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Breast cancer subtypes: implications for the treatment and survival of patients in Africa—a prospective cohort study from Mozambique

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ABSTRACT

Background Data regarding breast cancer epidemiology, treatment and survival in Africa are scarce. We aimed to assess the distribution of breast cancer subtypes in Mozambique and its impact on patients' treatment and survival. The concordance of biomarker assessment between cytological and histological samples was also evaluated.

Methods Prospective cohort study including 210 patients diagnosed between January 2015 and August 2017, followed to November 2019. Clinicopathological characteristics, treatment, 3-year overall survival (OS) and disease-free survival (DFS) were compared across classic tumour subtypes (oestrogen receptor (ER)-positive/human epidermal growth factor receptor 2 (HER2)-negative, HER2-positive and triple-negative breast cancer (TNBC)) and surrogate intrinsic subtypes (St. Gallen classification). Concordance was measured using Cohen's κ statistics.

Results A total of 51% of patients had ER-positive/HER2-negative tumours, 24% HER2-positive and 25% TNBC. Concordance between cytological and histological samples regarding ER and HER2 status was substantial (κ=0.762 and κ=0.603, respectively). There were no significant differences across subtypes regarding clinical characteristics and treatment, except for HIV positivity and high histological grade (more prevalent among TNBC) or endocrine therapy (higher use among ER-positive/HER2-negative and HER2-positive patients). Three-year OS was 52.5% (95% CI, 44.3% to 60.0%), being higher in ER-positive/HER2-negative (61.1%) compared with HER2-positive (53.2%) and TNBC (31.9%) patients. Adjusted HRs were 1.96 (95% CI, 1.13 to 3.39) among HER2-positive and 3.10 (95% CI, 1.81 to 5.31) among TNBC versus ER-positive/HER2-negative patients. Three-year DFS was 46.6% (95% CI, 38.0% to 54.8%), being lower among TNBC versus ER-positive/HER2-negative patients (HR 2.91; 95% CI, 1.64 to 5.16). Results were similar between surrogate intrinsic subtypes.

Conclusion There was a high proportion of HER2-positive and TNBC among Mozambican patients and their survival was poor compared with ER-positive/HER2-negative patients, partly due to the limited treatment options. A systematic assessment of ER, PR and HER2 status is feasible and may help tailoring and optimise the treatment of patients with breast cancer in low-resource settings, potentially leading to survival gains in this underserved population.

Key questions

What is already known about this subject?

- Breast cancer incidence and mortality rates have been increasing over the last decades in developing countries, including sub-Saharan Africa. Part of this high mortality has been attributed to the large proportion of cases of triple-negative breast cancer (TNBC) and lower proportion of the oestrogen receptor (ER)-positive/human epidermal growth factor receptor 2 (HER2)-negative subtype among African populations compared with Western countries. Nonetheless, there is a paucity of data regarding treatment and survival according to the different breast cancer subtypes in Africa.

What does this study add?

- Nearly half of patients with breast cancer in Mozambique had HER2-positive or TNBC and their treatment was similar across subtypes. Part of these subtypes were determined in cytological samples and we demonstrated, for the first time in Africa, that this is a feasible method for their assessment. Survival was poor, especially among HER2-positive and TNBC patients, who had a twofold and threefold increase in the risk of death versus ER-positive/HER2-negative patients, respectively.

How might this impact on clinical practice?

- Our study has clinical and health policy implications for the management of breast cancer in Africa. Our results highlight the need and the feasibility of the universal assessment of ER, PR and HER2 status on breast tumours, even in low-resource settings, as this may optimise the use of systemic treatments, potentially leading to important survival gains in this underserved population.

INTRODUCTION

Breast cancer incidence rates have been increasing over the last decades, especially in developing countries.[1] In Mozambique, it is now the second most incident cancer among Maputo City women, with a crude incidence rate of 8.6/100 000 women.[2] Furthermore, age-standardised mortality rates are greater in low/medium-income than in high-income countries (14.9 versus 11.6/100 000 women, respectively).[1] In sub-Saharan Africa, these differences have been attributed to the high proportion of patients with locally advanced/metastatic disease[3] and to poor access to diagnosis and treatments.[4]

However, breast cancer is a heterogeneous entity, comprising four molecular subtypes (luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) overexpression, basal), with different clinical courses, prognosis and treatment.[5 6] As this molecular determination is not widely available, a surrogate classification based on immunohistochemistry and in situ hybridisation (ISH) assessments of the oestrogen receptor (ER), the progesterone receptor (PR), HER2 and Ki67 biomarkers was proposed at the St. Gallen conference.[7] Nonetheless, in clinical practice and trials, breast tumours are still classified according to the 'classic' definition of 'ER-positive/HER2-negative', 'HER2-positive' and triple-negative breast cancer (TNBC; ER-negative/PR-negative/HER2-negative).

These biomarker determinations are usually carried out on surgically excised specimens or core needle biopsies.[8 9] Nevertheless, in developing countries, fine needle aspiration cytology (FNAC) may be an appropriate resource to deal with the difficulties of inadequate pathology services, as it is a cheaper and less invasive diagnostic method.[10] Additionally, studies from high-income countries demonstrated that biomarker assessment can be performed on cell blocks taken from FNAC, with concordances around 96%–98% for ER and 96% for HER2 when compared with histological samples.[11 12]

A meta-analysis showed that there is a higher proportion of cases of TNBC (21%) and a lower proportion of the ER-positive/HER2-negative subtype (52%) among African populations[13] compared with Western countries, where ER-positive/HER2-negative tumours represent more than 70% of cases.[14] Nonetheless, there is a wide variation in these proportions among different African studies.[13] Additionally, there is a paucity of data regarding breast cancer treatment and survival according to the different subtypes in African countries.[15–19] This knowledge may allow for a better management and organisation of healthcare services in this low-resource setting, translating into improved outcomes for patients with breast cancer.

Thus, this study aimed to assess the distribution of breast cancer subtypes in Mozambique, and its impact on treatment and survival. The concordance between biomarker and subtype assessment in cell blocks and histological samples was also evaluated.

