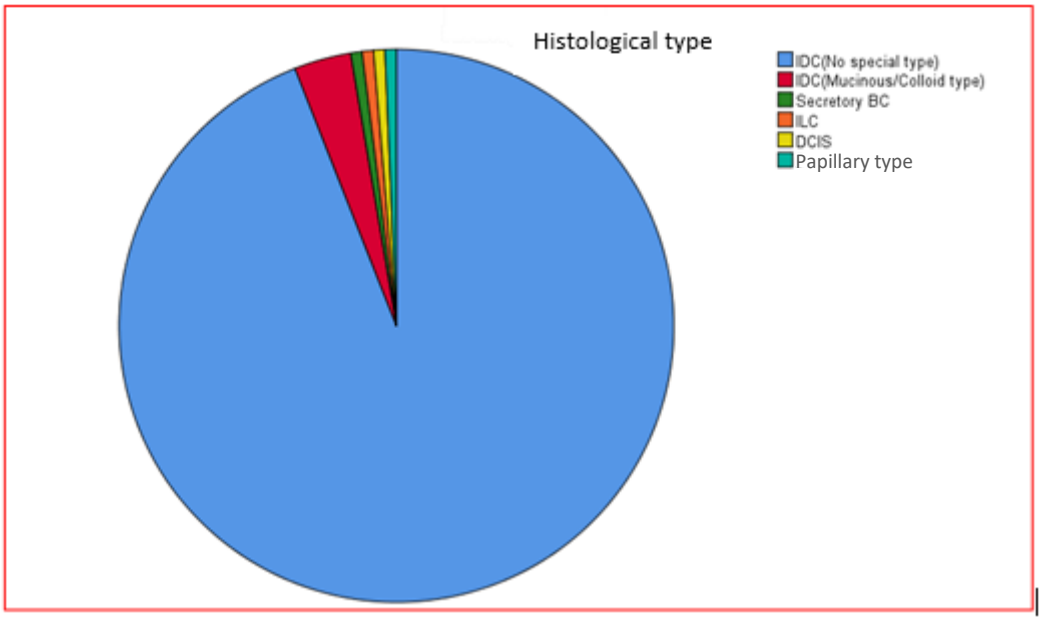


Figure 3: Pie chart reflecting percentage distribution of histological types of 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

4.2.4 Tumor Size

Tumor size was categorically arranged into groups depending on size. Table 3 shows the frequency distribution of the observed tumor ranges. Over two thirds (65.6%) of patients did not have their tumor size determined because of the heterogeneity in collecting the sample. Pathologists report that methods such as Trucut needle biopsy are the most favourable collection methods currently used as they reduce surgical work. However, these methods also limit the ability to sample a complete tumor to be measured for size. Cases with recorded tumor size showed an average tumor size of 3.36 ± 1.93cm.

Table 3: Distribution of Tumor sizes per size range in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

Tumor Size		
	Frequency	Percent
Not reported	99	65.6
<1 cm	20	13.2
1.1- 2 cm	8	5.3
2.1-3 cm	8	5.3
3.1- 4 cm	6	4.0
4.1- 5 cm	3	2.0
5.1- 6 cm	3	2.0
>6 cm	4	2.6
Total	151	100.0

4.2.5 Tumor Grade

Tumor grade was determined by pathologists at the respective institutions using the modified Bloom-Richardson TNM staging. Grade II breast tumors were the most prevalent in the population, with Grade I as the least frequent. Table 2 shows the frequencies of each tumor grade in the study population.

Table 4: Frequencies and percentages of histologic Tumor grade in 151 breast cancer cases seen at Polokwane Lancet Laboratories and Mankweng Hospital in Limpopo Province.

Tumor Grade		
	Frequency	Percent
Precluded	7	4.6
Grade I	5	3.3
Grade II	78	51.7
Grade III	61	40.4
Total	151	100.0

4.2.6 Lymph Node Status

The lymph node status reflects whether or not the pathologist identified cancerous cells in the lymph nodes obtained from the tissue specimen. In cases where the lymph node status was not assessed in the collected specimen, Table 5 of frequencies reflects these as “not recorded”. Positive cases were those whose lymph node status was reported in the pathology report and were certified to have cancer cells. Negative cases were those whose lymph node status was reported not to show cancer metastasis. Cases that showed reactive hyperplasia are those cases in which the cancer cells have entered a stage of becoming cancerous, but are not completely cancerous yet.

Table 5: Frequencies and percentages of lymph node status in 151 breast cancer cases seen at Polokwane Lancet Laboratories and Mankweng Hospital in Limpopo Province.

Lymph node Status		
	Frequency	Percent
Not reported	52	34.4
Negative	12	7.9
Positive	66	43.7
Shows reactive hyperplasia	21	13.9
Total	151	100.0

