BREAST CANCER: MOLECULAR CLASSIFICATION USING IMMUNOHISTOCHEMICAL EXPRESSION AT THE NATIONAL HEALTH LABORATORY SERVICE (NHLS), CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH), JOHANNESBURG.

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

of

Master of Medicine in Anatomical Pathology

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DECLARATION

I, Matomola Mwange, declare that this research report is my work. It is submitted for the degree of Master of Medicine in Anatomical Pathology at the University of the Witwatersrand, Johannesburg. This research report has not been submitted before for any degree or examination at this or any other university.

Signed

On this……9……day of ……December……2021

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I would like to thank my supervisor, Dr M Louw, for her support and guidance throughout this process. Your expertise and knowledge in this field are invaluable and helped enrich this study.

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To my colleagues who have walked this long and challenging journey with me, thank you.

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PRESENTATIONS ARISING FROM THIS PROJECT

None.

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ABSTRACT

Background: Breast cancer is a heterogeneous disease, and the current histological classification fails to differentiate cancers with the same histologic patterns that may biologically behave differently. Accurate grouping of breast cancers into clinically relevant molecular subtypes is essential for therapeutic/oncologic decision making and optimal patient care. We set to use immunohistochemistry, an affordable, fast method that requires no ultra-specialised laboratory facilities to determine the molecular subtypes of breast cancer and their distribution in our population group at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), National Health Laboratory Service (NHLS), Johannesburg.

Methods: A Systemised Nomenclature of Medicine based search was performed to retrieve all diagnosed cases of breast cancers (on core biopsies) over one year on the database of the NHLS histology laboratory at Charlotte Maxeke Johannesburg Academic Hospital. The search included the periods from January 1, 2019, to December 31, 2019. The following data was extracted from the histology reports and captured – Age, tumour grade, histological subtype and IHC expression of ER, PR, HER-2 and Ki67 proliferative index. Medians and inter-ranges (IQR) for the continuous variables with skewed distribution. Frequencies and percentages were used to summarise the distribution of categorical variables, whereas the median and interquartile range were used for continuous variables.

Results: A total of 265 diagnosed cases of breast cancers on core biopsies were available for analysis. The median age was 53 years (IQR 41-63), and 53% (n=140) were

aged >50 years. Most tumours were either in the grade 2 or grade 3 category, 49.4% (n=131) and 44.5% (n=118) respectively. Per histological classification, the invasive breast cancer of no special type (IBC, NST) was by far the commonest (n=236, 89.1%), and approximately 8% (n=20) had invasive breast carcinoma of special type. Histological subtype differed by median age and tumour grade: patients with invasive lobular carcinoma had the highest median age, p=0.030 and having IBC, NST was associated with advanced tumour grade (p=0.004). The molecular subtype classifications of the breast cancers were as follows: Luminal B was the highest (n=113, 42.6%), followed by Luminal A (n=79, 29.8%), Basal (n=50, 18.9%), and the smallest proportion was in the HER2 overexpression group (n=23, 8.7%). The age group distribution was not different across the molecular subtypes (p=0.377), but the molecular subtype differed by tumour grade (p<0.001). The proportion of patients with IBC, NST, who had advanced tumour grade was significantly higher than that for the IBC, ST subtype, 46.2% vs 31.0%, p=0.017.

Conclusion: Based on the findings of this single centre study in South Africa, the aggressive molecular subtypes of breast cancer were not the predominant types. Scaling up early diagnosis and treatment as presentation to care is often late may improve breast cancer management outcomes, including survival in low-income countries like South Africa.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Breast cancer is estimated to be the most common cancer worldwide, accounting for about 17.5 million cancer cases and 9 million deaths in 2015 (1). According to the National South African Cancer Registry (2017), breast cancer accounts for approximately 23% of all cancers, with an average age of 37 years and an estimated lifetime risk of 1:25 (2). The cancer burden in Africa has increased, primarily due to lifestyle changes associated with economic development, increase in life expectancy and ,more prolonged survival of patients living with HIV (PLWHIV) receiving antiretroviral therapy (3). PLWHIV have a substantially increased risk of developing cancer as compared to the general population (3). Sub-Saharan African countries (SSA) are currently experiencing the 'double burden' of significant infectious and chronic diseases, and tumours including malignant tumours, in the epidemiological transition (4).

Additionally, these countries also face difficulties in cancer diagnosis, stage assessment and access to cancer drugs at affordable prices (3). It is, therefore, crucial to carefully evaluate the profiles of oncological diseases to prevent suboptimal medical management of patients, bad practices and inadequate allocation of financial resources (3). Breast cancer is the most common cause of death from cancer in women (5). Breast cancer represents a molecularly heterogeneous group of diseases and comprises numerous distinct biological entities, often associated with specific clinical behaviour and pathological features (6,7). In Sub-Saharan Africa, breast cancer clinical presentation

differs from that of high-income countries as the disease tends to be late-stage at presentation, occurs in a higher proportion of young patients and has a very high mortality rate (8).

The mainstay of breast cancer grouping assessment seeks to answer: How bad the tumour is (typing and grading) and how extensive the tumour is (staging) (9). The type and grading are based on the histologic subtypes and grades detailed in the World Health Organization (WHO) tumour classification guide (10). In contrast, the tumour size, nodal status and distant metastasis speaks to the staging (9). Accurate grouping of breast cancers into clinically relevant subtypes is essential for therapeutic/oncologic decision making (11). Perou and colleagues first proposed the molecular classification of breast cancer based on gene expression profiles in 2000 (12). Gene expression profile studies showed that levels of estrogen (ER), progesterone (PR) (both being hormone receptors) and Human Epidermal Growth Factor Receptor 2 (HER-2) overexpression could be used to characterize tumours of different subtypes: Luminal A, Luminal B, HER-2 overexpressed and triple-negative breast cancers (7). Identifying these breast cancer molecular subtypes provides essential information on the prognosis and response to treatment (13).

Numerous studies have shown the possibility of reproducing this molecular classification using simple immunohistochemical tests based on the expression of ER, PR, HER-2, Ki-67, and high and low molecular weight cytokeratin biomarkers, such as CK5/6 (13).

1.2 Epidemiology

The available estimates demonstrate that breast cancer incidence and mortality are rising in low- and middle-income countries (LMICs), including Africa. However, high-quality data from LMICs (and particularly from sub-Saharan Africa) are largely lacking. Case fatality rates from breast cancer appear to be substantially higher in LMICs than in high-income countries (GLOBOCAN 2012) (14). Figure 1 shows estimates from the GLOBOCAN project, run by the International Agency for Research on Cancer (http://www.iarc.fr).