METHODS

Setting

Mozambique is a low-income country in eastern sub-Saharan Africa, with 28 million inhabitants.[20] Around 13% of adults aged 15–49 years are infected by HIV,[21] but the country is now facing an increase in non-communicable diseases, such as cardiovascular disease and cancer.[22] The free-of-charge public health system is the largest healthcare provider, but has few resources for cancer care.

Until 2016, there were only three Pathology Departments in the country, one in each of the Central Hospitals: in Maputo (the capital city), Beira and Nampula. The Pathology Department of the Maputo Central Hospital (MCH) is the national reference department and it has a dedicated FNAC clinic since 1996.[23] This department also centralises the performance of immunohistochemistry, but there are frequent ruptures in reagent supplies. Thus, a research grant allowed for the acquisition of most reagents for the assessment of ER, PR, HER2 and Ki67 that were used in this study.

There are two Medical Oncology Units (in Nampula and Maputo), where patients have access to anthracyclines, cyclophosphamide, taxanes, methotrexate and tamoxifen, although with occasional interruptions in supply. Trastuzumab and aromatase inhibitors are not available. The Radiotherapy Unit opened in August 2019 and a multidisciplinary tumour board meeting for breast cancer was created in March 2016. Treatment decisions at the multidisciplinary tumour board are usually based on the European Society for Medical Oncology breast cancer guidelines,[6] although adapted to the available resources.

Study design

The prospective Moza-BC cohort study included consecutive incident cases of breast cancer, with a pathological diagnosis performed in one of the three Central Hospitals of Mozambique, from January 2015 to August 2017 (online supplementary figure 1). Data on sociodemographic, clinicopathological characteristics, treatment and survival of patients followed in the Oncology Unit of the MCH were prospectively collected until July 2018. Survival data were updated through the MCH Cancer Registry, health records and telephone interviews in November 2019. Patients without treatment/follow-up data were mostly from the Centre/North of the country, but there were no significant differences in clinicopathological characteristics in relation to those followed at the MCH (online supplementary table 1).

Breast tissue samples were collected by FNAC, surgical biopsy and breast surgery, and infrequently by core needle biopsy. Handling of histological specimens was standardised in the three hospitals according to the College of American Pathologists' recommendations.[8] Cell blocks were created from the aspirated FNAC material using HistoGel (Thermo Scientific, USA), according to the manufacturer's instructions. Cell blocks were sent to the MCH and those with adequate cellularity (≥100 cells) were selected for biomarker assessment. Immunostaining

of both cell blocks and histological samples was manually performed at the MCH, using the UltraVision Detection System anti-Polyvalent, horseradish peroxidase (HRP) (ready-to-use, Thermo Scientific) and Quanto Detection System HRP DAB (Thermo Scientific). Anti-ER, anti-PR, anti-HER2 and anti-Ki67 antibodies (clones SP1, SP2, SP3 and SP6, respectively; Thermo Scientific) were used. Expression of ER, PR, HER2 and Ki67 were assessed as described in the literature.[8 9 24] Cases with a HER2 immunohistochemistry score of 2+ (HER2 equivocal) were submitted to silver-ISH at the Centro Hospitalar Universitário de São João, Portugal. Two pathologists from the MCH determined the pathology diagnosis and biomarker assessment independently. Approximately 10% of cell blocks were sent for quality control to Portugal, where they were restained and reassessed by a third pathologist.

Tumours were grouped according to the 'classic' classification into ER-positive/HER2-negative, HER2-positive (ER-positive/HER2-positive and ER-negative/HER2-positive) and TNBC. Tumours were also classified into surrogate intrinsic subtypes, according to the 2015 St. Gallen Consensus[7]: luminal A-like (ER-positive, PR positivity ≥20%, HER2-negative and Ki67 ≤29%), luminal B-like (ER-positive/HER2-negative and either PR positivity <20% or Ki67 >29%, or ER-positive/HER2-positive), HER2-enriched (ER-negative/PR-negative/HER2-positive) and basal-like (ER-negative/PR-negative/HER2-negative). Due to the absence of validated local reference data, the Ki67 cut-off of 29% was used based on the international recommendations.[7] Among cases with both histological and cytological samples, in case of disagreement in subtype classification, the sample with the largest expression of biomarkers was selected, as it dictated treatment. Histological grade was assessed according to the Elston-Ellis definition.[25]

Staging was classified by the American Joint Committee on Cancer tumour, node, metastasis 7th edition.[26] Clinical staging was usually performed using physical examination, mammography/breast ultrasound, chest X-ray and abdominal ultrasound.

Statistical analysis

Baseline patient, tumour and treatment characteristics were compared across subtypes using t-test and analysis of variance for continuous variables, and χ^2 and Fisher exact tests for categorical variables. Overall survival (OS) was defined as time from diagnosis (date of pathological confirmation of breast cancer) until death by any cause. Among patients with early stage disease (stage I–III), disease-free survival (DFS) was defined as time from diagnosis until locoregional or distant relapse, second primary malignancy or death by any cause. Survival analyses were performed using the Kaplan-Meier estimator. Comparisons of survival across subtypes were accomplished through log-rank tests, and adjusted HRs and the corresponding 95% CIs computed using Cox proportional hazards regression.

Agreement of biomarker assessment between cell blocks and histological samples was measured using Cohen's κ statistics and classified as fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) and almost perfect (0.81–1).[27]Spearman's rank order coefficients were calculated for the correlation of ER and PR expressions in percentage for cell blocks versus histological samples. Analyses were carried out in STATA V.15 (Stata, College Station, Texas, USA). All tests were two-sided and a p-value of <0.05 was considered significant. Changes in subtype classification were illustrated using Sankey diagrams created in R (V,3.6.0) with the flipPlots package (V.1.2.0). The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) consensus was followed for the reporting of this manuscript.[28]

RESULTS

Among the 262 patients included in the Moza-BC cohort, 210 had tissue available for biomarker assessment (online supplementary figure 1): 108 (51%) had ER-positive/HER2-negative disease, 50 (24%) HER2-positive tumours and 52 (25%) TNBC. Using the St. Gallen classification of surrogate intrinsic subtypes, 28 (13%) patients had luminal A-like, 103 (49%) luminal B-like, 27 (13%) HER2-enriched and 52 (25%) basal-like tumours. Overall, 62% of tumours were ER-positive.