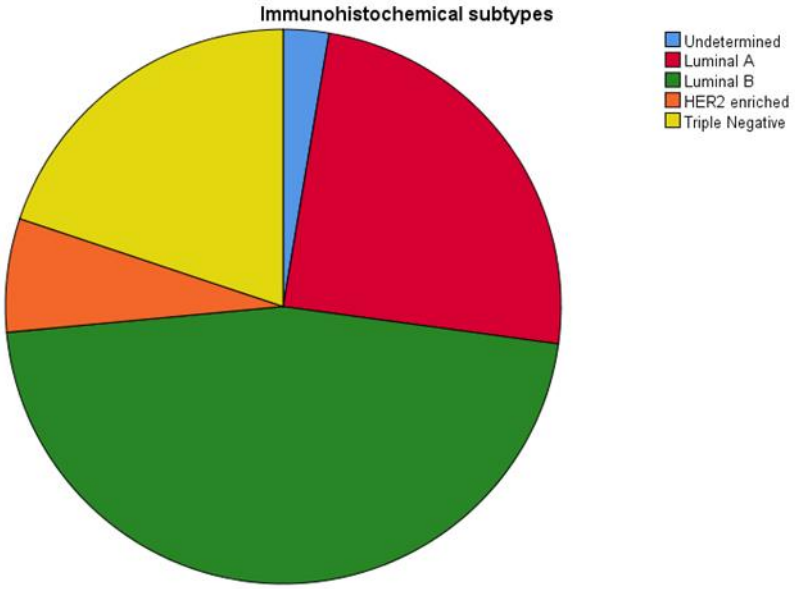
4.2.7 Immunohistochemical Subtypes

The immunohistochemical subtypes were determined based on the molecular markers in accordance with Table 1 on page 19. Luminal B was found to be the most prevalent subtype with a frequency of 46.4%. HER2- enriched was the least frequent at 6.6% (10 patients). Three cases (2.0%) were classified as undetermined as they did not fit any of the St. Gallen Guidelines of classification. Table 6 and Figure 4 outline the frequencies of the immunohistochemical subtypes of breast cancer in the study.

Table 6: Frequencies and percentages of immunohistochemical subtypes of 151 breast cancer cases seen at Lancet laboratories and Mankweng hospital, Limpopo province.

	Frequency	Percent
Undetermined	4	2.6
Luminal A	37	24.5
Luminal B	70	46.4
HER2 enriched	10	6.6
Triple Negative	30	19.9

Figure 4: Pie chart of percentages of immunohistochemical subtypes in 151 breast cancer cases seen at Lancet laboratories and Mankweng hospital, Limpopo province.



4.2.8 Immunohistochemical Subtypes and Age

A one-way ANOVA analysis showed that there is no significant difference in the mean age of diagnosis for each of the immunohistochemical subtypes (p=0.774) (see Table 7). All immunohistochemical subtypes had most of their cases occur in the ranges 41-50 and 51-60 years of age. Luminal A had 72.97% of its cases in the aforementioned ranges age, Luminal B had 57.14 %, HER2- enriched had 90% and Triple Negative subtype had 63.33 % of its cases in these age categories (see Table 8). Chi- square analysis revealed no significant difference between age ranges amongst different subtypes (likelihood ratio p=0.70).

Table 7: Mean age at diagnosis for each immunohistochemical subtype in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

	Immunohistochemical subtypes					
Immunohistochemical subtype	Undetermined	Luminal A	Luminal B	HER2-enriched	Triple negative	Total
Mean	52.50	50.92	49.61	54.20	49.47	50.28
N	4	37	70	10	30	151
Std. Deviation	6.245	10.830	12.368	8.879	11.572	11.468

Table 8 Age group frequencies and percentages amongst immunohistochemical subtypes in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province

		Immunohistochemical subtypes				
		Undetermined	Luminal A	Luminal B	HER2-enriched	Triple Negative
		n (%)	n (%)	n (%)	n (%)	n (%)
Age Groups	<30	0(0)	1(2.70)	0(0.00)	0(0.00)	1(3.33)
	31-40	0(0)	3(8.11)	16(22.86)	0(0.00)	4(13.33)
	41-50	2(50)	17(45.95)	26(37.14)	5(50.00)	14(46.67)
	51-60	2(50)	10(27.03)	14(20.00)	4(40.00)	5(16.67)
	61-70	0(0)	4(10.81)	10(14.29)	1(10.00)	4(13.33)
	71-80	0(0)	2(5.41)	3(4.29)	0(0.00)	2(6.67)
	81-90	0(0)	0(0.00)	1(1.43)	0(0.00)	0(0.00)
Total		4	37	70	10	30

4.2.9 Immunohistochemical Subtypes and Histological type

Cross-tabulation showed that a majority of all the histochemical subtypes are of the IDC (No special type) (see Table 9). Luminal A had IDC (Mucinous/Colloid type) as the second most prevalent histological type, with none of its cases being DCIS or Secretory breast cancer. Luminal B breast cancers had IDC (Mucinous/Colloid) and Secretory breast cancers equally as the second most frequent subtypes, and none of the cases were DCIS, ILC or IDC (Papillary type). HER2- enriched breast cancers had both IDCI and IDC (Mucinous/Colloid type) as the second most frequent histological

types, with none of the cases being ILC, Secretory breast cancer or IDC (Papillary type). All Triple negative breast cancers were also IDC (No special type). No significant difference was observed in the histological type distribution amongst the immunohistochemical subtypes (likelihood ratio p=0.51).