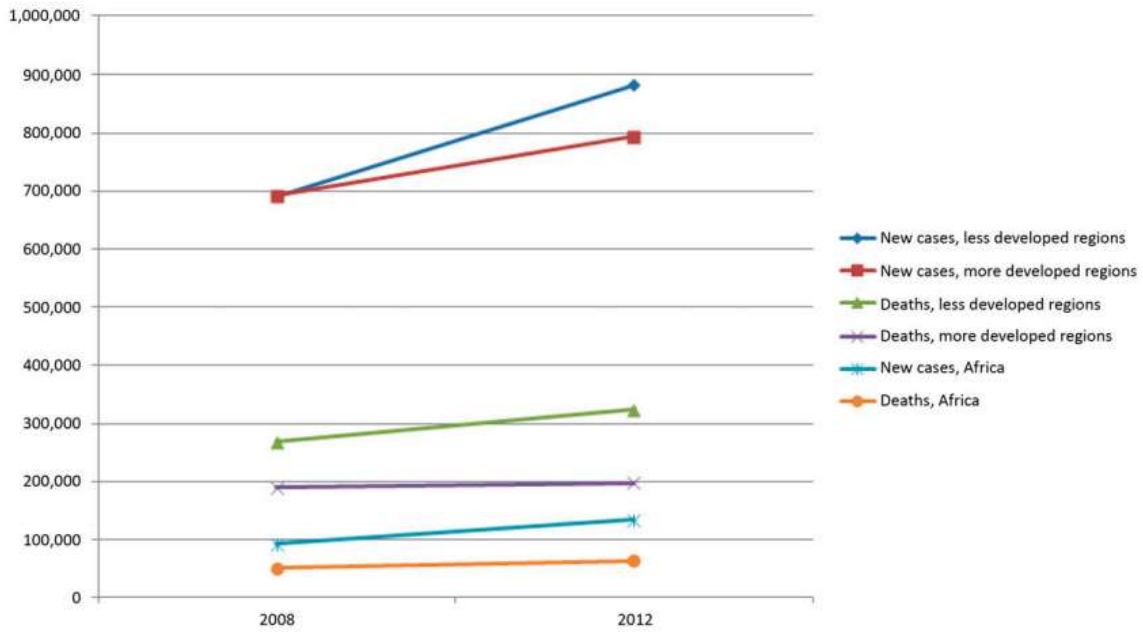
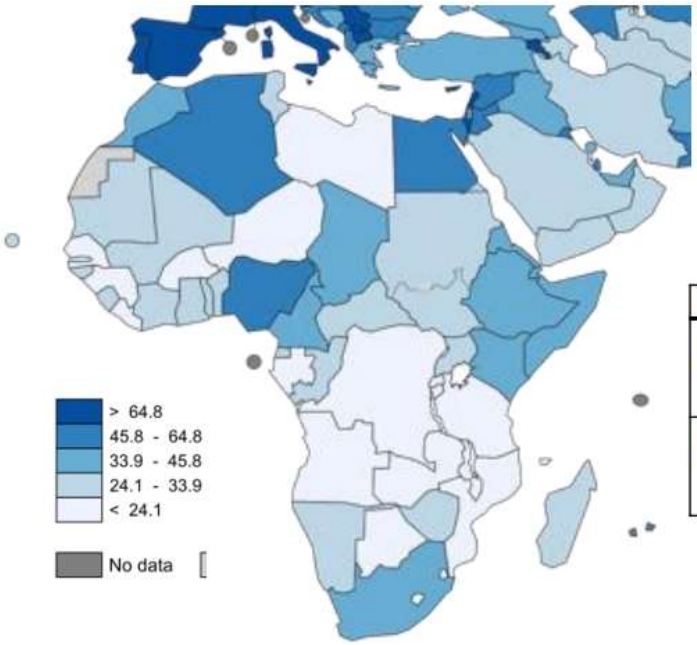


Figure 1 GLOBOCAN estimates of breast cancer incidence and mortality worldwide and in Africa in 2008 and 2012. (5)

Breast cancer is the leading cause of death among the female population worldwide, as well as in the majority of Africa, according to data from 26 African countries (15). In Africa, breast cancer is responsible for one in four cancer diagnoses and one in five cancer deaths in women (15). The reported incidence per 100,000 women in sub-Saharan Africa is 13.5 - 30, 38.9 in Western Africa, 30.4 in Eastern Africa and 26.8 in Central Africa (15). Breast cancer in African women tends to present at a younger age - with the overall mean age of presentation in West African women being 35 to 40 years. This is 10 to 15 years earlier than in women of western descent (15). Figure 2 shows the estimated age-standardized breast cancer incidence rates per 100,000 in Africa.



Region	North	West	East	Mid	South
Incidence (ASR) /100,000	34.4	38.6	30.4	26.8	38.9
Mortality (ASR) /100,000	17.4	20.0	15.6	14.9	15.5

Figure 2 Estimated age-standardised breast cancer incidence rates per 100,000 in Africa

Doren and colleagues showed traditional risk factors include:

1. Age: breast cancer incidence increases with advancing age in women of western decent but presents much earlier in African women.
2. Gender: breast cancer is more prevalent in women.
3. Personal history of breast cancer: A history of cancer in one breast increases the likelihood of a second primary cancer in the contralateral breast.
4. Histologic factors: Histologic abnormalities include lobular carcinoma in situ (LCIS), ductal carcinoma in situ and proliferative changes with atypia.
5. Family history and genetic factors: there is a 2- to 3-fold increased risk of developing breast cancer in an individual if known to have a first-degree relative with breast cancer. BRCA1 and BRCA2 gene mutations infer an increased breast cancer susceptibility.
6. Reproductive risk factors: estrogen exposure, the onset of menarche before 12 years of age, first live childbirth after 30 years of age, nulliparity and menopause after age 55 years have been associated with breast cancer.
7. Exogenous hormone use: Therapeutic or supplemental estrogen and progesterone have been implicated in breast cancer development.

1.3 Pathophysiology

The breast is a complex tubuloalveolar organ fixed within asymmetrical connective tissue, containing terminal duct lobules with stratified epithelium bordered by a basement membrane (16). The stratified epithelium comprises two cell populations - myoepithelial and epithelial, distinguishable through immunohistochemical staining with antibodies against myosin or cytokeratins (16). The stroma consists of blood vessels, lymphatics, and stromal cells.

Breast cancer develops due to damage to deoxyribonucleic acid (DNA), hereditary mutations and exposure to estrogen (16). Mutations in genes such as P53, BRCA1/BRCA2 or pathways such as RAS/MEK/ERK pathway and PI3K/AKT have been implicated in the development of breast cancer.

1.4 Types of breast cancer

Broadly, breast cancer is subdivided into either non-invasive or invasive cancers.

Non-invasive cancers: This cancer has not extended beyond the lobule or ducts where it is situated (17). Ductal carcinoma in-situ (DCIS) and lobular carcinoma in-situ (LCIS) are examples (16). A myoepithelial cell layer surrounds both DCIS and LCIS. DCIS is a precancerous lesion (16).

Invasive cancers: This cancer is no longer bound by the myoepithelial cells and has invaded the stroma surrounding the ducts and lobules (16). Invasive breast cancer of no special type (NST), formally known as ductal carcinoma, is the most common. Other breast cancers of special type include tubular carcinoma, medullary carcinoma, mucinous carcinoma, inflammatory carcinoma, cribriform, micropapillary, papillary, metaplastic and apocrine carcinomas (16,18).

Breast cancer is now considered a disease that has very high heterogeneity and hence different approaches in classification. The classifications range from histopathological subtypes, subtypes derived from immunohistochemical markers and classification by distinct molecular profiles. See Figure 3.