Patients were young (median age: 48.0 years), mostly premenopausal (53%), overweight/obese (62%), black (98%) and lived in the South of Mozambique (87%). There were 45 (25%) HIV-positive patients and 74% were diagnosed with stage III/IV disease (table 1). HIV-positive patients had a higher proportion of TNBC compared with HIV-negative patients (33% versus 20% respectively, p=0.048). TNBC tumours were more likely grade 3, but there were no differences in stage distribution. Results were similar across surrogate intrinsic subtypes (online supplementary table 2).

Clinical management

There were no differences in terms of time from symptoms to diagnosis, the type of first treatment received or in time from diagnosis to treatment across classic subtypes (table 2). More than 80% of patients underwent surgery and the proportion was higher among those with HER2-positive tumours. Surgical treatments were mostly mastectomies, as only 5% of patients had access to radiotherapy.

More than 90% of patients received chemotherapy, including patients with ER-positive/HER2-negative tumours (96%) and luminal A-like tumours (96%; online supplementary table 3). Among early patients with breast cancer, 104 (73%) received neoadjuvant chemotherapy, but only 3 (2%) achieved a pathological complete response (pCR) in the breast and lymph nodes (ypT0/is, ypN0). TNBC and HER2-positive tumours were less likely to be downstaged under neoadjuvant chemotherapy compared with ER-positive/HER2-negative tumours. Among ER-positive/HER2-negative patients with de novo stage IV disease

Table 1 Patients' baseline characteristics according to each classic breast cancer subtype

	ER-positive/HER2-negative	HER2-positive	TNBC	
	n=108	n=50	n=52	P value
Age in years (n, %)				0.702
<40	25 (23.8)	13 (26.0)	14 (26.9)	
40–49	32 (30.5)	12 (24.0)	12 (23.1)	
50–59	23 (21.9)	14 (28.0)	9 (17.3)	
≥60	25 (23.8)	11 (22.0)	17 (32.7)	
Missing	3	0	0	
Race (n, %)				0.603
Black	105 (98.1)	49 (98.0)	52 (100)	
Other*	2 (1.9)	1 (2.0)	0	
Missing	1	0	0	
Education in years (n, %)				0.253
0	20 (27.8)	7 (17.9)	5 (14.3)	
1–4	9 (12.5)	3 (7.7)	7 (20.0)	
>4	43 (59.7)	29 (74.4)	23 (65.7)	
Missing	36	11	17	
Place of residence (n, %)				0.178
South (including Maputo)	90 (89.1)	43 (89.6)	37 (78.7)	
Centre/North	11 (10.9)	5 (10.4)	10 (21.3)	
Missing	7	2	5	
Menopausal status (n, %)				0.916
Premenopausal	48 (52.7)	24 (54.5)	20 (50.0)	
Postmenopausal	43 (47.3)	20 (45.5)	20 (50.0)	
Missing	17	6	12	
Body mass index (n, %)				0.255
Under/normal weight (<25 kg/m^2)	36 (42.9)	15 (38.5)	10 (27.0)	
Overweight/obese (≥25 kg/m^2)	48 (57.1)	24 (61.5)	27 (73.0)	
Missing	24	11	15	
HIV status (n, %)†				**0.043**
Negative/unknown	67 (73.6)	38 (86.4)	25 (62.5)	
Positive	24 (26.4)	6 (13.6)	15 (37.5)	
Missing	17	6	12	
Tumour characteristics (clinical staging) (n, %)				0.513
cT1	3 (2.8)	3 (6.1)	1 (1.9)	
cT2	27 (25.2)	15 (30.6)	14 (26.9)	
cT3	29 (27.1)	17 (34.7)	18 (34.6)	
cT4	48 (44.9)	14 (28.6)	19 (36.5)	
Missing	*	*	0	
Lymph node status (clinical staging) (n, %)				0.095
cN0	27 (27.6)	20 (44.4)	12 (26.7)	
cN+	71 (72.4)	25 (55.6)	33 (73.3)	
Missing	10	5	7	
Tumour characteristics (pathological staging) (n, %)				0.672
(y)pT0/Tis	4 (5.1)	1 (2.4)	2 (5.9)	
(y)pT1	15 (19.2)	8 (19.0)	7 (20.6)	
(y)pT2	31 (39.7)	21 (50.0)	10 (29.4)	
(y)pT3	15 (19.2)	7 (16.7)	11 (32.4)	
(y)pT4	13 (16.7)	5 (11.9)	4 (11.8)	
Missing	30	8	8	

Continued

Table 1 Continued

	ER-positive/HER2-negative	HER2-positive	TNBC	
	n=108	n=50	n=52	P value
Lymph node status (pathological staging) (n, %)				0.512
(y)pN0	13 (21.7)	11 (29.7)	9 (31.0)	
(y)pN1	26 (43.3)	10 (27.0)	11 (37.9)	
(y)pN2	15 (25.0)	9 (24.3)	4 (13.8)	
(y)pN3	6 (10.0)	7 (18.9)	5 (17.2)	
Missing	48	13	23	
Median tumour size at surgery in millimetres (median, range)	40 (0–180)	40 (2.5–134)	45 (0–180)	0.115
Missing	31	8	18	
Multifocal tumours at surgery (n, %)				0.974
No	64 (88.9)	35 (89.7)	28 (90.3)	
Yes	8 (11.1)	4 (10.3)	3 (9.7)	
Missing	36	11	21	
Lymphovascular invasion at surgery (n, %)				0.596
No	14 (22.6)	12 (30.8)	9 (30.0)	
Yes	48 (77.4)	27 (69.2)	21 (70.0)	
Missing	46	11	22	
Neural invasion at surgery (n, %)				0.642
No	47 (75.8)	29 (74.4)	25 (83.3)	
Yes	15 (24.2)	10 (25.6)	5 (16.7)	
Missing	46	11	22	
Histological grade at surgery (n, %)				**0.001**
1	14 (19.2)	16 (40.0)	5 (16.1)	
2	42 (57.5)	13 (32.5)	9 (29.0)	
3	17 (23.3)	11 (27.5)	17 (54.8)	
Missing	35	10	21	
Histological subtype at surgery (n, %)				0.749
No residual tumour/in situ carcinoma	4 (5.2)	1 (2.4)	2 (5.7)	
Invasive ductal carcinoma (NST)	64 (82.1)	36 (87.8)	31 (88.6)	
Other invasive subtypes‡	10 (12.8)	4 (9.8)	2 (5.7)	
Missing	30	9	17	
Stage at diagnosis (n, %)				0.153
I	1 (1.1)	1 (2.3)	1 (2.5)	
II	14 (15.4)	15 (34.1)	12 (30.0)	
III	58 (63.7)	23 (52.3)	18 (45.0)	
IV	18 (19.8)	5 (11.4)	9 (22.5)	
Missing§	17	6	12	