Table 9: Histological type frequencies and percentages amongst immunohistochemical subtypes in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

		Immunohistochemical subtypes				
		Undetermined	Luminal A	Luminal B	HER2-enriched	Triple Negative
		n (%)	n (%)	n ()	n (%)	n (%)
Histological type	IDC (No special type)	4(100)	32(86.5)	68(97.1)	8(80)	30(100)
	IDC (Mucinous/Colloid type)	0(0.0)	3(8.1)	1(1.4)	1(10)	0(0.0)
	Secretory BC	0(0.0)	0(0.0)	1(1.4)	0(0.0)	0(0.0)
	ILC	0(0.0)	1(2.7)	0(0.0)	0(0.0)	0(0.0)
	DCIS	0(0.0)	0(0.0)	0(0.0)	1(10)	0(0.0)
	IDC (Papillary type)	0(0.0)	1(2.7)	0(0.0)	0(0.0)	0(0.0)
Total		4	37	70	10	30

4.2.10 Immunohistochemical Subtypes and Tumor size

All immunohistochemical subtypes had most of their tumors being <1 cm in diameter. No significant difference was observed in tumor size amongst the immunohistochemical subtypes (likelihood ratio p=0.49) (see Table 10)

Table 10: Tumor size distribution frequencies amongst immunohistochemical subtypes in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

		Immunohistochemical subtypes				
		Undetermined	Luminal A	Luminal B	HER2-enriched	Triple Negative
		n (%)	n (%)	n (%)	n (%)	n (%)
Tumor size	Not reported	1(25.0)	24(64.9)	50(71.4)	6(60.0)	18(60.0)
	<1 cm	0(0.0)	6(16.2)	6(8.6)	2(20.0)	6(20.0)
	1.1- 2 cm	1(25.0)	1(2.7)	4(5.7)	0(0.0)	2(6.7)
	2.1-3 cm	0(0.0)	2(5.4)	3(4.3)	2(20.0)	1(3.3)
	3.1- 4 cm	1(25.0)	2(5.4)	1(1.4)	0(0.0)	2(6.7)
	4.1- 5 cm	0(0.0)	1(2.7)	2(2.9)	0(0.0)	0(0.0)
	5.1- 6 cm	0(0.0)	0(0.0)	3(4.3)	0(0.0)	0(0.0)
	>6 cm	1(25.0)	1(2.7)	1(1.4)	0(0.0)	1(3.3)
Total		4	37	70	10	30

4.2.11 Immunohistochemical Subtypes and Tumor grade

Luminal A and Luminal B histochemical subtypes had most of its tumors in the Grade II category. HER2-enriched and Triple negative had most of its tumors as Grade III (see Table 11). A significant difference was observed in the association between immunohistochemical subtypes and tumor grade (likelihood ratio p=0.000365).

Table 11: Tumor grade distribution frequencies amongst immunohistochemical subtypes in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

		Immunohistochemical subtypes				
		Undetermined	Luminal A	Luminal B	HER2-enriched	Triple Negative
		n (%)	n (%)	n (%)	n (%)	n (%)
Tumor grade	Precluded	0(0.0)	2(5.4)	5(7.5)	0(0.0)	0(0.0)
	Grade I	0(0.0)	2(5.4)	1(1.4)	1(10.0)	1(3.3)
	Grade II	3(75.0)	24(64.9)	43(61.4)	1(10.0)	7(23.3)
	Grade III	1(25.0)	9(24.3)	21(30.0)	8(80.0)	22(73.3)
Total		4	37	70	10	30

4.2.12 Immunohistochemical Subtypes and Lymph Node Status

Most of the cases of Luminal A, Luminal B and HER2- enriched had lymph nodes that showed cancer metastasis (as shown in Table 12). HER2- enriched breast cancers had an equal number of cases being negative and showing reactive hyperplasia. There was no significant difference in lymph node status amongst the immunohistochemical subtypes (likelihood ratio p=0.4).

Table 12: Lymph node status distribution frequencies amongst immunohistochemical subtypes in 151 breast cancer cases seen at Lancet Laboratories and Mankweng Hospital, Limpopo Province.

		Immunohistochemical subtypes				
		Undetermined	Luminal A	Luminal B	HER2-enriched	Triple Negative
		n (%)	n (%)	n (%)	n (%)	n (%)
Lymph node status	Not reported	1(25.0)	14(37.8)	21(30.0)	2(20.0)	14(46.7)
	Negative	1(25.0)	2(5.4)	5(7.1)	1(10.0)	32(10.0)
	Positive	2(50.0)	14(37.8)	33(47.1)	7(70.0)	10(33.3)
	Shows reactive hyperplasia	0(0.0)	7(18.9)	11(15.7)	0(0.0)	32(10.0)
Total		4	37	70	10	30

CHAPTER 5
DISCUSSION

This chapter discusses the research results in line with the research aim, objectives and literature.

The determination of immunohistochemical subtypes through molecular markers ER, PR, HER2 and Ki-67 is pivotal in the effective management of breast cancer. Equally important for treatment and prognosis outcomes is assessment of clinicopathological characteristics of breast cancer. Considering this, research on the relationship between the immunohistochemical subtypes and clinicopathological characteristics of breast cancer has become important in continued patient management.

Several studies have investigated the association between immunohistochemical subtypes and various clinicopathological characteristics in South Africa, Africa and other parts of the world. Van Bogaert conducted the only study published on the immunohistochemical profiles of breast cancer amongst women in Limpopo Province in 2013. The study excluded patients seen at private histopathological facilities. In the study, Van Bogaert did not correlate the immunohistochemical subtypes with any clinicopathological features of breast cancer, or give demographic information such as age of diagnosis. The aim of this study was to determine the immunohistochemical subtypes of breast cancer and to correlate them to clinicopathological characteristics. This was conducted in retrospect on 151 patients who were diagnosed with breast cancer between 2015 and 2020 in Polokwane Lancet Laboratories and Mankweng Hospital.