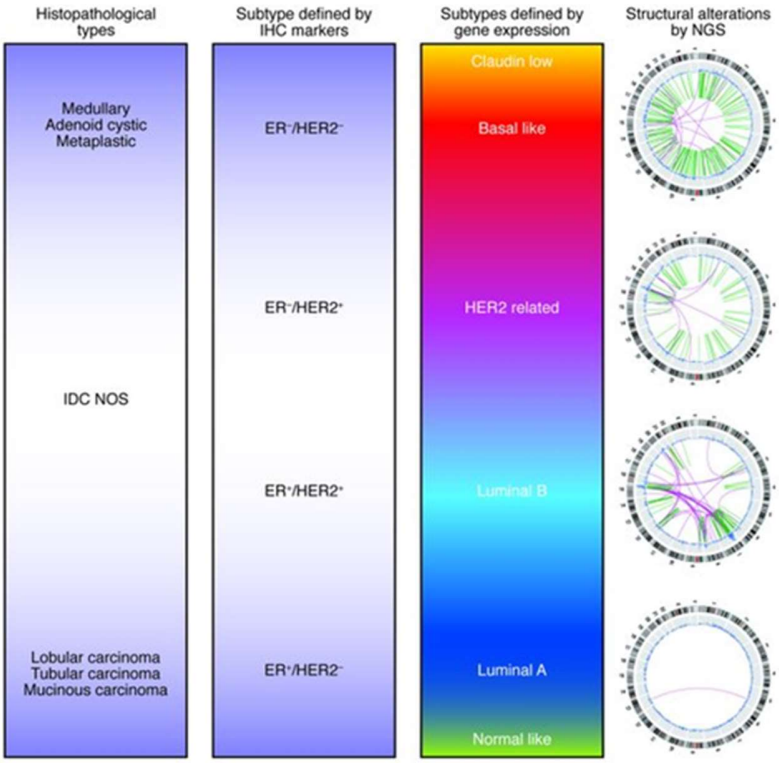


Figure 3 Subtypes of breast cancer

1.4.1 Traditional classification of breast cancer

Histologic Type: Histologic classification is based on the pathologic growth pattern – including tumour cell type, extracellular secretions (e.g. mucinous carcinoma), architectural features (e.g. papillary carcinoma) and immunohistochemical profile (9). There are more than 20 different histologic types of invasive breast cancers; the most common is Invasive Breast Cancer of No Special Type (IBC-NST) – accounting for 70% to 80% of all invasive cancers (9). Invasive lobular carcinoma (ILC) follows breast carcinoma accounting for 10% of all invasive cancers (9). Less common histologic subtypes include mucinous, cribriform, micropapillary, papillary, tubular, medullary, metaplastic and apocrine carcinomas (18). This classification is limited as it does not fully reflect the biological heterogeneity of breast cancers. The drawback of histologic typing is that it represents a broad categorization and fails to differentiate cancers with the same histologic patterns that may have different biological behaviour (9).

Grade: The grade encompasses the microscopic assessment of histologic differentiation (9). It assesses tubule formation, nuclear pleomorphism and proliferation as indicated by the mitotic index (9). The current, widely accepted Bloom Richardson grading evaluates each parameter with a numerical scoring of 1 to 3 and produces a summation score for grade assignment (9). Grading is an essential prognostic factor (19). Assessment of the above parameters may fail to capture the varied clinical courses of individual breast cancers (9). In this era of personalised medicine, a better understanding and classification is called for.

1.4.2 Immunophenotype assessment classification

Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor 2 (HER-2) assessment using immunohistochemistry is routine in breast cancer management (9). ER and PR are nuclear sex steroid receptors that stimulate the growth of healthy and neoplastic breast epithelium (9). These hormone receptors are expressed in about 75% of all breast cancers (9). ER and PR are assessed immunohistochemically with a 1% nuclear expression cut-off in tumour cells being positive (20). The majority of ER-positive cancers also tend to be PR-positive, although a small number can show single receptor positivity (9). Tumours showing individual receptor expression are more aggressive and less responsive to hormonal therapy (9).

HER-2 overexpression with the amplification of the corresponding gene on 17q occurs in 15% of all breast cancers (9). A combination of immunohistochemical and fluorescence in-situ hybridisation (FISH) is used to assess HER-2 status (9). HER-2 positivity is defined by >10% of tumour cells showing intense complete and circumferential staining or HER2:CEP17 ratio >=2 using FISH (9). The presence or absence of these receptors on the tumour cell surface serves as prognostic markers and essential predictive factors for hormonal/anti-HER2-targeted therapy and is also associated with conditional gene expression in the tumour cell itself (9,21).

Based on these genetically determined expressions in the tumour cells, five molecular subtypes of breast cancer were classified at the St. Gallen International Expert Consensus in 2011 (21).

1.4.3 Molecular classification of breast cancer

Tsang et al., through high-throughput technologies, provided evidence of breast cancer heterogeneity at the molecular level, leading to changes in the paradigms of breast cancer biology. Their study demonstrated the relevance of the immunophenotypic classification by hormone receptors and HER-2 status, in line with the St. Gallen International Expert Consensus in 2011 (9). This consensus classified five intrinsic subtypes by hierarchical clustering: Luminal A, Luminal B, HER-2 over-expression, Basal and Normal like subtypes (9,21). See Table 1.

Table 1 Summary of the breast cancer molecular subtypes

Intrinsic subtype	**IHC status**	**Grade**	**Outcome**	**Prevalence**
Luminal A	[ER+ | PR+] HER2-KI67-	1|2	Good	23.7% [p1] [10]
Luminal B	[ER+ | PR+] HER2-KI67+	2|3	Intermediate	38.8% [p1] [10]
	[ER+ | PR+] HER2+KI67+		| Poor	14% [p1] [10]
HER2 over-expression	[ER-PR-] HER2+	2|3	Poor	11.2% [p1] [10]
Basal	[ER-PR-] HER2-, basal marker+	3	Poor	12.3% [p1] [10]
Normal-like	[ER+ | PR+] HER2-KI67-	1|2|3	Intermediate	7.8% [p2] [15]

Other authors have defined six subtypes - Luminal A, Luminal B, HER-2 positive, basal-like (triple-negative), normal breast cell-like and Claudin- low, but the classification encompassing five molecular subtypes remains the most frequently used (22).

A putative model linking human mammary epithelial hierarchy to molecular cancer subtype has been put forward. According to the model, the transformation of a normal stem or progenitor cell can give rise to a heterogeneous cancer population. Alternatively, e- a differentiated cancer cell can acquire stem cell-like features within a heterogeneous tumour, contributing to the molecular heterogeneity of breast cancer (23). See Figure 4.