P values in bold are considered to be statistically significant (<0.05).
*Includes mixed and Indian race.
†Seven patients had unknown HIV status; among HIV-positive patients, 31 (69%) had been previously diagnosed; the median time since HIV diagnosis was 3.93 years (range: 0.1–11.7); 41 (91%) patients were under ART when starting chemotherapy, mostly with the TDF+3TC+EFV regimen (28 patients); the median time under ART was 2 years (range 0.1–11.7); the median CD4+ cell count was 448 cells/µL (range 43–1104 cells/µL) and 39 (87%) patients had a CD4+ cell count >200/µL.
‡Includes lobular, mixed, papillary, squamous cell carcinoma, metaplastic and mucinous breast cancer.
§Includes the 35 patients for whom there is available cT/N and/or (y)pT/N status, but without information regarding the presence of metastases.
ART, antiretroviral treatment; cT/N, clinical tumor status and clinical lymph node status; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; NST, no special type; ;TDF+3TC+EFV, tenofovir + lamivudine + efavirenz; TNBC, triple-negative breast cancer; (y)pT/N, pathological tumor status and pathological lymph node status.

(n=18), one patient did not receive any treatment and all the others received chemotherapy as the first-line systemic treatment; among them, only eight patients ever received endocrine therapy. In ER-negative patients, 19 (31%) were given endocrine therapy, as biomarker results were not yet available at the time of prescription.

Survival

After a median follow-up of 38.3 months, only 16 (9%) patients were lost to follow-up as their last contact with the hospital was >12 months before the survival cut-off date, but they were still included in the analysis. Globally, 3-year OS was 52.5% (95% CI, 44.3% to 60.1%),

Table 2 Breast cancer clinical management according to each classic subtype

	ER-positive/HER2-negative	HER2-positive	TNBC	
	n=108	n=50	n=52	P value
Time from first symptom until diagnosis (n, %)				0.468
<180 days	30 (50.8)	13 (48.1)	13 (65.0)	
≥180 days	29 (49.2)	14 (51.9)	7 (35.0)	
Missing	49	23	32	
Timing of diagnosis (n, %)				0.729
Pre-MTB implementation	46 (50.5)	22 (50.0)	23 (57.5)	
Post-MTB implementation	45 (49.5)	22 (50.0)	17 (42.5)	
Not applicable*	17	6	12	
Type of first treatment received (n, %)				0.069
No treatment received	1 (1.1)	0	2 (5.0)	
Surgery	17 (18.9)	14 (31.8)	14 (35.0)	
Chemotherapy/endocrine therapy†	72 (80.0)	30 (68.2)	24 (60.0)	
Missing	18	6	12	
Time from diagnosis until first treatment (n, %)				0.083
<45 days	36 (40.4)	26 (59.1)	21 (55.3)	
≥45 days	53 (59.6)	18 (40.9)	17 (44.7)	
Missing	19	6	14	
Surgery (ever) (n, %)				**0.030**
No	17 (18.3)	1 (2.2)	6 (15.0)	
Yes	76 (81.7)	45 (97.8)	34 (85.0)	
Missing	15	4	12	
Surgical intent (n, %)‡				0.971
Diagnostic	3 (3.9)	1 (2.2)	1 (2.9)	
Curative	61 (80.3)	37 (82.2)	28 (82.4)	
Palliative	8 (10.5)	4 (8.9)	2 (5.9)	
Unknown	4 (5.3)	3 (6.7)	3 (8.8)	
Type of breast surgery (n, %)§				
Total mastectomy	70 (92.1)	41 (91.1)	33 (97.1)	0.553
Tumourectomy	6 (7.9)	4 (8.9)	1 (2.9)	
Status of surgical margins (n, %)				0.431
Clean	64 (91.4)	36 (92.3)	26 (83.9)	
Positive	6 (8.6)	3 (7.7)	5 (16.1)	
Missing	6	6	3	
Axillary surgery—type (n, %)¶				
Axillary dissection	66 (98.5)	40 (88.9)	31 (100)	0.586
Sentinel lymph node biopsy	1 (1.5)	0	0	
Not done/missing	9	5	3	
Axillary surgery—completeness (n, %)**				0.710
Not done/no isolated lymph nodes	9 (11.8)	5 (11.9)	3 (9.1)	
Incomplete	21 (27.6)	7 (16.7)	9 (27.3)	
Complete	46 (60.5)	30 (71.4)	21 (63.6)	
Missing	0	3	1	
Chemotherapy (ever) (n, %)				0.413
No	4 (4.4)	2 (4.5)	4 (10.0)	
Yes	87 (95.6)	42 (95.5)	36 (90.0)	
Missing	17	6	12	

