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FMUP FACULDADE DE MEDICINA
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BREAST CANCER HETEROGENEITY

ETIOLOGY, CLINICAL MANAGEMENT, USE OF HEALTH RESOURCES AND SURVIVAL

MARIANA DA ROCHA ALMEIDA BRANDÃO

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Acronyms and Abbreviations

aHR	Adjusted hazard ratio
aOR	Adjusted odds ratio
ASCO	American Society of Clinical Oncology
BMI	Body mass index
CAP	College of American Pathologists
CI	Confidence interval
DALY	Disability-adjusted life-year
ER	Estrogen receptor
ESMO	European Society for Medical Oncology
FNAC	Fine-needle aspiration cytology
GDP	Gross domestic product
HR	Hormone receptor
IAEA	International Atomic Energy Agency
IC	Intervalos de confiança
IDFS	invasive disease-free survival
Int$	International dollars
IPO-Porto	Instituto Português de Oncologia do Porto Francisco Gentil
ISH	*in situ* hybridization
Moza-BC	Mozambican Breast Cancer cohort
MTB	Multidisciplinary tumor board
NEON-BC	Neuro-oncological complications of breast cancer cohort
PARP	Poly-ADP ribose polymerase
pHR	Pooled hazard ratio
pOR	Pooled odds ratio
PR	Progesterone receptor
QALY	Quality-adjusted life year
SEER	Surveillance, Epidemiology, and End Results database
SLNB	Sentinel-lymph node biopsy
TNBC	Triple-negative breast cancer
US	United States of America
WHO	World Health Organization
WHO-STEPS	World Health Organization STEPwise approach to Surveillance Noncommunicable Disease Risk Factor Survey
WLWH	Women living with HIV

SUMMARY

Breast cancer is the most frequent malignancy among women in the world. It is also the most frequent cause of cancer death in women in developing regions and the second cause among women from developed countries. Yet, patients with breast cancer are a heterogeneous group, due to socio-economic factors, clinical characteristics and their tumor biology, all of which influence etiology, care and survival.

This heterogeneity creates different challenges in each setting. In low-income countries, diagnosis delays, advanced stage at presentation, presence of comorbidities, such as HIV infection, and low availability of resources, coupled with their sub-optimal use, contribute to lower survival compared to Western countries. Nonetheless, there are still few data concerning risk factors, clinical management and survival of women with breast cancer in low-income countries, especially in Sub-Saharan Africa.

On the other hand, in high-income countries, the short time to diagnosis and the availability of effective therapy lead to other kind of challenges. The increasing number of cancer survivors, who may develop a second primary malignancy and might need different treatments, requires the study of clinical management and outcomes among these patients. Additionally, due to several new treatment options, the cost of breast cancer care is rising. Hence, there is the need to evaluate health resource use according to tumor subtypes, which is essential to determine the economical evaluations of innovative products.

Therefore, this thesis aimed to assess the impact of breast cancer heterogeneity on etiology, clinical management, health resource use and prognosis in different settings: in a high-income, developed country (Portugal) and in a low-income, developing country (Mozambique). This objective was accomplished through:

1. Analysis of two prospective cohorts of patients with newly diagnosed breast cancer:
    a. The “NEON-BC – Neuro-oncological complications of breast cancer” cohort, from Portugal, that included women with newly diagnosed stage I-III breast cancer in 2012, followed at the Instituto Português de Oncologia do Porto Francisco Gentil during the first five years after diagnosis;
    b. The “Moza-BC – Mozambican Breast Cancer” cohort, which recruited Mozambican women consecutively diagnosed with breast cancer in the three main Hospitals of Mozambique (Maputo, Beira and Nampula Central Hospitals) between January/2015 and August/2017, and who were followed until November/2019;

    In both cohorts, participants were divided according to their tumor’s biomarkers into the classic subtypes (hormone receptor [HR]-positive/HER2-negative, HER2-positive and triple-negative breast

cancer [TNBC]) and the surrogate intrinsic subtypes (luminal A-*like*, luminal B-*like*, HER2-enriched and basal-*like*);

2. A case-control analysis including cases from the Moza-BC cohort and controls from the general population, evaluated as part of the population-based "World Health Organization STEPwise approach to Surveillance Noncommunicable Disease Risk Factor Survey" (WHO-STEPS), conducted in Mozambique between 2014 and 2015;
3. A systematic review and meta-analysis, also including data from the Moza-BC cohort.