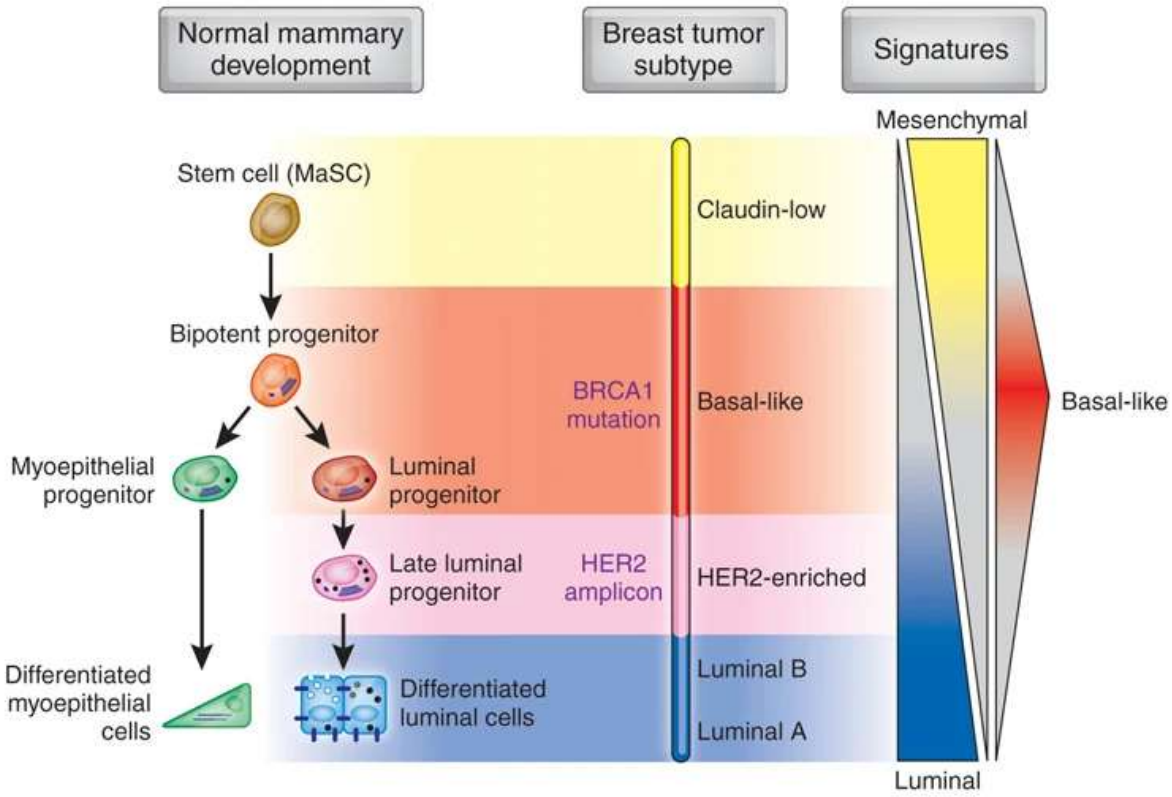


Figure 4 Putative model to explain breast cancer molecular signatures.

Luminal tumours

Luminal tumours are the most common subtypes within breast cancer and express hormone receptors with expression profiles reminiscent of breast luminal epithelial (11). The two subtypes are luminal A and luminal B. Their usual routine immunophenotype is ER+, PR+ and HER2- (ER-positive, PR-positive, HER-2 negative) (11). Sorlie et al. found that these subtypes generally carry a good prognosis, although luminal B tumours showed a significantly worse prognosis. Luminal A tumours can be adequately treated with endocrine therapy, while luminal B tumours tend to be more proliferative and respond to combined chemotherapy and hormonal treatment therapeutic strategies (11). Studies by Fernando and colleagues involving 179 cases found 25.7% were luminal A like and 27% were luminal B like (19,3% luminal B and HER-2 negative and 7.9% luminal B and HER-2 positive) (3). Kondov et al. showed 26.5% luminal A-like and 55.4% luminal B-like (31,38% luminal B and HER-2 negative and 24,14% luminal B and HER-2 positive) intrinsic subtypes from 290 cases (21).

Luminal A is the most common molecular subtype, accounting for 40% to 50% of invasive breast cancers and are typically low grade with a good prognosis (9). Luminal A is differentiated from the Luminal B subtype on the bases of the Ki67 proliferative index (>20%) and co-expression of HER-2 (positive or negative) on IHC:

- Luminal A: ER+, PR+, HER2−, Ki67 < 20%
- Luminal B: HER-2 negative: ER+ and/or PR +, HER2 -, Ki67 ≥ 20%)
- Luminal B: HER-2 positive: ER+ and/or PR +, HER-2 +

(9,24)

HER2 over-expression tumours

These tumours are identified with either ER-, PR- and HER2+ (ER-negative, PR-negative, HER-2 positive) by immunostaining or fluorescence in-situ hybridisation (FISH) for HER-2 over-expression when HER-2 IHC is equivocal (+2) (25). These tumours tend to be grade 3 and have a poor prognosis (11). HER-2 monoclonal antibody therapy has shown to be effective in this breast cancer subtype (11). Studies by Fernando and colleagues involving 179 cases found 15.7% were HER-2 positive (3) while, Kondov et al. showed 8.62% HER-2 positive cases (21).

Basal tumours

The basal subtype has expression profiles mimicking healthy breast myoepithelial cells or basal epithelial cells of other parts of the body and account for up to 90% of triple-negative breast cancer cases (11). This subtype follows an aggressive clinical course, lacks any form of standard targeted systemic therapy, is associated with lower disease-specific survival and has a higher risk of local and regional relapse (11). The IHC profile is:

- Triple-negative: ER−, PR− and HER2− (ER-negative, PR-negative, HER2 negative).

Fernando and colleagues found 31.4% were triple-negative, while Kondov et al. showed 9.31% (3,21).

Ki67

Ki67 index suffers from poor interobserver reproducibility; despite this, it is one of the most important prognostic markers used by oncologists in selecting therapy (26). Ki67 correlates with the mitotic activity and the S phase of the cell cycle, while non-neoplastic breast tissue cells have a proliferative activity of about 3% (21). Higher Ki67 proliferative index correlates with young age, larger tumours, positive lymph nodes, negative estrogen receptors and positive HER-2 receptors status (27). Bustreo et al. investigated 1688 female patients with primary breast cancers to establish the optimal Ki67 cut-offs and found that at the 20% Ki67 cut-off, one could reliably:

- Discriminate patients at low or high risk of recurrence and death
- Stratify patients at higher risk and eligible to adjuvant chemotherapy before hormone therapy.
- They also showed that the Ki67 proliferative index, when combined with tumour size and the number of metastatic lymph nodes, was highly predictive of disease-specific survival and disease-free interval (26).

CHAPTER TWO: STUDY AIMS AND OBJECTIVES

2.1 Aims

This study aimed to determine the molecular subtypes of breast cancer (on core biopsies) and their distribution at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), National Health Laboratory Services (NHLS), using immunohistochemistry, which is affordable, fast and requires no ultra-specialised laboratory facility.

2.2 Objectives

- To determine the distribution and relationship of breast cancer regarding age, histologic type, and Bloom and Richardson grade.

- To classify breast cancers into molecular/intrinsic subtypes (Luminal A, Luminal B, HER2 overexpressed and Basal) based on the IHC expression (ER, PR, HER-2 and Ki-67 proliferative index).

- To determine the relationship between age and grade with the molecular subtypes.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

A cross-sectional study of samples of diagnosed cases of breast cancers (on core biopsies) over one year at the CMJAH, period ranging from January 1, 2019, to December 31, 2019.

3.2 Study setting

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), previously known as Johannesburg General Hospital, is located in Parktown, Johannesburg, Gauteng. The hospital is the principal teaching hospital for the University of the Witwatersrand, Faculty of Health Sciences. The hospital receives referrals from several hospitals in its catchment area. The National Health Laboratory Service (NHLS) anatomical pathology department, based in CMJAH, is the largest in continental Africa. The NHLS is the sole provider of laboratory diagnostic tests to state hospitals. This fully computerised NHLS academic laboratory is accredited by the South African National Accreditation System, which performs annual quality-control checks.