The age at diagnosis of a breast cancer patient is important in both research and clinical practice. The risk of breast cancer is set to increase with age and the onset of menopause (Surakasula, Nagarjunapu & Raghavaiah, 2014). In this study, the age at diagnosis was collected for all 151 cases, and the mean age at diagnosis was determined. The mean age of cancer diagnosis was found to be 50.32 ± 11.40 standard deviation, with a range of 29 to 90 years amongst the study cohort. The results show consistency in the average age of cancer diagnosis amongst populations in Africa. Studies conducted by Adani-Ife, Amegbor, Doh and Darre on 117 women in Togo; Sengal, Haj-Mukhtar, Ehaj, Bedri, Kantehadt and Muhamedani on 678 women in Sudan; and in Eritrea by McCormack, Mckenzie, Foerster, Zietsman, Galukande *et*

al. on 2228 women from Ethiopia, Kenya, Mauritius, Mozambique, Uganda, Zimbabwe, Namibia, Nigeria, Mali, Ghana, Gambia and South Africa show that the estimated mean age at diagnosis of breast cancer ranges between 50 and 60 amongst African women. When compared with European studies, a difference of approximately ten years was observed in the average age of cancer diagnosis. A Belgian study conducted on 358 European women by Preat, Simon and Noel in 2014 reported the mean age of diagnosis of breast cancer in European women as 60 ± 13.0 standard deviation. A Swiss study conducted on 1214 women in Switzerland in 2009 by Spitale, Mazzola, Soldini, Mazzachei and Bordon reported a mean age of 62.7± 14.4 standard deviation. These contrasts indicate early incidences of breast cancer in African women than those in Europe. Comparative studies have been conducted amongst African-American women with women of European descent. The studies indicate that the mean age at diagnosis is lower in African American women than in women of European descent (Yedjou, Sims, Miele, Noubissi, Lowe *et al.*, 2019; Danforth, 2013; Anderson, Rosenberg, Menashe, Mitani & Pfeiffer, 2008; Hausauer, Keegan & Clarke; 2007).

Furthermore, this study found that over half of the cases (57.4 %) in the study cohort were diagnosed with breast cancer at age 50 or before. Diagnosis of breast cancer before age 50 together with a family history of breast cancer suggests genetic influences on the development of the cancer, namely, mutations in *BRACA 1* and *BRACA 2* genes (Godet & Gilkes, 2017). Based on this outcome, it can be suggested that hereditary genetic mutations are amongst the causes of breast cancer in this study cohort. This outcome speaks to the need for genetic testing and further research on the genetic influences of breast cancer in Limpopo Province. The lower mean age at diagnosis of the study cohort indicates the need for early screening and testing of breast cancer amongst women in Limpopo Province, as well as the need for comparative research on the onset of breast cancer in black versus white women in South Africa for the purpose of targeted screening and therapies.

The study further found no significant relationship between the age of diagnosis and the immunohistochemical subtype. That is, the age of a patient does not contribute to whether or not the patient is diagnosed with Luminal A, Luminal B, Triple negative or HER2- enriched breast cancer. Studies that also reported a similar outcome include those by Adani-Ife, Amegbor, Doh and Darre (2020), McCormack *et al.* (2013) and

Gakinya (2010). However, other studies have observed a significant correlation between immunohistochemical subtypes and age (Sengal *et al.*, 2017; Widodo, Dwianingsih, Triningsih, Utoro & Soeripto, 2014; Spitale *et al.*, 2009). The contrast in results can be explained by differences in sample sizes, noting that smaller sample sizes tend to show an insignificant relationship between age and immunohistochemical subtypes. The contrasts can further be explained by variations in how age is grouped.

The molecular markers ER, PR and HER2 have been widely used to determine treatment options for breast cancer patients. Furthermore, they are used in the classification of breast cancer into immunohistochemical subtypes, hence the need for attention in this study. All 151 cases had been histologically confirmed for ER, PR and HER2 as a requirement for cancer diagnosis and treatment in the respective institutions. The results showed ER+, PR+ and HER2+ prevalence of 73.5%, 62.3% and 26.5% respectively. These results show consistency with South African studies by Kakudji, Mwila, Burger, du Plessis and Naidu (2018) and McCormack *et al.* (2013), which showed the same order of prevalence for ER+, PR+ and HER2+ tumors, ER+ tumors being the most prevalent, followed by PR+ and HER2+. These results indicate that over 50% of the study cohort may benefit from adjuvant and/or neoadjuvant endocrine therapy, with drugs such as Tamoxifen, Falvestrant or aromatase inhibitors, depending on patient age and whether or not they have reached menopause.

Data on the level of Ki-67 was also collected in this study because of the classification guidelines used to determine the immunohistochemical subtypes (see Table 1). The appropriate cutoff value of Ki-67 is still widely debated internationally, hence ongoing research. In this study, a cutoff value of ≤14 %/>15% was used to classify the Ki-67 level as being low (≤ 14%) or high (>15%). Results showed that more than half of the patients (54.3%) have a Ki-67 level >15%. Studies have correlated a high Ki-67 index with adverse prognostic factors, including high tumor grade, which points out rapid cancer progression (Madani, Payandeh, Sadeghi, Motamed & Sadeghi, 2016). Based on these results, we concluded that over 50% of cancer cases in Limpopo Province may present with unfavourable prognostic factors because of high levels of Ki-67(>15%).

