Profile of molecular subtyping of breast cancer and clinico-pathological features in Mankweng Hospital breast oncology clinic, Limpopo Province, South Africa

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Background. Breast cancer is recognised as a heterogeneous disorder, comprising a number of subcategories of several cellular compositions, molecular alterations as well as clinical behaviour. Across the world, research has been able to show that the most common molecular subtype is luminal A, followed by triple negative and human epidermal receptor 2 (HER2)-positive (non-luminal) subtype. However, another study found that the most common molecular subtype was HER2/neu amplification, suggesting that subtypes case frequencies differ in different people.

Objectives. To determine the case frequency of molecular subtypes of breast cancers and the associated clinicopathological features in women from Limpopo Province, South Africa.

Method. We performed a retrospective, cross-sectional descriptive study from July 2021 - June 2021 at Mankweng Hospital breast oncology clinic. The inclusion criterion was all women with histologically confirmed breast cancer.

Result. We identified 222 women who met the inclusion criteria, and the age range (median) was 25 - 91 (54.8) years. The majority of the women came from the Vhembe district (28%; *n*=62), followed by Capricorn district (26%; *n*=59), Mopani district (17%; *n*=38), Sekhukhune district (16%; *n*=35), and Waterberg district (13%; *n*=28). Histology revealed that the most common type was invasive ductal carcinoma (no special type; 91.44%; *n*=203), followed by invasive mucinous carcinoma (4%; *n*=9). The most predominant molecular subtype was luminal B (48.19%; *n*=107), followed by luminal A (22.97%; *n*=51), triple negative (17.12%; *n*=38) and 11.75% (*n*=26) overexpressed HER2. More than one-third of the cancers were HER2-positive (40.54%; *n*=90), and 59.46% (*n*=132) were HER2-negative. The majority of patients presented with late-stage cancer (62.16%; *n*=138), and the rest presented with early-stage (I and II) disease (37.84%; *n*=84).

Conclusion. The majority of our patients had luminal subtypes and hormonal receptor-positive breast cancers, which should be associated with very good clinical outcomes. However, the majority of patients presented late with advanced-stage disease and high Ki-67 expression. Therefore, research is required to help us understand why in our context patients present late with advanced-stage disease.

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Breast cancer is the most common cancer in women and the second most common cancer overall, worldwide.[1] Over the years, the demographics have changed, lifespan has increased and in turn, there has been an increase in the aged population. These changes have also been accompanied by an increase in the number of individuals diagnosed with breast cancer, even more so in developing countries.[2] The increase in the incidence of breast cancer has not been accompanied by an increase in mortality, suggesting that there has been aggressive screening and detection of breast cancer, coupled with better clinical management of breast cancer patients.[3]

Breast cancer is recognised as a heterogeneous disorder, comprising a number of subcategories of cellular compositions, molecular alterations as well as clinical behaviour.[4] Phenotypically identical breast tumours, which are histologically similar, can present with a wide spectrum of clinical outcomes and response to therapy. Over the years, due to the availability of hormone-receptors and human epithelial growth factor receptor 2(HER2/neu)-based determination techniques, classification has been based on immunohistochemistry, genetic and molecular findings.[4,5] In the past, breast cancer was classified based on clinicopathological features such as tumour stage and grade, and other morphological features such as histological types, proliferation status and lymph vascular infiltration. However, this alone could not account for the differences in prognosis and response to therapy.[6] With the discovery of molecular classification >30 years ago, there has been an appreciation of genetic diversity in breast cancer.[4] This has led to molecular classification systems being recognised and appreciated as a useful tool for predicting the response to treatment and to predict prognosis and guide therapy in patients with breast cancer.[7]

Classifying breast cancer into varied molecular subtypes in an important step in therapeutic decision-making. Classic immunohistochemistry markers such as hormone receptors including oestrogen receptor (ER) and progesterone receptor (PR) along with HER2, play a vital role in molecular subtyping.[5] Newer methods including gene expression profiling using DNA microarrays have also been developed, which are therapeutically important in molecular classification.[4] However, immunohistochemical analysis based on hormone receptors and HER2 is preferable and commonly used in clinical practice as this method has been found to be easy, cost effective and provides similar results for molecular subtypes.[7]