A Systemised Nomenclature of Medicine (SNOMED) based search was performed to retrieve all diagnosed cases of breast cancers (on core biopsies) over a one-year period

on the database of the NHLS histology laboratory at Charlotte Maxeke Johannesburg Academic Hospital. The search included the periods from January 1, 2019, to December 31, 2019. The following data was extracted from the histology reports and captured – Age, tumour grade, histological subtype and IHC expression of ER, PR, HER-2 and Ki67 proliferative index.

3.3 Eligibility criteria

3.3.1 Inclusion criteria

- Breast cancer biopsy reports with IHC details of ER, PR, HER-2 and Ki67 proliferative index.
- Breast samples from both males and females.

3.3.2 Exclusion criteria

- Histology reports with no IHC details of ER, PR, HER-2 and Ki67 proliferative index.
- IHC determined HER-2 equivocal (+2) with no FISH results.
- Resection specimens as this may duplicate the data.
- Ductal carcinoma in-situ (DCIS) and lobular carcinoma in-situ (LCIS)

3.4 Variables and definitions

The variable descriptions are as below.

Variable	Description
Age	Demographic variable measured in years as a continuous variable and in the following categories: 18-34 years; 35-50 years, and >50 years.
Tumour grade	The Bloom and Richardson grading system was used. Grade 1, Grade 2 and Grade 3 categories. Grade 3 being the highest or worst score.
Histological subtype	The following grouping consisting of Invasive Breast Cancer – No Special Type (IBC-NST), Invasive Breast Cancer and Invasive lobular carcinoma (ILC)
Molecular subtype	Five intrinsic subtypes by hierarchical clustering: Luminal A, Luminal B, HER-2 over-expression, Basal and Normal like subtypes.

3.5 Sampling and Sample size calculation

Cases were retrieved from the database of the NHLS histology laboratory at Charlotte Maxeke Johannesburg Academic Hospital. The formula for sample size estimation of a descriptive prevalence study was used (Oxford Handbook of Epidemiology for Clinicians, Oxford University Press, 2012): $n=Z^2P(1-P)/d^2$ where n is the sample size, Z is the statistic corresponding to the level of confidence, P is expected prevalence, and d is precision (corresponding to effect size). The level of confidence is 95% confidence

interval (CI). Assuming a 5 % level of significance, the use of a two-sided test in the analysis and varying molecular subtype prevalence levels of between 10% and 30% allowing for a generous marginal error of that does not exceed more than 5% with 95% confidence level, Table 1 presents these estimated minimum adjusted sample size estimates. The final adjusted sample size estimate assumes a potential specimen loss rate of up to 10% (i.e. up to 10% of selected specimens may not be suitable for analysis). Per the assumptions described and illustrated in Table 2, the sample size of 265 cases in our database for the study period was deemed reasonably sufficient.

Table 2 Sample size estimation

Parameter	Sample size1	Sample size 2	Sample size 3
Adjusted Sample size	**154**	**271**	**356**
Sample size	139	246	323
Prevalence	10%	20%	30%
level of significance	5%	5%	5%
Power	80%	80%	80%

3.6 Data collection and Laboratory Methods

Data were entered in a Microsoft Excel spreadsheet from the NHLS database and exported to STATA version 13 (Stata Corp., College Station, TX, USA) for analysis.

The classification of the tumours into their molecular subtypes was performed on retrieving histological reports of the cases that met the inclusion criteria. Staining intensity was scored as 0 (none), 1 (weak), 2 (moderate), or 3 (strong). For ER and PR scores, specimens with <1% nuclei staining were considered negative (score 0), and those with weak (1% to 10%, score 1), moderate (10% to 33%, score 2), and strong (>33%, score 3) staining were classified as hormone-receptor-positive, denoted ERP and PRP respectively. No weak or moderate HER2 staining was considered. For HER2 analysis scores of 0 or 1 were considered HER2 negative (HER2N), and a score of 3 as HER2 positive (HER2+). A positive control sample was included in all batches. Breast cancer subtypes were also classified as luminal A (ERP and/or PRP, HER2N), luminal B (ERP and/or PRP, HER2P), HER2-positive enriched (HER2P, ERN, and PRN), and triple-negative (TRN: HER2N, ERN, and PRN), a previously used classification (25). The NHLS laboratory provides the histopathology report to the breast clinic in electronic format; the receptor-staining scores are then entered into the patient database.

3.7 Statistical analysis

Means and standard deviations (SD) were described for the continuous variables with normal distribution and medians and inter-ranges (IQR) for the continuous variables with skewed distribution. The Shapiro-Wilk test was used to test for normality. The student t-test or the nonparametric Mann-Whitney-U test was used to determine the difference between groups, respectively. Frequencies and percentages were used for categorical variables. The differences were determined by Pearson's Chi-square or a Fisher exact test where cell frequencies <5. The analysis of variance method was used to compare the differences when groups were>2. A 2-sided P value of <0.05 was considered statistically significant.

3.8 Ethical considerations

Patient anonymity was always maintained using a randomly assigned linked code. Data capture sheets did not contain any identifiable patient particulars. The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Appendix A). The NHLS Corporate Datawarehouse granted permission to access the laboratory database.

CHAPTER FOUR: RESULTS

A total of 265 diagnosed cases of breast cancers on core biopsies over one year from the database of the NHLS histology laboratory at Charlotte Maxeke Johannesburg Academic Hospital were analysed. The search included the periods from January 1, 2019, to December 31, 2019.

4.1 Baseline demographic and clinicopathological characteristics

The baseline characteristic of patients is shown in Table 3. The median age was 53 years (IQR 41 to 63 years), just above half of the patients were aged > 50 years (n=140, 53.0%), 38.5% (n=101) were aged between 35 and 50 years and just less than one in ten patients were aged between 18 to 34 years (n=23, 8.7%). Most tumours were either in the grade 2 or grade 3 tumour grades, 49.4% (n=131) and 44.5% (n=118) respectively. Only 16 tumours (6.0%) were grade 1.

According to histological subtype, the invasive breast cancer, no-special type (IBC, NST), was the commonest (n=236, 89.1%). Approximately 7.6% (n=20) had the invasive breast carcinoma, special type and 3.4% (n=9) had invasive lobular carcinoma. Carcinoma of mucinous morphology dominated the special types (n=8), while there were four cases each of metaplastic and medullary carcinoma. Other subtypes included glycogen rich clear cells, those with cribriform and micropapillary features, signet ring cell differentiation, and tubular morphology (each had one case each).

When classified by molecular subtype: Luminal B was the highest (n=113, 42.6%) of which 40 out of 113 (35.4%) also expressed HER2 (Luminal B/HER2+), followed by Luminal A (n=79, 29.8%), Basal (n=50, 18.9%), and the minor proportion was in the HER2 overexpression group (n=23, 8.7%).