The following paragraphs describe the specific objectives of the thesis, along with the corresponding methods and main results:

1. **To assess the relation between risk factors and the occurrence of breast cancer, overall and by each tumor subtype in women from Mozambique (*Paper I*);**

   The associations between education, number of births, height, weight, body-mass index (BMI) and breast cancer risk among 138 cases (participants from the Moza-BC cohort) and 638 controls from the general population (from a WHO-STEPS survey), were investigated. Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were estimated using multivariable logistic regression.

   Multiparity (≥6 *vs* 0-1 live births) was a protective factor for the development of HR-positive (aOR 0.22 [95% CI, 0.08-0.64]) and HR-positive/HER2-negative tumors (aOR 0.20 [0.06-0.68]), while a higher educational level (≥8 *vs* 0 schooling years) increased breast cancer risk across all subtypes (overall aOR 1.98 [1.04-3.80]). Higher weight and BMI were associated with a higher breast cancer risk among post-menopausal women (per 1-Kg-increase: aOR 1.05 [1.02-1.08]; per 1-Kg/m^2-increase: 1.11 [1.04-1.18], respectively), but were protective in pre-menopausal women (aOR 0.98 [0.96-0.99]; 0.95 [0.91-0.99], respectively), regardless of subtype. Higher height increased the risk of HR-negative tumors in post-menopause (per 10-cm-increase: aOR 2.81 [1.41-6.03]).

2. **To assess the distribution of breast cancer subtypes, and its impact on patients' treatment and survival in Mozambique (*Paper II*);**

   We included 210 women from the Moza-BC cohort. Clinico-pathological characteristics, treatment, and three-year overall survival and disease-free survival were compared across classic tumor subtypes and surrogate intrinsic subtypes. Cox proportional hazards regression was used to compute aHR and 95% CI.

A total of 51% of patients had HR-positive/HER2-negative tumors, 24% HER2-positive and 25% TNBC; 13% of women had luminal A-*like*, 49% luminal B-*like*, 13% HER2-enriched and 25% basal-*like* tumors. There were no significant differences across subtypes regarding clinical characteristics and treatment, except for HIV-positivity and histological grade III, which were more prevalent among TNBC; and endocrine therapy, which was used more often among patients with HR-positive/HER2-negative and HER2-positive disease. Three-year overall survival was 52.5%, being higher in HR-positive/HER2-negative (61.1%) compared to HER2-positive (53.2%) and TNBC (31.9%) patients. Adjusted hazard ratios (aHR) were 1.96 (95% CI, 1.13-3.39) among HER2-positive and 3.10 (1.81-5.31) among TNBC *vs* HR-positive/HER2-negative patients. Three-year disease-free survival was 46.6%, being lower among TNBC compared to patients with HR-positive/HER2-negative tumors (aHR 2.91 [1.64-5.16]). The results obtained were similar between surrogate intrinsic subtypes.

3. **To quantify the relation between breast cancer subtypes and clinical management and health resource use among women with early breast cancer from Portugal (*Paper III*);**

   We included 703 women from the NEON-BC cohort. The use of health resources during the first three years after diagnosis was obtained through clinical and administrative records. Costs were computed for each individual patient, and compared according to stage and subtype.

   The cost of early breast cancer care was mainly driven by the tumor subtype and, to a lesser extent, by stage. The median cost of care was four-fold higher among patients with HER2-positive tumors (41 513€/patient) compared to those with HR-positive/HER2-negative (10 540€/patient) and TNBC (11 224€/patient). Similar differences were observed across surrogate intrinsic subtypes.