Continued

Table 2 Continued

	ER-positive/HER2-negative	HER2-positive	TNBC	
	n=108	n=50	n=52	P value
Intent of first-line CT (n, %)††				0.075
Neoadjuvant only	16 (18.4)	2 (4.8)	2 (5.6)	
Neoadjuvant+adjuvant	42 (48.3)	25 (59.5)	16 (44.4)	
Adjuvant only	12 (13.8)	10 (23.8)	10 (27.8)	
Palliative	17 (19.5)	5 (11.9)	8 (22.2)	
Neoadjuvant CT—outcome (n, %)				**0.017**
Same stage	14 (24.1)	13 (48.1)	10 (55.6)	
Upstaging	7 (12.1)	6 (22.2)	1 (5.6)	
Downstaging	26 (44.8)	8 (29.6)	6 (33.3)	
Unknown‡‡	11 (19.0)	0	1 (5.6)	
pCR rate after neoadjuvant CT (n, %)				0.825
No pCR	55 (93.2)	26 (96.3)	16 (88.9)	
pCR only in the breast (ypT0/is)	2 (3.4)	1 (3.7)	1 (5.6)	
pCR in the breast and lymph nodes (ypT0/is, ypN0)	2 (3.4)	0	1 (5.6)	
Type of first-line CT regimen (n, %)††				0.086
Anthracycline-based only	43 (49.4)	13 (31.0)	20 (55.6)	
Anthracyclines+taxanes based	40 (46.0)	28 (66.7)	15 (41.7)	
Other§§	4 (4.6)	1 (2.4)	1 (2.8)	
First-line CT dose intensity (n, %)††				0.263
<85%	47 (57.3)	20 (50.0)	24 (68.6)	
≥85%	35 (42.7)	20 (50.0)	11 (31.4)	
Missing	5	2	1	
Cumulative dose of doxorubicin in mg/m^2 (median, range)	240 (60–420)	240 (120–360)	240 (120–360)	0.75
Endocrine therapy (ever) (n, %)				**<**0.001
No	30 (33.3)	19 (43.2)	31 (79.5)	
Yes	60 (66.7)	25 (56.8)	8 (20.5)	
Missing	18	6	13	
Radiotherapy (ever) (n, %)				0.358
No	86 (95.6)	40 (90.9)	39 (97.5)	
Yes	4 (4.4)	4 (9.1)	1 (2.5)	
Missing	18	6	12	

P values in bold are considered to be statistically significant (<0.05).
*Not applicable as patients were not treated/followed at the Maputo Central Hospital and, therefore, were not discussed by the multidisciplinary tumour board.
†One patient received endocrine therapy as first treatment, who had a luminal B-like tumour.
‡Patients submitted to a surgical biopsy with diagnostic intent followed by a tumourectomy or a mastectomy with curative intent were included in the 'Curative' intent group.
§Patients submitted to a tumourectomy followed by a mastectomy were included in the 'Mastectomy' group.
¶One patient received a sentinel lymph node biopsy followed by an axillary dissection and was, therefore, included in the 'Axillary dissection' group.
**Among patients receiving any type of breast surgery (n=155). Not done: not done or no isolated lymph nodes; incomplete: 1–5 isolated lymph nodes (in case of axillary lymph node dissection); complete: ≥6 isolated lymph nodes (in case of axillary lymph node dissection) or ≥1 isolated lymph nodes with ≤2 positive lymph nodes (in case of sentinel lymph node biopsy).
††First line of chemotherapy that the patient received includes neoadjuvant, adjuvant or palliative treatment. If the patient received part of chemotherapy as neoadjuvant (eg, AC regimen), and another part as adjuvant chemotherapy (eg, paclitaxel), the type of regimen and dose intensity refer to the entire scheme (neoadjuvant plus adjuvant).
‡‡Cases in whom there were missing data regarding clinical staging or patient-abandoned treatment.
§§Includes: taxane-based CT (three patients), non-anthracycline/non-taxane-based CT (two patients) and unknown regimen (one patient). The preferred anthracycline-containing regimen was AC (cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 every 3 weeks) and the preferred taxane used was paclitaxel (175 mg/m^2 every 3 weeks); dose-dense regimens were not used due to the unpredictable availability of granulocyte-colony stimulating factors.
CT, chemotherapy; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; MTB, multidisciplinary tumour board; pCR, pathological complete response; TNBC, triple-negative breast cancer.