Molecular markers were then used to classify different cases into immunohistochemical subtypes, as mentioned above. The distribution of the different immunohistochemical subtypes of breast cancer has been described for different populations globally, including populations in Asia, Europe, America as well as Africa. South African studies have also quantified this distribution, and a contrast of results has been observed, both in South Africa and globally. This study is the second, following that of van Bogaert in 2013, to research the immunohistochemical subtypes of breast cancer in Limpopo Province, South Africa. The results indicate that Luminal B subtype is the most predominant in the study population, with a prevalence of 46.4%. Over 20% (24.5%) of the cases were classified as being of the Luminal A subtype, followed by 19.9% being Triple negative, and 6.6% of cases were of the HER2-enriched subtype. A total of 2.6 % cases were classified as undetermined as they did not fit into any of the provided guidelines of classification (see Table 1). These cases were ER-/PR+.

Several studies have shown Luminal B as the most prevalent subtype amongst the Asian populations (Shahid & Galzar, 2018; San *et al.*, 2017), European populations (Kondov, Milenkovikj, Kondov, Petrushevska, Basheska *et al.*, 2018; Preat, Simon & Noel, 2014), American populations (Yabar, Melendez, Munoz, Deneo, Freire *et al.*, 2017) and African populations (Brandao, Guisseve, Bata, Alberto, Ferro *et al.*, 2020). Locally, a study by Kakudji, Mwila, Burger, du Plessis and Naidu conducted on 134 women at Potchefstroom Hospital in North West in 2018 also shows agreement with this study, indicating Luminal B as the most prevalent immunohistochemical subtype. However, some local studies, including a study by van Bogaert conducted in 2013 on 769 women in Limpopo Province, and a study by McCormack, Joffe, van den Berg, Broez and dos Santos Silva conducted in 2012 on 1218 women in Gauteng Province, have observed different results on the most prevalent immunohistochemical subtype. These studies have observed Luminal A as the most prevalent. This variation in results may be because in other studies, unlike this one and supporting studies, the criteria for classifying the immunohistochemical subtypes did not employ the level of Ki-67 as a cut-off factor to distinguish Luminal A subtypes that are HER2 negative. Furthermore, even some studies that do employ the Ki-67 level as a classification criterion have a different cut-off level, hence the variations in the dominant subtype between Luminal A and Luminal B.

Research data showed that most of the cases of breast cancers in Limpopo Province (65.6 %) do not have the tumor size determined or reported because of the method of sample collection. During the data collection process, patient records showed that the preferred method of sample collection is a TRUCUT needle biopsy. Pathologists at the respective institutions report that this is because the method is less invasive, less expensive, has a low complication rate and is much simpler to perform than other forms of sample collection such as mastectomy. However, some data on tumor size were collected in the study. The data revealed that most tumors were <1cm in size (13.6%). A little over ten percent (10.6% %) of the tumors were between 1.1 and 3 cm in diameter, while 2.6% of the tumors were >6cm in diameter. The small sample size gives a limitation to the meaning of the results, but it can be suggested that more patients in the study cohort present with tumors less than 1cm in diameter. For this study cohort, this points to the notion that breast cancers diagnosed in Limpopo Province are diagnosed early in their development, hence the small tumors at diagnosis.

Considering the relationship between tumor size and immunohistochemical types, this study found no significant difference in the tumor size amongst the immunohistochemical subtypes. The study did find that most of the tumors were less than 1 cm in diameter for all the histochemical subtypes. These findings are consistent with findings by Adani-Ife, Amegbor, Doh and Darre (2020), So, Han and Seo (2016), Widodo *et al.* (2014) and Gakinya (2010).

This study also looked at the histological type of breast cancer as a clinicopathological characteristic of breast cancer. The determination of breast cancer histological type is important in research because of its association with distinct presentations and outcomes of the disease (Weigelt, Geyer & Reis-Filho, 2010). Knowledge of the histological type enables medical practitioners and researchers to determine disease prognosis. This study found that IDC (No special type) was the most predominant histological type, with a prevalence of 94.0%. This finding was consistent with African (Adeniji, Dawodu, Habeebu, Oyekan, Bashir *et al.*, 2020; Adani-Ife, Amegbor, Doh & Darre, 2020; Alwan & Tawfeeq, 2019; Sengal *et al*., 2017), Asian (Shahid & Gulzar, 2018; San, *et al.*, 2017; Sohn, Han & Seo, 2016; Liu, Ren, Yan, Zhang, Luo, *et al.*, 2015); European (Yabar, Melendez, Munoz, Deneo, Freire *et al.*, 2017) and American (Porro, Mrazek, Washington & Chao, 2014) studies, which also indicate that IDC

(NST) is the predominant histological type of breast cancer. With this finding and the backing research papers, it can be said that there is global consistency in terms of the predominant breast cancer histological type amongst African, Asian, European and American patients in spite of race or geographical location.

This research also found IDC (Colloid/Mucinous type) as the second most prevalent subtype (3.3%), with the other subtypes (IDC (Papillary type), ILC, DCIS and Secretory breast cancer) being the least predominant histological types of breast cancer, with a prevalence of 0.7% each. Some studies have reported ILC as the second prevalent histological type of breast cancer after IDC (No special type) cancer, as opposed to findings in this study (Amegbor, Doh & Darre,2020; Shahi & Gulzar, 2018; Alwan, Kerr, Al-Okati, Pazella, Furat & Tawfeeq, 2018). Several reasons for this variation have been noted, including sample size variations and differences in classification schemes (some researchers combine IDC (NST) and IDC (mucinous/papillary) as one IDC class). This indicates the need for further research and standardising of research methods and classification schemata.