4. **To compare health resource use and the cost of care of women with early breast cancer in a high-income (Portugal) *vs* a low-income country (Mozambique), overall and according to stage and subtype (*Paper IV*);**

   Using data from the prospective NEON-BC cohort (n=703) and the Moza-BC cohort (n=162), we compared the cost of care of women with early breast cancer during the first three years after diagnosis, between Portugal and Mozambique. Local currency prices were converted to 2019 international dollars (Int$) using purchasing power parity exchange rates for household consumption.

   The median cost of care was 18 533 Int$/patient *vs* 2888 Int$/patient among Portuguese and Mozambican patients, respectively. The cost rose with increasing stage only in Portuguese patients, among whom there was also a great disparity according to subtypes, with a median cost of 66 811 Int$/patient with a HER2-positive tumor. Nearly half of Mozambican resources were used in medical

tests and only 22% in systemic therapy. In Portugal, systemic therapy accounted for 35%, while medical tests for 11% of the healthcare cost; among patients with HER2-positive tumors, systemic therapy corresponded to 69% of costs. Overall, surgery accounted for 17% and 18% of the total cost in Portugal and Mozambique, respectively.

5. **To quantify the association between a previous cancer diagnosis and clinical management, healthcare use and survival of women with early breast cancer from a high-income setting (*Paper V*);**

   We classified women included in the NEON-BC cohort according to the existence of a previous cancer (breast or other). Data regarding healthcare use during the first year and five-year relapse-free survival were obtained through clinical and administrative records. We computed aOR and 95% CI to quantify the association between a previous cancer diagnosis and treatment and healthcare use; and aHR and 95% CI for recurrence or death by any cause according to a previous cancer diagnosis.

   Among 681 women with a breast cancer diagnosed in 2012, 21 had a previous breast cancer diagnosis and 32 had a previous non-breast cancer. The latter were less likely to receive anthracycline-based combination chemotherapy (aOR 0.03 [95% CI, 0.01-0.20]). The former had higher odds of mastectomy (aOR 4.17 [1.36-12.72]) and genetic testing (aOR 14.82 [3.60-61.01]). Five-year relapse-free survival aHR were 2.75 (0.79-9.52) and 0.52 (0.07-3.89) for previous breast cancer and non-breast cancer, respectively.

6. **To evaluate the impact on survival and the cost-effectiveness of a multidisciplinary tumor board for breast cancer in a low-resource setting (*Paper VI*);**

   Among 205 women from the Moza-BC cohort, 98 were included before and 107 after the implementation of the multidisciplinary tumor board (MTB). Pre- and post-MTB implementation subcohorts were compared for clinical characteristics, treatments and overall survival. Cox proportional hazards regression was used to compute aHR and 95% CI. The impact of MTB implementation on the cost per quality-adjusted life year (QALY) was estimated from the provider perspective.

   No significant differences between pre- and post-MTB subcohorts were found regarding clinical characteristics or treatments received. Among patients with early breast cancer (stage 0-III; n=163), the three-year overall survival (95% CI) was 48.0% (35.9-59.1) in the pre-MTB and 73.0% (61.3-81.6) in the post-MTB subcohort – aHR 0.47 (0.27-0.81). The absolute three-year mean cost increase was 120 US dollars/patient and the incremental cost-effectiveness ratio was 803 US dollars/QALY, corresponding to 1.6 times the gross domestic product per capita of Mozambique.

7. **To measure the impact of HIV infection in the clinical presentation and prognosis of women diagnosed with breast cancer from both high- and low-income countries (*Paper VII*);**

   We performed a systematic review and meta-analysis using MEDLINE, Scopus, ISI Web of Knowledge, LILACS, SciELO and conference abstracts up to 1st January 2020. Cross-sectional and cohort studies comparing baseline characteristics (stage and/or subtypes) and/or overall survival of women living with HIV (WLWH) *vs* HIV-negative women with breast cancer were included. Random-effects meta-analyses were performed to estimate summary statistics (pooled odds ratio [pOR] and pooled hazard ratios [pHR]) and subgroup analyses according to world region.