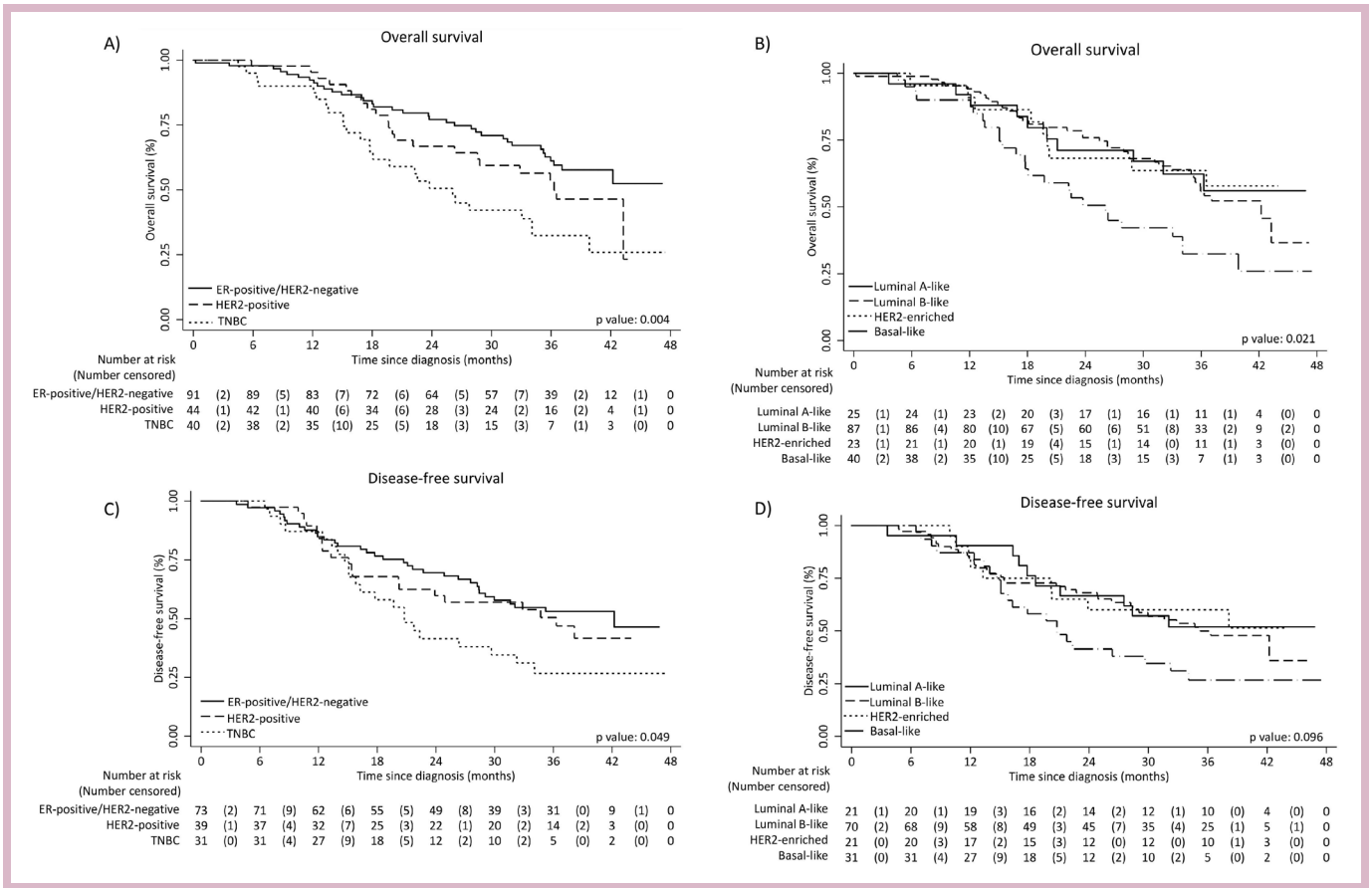

Figure 1 Kaplan-Meier curves for overall survival and disease-free survival according to classic subtypes (panels A and C) and surrogate intrinsic subtypes (panels B and D). ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

being higher in the ER-positive/HER2-negative subgroup (61.1%; 95% CI, 49.5% to 70.9%) compared with HER2-positive (53.1%; 95% CI, 36.5% to 67.3%) and TNBC (32.4%, 95% CI, 17.8% to 47.9%) (figure 1). Adjusting for prognostic factors, OS was significantly worse among HER2-positive (HR 1.96; 95% CI, 1.13 to 3.39) and TNBC (HR 3.10; 95% CI, 1.81 to 5.31) versus ER-positive/HER2-negative patients (table 3). Patients with luminal A-like and HER2-enriched subtypes appeared to have a better 3-year OS (62.3% and 63.6%, respectively) than those with luminal B-like (55.9%) or basal-like (32.4%) disease, but no significant differences were observed in the adjusted analysis.

There were 75 DFS events, consisting of locoregional relapse (n=27), distant relapse (n=12), both locoregional and distant relapse (n=8), second primary cancer (n=1) and death (n=27). Three-year DFS was 46.7% (95% CI, 38.1% to 54.9%), being significantly lower among patients with TNBC (26.7%; 95% CI, 12.2% to 43.6%) compared with ER-positive/HER2-negative (53.1%; 95% CI, 40.7% to 64.0%) or HER2-positive patients (50.5%; 95% CI, 33.2% to 65.5%) (figure 1). Results were similar across surrogate intrinsic subtypes.

When separately analysing patients with stage I–II and stage III–IV diseases, OS and DFS differences across subtypes were more pronounced among those with stage III–IV breast cancer (online supplementary table 4).

Biomarker/subtype concordance and quality control

Among the 51 patients with paired cell blocks/histological samples, the observed concordance was 88.2% for ER (κ=0.762), 80.4% for PR (κ=0.574), 83.7% for HER2 (κ=0.603) and 76.1% for Ki67 status (κ=0.271) (online supplementary table 5). Spearman's correlation σ was 0.749 for ER expression and 0.625 for PR expression in percentage, from 0 to 100%. Concordance was 78.4% (κ=0.661) for the classic subtype classification and 82.4% (κ=0.732) for the surrogate intrinsic subtype classification (figure 2).

Out of the 109 cell blocks, 15 (14%) were sent to quality control. Concordance was 93.3% for ER and PR status (κ=0.842 and κ=0.857, respectively), 80.0% (κ=0.541) for HER2 immunohistochemical score and 50.0% (κ=0.248) for Ki67 (online supplementary table 6).

DISCUSSION

This prospective study showed that nearly half of patients with breast cancer in Mozambique had TNBC or HER2-positive disease and that only 62% of tumours were

Table 3 Overall survival and disease-free survival estimates and multivariable analysis, according to classic and surrogate intrinsic subtypes

	n	3-year overall survival (95% CI)	P value*	Adjusted HR (95% CI)†
3-year overall survival				
Classic subtypes				
ER-positive/HER2-negative	91	61.1 (49.5 to 70.9)	**0.004**	1
HER2-positive	44	53.1 (36.5 to 67.3)		**1.96 (1.13 to 3.39)**
TNBC	40	32.4 (17.8 to 47.9)		**3.10 (1.81 to 5.31)**
Surrogate intrinsic subtypes				
Luminal A-like	25	62.3 (39.8 to 78.4)	**0.021**	*1
Luminal B-like	87	55.9 (43.8 to 66.3)		0.69 (0.33 to 1.44)
HER2-enriched	23	63.6 (40.3 to 79.9)		1.09 (0.43 to 2.74)
Basal-like	40	32.4 (17.8 to 47.9)		1.96 (0.93 to 4.12)
	n	**3-year disease-free survival‡**	**P value***	**AdjustedHR (95% CI)†**
Classic subtypes				
ER-positive/HER2-negative	73	53.1 (40.7 to 64.0)	**0.049**	*1
HER2-positive	39	50.5 (33.2 to 65.5)		1.61 (0.91 to 2.85)
TNBC	31	26.7 (12.2 to 43.6)		**2.91 (1.64 to 5.16)**
Surrogate intrinsic subtypes				
Luminal A-like	21	52.0 (29.1 to 70.6)	0.096	*1
Luminal B-like	70	49.9 (37.1 to 61.4)		0.91 (0.44 to 1.88)
HER2-enriched	21	60.0 (35.7 to 77.6)		1.15 (0.44 to 3.02)
Basal-like	31	26.7 (12.2 to 43.6)		**2.38 (1.10 to 5.13)**

Values in bold are considered to be statistically significant.
*p value for the univariate survival analysis.
†Adjusted for age (<40 versus 40–49 versus 50–59 versus ≥60 years), HIV status (negative/unknown versus positive), stage at diagnosis (0–II versus III versus IV) and date of diagnosis (pre- versus post-multidisciplinary tumour board implementation).
‡There were 75 disease-free survival events, consisting of locoregional relapse (n=27), distant relapse (n=12), both locoregional and distant relapse (n=8), second primary cancer (n=1) and death (n=27).
ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

ER-positive. Part of these subtypes were determined in cell blocks and we demonstrated, for the first time in Africa, that this is a feasible method for their assessment.