There are five main intrinsic or molecular subtypes of breast cancer that are based on the genes a cancer expresses. According to the St Gallen Consensus 2011,[8] molecular subtypes of breast cancer are classified based on immunohistochemistry into luminal A (ER+/PR+/HER2–/Ki-67low), luminal B (ER+/PR+/HER2–/+/Ki-67high), HER2-overexpression (ER–/PR–/HER2+) and triple negative breast cancers (TNBCs; ER–/PR–/HER2–).[8] Molecular subtypes can be

broadened and explained thoroughly as follows:[9-11] luminal A breast cancer is hormone-receptor positive (ER and/or PR-positive), HER2-negative, and has low levels of the Ki-67, which helps control how fast cancer cells grow. Luminal A cancers are low-grade, tend to grow slowly and have the best prognosis. Luminal B (HER2-negative or HER2-positive) breast cancer is hormone-receptor positive (ER and/or PR-positive), and either HER2-positive or HER2-negative with high levels of Ki-67. Luminal B cancers generally grow slightly faster than luminal A cancers and their prognosis is slightly worse. Triple-negative/basal-like breast cancer is hormone-receptor negative (ER and PR-negative) and HER2-negative. This type of cancer is more common in women with *brca1* gene mutations. Researchers are unsure why this type of cancer is also more common among younger and Black women.[12] HER2-enriched breast cancer is hormone-receptor negative (ER and PR-negative) and HER2-positive. HER2-enriched cancers tend to grow faster than luminal cancers and can have a worse prognosis, but they are often successfully treated with targeted therapies aimed at the HER2 protein, such as trastuzumab, pertuzumab, lapatinib, neratinib, and T-DM1 or ado-trastuzumab emtansine. Ki-67 is an indicator of tumour proliferation and an elevated/high Ki-67 is associated with poor prognosis. In the absence of reliable measurement of Ki-67, it has been proposed that an alternative method such as tumour grade can be used to distinguish between luminal A and luminal B (HER2-negative) subtypes.[8] Among the five subtypes of breast cancer, the distribution of pathological grades showed a significant difference ($p<0.001$),[13] suggesting that it is of therapeutic importance.[8] Systemic therapy and management strategies for patients with breast cancer have been developed based on molecular subtypes. Endocrine receptor-positive cancers have been proven to have good clinical response to endocrine therapy, while non-endocrine receptor cancers have been shown to respond to cytotoxic therapy.

It is important to identify patients benefiting from hormonal therapy and treatment targeting the HER-2/neu receptors. To achieve this, it is important to determine the correlation of HER-2/neu expression and hormone (ER, PR) receptor status in relation to the clinicopathological features of breast cancer. Histological subtypes of breast cancer refer to the growth pattern of the tumours. These patterns have been associated with distinctive clinical presentations and/or outcomes. To date, the most common subtype is invasive ductal carcinoma of not otherwise specified (IDC-NOS) or of no special type (IDC-NST).[15] Special types of breast cancer account for up to 25% of breast cancers and up to 17 distinct types have been described by the World Health Organization.[15] There has been no clear correlation between histological subtypes and clinical behaviour of these tumours, hence the molecular array analysis has allowed for more understanding on tumour behaviour than just looking at histological subtypes.

The main objective of the present study was to determine the case frequency of breast cancer molecular subtypes using immunohistochemistry and the associated clinicopathological features of women at Mankweng Hospital breast oncology clinic, Limpopo Province.

Methods

The present study is a retrospective, cross-sectional descriptive study of breast cancer patients who presented at Mankweng Hospital breast oncology clinic between July 2020 and June 2021. All patients with histologically confirmed breast cancer were included in the present study. Patients with missing information, including incomplete immunohistochemistry profile and males, were excluded from the present study. The clinic registers were used as a starting point for data collection. Patients' files and histology were retrieved from the hospital archive to compile the data bank. The collected data were transferred to a password-protected data bank in Excel spreadsheet (Microsoft Corp., USA). The following data were retrieved from the patients' files: age, district, type of breast cancer, staging, grading (according to the Nottingham modification of the Bloom-Richardson system),[5] Ki-67 index, and oestrogen, progesterone and HER2 receptor status.