   We included 18 studies (four from North America, 14 from Sub-Saharan Africa – including the Moza-BC cohort), with 3,174 WLWH and 2,394,598 HIV-negative women. WLWH from North America and Sub-Saharan Africa were more likely to present with stage III/IV disease compared to HIV-negative women – pOR 1.76 (95% CI, 1.58-1.95) and pOR 1.23 (1.06-1.42), respectively. WLWH from Sub-Saharan Africa were also less likely to have HR-positive/HER2-negative tumors (pOR 0.81 [0.66-0.99]). After adjustment, WLWH had worse overall survival compared to HIV-negative women, both in North America (pHR 2.45 [1.11-5.41]) and in Sub-Saharan Africa (pHR 1.43 [1.06-1.92]).

In conclusion, this thesis showed how breast cancer heterogeneity, at multiple levels, influences etiology, clinical management and prognosis. We have unraveled for the first time the etiological heterogeneity of breast cancer according to tumor subtypes in a Sub-Saharan African population. Moreover, we demonstrated that the proportion of TNBC is indeed higher among patients from Mozambique and that, despite the overall dismal prognosis of patients in this low-resource setting, having a TNBC determines an even worse survival. We have also seen that tumor subtype is the main determinant of health resource use and cost of care in Portugal, while it has virtually no impact in treatment tailoring in Mozambique – which has an underfunding of breast cancer care.

We verified that having breast cancer as a second primary malignancy did not lead to major overall changes in care and that had no significant impact on relapse-free survival among women from Portugal. In terms of healthcare management, we saw that the implementation of a multidisciplinary tumor board increased survival among women with early-stage breast cancer from Mozambique and demonstrated for the first time its cost-effectiveness in a low-income setting. Finally, we showed that HIV infection is associated with more advanced breast cancer stage at diagnosis and worse overall survival, both in high- and low-income countries.

Stakeholders across the world should move away from the vision of breast cancer as a single disease and understand that women with breast cancer are a very heterogeneous group, with different risk factors and diverse needs in terms of diagnosis, treatment and follow-up. This finer understanding of its

complexity, both at the molecular, and at the patient and country level, may inform the design of better policies in terms of resource allocation and healthcare organization, translating into a more effective delivery of breast cancer care and, ultimately, in improved patient outcomes.

RESUMO

O cancro da mama é a neoplasia maligna mais frequente na mulher, a primeira causa de morte por cancro nas mulheres dos países em desenvolvimento e a segunda causa nos países desenvolvidos. Contudo, as doentes com cancro de mama constituem um grupo heterogéneo, devido a fatores socio-económicos, características clínicas e biologia tumoral, que têm influência na etiologia, tratamento e sobrevivência.

Essa heterogeneidade coloca diferentes desafios em cada contexto. Em países de baixo rendimento, os atrasos no diagnóstico, o estádio avançado à apresentação, a presença de comorbilidades, como a infeção pelo HIV, e a baixa disponibilidade de recursos, aliada à sua utilização sub-ótima, contribuem para uma menor sobrevivência das doentes, em comparação com o observado nos países ocidentais. No entanto, existem poucos estudos sobre os fatores de risco, gestão clínica e sobrevivência das mulheres com cancro de mama em países de baixo rendimento, especialmente da África Subsaariana.

Por outro lado, em países de alto rendimento, o diagnóstico atempado e o fácil acesso a tratamentos eficazes resultam noutro tipo de desafios. O número crescente de sobreviventes de cancro, que podem desenvolver uma segunda neoplasia maligna primária e, portanto, exigir um tratamento diferente, requer o estudo da gestão clínica e dos resultados clínicos nestes doentes. Além disso, devido às várias opções terapêuticas existentes, o custo de tratamento do cancro da mama está a aumentar, sendo então necessário estudar a utilização de recursos de saúde de acordo com os subtipos tumorais, que são essenciais para as avaliações económicas de produtos inovadores.