Table 3 Baseline demographic and clinicopathological characteristics of breast cancer cases NHLS histology laboratory at Charlotte Maxeke Johannesburg Academic Hospital, 2019

Characteristic	**Summary**
Age (years), median (IQR)	53 (41 – 63)
Age group (years), n (%)	
18 – 34	23 (8.7%)
35 - 50	101 (38.3%)
>50	140 (53.0%)
Tumour grade	
Grade 1	16 (6.0%)
Grade 2	131 (49.4%)
Grade 3	118 (44.5%)
Histological subtypes	
Invasive lobular carcinoma	9 (3.4%)
Invasive breast cancer, special type	20 (7.6%)
Invasive breast cancer, no-special type	236 (89.1%)
Molecular subtype	
Basal	50 (18.9%)
Luminal A	79 (29.8%)
Luminal B (Luminal B / HER2 + = 40)	113 (42.6%)
HER2 over expression	23 (8.7%)

4.2 Distribution of histological subtypes by age and tumour grade

Patients with invasive lobular carcinoma (ILC) had a higher median age than the other subtypes, the median age of 70 years (54 – 75) compared to median age for 53 years (IQR 46-58) and 52 years (IQR 41-63) for invasive breast cancer, special type (IBC, ST) and invasive breast cancer of no special type (IBC, NST) respectively. This was statistically significant (p=0.03). Approximately 78% (n=7) of patients with ILC were above age 50 years, 60% for IBC, ST(n=12) and 52% for IBC, NST (n=121) (p=0.517). See Table 4 and Figure 5.

Seven out of nine patients with ILC had tumour grade 2. Tumour grade 3 was highest in patients with IBC, NST (46.2%, n=109) followed by IBC, ST subtype (40%, n=8), and the least was ILC (11.1%, n=1). This difference in tumour grade by subtype was statistically significant, p=0.018. Worsening tumour grade was significantly associated with higher odds of having IBC, NST (Odds ratio 5.5, 95%CI 1.6-19.3, p=0.008) and Odds ratio 3.5, 95%CI 1.1-11.5, p=0.038 for grade 3 and 2 respectively compared to grade 1.
See Table 4 and Figure 6.

Table 4 Distribution of histological subtypes by age and tumour grade

Characteristic	Invasive lobular carcinoma	Invasive breast cancer, special type	Invasive breast cancer, no special type	P-value
Median age (IQR)	70 (54 – 75)	53 (46 – 58)	52 (41 – 63)	**0.030**
Age group, n (%)				0.517
18 – 34	0 (0.0%)	1 (5.0%)	22 (9.4%)	
35 – 50	2 (22.2%)	7 (35.0%)	92 (39.2%)	
>50	7 (77.8%)	12 (60.0%)	121 (51.5%)	
Tumour grade, n (%)				**0.004**
Grade 1	1 (11.1%)	4 (20.0%)	11 (4.7%)	
Grade 2	7 (77.8%)	8 (40.0%)	116 (49.2%)	
Grade 3	1 (11.1%)	8 (40.0%)	109 (46.2%)	