Association analysis with the likelihood ratio showed that there is no significant difference or association between histological type of breast cancer and the type of immunohistochemical subtype. This means that having a certain immunohistochemical subtype of breast cancer does not significantly affect the histological type that the breast cancer is, but the reverse is true. Sohn, Han and Seo (2016) and Gakinya (2010) have also observed these same findings.

Breast cancer tumors are given a grade depending on how different the tumor cells are from normal cells and how fast they are growing. Tumor grade is important in determining treatment options when considered alongside other clinicopathological factors (Oluogun, Adedokun, Oyenike & Adeyeba, 2019). The study results showed that grade II tumors were the predominant type of tumors in the study cohort, accounting for 51.7% of the study population tumors. This finding agrees with those by Adani-Ife *et al.* (2020) and Oluogun, Adedokun, Oyenike and Adeyeba (2019). This was followed by Grade III tumors at 40.4% and Grade I tumors being the least predominant at 3.3 %. Over 4% (4.6 %) of the cases had a precluded tumor grade due to tumor heterogeneity or insufficient sample.

This study found a significant relationship between immunohistochemical subtypes and the tumor grade. Analysis shows that less aggressive subtypes of breast cancer, Luminal A and Luminal B are associated with a lower tumor grade of grade II, while the more aggressive subtypes of breast cancer HER2- enriched and Triple negative tend to associate with the more undesired grade III tumors. This agrees with reports that Luminal A and Luminal B subtypes are associated with less harsh clinicopathological characteristics as opposed to the more aggressive HER2- enriched and Triple negative subtypes (Oluogun, Adedokun, Oyenike & Adeyeba, 2019; Bennis *et al.*, 2012).

Lastly, the results showed that a majority of the breast cancer cases in the study cohort have lymph node involvement (43.7%) as opposed to the lesser 7.9 % cases, which were negative. Over 10% (13.9 %) of the cases presented with reactive hyperplasia, while 34.4 % had no information on the lymph node status. When corrected for the unreported cases, it is shown that over 60% (66%) of the breast cancer cases in the study population present with lymph node metastasis. This finding is consistent with findings by Kakudji, Mwila, Burger, du Plessis and Naidu (2020), Elidrissi Errahhali *et al.* (2017) and Sengal *et al.* (2017), who also found that a great number of breast cancer cases with known lymph node status showed lymph node involvement.

The difference in lymph node involvement amongst the immunohistochemical subtypes was found to be insignificant. This finding is consistent with findings by Sengal *et al.* (2017). Although a significant difference was not seen, it was observed that Triple negative breast cancers had less lymph node involvement (more negative cases than positive) than the other three subtypes.

CHAPTER 6

CONCLUSION

Evaluation of molecular markers and thus immunohistochemical subtypes and clinicopathological characteristics is an ongoing and important subject of study in the field of breast cancer.

This study found that most cases of breast cancer are diagnosed at an age less than 50, which is an approximate decade earlier than breast cancer cases in Europe, but is consistent and in line with African patterns. It was also found that most of the cases are of IDC (no special type), of Luminal B subtype, have tumors less than 1 cm in diameter, and the tumors are predominantly of Grade II. There was no significant association between immunohistochemical subtypes of breast cancer and age, tumor size, tumor grade, lymph node status or histological type of cancer. However, this research pointed out that aggressive immunohistochemical subtypes of breast cancer, that is, HER2- enriched and Triple negative breast cancers are more likely to have a higher tumor grade (Grade II or III) compared to Luminal A and Luminal B (Grade I or Grade II). The results of this study, in contrast with other published research papers, indicate that breast cancer patients in Limpopo Province have a high Ki-67 level. In such a case, the breast cancer tends to proliferate rapidly and metastasises. This is supported by the finding that most of the cases in the study also have lymph node involvement, with others showing the pre-phase of this invasion, reactive hyperplasia. This suggests that breast cancer cases in Limpopo Province develop rapidly and can metastasise quickly.

Some variation and inconsistencies have been observed between this study and other studies of the same nature. These variations may be a result of heterogeneity in methodologies of categorising, and thus analysing data such as age and tumor size, classification criteria for immunohistochemical subtypes based on the hormonal markers, sample size and mode of data collection. These variations, therefore, warrant the need for further research on the current topic and standardisation of data collection methods and analysis approach to obtain consistent results.

To the best of our knowledge, only one study was published on the immunohistochemical subtypes of breast cancer in Limpopo Province by Van Bogaert in 2013. However, the study did not report on the age of diagnosis of patients or

clinicopathological characteristics. It also did not outline when the data was collected. This current study was aimed at determining the immunohistochemical subtypes of breast cancer in Limpopo Province and to associate them with clinicopathological and sociodemographic characteristics.