Statistical analysis

Data were analysed using Statistica software (version 7.1; TIBCO software, USA) and SPSS version 23 (IBM Corp., USA). The patient demographics were summarised using descriptive statistics. Dependent variables that were normally distributed were summarised using means and standard deviations. The comparison between normally distributed variables was done by performing the Student's *t*-test and analysis of variance was used to compare means between more than two groups. Correlations between continuous variables was done using regression modelling and Pearson's coefficient was calculated. A $p<0.05$ was considered statistically significant.

Study setting

Mankweng Hospital breast oncology unit is situated in Turfloop/Sovenga, Limpopo Province, South Africa. It is a tertiary hospital providing breast oncology services, among other services, to all the population of the Limpopo Province. Ethical approval was obtained from the Research Ethics Committee of the Polokwane/Mankweng Hospital complex (ref. no. PMREC 26 MAY UL 2021/C).

Results

We identified a total of 222 women who met the inclusion criteria into the present study. The majority of the patients (25%) were in the 50 - 60 years age group, followed by 40 - 50 years (24%), 60 - 70 years age group (18%), and 30 - 40 years age group (14%) (Fig. 1). The results show that the majority of affected patients were >50 years; however, there was also quite a good number of young patients.

The most common subtype in the present study was luminal B (48%; HER2-positive (29%) and HER2-negative (19%)), followed by luminal A (23%), triple negative (17.12%) and HER2-overexpression/non-luminal (12%) (Fig. 2). This correlates with findings from most studies that have shown that luminal types of breast cancer are the most common molecular subtype. The majority of luminal B (31%; $n=69$) and HER2-negative (33%; $n=73$) cancers were detected in late stages.

The majority of patients had oestrogen receptor (ER)-positive (68%; $n=151$) cancer, followed by progesterone receptor (PR)-positive (59%; $n=132$) cancer in our present study. With regards to HER2 status, the majority of patients had cancers that were HER2-negative ($n=132$; 59%) compared with individuals with HER2-positive cancers.

The majority of the patients in the present study had disease at Grade 2 (61%; $n=134$) followed by Grade 3 (29%; $n=63$) and Grade 1 (10%; $n=22$) (Table 1). For Grade 1, the most dominant subtypes was luminal A (6%; $n=14$), and for Grade 3, triple negative breast cancer was more prevalent (13%; $n=28$; $p<0.0001$). This evidence shows that the luminal types of breast cancer are less aggressive that the non-luminal cancers.

We found that luminal A cancers were predominant for low (<10%) Ki-67 index (10%; $n=22$), followed by luminal B (4%; $n=8$), HER2 (3%; $n=6$) and triple negative (1%; $n=3$) ($p<0.0001$). This evidence further suggests that luminal A is associated with better prognosis and good clinical outcomes as compared with the other subtypes. For the high (>20%) Ki-67 index, we found that luminal B was the most dominant (20%; $n=44$), followed by triple negative cancer (15%; $n=33$), HER2 (6%; $n=14$) and luminal A (<1%; n=1; $p<0.0001$). This further shows the aggressiveness of luminal B subtype and poor clinical outcomes. High Ki-67 was found in patients mostly in the late stages of disease (28%; $n=63$). Although Ki-67 index is a valuable biomarker

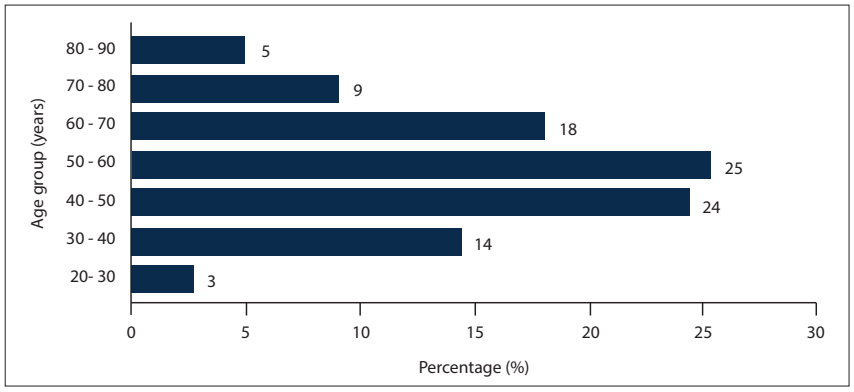

Fig. 1. Age of the patients presenting at Mankweng breast oncology clinic between July 2020 and June 2021.