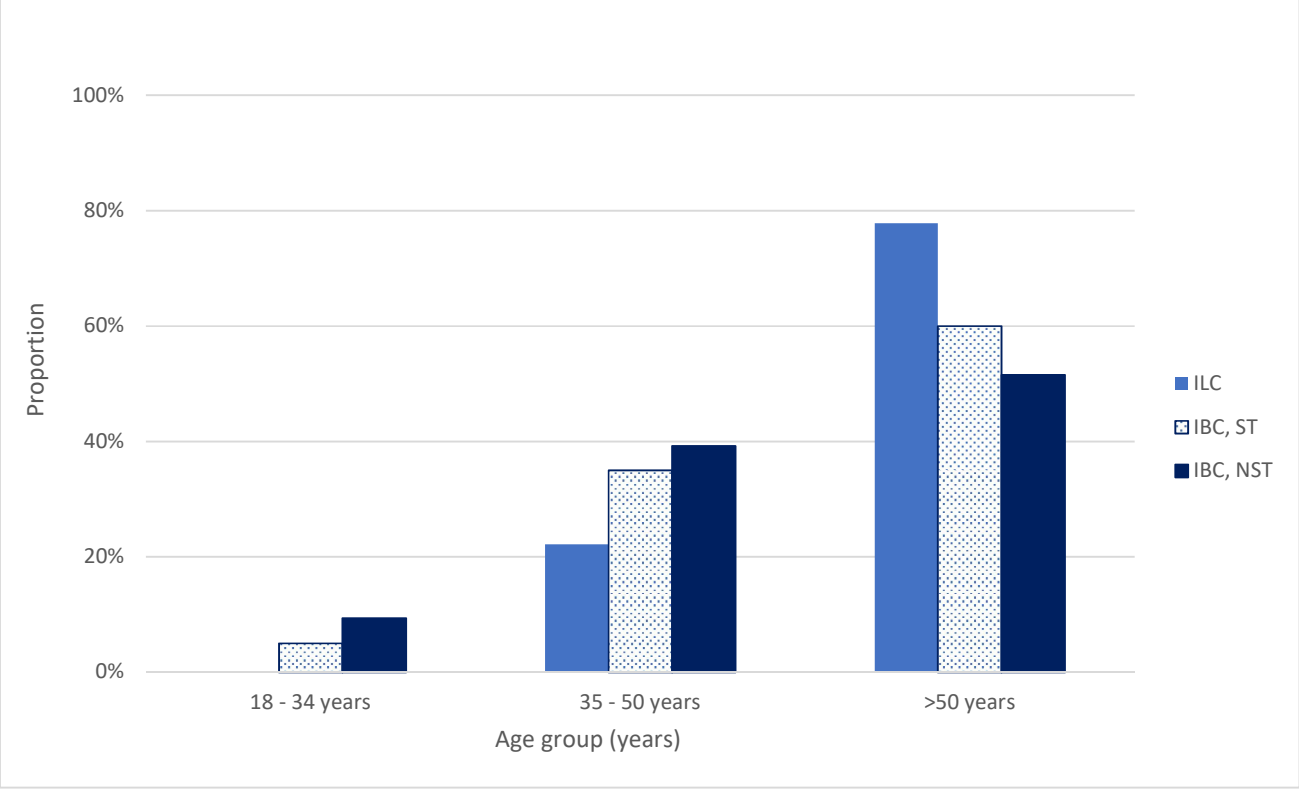


Figure 5 Distribution of histological subtypes by age

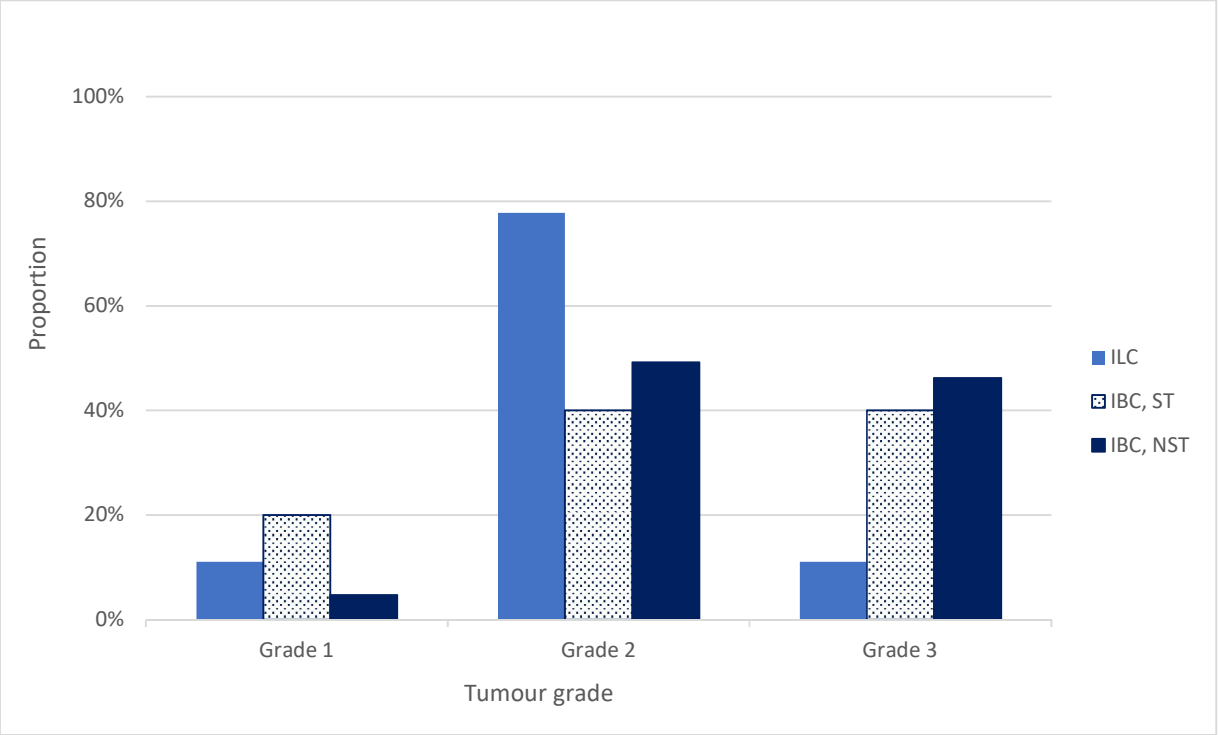


Figure 6 Distribution of histological subtypes by tumour grade

4.3 Distribution of molecular subtypes by age and tumour grade

The median age was broadly similar across all molecular subtypes (p=0.083). See Table 4. Generally, most patients were in the 35 years or above age group (>80%), with around or just above one in two being in the greater than 50-year age group across all molecular subtypes. This age distribution was not different across the subtypes (p=0.377).

The occurrence of advanced tumour grades differed by molecular subtype, which was statistically significant, p<0.001. Order of molecular subtype with the highest proportion

of advanced tumour grade (grade 3) to the lowest was as follows: basal (83.7%), HER2 (60.9%), Luminal B (46.0%) and Luminal A (12.7%). See Table 5 and Figure 8.

Table 5 Distribution of molecular subtypes by age and tumour grade

Characteristic	**Basal**	**HER2 overexpression**	**Luminal A**	**Luminal B***	**P-value**
Median age (IQR)	52.5 (44 – 64)	48.5 (40 – 63)	55 (46 – 67)	52 (40 – 60)	0.083
Age group					0.377
18 – 34	2 (4.0%)	4 (18.2%)	4 (5.1%)	13 (11.5%)	
35 – 50	20 (40.0%)	7 (31.8%)	31 (39.2%)	43 (38.1%)	
>50	28 (56.0%)	11 (50.0%)	44 (55.7%)	57 (50.4%)	
Tumour grade					**<0.001**
Grade 1	0 (0.0%)	0 (0.0%)	15 (19.0%)	1 (0.9%)	
Grade 2	8 (16.3%)	8 (34.8%)	54 (68.4%)	60 (53.1%)	
Grade 3	41 (83.7%)	14 (60.9%)	10 (12.7%)	52 (46.0%)	

* Luminal B/HER2+: grade 1 = 1; grade 2 = 18 and grade 3 = 21.