The study successfully evaluated the above-mentioned association, factoring in age of patients diagnosed and when the data was collected. Although success was met in this regard, there were limitations to the present study as well. Factors such as relying only on data in the records and not retrieving new and additional data from stored samples and patients, and usage of a small sample size due to time constraints imparted some limitations to the study. COVID-19 regulations also impaired this study as some areas of Mankweng Hospital where data could be collected were not easily accessible. The nature of how data was recorded in the respective institutions also limited the study in that some of the indices, especially further demographic information of patients, were not on record. This was seen predominantly on records from the private sector.

In conclusion, this study was able to shed light on breast cancer subtypes in Limpopo private and public sectors, thus informing the population on the average age of breast cancer onset, immunohistochemical subtypes of breast cancer and clinicopathological characteristics. Considering the whole research, we recommend early screening of breast cancer in Limpopo Province and further research on factors resulting in the early onset of breast cancer in this population.

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APPENDICES

Appendix 1. Data Collection Tool.

Medical Record Data Collection Form on Histopathological profile		
Assigned Record Number		
Date of Diagnosis		
Personal Information		
Variable	Description	
Age at Diagnosis		
Menopausal Status		
Ethnicity		
Race		
Medical Facility		
Geographic Region		
Histochemical Profile		
Variable	Description	Level
ER Status		
PR Status		
Ki-67 Status		
HER2 Status		
Breast Cancer Classification		
Variable	Description	
Luminal A		
Luminal B		
HER2		
Triple Negative		
Clinicopathological charectaristics		
Grade		
Tumor size		
Lympnode status		
Resection margins		
Lymphovascular involvement		
Tubular growth		
Histological subtype		

Appendix 2. Letters of request for data.

2.1 Limpopo department of health

Room 12 BLOCK F2

MBH Residence

University of Limpopo

PO Box X1106

Sovenga

0727

The Manager (Research Office)

Limpopo Department of Health

College Avenue

Hospital Park

0699

RE: REQUEST FOR ETHICAL CLEARANCE TO USE MEDICAL RECORDS FOR RESEARCH

To Whom It May Concern,

This letter servers as a request for permission to use medical records of breast cancer patients held by Mankweng Hospital and Lancet Laboratories for the purpose of research. A student at the University of Limpopo, Peka L.N.J, will conduct the research as a requirement for MSc in Medical Sciences at the University of Limpopo for the academic years 2019/2020. The research will be supervised by Dr G.A Adefolaju of the University of Limpopo, Prof K.E Scholtz of the University of Limpopo and Dr F Ooko of Mankweng Hospital.

The aim of the research is to determine the molecular subtypes of breast cancer that are treated in the public and private health sectors in Limpopo Province and associate them to *clinicopathological* characteristics and demographical information to identify which sector sees more cancer patients, hence the request to use data held by the institution. The inclusion and exclusion criteria are as follows:

Inclusion Criteria:

Records of female patients diagnosed with breast cancer during the period of January 2012 to December 2018 and referred to Lancet histopathology laboratory and the records have data on histological confirmation and hormone receptor status.

Exclusion Criteria:

Breast cancer data from male patients will be excluded from this study. All records with incomplete data relevant to the present study will be excluded from this study. Records before 2012 will be excluded from this study because from acquired information from the institutions, such complete records only date back to 2012.
Attached is the data collection tool that outlines the information that will be sought from the records. For further inquiries and concerns, please contact Dr GA Adefolaju on 0152683281 0786462774.

Hope you find the above in order.
Yours faithfully,
Peka L.N.J
Call: 0681518748/Email: lenojopeka@gmail.com

2.2. Polokwane Lancet Laboratories

Room 12 BLOCK F2

MBH Residence

University of Limpopo

PO Box X1106

Sovenga

0727

The Manager

Polokwane Lancet Laboratories

44a Grobler street

Polokwane

0699

RE: REQUEST TO USE MEDICAL RECORDS FOR RESEARCH PROJECT

To Whom It May Concern,

This letter serves as a request for permission to use medical records of breast cancer patients held by the institution for the purpose of research. A student from the University of Limpopo, Ms. L.N.J. Peka, will conduct the research as a requirement for an MSc in Medical Sciences at the University of Limpopo for the academic years 2019/2020. The research will be supervised by Dr G.A. Adefolaju from the University of Limpopo, Prof K.E. Scholtz from the University of Limpopo, and Dr F. Ooko from Mankweng Hospital.

The aim of the research is to determine the molecular subtypes of breast cancers treated in the public and private health sectors in Limpopo Province and associate them with *clinicopathological* characteristics and demographics. The inclusion and exclusion criteria are as follows:

Inclusion Criteria:

Records of female patients diagnosed with breast cancer during the period of January 2012 to December 2018, referred to Lancet histopathology laboratory, with records including data on histological confirmation and hormone receptor status.

Exclusion Criteria:

Breast cancer data from male patients will be excluded from this study. All records with incomplete data relevant to the present study will be excluded from this study.

The data collection tool, study proposal and ethical clearance certificate are attached for your perusal.

For any further inquiries and concerns, please contact Dr GA Adefolaju on 0152683281/0786462774.

I hope you find the above in order.

Yours faithfully,

L.N.J. Peka
Call: 0681518748
Email: lenojopeka@gmail.com

2.3 Mankweng Hospital

Room 12 BLOCK F2

MBH Residence

University of Limpopo

PO Box X1106

Sovenga

0727

Clinical manager

Mankweng Hospital

Private Bag X1117

Sovenga

0727

RE: REQUEST TO USE MEDICAL RECORDS FOR RESEARCH PROJECT

To Whom It May Concern,

This letter serves as a request for permission to use medical records of breast cancer patients held by the institution for the purpose of research. A student from the University of Limpopo, Ms. L.N.J. Peka, will conduct the research as a requirement for an MSc in Medical Sciences at the University of Limpopo for the academic years 2019/2020. The research will be supervised by Dr G.A. Adefolaju from the University of Limpopo, Prof K.E. Scholtz from the University of Limpopo, and Dr F. Ooko from Mankweng Hospital.