Assim, esta tese teve como objetivo avaliar o impacto da heterogeneidade do cancro da mama na etiologia, gestão clínica, uso de recursos de saúde e prognóstico em diferentes contextos: num país desenvolvido, de alto rendimento (Portugal), e num país em desenvolvimento, de baixa rendimento (Moçambique). Este objetivo foi alcançado através de:

1. Análise de duas coortes prospetivas de doentes com cancro da mama recém-diagnosticado:
    a. A coorte NEON-BC, de Portugal, que incluiu mulheres com diagnóstico de cancro da mama em estádio I-III em 2012, seguidas no Instituto Português de Oncologia do Porto Francisco Gentil durante os primeiros cinco anos após o diagnóstico;
    b. A coorte Moza-BC, que recrutou mulheres moçambicanas com diagnóstico de cancro da mama realizado nos três principais Hospitais de Moçambique (Hospitais Centrais de Maputo, Beira e Nampula), entre janeiro de 2015 e agosto de 2017, que foram seguidas até novembro de 2019;

    Em ambas as coortes, as participantes foram divididas de acordo com os biomarcadores do seu tumor em: i) subtipos tumorais clássicos (recetores hormonais [HR]-positivos/HER2-negativo, HER2-positivo

e cancro da mama triplo-negativo [TNBC]) e ii) subtipos tumorais intrínsecos (luminal A-*like*, luminal B-*like*, HER2-enriquecido e basal-*like*);

2. Análise de caso-controlo com casos da coorte Moza-BC e controlos da população geral, avaliados no âmbito do " World Health Organization STEPwise approach to Surveillance Noncommunicable Disease Risk Factor Survey" (WHO-STEPS), realizado em Moçambique entre 2014 e 2015;
3. Uma revisão sistemática e meta-análise, incluindo também dados da coorte Moza-BC.

Os parágrafos seguintes descrevem os objetivos específicos da tese, juntamente com os métodos e principais resultados correspondentes:

1. **Avaliar a relação entre fatores de risco e a ocorrência de cancro da mama, em geral e de acordo com cada subtipo tumoral em mulheres moçambicanas (*Artigo I*);**

   Investigámos as associações entre escolaridade, número de nados-vivos, altura, peso, índice de massa corporal (BMI), obesidade (BMI ≥30 kg/m^2) e a ocorrência de cancro da mama, através de um desenho caso-controlo, com 138 casos da coorte Moza-BC e 638 controlos da população geral, provenientes do inquérito WHO-STEPS. Foram estimados odds ratios ajustados (aOR) e intervalos de confiança a 95% (IC 95%) através de modelos de regressão logística condicional.

   A multiparidade (≥6 *vs* 0-1 nados-vivos) é um fator de proteção para o desenvolvimento de tumores HR-positivos (aOR 0,22 [IC 95%, 0,08-0,64]) e HR-positivos/HER2-negativos (aOR 0,20 [0,06-0,68]), enquanto que uma maior escolaridade (≥8 *vs* 0 anos de escolaridade) aumenta o risco de todos os subtipos tumorais (aOR 1,98 [1,04-3,80]). Peso e BMI mais elevados associam-se a um maior risco de cancro da mama em mulheres pós-menopáusicas (por aumento de 1 kg: aOR 1,05 [1,02-1,08]; por aumento de 1 kg/m^2: 1,11 [1,04-1,18], respetivamente), mas são protetores em mulheres pré-menopáusicas (aOR 0,98 [0,96-0,99]; 0,95 [0,91-0,99], respetivamente), para todos os subtipos. Uma altura mais elevada associa-se a uma maior risco de desenvolvimento de tumores HR-negativos na pós-menopausa (por aumento de 10 cm: aOR 2,81 [1,41-6,03]).