There were no striking differences in baseline characteristics or treatment across subtypes, except for HIV status, histological grade or endocrine therapy. The association between TNBC and high histological grade is well-known[29]; however, it is intriguing between TNBC and HIV-positive status. TNBC is the most immunogenic breast cancer subtype[30 31] and, therefore, patients with a compromised immune system may be more susceptible to its development. Nonetheless, a previous meta-analysis has not demonstrated a significant difference in TNBC proportion among HIV-positive versus HIV-negative patients.[32]

Neoadjuvant chemotherapy was prescribed to 73% of early patients with breast cancer, which is much higher than what has been reported in South Africa (35%–62%),[16 17] Rwanda (48%)[33]or stage III patients from the USA (42%).[34] A report from South Africa showed that 64% of patients receiving neoadjuvant chemotherapy had a clinically significant response,[16] which is better than our results. In our study, the pCR rate was also low: 2% in this analysis and 7% if considering patients from the Moza-BC cohort having pCR but not included in this analysis (online supplementary figure 1). These disappointing results may be explained by the overall low chemotherapy dose intensity, absence of targeted anti-HER2 therapy (ie, trastuzumab) and the generalised use of 'sandwich' chemotherapy regimens (neoadjuvant plus adjuvant).

Prognosis was dismal, as almost 50% of patients had died within 3 years following diagnosis. This is in line with a pooled analysis from several sub-Saharan African cancer registries showing an overall 3-year relative survival of 66%.[35] Furthermore, we observed that despite the global poor prognosis, survival was even worse among patients with HER2-positive tumours and TNBC, who had a twofold and threefold increase in the risk of death versus ER-positive/HER2-negative patients, respectively.

Our cohort study has many advantages compared with other African reports.[15–19] We used ISH to assess HER2-equivocal cases and evaluated Ki67, and as such were able to categorise surrogate intrinsic subtypes according to the St. Gallen classification, which is often used to

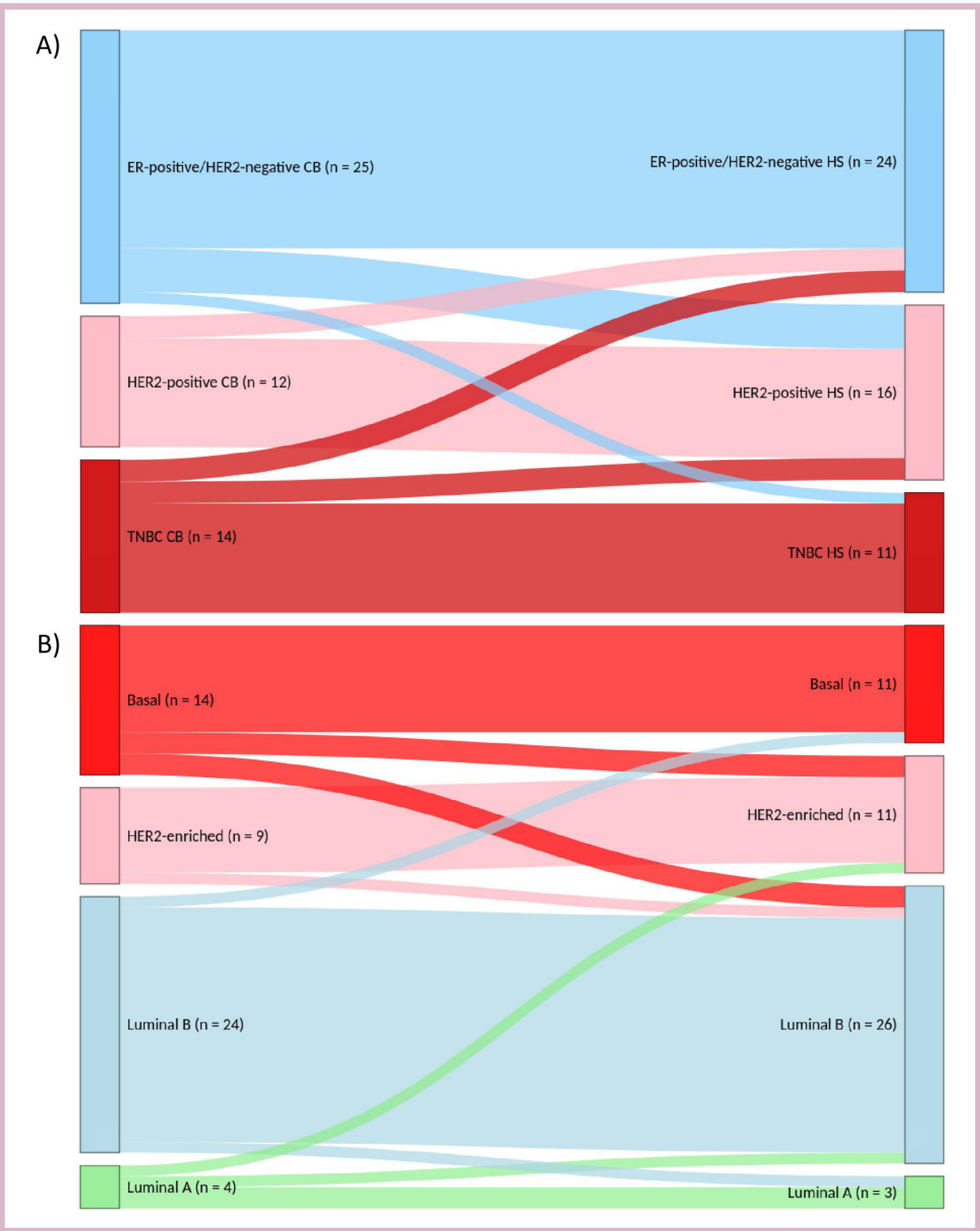

Figure 2 Sankey diagrams showing the reclassification of classic subtypes (panel A) and surrogate intrinsic subtypes (panel B) between CBs versus paired histological specimens (n=51). CB, cell blocks; ER: oestrogen receptor; HER2, human epidermal growth factor receptor 2; HS, histological samples; TNBC, triple-negatives breast cancer.

determine early breast cancer treatment.[6 7] Many African series did not use ISH and classified HER2-equivocal cases as 'HER2-negative' or excluded them, which may have underestimated the prevalence of the HER2-positive subtype.[15 16 18 36 37] Additionally, most African series did not assess Ki67, and thus luminal A-like was classified as 'ER-positive/HER2-negative' and luminal B-like as 'ER-positive/HER2-positive',[13 36 37] which differs from the St. Gallen definition.[7] Moreover, we not only assessed biomarkers in histological samples, but also in cytological

samples, which were frequently excluded from other series.[19 37 38] Although only 109 out of 159 cell blocks (69%) had sufficient cellularity for immunohistochemistry assessment, we found that concordance between cell blocks and histological samples was substantial for ER and HER2 status, supporting its use in assessing breast cancer biomarkers in low-resource settings. The exception was the low concordance for Ki67; however, its low reproducibility is well-known.[39] Nonetheless, further research is still needed for a better understanding of the reliability of ER and HER2 immunohistochemistry evaluation in breast cancer cell blocks in low-resource settings.