The aim of the research is to determine the molecular subtypes of breast cancers treated in the public and private health sectors in Limpopo Province and associate them with *clinicopathological* characteristics and demographics. The inclusion and exclusion criteria are as follows:

Inclusion Criteria:

Records of female patients diagnosed with breast cancer during the period of January 2012 to December 2018, referred to Lancet histopathology laboratory, with records including data on histological confirmation and hormone receptor status.

Exclusion Criteria:

Breast cancer data from male patients will be excluded from this study. All records with incomplete data relevant to the present study will be excluded from this study.

The data collection tool, study proposal and ethical clearance certificate are attached for your perusal.

For any further inquiries and concerns, please contact Dr GA Adefolaju on 0152683281/0786462774.

I hope you find the above in order.

Yours faithfully,

L.N.J. Peka

Call: 0681518748

Email: lenojopeka@gmail.com

Appendix 3. Ethical Approval Letters.

3.1 Turfloop Research Ethics Committee (TREC)

University of Limpopo
Department of Research Administration and Development
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 3935, Fax: (015) 268 2306, Email: anastasia.ngobe@ul.ac.za

TURFLOOP RESEARCH ETHICS COMMITTEE

ETHICS CLEARANCE CERTIFICATE

MEETING:	**24 April 2020**
PROJECT NUMBER:	**TREC/91/2020: PG**
PROJECT:	
Title:	Immunohistochemical Subtypes of Breast Cancer and Their Association with Demographic and Clinico-Pathological Characteristics in The Limpopo Province
Researcher:	LNJ Peka
Supervisor:	Dr GA Adefolaju
Co-Supervisor/s:	Prof KE Scholtz
	Dr F Ooko
School:	Health Care Sciences
Degree:	Master of Science in Medical Science

PROF P MASOKO
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

Note:

i) **This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.**

ii) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.**

iii) **PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

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3.2 Polokwane-Mankweng Research Ethics committee

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT

ENQUIRIES: Mr MA POOPEDI **DATE: 08 October 2020**

MANAGER: CLINICAL RESEARCH

PIETERSBURG/MANKWENG

RESEARCH ETHICS COMMITTEE (PMREC)

ananiaspoopedi@gmail.com

REFERENCE	**: PMREC 29 July 2020 UL 2020/B**
Date	**: 29 July 2020**
RESEARCHER	**: Ms LNJ Peka**
	(PRINCIPAL INVESTIGATOR)
RESEARCH	**: Post-Graduate Research**
DEPARTMENT	**: Healthcare Sciences**

Project Title: Immunohistochemical subtypes of breast cancer and their association with demographic and clinico-pathological characteristics in the Limpopo province.

Approval Status: Approved

NB: A yearly progressed report is required from the applicant until the project is finished.

Kind Regards

Dr W Holtshousen

Interim-Chairperson: Pietersburg/Mankweng Complex Research Ethics Committee

School of Medicine

University of Limpopo

REC 300408-006

3.3. Department of Health Approval letter

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH

Ref : LP -2020-06-018
Enquires : K. Letseparela
Tel : 015-293 6028
Email : Kurhula.Hlomane@dhsd.limpopo.gov.za

Ms LNJ Peka

PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES

Your Study Topic as indicated below;

Immunohistochemical subtypes of breast cancer and their association with demographic and clinic-pathological characteristics in Limpopo Province

1. Permission to conduct research study as per your research proposal is hereby Granted.
2. Kindly note the following:
   a. Present this letter of permission to the institution supervisor/s a week before the study is conducted.
   b. In the course of your study, there should be no action that disrupts the routine services, or incur any cost on the Department.
   c. After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   d. The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   e. The approval is only valid for a 1-year period.
   f. If the proposal has been amended, a new approval should be sought from the Department of Health
   g. Kindly note that, the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated

Head of Department

24/07/2020
Date

Private Bag X9302 Polokwane
Fidel Castro Ruz House, 18 College Street. Polokwane 0700. Tel: 015 293 6000/12. Fax: 015 293 6211.
Website: http/www.limpopo.gov.za

The heartland of Southern Africa – Development is about people!

Appendix 4. Editorial certificate

University of Limpopo
Department of Physiology and Environmental Health
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 4010, Cell: (076) 894 6472, Email: agosi.malatji@ul.ac.za

15 July 2021

Dear Sir/Madam,

SUBJECT: RESEARCH DISSERTATION EDITING

This letter certifies that the dissertation titled, '**Immunohistochemical Subtypes of Breast Cancer and their Association with Demographic and Clinicopathological Characteristics in the Limpopo Province**' by LNJ Peka has been edited as a copy. Additionally, should there not be further amendments, I am satisfied with the grammaticality of the document regarding its observance to the editorial values in the following aspects:

- Consistency
- Cohesion
- Clarity
- Conciseness

I hope you find the above in order.

Best regards,

Mr PA Malatji (MA – UL)

Scholar/Lecturer

Finding solutions for Africa