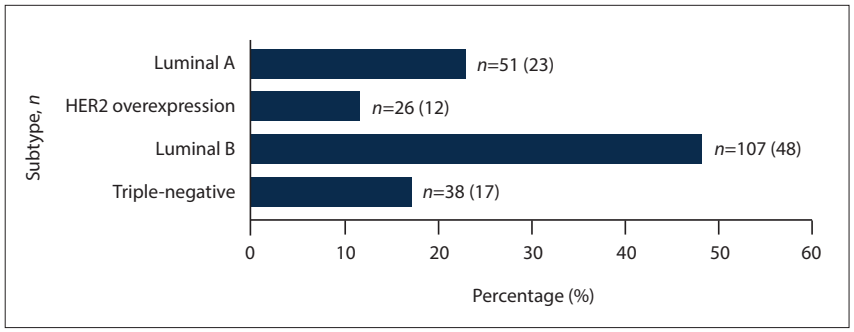

Fig. 2. Molecular subtypes of breast cancers seen in patients presenting at Mankweng breast oncology clinic between July 2020 and June 2021 (N=222). The subtypes are expressed in actual numbers as well as percentages.

in breast cancer, no independent prognostic significance of Ki-67 has been established.[22]

The majority of the patients in the present study were from Vhembe district (28%), followed by Capricorn district (26%), Mopani district (17%), Sekhukhune district (16%), and Waterberg (13%) (Table 1).

On the molecular subtype classification, it is evident that luminal B is the most common subtype (48%), followed by luminal A (23%), then triple negative (17%) and the least prevalent was HER2-overexpression molecular subtype (Table 2).

Discussion

According to histological type, the most common cancer in our present study was the invasive ductal carcinoma (no special type; 91.4%). Looking at a 10-year retrospective study done in South-Western Nigerian[16] women, the most common histological type was invasive ductal carcinoma of no special type (88.9%). Although early diagnosis is a key determinant factor for breast cancer survival, delay in presentation and advanced-stage diagnosis are common challenges in developing countries.[17] The majority of patients (62.16%) in our study presented at late stage (III and IV). This can be attributed

Table 1. Descriptive summary of breast cancer patients (*N*=222)

	n (%)	Early stage (I & II), *n* (%)	Late stage (III & IV), *n* (%)	*p*-value
Age, 25 - 91 years	222 (100)	83	137	
<50	93 (42)	33 (15)	60 (27)	0.5800
>50	129 (58)	51 (23)	78 (35)	
Molecular subtype				
Luminal A	51 (22.97)	24 (11)	27 (12)	0.04
Luminal B	107 (48.19) (HER2-negative = 43 (19))	38 (17)	69 (31)	
HER2-overexpression without ER/PR	26 (11.71)	5 (2)	21(9)	
Triple negative	38 (17.12)	17 (8)	21 (9)	
HER2 status				
HER2+	90 (40.54)	25 (11)	65 (29)	0.0107
HER2–	132 (59.46)	59 (27)	73 (33)	
Grade				
Grade 1	23 (10)	7 (3)	15 (7)	0.7301
Grade 2	136 (61)	51 (23)	83 (38)	
Grade 3	63 (29)	26 (12)	37 (17)	
Ki-67 index				
Low	40 (18)	16 (7)	24 (11)	0.2471
Intermediate	90 (40.54)	39 (18)	51 (23)	
High	92 (41.44)	29 (13)	63 (28)	
District				
Sekhukhune	35 (16)	-	-	-
Mopani	38 (17)	-	-	
Capricorn	59 (26)	-	-	
Waterberg	28 (13)	-	-	
Vhembe	62 (28)	-	-	

HER2 = human epidermal receptor 2; ER = oestrogen receptor; PR = progesterone receptor.