2. **Avaliar a distribuição dos subtipos de cancro da mama e o seu impacto no tratamento e sobrevivência de doentes moçambicanas (*Artigo II*);**

   Foram incluídas 210 mulheres da coorte Moza-BC. As características clínico-patológicas, o tratamento, a sobrevivência global e a sobrevivência livre de doença aos três anos foram comparados de acordo com os subtipos clássicos e com os subtipos intrínsecos. Foram calculados hazard ratios ajustados (aHRs) e IC 95% por regressão de Cox.

Em relação aos subtipos clássicos, 51% das doentes apresentaram tumores HR-positivos/HER2-negativo, 24% HER2-positivo e 25% TNBC; quanto aos subtipos intrínsecos, 13% das mulheres tinham tumores luminal A-*like*, 49% luminal B-*like*, 13% HER2-enriquecido e 25% basal-*like*. Não houve diferenças significativas entre os subtipos em relação às características clínicas e ao tratamento, exceto no que diz respeito à infeção por HIV e ao grau histológico III, que foram mais frequentes entre as mulheres com TNBC, e à hormonoterapia, que foi mais utilizada pelas doentes com tumores HR-positivos/HER2-negativo e HER2-positivo. A sobrevivência observada aos três anos foi de 52,5%, sendo mais elevada nas doentes com tumores HR-positivos/HER2-negativo (61,1%) em comparação com aquelas com os subtipos HER2-positivo (53,2%) e TNBC (31,9%). Os aHR foram 1,96 (IC 95%, 1,13-3,39) para as doentes com tumores HER2-positivo e 3,10 (1,81-5,31) para aquelas com TNBC, relativamente às mulheres com tumores HR-positivos/HER2-negativo. A sobrevivência livre de doença aos três anos foi de 46,6%, sendo menor nas mulheres com TNBC em comparação com as doentes com tumores HR-positivos/HER2-negativo (aHR 2,91 [1,64-5,16]). A análise de acordo com os subtipos tumorais intrínsecos produziu resultados semelhantes.

3. **Quantificar a relação entre os subtipos de cancro da mama e a gestão clínica e utilização de recursos de saúde por mulheres com cancro da mama precoce em Portugal (*Artigo III*);**

   Foram incluídas 703 mulheres da coorte NEON-BC. A utilização de recursos de saúde nos primeiros três anos após o diagnóstico foi obtida através de registos clínicos e administrativos. Os custos foram calculados individualmente para cada doente e comparados de acordo com o estádio ao diagnóstico e subtipo tumoral.

   O custo do diagnóstico e tratamento do cancro da mama precoce foi influenciado sobretudo pelo subtipo tumoral e, em menor extensão, pelo estádio. O custo mediano do tratamento foi quatro vezes maior entre as doentes com tumores HER2-positivo (41 513€/doente) em comparação com as mulheres com tumores HR-positivos/HER2-negativo e TNBC (10 540€/doente e 11 224€/doente, respetivamente). A análise de acordo com os subtipos tumorais intrínsecos mostrou resultados semelhantes.

4. **Comparar a utilização de recursos de saúde e o custo dos cuidados de mulheres com cancro da mama precoce num país de alto rendimento (Portugal) *vs* um país de baixo rendimento (Moçambique), em geral e de acordo com o estádio e subtipo (*Artigo IV*);**

   Utilizando dados da coorte NEON-BC (n=703) e da coorte Moza-BC (n=162), comparámos o custo de diagnóstico e tratamento de mulheres com cancro da mama precoce, durante os primeiros três anos

após o diagnóstico, entre Portugal e Moçambique. Os preços em moeda local foram convertidos para dólares internacionais (Int$) de 2019, utilizando taxas de câmbio de paridade de poder de compra para consumo doméstico.