By classifying our tumour samples according to international standardised methods, we could reliably compare clinicopathological characteristics, treatment and survival of patients across the different breast cancer subtypes. Furthermore, we enriched our cohort with patients with stage III/IV tumours by using cell blocks, making our sample more representative of the 'real' breast cancer population. Moreover, unlike retrospective series with high losses to follow-up (up to 48%),[15–19] only 9% of our patients were considered lost.

Nonetheless, our study has limitations. This is a hospital-based study; however, OS was similar to African population-based estimates,[35] and this allowed for the description of survival according to breast tumour subtypes, which has not yet been estimated at a population-level in Africa. Despite the fact that we included patients from the three largest hospitals in Mozambique, detailed follow-up data were only available for patients treated at the MCH. Yet, this single-centre subcohort is similar to other African series in terms of young age at diagnosis,[40] high prevalence of HIV infection,[15] long delays between symptoms and diagnosis[41] and a large proportion of stage III/IV.[3] The limited staging options available (chest X-ray and abdominal ultrasound) may have led to an underestimation of the real incidence of stage IV disease, which may partly explain the low survival of our patients. Nonetheless, these are the staging examinations usually available in most African countries[4] and, therefore, our findings may be compared with the other African series.

When performing HER2 determination in cell blocks, there may be a risk of false positivity due to the tumour's in situ component. However, only 2 out of 49 cases switched from 'HER2-positive' in the cell block to 'HER2-negative' in the surgical sample. Nonetheless, both patients were submitted to neoadjuvant chemotherapy, which may have led to HER2 expression loss. In a large series from the USA, such potentially false-positive HER2 results on cell blocks were not observed.[11]

Our study has clinical and health policy implications for the management of breast cancer in Africa. Clinicians and stakeholders should move from a 'homogeneous' view of this disease and understand the importance of determining breast tumour subtypes before starting treatment. This is especially relevant in a setting with such a high proportion of stage III/IV, in which systemic therapy has a more predominant role. The WHO has recently included ER/PR and HER2 overexpression tests in the list of essential diagnostic tools.[42] Here, we demonstrated that this assessment can be made in low-resource settings, using cell blocks from FNAC, which are usually available and much less expensive than core needle biopsies.[23]

International societies increasingly recommend the use of neoadjuvant therapy, especially for HER2-positive tumours and TNBC.[6] Therefore, if the physician has access to biomarker determination and is aware that the patient has TNBC, neoadjuvant chemotherapy combining anthracyclines, taxanes and platinum salts could be recommended, as the addition of platinum salts increases the chance of achieving a pCR.[43] Even if there is no demonstrated long-term survival benefit from the addition of platinum, these inexpensive drugs could still be used to increase the chance of tumour downsizing, improving the proportion of clean surgical margins and breast-conserving surgeries. Moreover, it would be important to enhance chemotherapy dose intensity, by using adequate supportive treatments.

Over a quarter of our patients with early disease had HER2-positive tumours. The survival of these patients has substantially improved in Western countries, due to anti-HER2 therapy.[44] However, in Mozambique, like in most African countries, patients do not have access to these drugs,[45] which also contributes to their dismal prognosis. With the appearance of trastuzumab biosimilars,[46] it may now be possible to use them in the neoadjuvant setting to increase the likelihood of tumour downsizing and pCR, with impact in long-term survival.[47] Then, in the adjuvant setting, administration of trastuzumab for 9 weeks[48] to 6 months[49] may also be considered, instead of the standard 12-month regimen,[44] as it still might improve survival when compared with the absence of trastuzumab treatment.

Almost two-thirds of our patients had ER-positive tumours. However, if the tumour's ER/PR status is unknown, the physician may act 'on the safe side' and prefer chemotherapy over endocrine therapy for (neo) adjuvant/palliative treatment. This partly explains the very high use of chemotherapy among ER-positive/HER2-negative patients in our series (96%), as in most cases biomarker results were only available after systemic treatment had been already started. On the other hand, before this study, following chemotherapy, all patients with unknown ER status would receive endocrine therapy, which is a common situation across sub-Saharan Africa.[4] Therefore, it is paramount to test for ER/PR to adequately select patients who benefit from endocrine therapy—especially in the African setting, where the proportion of ER/PR-positive tumours is lower than in Western countries.[13] Thus, if the tumour's ER/PR status is known, this may lead to substantial savings, as an important proportion of patients would be spared from endocrine therapy and/or chemotherapy.

CONCLUSION

In this prospective cohort study, we found a high proportion of HER2-positive disease and TNBC among Mozambican patients. We demonstrated that biomarker assessment is feasible, even in patients undergoing FNAC. Globally, the 3-year OS was 52.5%, being even worse among patients with HER2-positive disease or TNBC. Our results highlight the need for the universal assessment of ER, PR and HER2 status on breast tumours, as this may optimise the use of systemic treatment, potentially leading to important survival gains in this underserved population.

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Ethics approval The National Health Bioethical Committee of Mozambique approved this study (reference number 226/CNBS/15). All participants provided written informed consent.

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Data availability statement Data are available on reasonable request. The datasets generated and analysed during the current study are not publicly available due to the fact that the included patients did not specifically provide their consent for public sharing of their data and that anonymisation, even if possible, is partially impaired by the fact that the majority of patients were treated in the same institution and diagnosed within a restricted period of time (January 2015–August 2017), with some of the groups being small. Nonetheless, data may be available from the corresponding author on reasonable request.

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