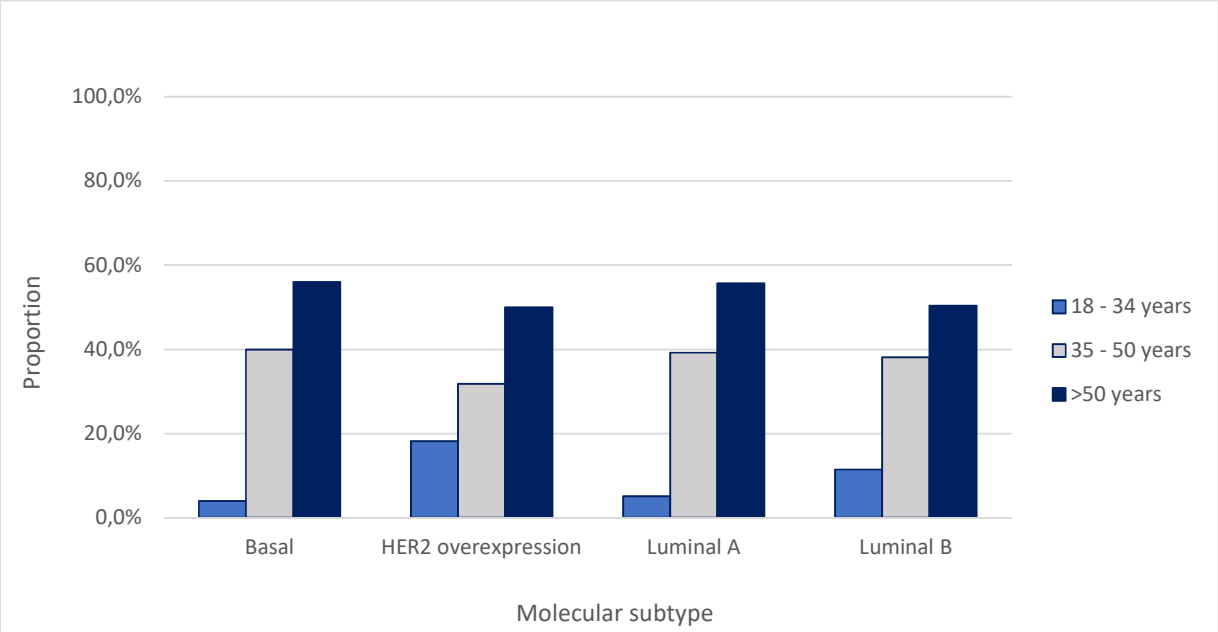


Figure 7 Distribution of molecular subtypes by age

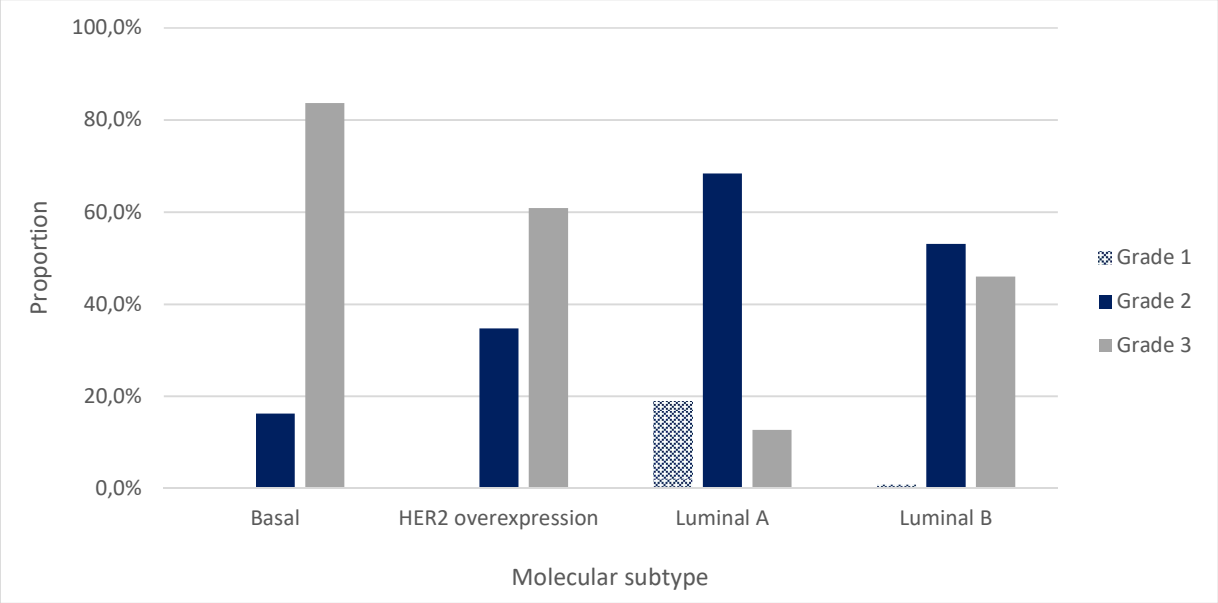


Figure 8 Distribution of molecular subtypes by tumour grade

CHAPTER FIVE: DISCUSSION

The breast cancer molecular subtype distribution in sub-Saharan African populations is still not well described. Therefore, in this study, we measured the distribution of the different molecular subtypes using immunohistochemical expression among patients presenting with breast cancer at the CMJAH, Johannesburg, South Africa, over one year.

Breast cancer is a heterogeneous disease, and the current histological classification fails to differentiate cancers with the same histologic patterns that may biologically behave differently. Accurate grouping of breast cancers into clinically relevant molecular subtypes is essential for targeted therapeutic/oncologic decision making and optimal patient care. The most prevalent molecular subtypes in this population were Luminal B and Luminal A subtypes, each with a prevalence of approximately four and three in ten. The more aggressive subtypes, Basal and HER2 overexpression groups, had a combined occurrence in the study population of slightly above one in four (18.9% and 8.7%, respectively). This observation suggests that late-stage aggressive breast cancer was not the predominant subtype in this population. The HER2 overexpression subtype shows faster growth than the Luminal subtypes and correspondingly carries a worse prognosis. The Basal subtype is the most aggressive and accounts for most triple-negative breast cancer cases and is associated with lower disease-specific survival and has a higher risk of local and regional relapse (10). The Basal and HER2 overexpression subtypes also showed significant association with the advanced tumour stage. These findings of the molecular subtype distribution in this study population are mainly similar to reports from

other populations in Southern and East Africa (28–31). However, a few reports from West Africa show a contrasting pattern of higher proportions of the aggressive tumour subtypes (Basal and HER2 overexpression), ranging from a combined 55% to 70% (32–34). In a large case series examining 1,218 women attending a public hospital in Soweto, South Africa, the Luminal subtypes dominated (combined 68%) (28). A similar pattern was reported in a significant national cancer registry data analysis in Namibia and South Africa (29). Additionally, a prospective single centre analysis of breast cancer patients in Kenya demonstrated a comparable prevalence of the aggressive cancer subtypes (31).

However, findings from 6 large centres in the southwest of Nigeria demonstrated divergent results (34). The most common molecular subtype in this large cohort of Nigerian breast cancer patients was the triple-negative phenotype, while Luminal A was represented in only 15% of this cohort. The distribution is also observed in some West African studies (32,33). The difference in findings may be partly due to population differences (Southern Africans are more diverse racially), selection bias (sicker patients are more likely to be the majority of persons identified) and heterogeneity in specimen collection or storage. A systematic review and meta-analysis of receptor-defined subtypes of breast cancer in indigenous African populations suggested that the study design, the quality of archival tissue, and the methodology of receptor testing contributed to the conflicting results (35). Sufficiently extensive multi-centre cross-sectional studies able to stratify by race/ethnicity and with standardised specimen collection/storage, diagnostic criteria are needed better to understand the distribution of molecular subtypes in SSA.

While the dominating molecular subtypes were the less aggressive forms, the study affirms the higher occurrence of early-onset female breast cancers observed in low/middle-income countries than in high-income countries and the late presentation compared to high-income countries (1,14,15). According to the United States National Cancer Institute's Surveillance, Epidemiology, and End Results Program, the median age of breast cancer diagnosis is 62 (36). In the United Kingdom 2018 cancer surveillance report, the group most affected by breast cancer in terms of the number of cases was 65 to 69-year-old women (36). The median age of the study population was 53 years, and this is at least about a decade less than reports from resourced countries but similar to studies conducted in other SSA and LMICs (refs). High mortality rates within five years of diagnosis are common in SSA and LMICs, and this could be attributable to late-stage at presentation (37,38). As seen in the proportion of advanced tumour grade (grade 3), a late presentation was prevalent in 94% of patients in this study. While there was no association between age and receptor status/molecular subtype demonstrated in this study, there was a statistically significant association between histological and molecular subtypes with tumour grade. The triple-negative (Basal) HER2 enriched tumours tended to be higher than the Luminal subtype.

Most invasive breast cancers have no special features and are classed as No Special Type. As previously described in the literature, Invasive breast carcinoma of no special type (IBC, NST) is the most prevalent histological type of invasive breast cancer (39). The observation was confirmed in this study population, where it was by far the commonest

(almost 90%). As expected, the occurrence of the no special type showed a significant association with advanced tumour grade.

The study findings should be interpreted in light of some limitations that include its small size, which may impact statistical power. As this was a single centre study at an academic quaternary hospital, there is a potential of referral bias and may be less representative of the South African population with breast cancer. In addition, the retrospective nature limits the availability of some crucial variables that may be useful in interpreting the results and carrying out extended statistical analysis to elucidate better how demographic and clinical characteristics may relate to molecular subtypes.

CHAPTER SIX: CONCLUSIONS

Based on the findings of this single centre study in South Africa, the aggressive molecular subtypes of breast cancer, i.e. Basal and HER2 overexpression subtypes, were not the predominant types, as several reports suggest. However, the early presentation of breast cancer in this setting is confirmed in this study. According to the GLOBOCAN 2012 Report, case fatality rates from breast cancer appear to be substantially higher in SSA and LMICs than in high-income countries (13). As the findings of this study do suggest that the local population has no predisposition to more aggressive breast cancer forms compared to the high-income countries, it is realistic that scale-up of interventions such as improving awareness by both patients and healthcare providers, increasing breast cancer screening rates and treatment coverage may potentially result in improved breast cancer survival rates in South Africa and other LMICs. Emphasis also needs to be placed on early diagnosis and treatment as the presentation to care is often late.

Low utilisation of immunohistochemistry in many low-resource settings impedes optimum treatment targeting and management outcomes in patients likely to benefit (31,40,41). Additionally, immunohistochemistry is inexpensive, fast, requires no ultra-specialised laboratory facilities and provides an affordable system/approach, especially for low/middle income countries. Therefore, there is a need to urgently upscale the use of immunohistochemistry diagnostic approaches in these settings.

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CHAPTER SEVEN: APPENDICES

Appendix A: Ethics Certificate

Appendix B: Data collection sheet

No.	Age	Tx grade	Hx subtype	ER	PR	HER-2	Ki67<20%	Mol. Subtype