Table 2. Histology type

Subtypes	*n* (%)
Invasive carcinoma (no special type): ductal	203 (91.44)
Invasive carcinoma: mucinous	9 (4)
Invasive carcinoma: neuroendocrine	2 (0.9)
Invasive carcinoma: micropapillary	1 (0.45)
Invasive carcinoma: tubular	1 (0.45)
Invasive carcinoma: lobular	1 (0.45)
Invasive carcinoma: metaplastic carcinoma (squamous cell carcinoma)	3 (1.35)
In situ carcinoma: ductal	2 (0.9)

to several factors including lack of education, seeking alternative methods such as traditional doctors, and fear of surgery.

The mean age at diagnosis of breast cancer has been found to be at ~40 - 50 years.[10,11] A South-Western Nigerian[16] study found the mean (range) age to be 49.7 (20 - 89) years and the most affected age group was 50 - 59 years. Similarly, a study from India[6] revealed that their patients were younger, with the average age at the time of diagnosis of 50.5 years and 7% of patients were <35 years compared with those in a Western case series. This has also been corroborated by other African studies that have also reported a mean age <50 years.[18] We found the mean (range) age at diagnosis to be 54.8 (25 - 91) years and patients <40 years of age accounted for 17% of individuals presenting to the clinic.

Breast cancer, particularly triple negative disease, was found in younger women with an age range of 40 - 60 years,[9,10] and patients usually presented at an advanced stage of disease.[12] A retrospective study conducted China[19] showed that young women ≤35 years were more likely to get basal cell-like/triple negative breast cancer (36.9%) compared with other subtypes,[19] indicating that younger individuals are more likely to get aggressive disease.[12,13]

In the present study, the prevalence of triple negative breast cancer was the same (9%) for all age groups (>50 and <50 years). Moreover, the majority of the patients presented with grade 2 and 3 cancers regardless of age group. This evidence is suggestive of the aggressive nature of triple negative breast cancer subtype; however, age does not seem to be a contributing factor.

Studies across the world have shown that the most common molecular subtype is luminal A, followed by triple negative and HER2-positive (non-luminal) subtype.[4,8,20] However, a study by Cardoso *et al.*[10] found that the most common molecular subtype was the HER2/neu amplification, suggesting that differences in case frequencies of subtypes do exist between different populations.

Hormone receptor-positive breast cancer cells have either ER or PR, or both. These breast cancers can be treated with hormone therapy drugs that lower oestrogen levels or block oestrogen receptors. Hormone receptor-positive cancers tend to grow more slowly than those that are hormone receptor-negative. Women with hormone receptor-positive cancers tend to have a better outlook in the short-term, but these cancers can sometimes come back many years after treatment.[21] Most of the patients in our study presented with advanced disease; however, the majority of the patients with advanced stage had non-luminal cancer, suggesting that indeed hormone receptor-positive tumours might have a better clinical outcome. We cannot comment much on these, as other confounding factors such as time of onset of symptoms and progression of disease were not looked at in the present study.

A study conducted in Indonesia[20] found that luminal A subtype of breast cancer was commonly found in women >50 years of age (p=0.028), low-grade cancer (p=0.09), negative lymph node metastasis (p=0.034) and stage III (p=0.017).[20] Even though the difference was insignificant, luminal A subtype breast cancer was mostly found in small size breast cancer (p=0.129). HER2-positive subtype breast cancer was more commonly diagnosed with large size, positive lymph node metastasis and poor grade. Triple negative/basal-like cancer was mostly diagnosed among <50 years old women.[20]

Study limitations

The present study was retrospective and some patients had missing records. We also observed poor note-keeping and inability to trace patients' histology results.

Conclusion

The majority of our patients had luminal subtypes and hormonal receptor-positive breast cancers, which should be associated with very good clinical outcomes. However, the majority of patients presented late with advanced-stage disease and high Ki-67 expression. Therefore, research is required to help us understand why in our context patients present late with advanced-stage disease.

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