O custo mediano dos cuidados foi de 18 533 Int$/doente *vs* 2888 Int$/doente nas doentes portuguesas e moçambicanas, respetivamente. O custo foi mais elevado nos estádios mais avançados, mas apenas entre as doentes portuguesas, entre as quais se verificou também uma grande disparidade de custos de acordo com os subtipos (mediana de 66 811 Int$/doente com tumor HER2-positivo). Quase metade dos recursos moçambicanos foram usados em exames médicos e apenas 22% em tratamentos sistémicos. Em Portugal, a terapêutica sistémica foi responsável por 35% dos custos e os exames médicos por 11%; nas doentes com o subtipo HER2-positivo, os tratamentos sistémicos corresponderam a 69% dos custos. A cirurgia contribuiu para 17% e 18% do custo total em Portugal e em Moçambique, respetivamente.

5. **Quantificar a associação entre um diagnóstico prévio de cancro e a gestão clínica, uso de cuidados de saúde e sobrevivência de mulheres com cancro da mama precoce, num país de alto rendimento (*Artigo V*);**

   As mulheres incluídas na coorte NEON-BC foram classificadas de acordo com a existência de cancro prévio (da mama ou outro). Foram obtidos dados sobre o uso de cuidados de saúde durante o primeiro ano e a sobrevivência livre de recidiva aos cinco anos a partir de registos clínicos e administrativos. Calculámos aOR e IC 95% para quantificar a associação entre um diagnóstico prévio de cancro e os tratamentos e uso de cuidados de saúde, e aHR e IC 95% para recorrência ou morte por qualquer causa, de acordo com um diagnóstico prévio de cancro.

   Entre 681 mulheres com cancro da mama diagnosticado em 2012, 21 tinham um diagnóstico prévio de cancro da mama e 32 tinham uma outra neoplasia prévia. A quimioterapia à base de antraciclinas foi menos frequente neste segundo grupo (aOR 0,03 [IC 95%, 0,01-0,20]), enquanto o primeiro grupo apresentou uma maior frequência de mastectomia (aOR 4,17 [1,36-12,72]) e de realização de testes genéticos (aOR 14,82 [3,60-61,01]). Os aHR de sobrevivência livre de recidiva aos cinco anos foram 2,75 (0,79-9,52) para as doentes com diagnóstico prévio de cancro da mama e 0,52 (0,07-3,89) para as doentes com outra neoplasia prévia.

6. **Avaliar o impacto na sobrevivência e a relação custo-efetividade de uma consulta de grupo multidisciplinar para cancro da mama num país de baixo rendimento (*Artigo VI*);**

Das 205 mulheres da coorte Moza-BC, 98 foram incluídas antes e 107 após a implementação da consulta de grupo multidisciplinar (MTB). As sub-coortes recrutadas nos períodos pré- e pós-implementação da MTB foram comparadas de acordo com as características clínicas, tratamentos e sobrevivência global. Foram calculados aHRs e IC 95% por regressão de Cox. O impacto da implementação da MTB no "custo por ano de vida ajustado pela qualidade" (QALY) foi estimado a partir da perspetiva do provedor.

Não encontrámos diferenças significativas entre as sub-coortes pré- e pós-MTB em relação às características clínicas ou aos tratamentos recebidos. Entre as doentes com cancro da mama em estádio precoce (estádio 0-III; n=163), a sobrevivência global aos três anos (IC 95%) foi de 48,0% (35,9-59,1) na sub-coorte pré-MTB e de 73,0% (61,3-81,6) na sub-coorte pós-MTB – aHR 0,47 (0,27-0,81). O aumento absoluto do custo médio de diagnóstico e tratamento aos três anos foi de 120 dólares americanos/doente e o rácio custo-eficácia incremental foi de 803 dólares americanos/QALY, correspondendo a 1,6 vezes o produto interno bruto per capita de Moçambique.

7. **Medir o impacto da infeção pelo HIV na apresentação clínica e prognóstico de mulheres com diagnóstico de cancro da mama de países de alto e baixo rendimento (*Artigo VII*);**

Realizámos uma revisão sistemática e meta-análise, incluindo estudos identificados através de pesquisas efetuadas nas bases de dados eletrónicas MEDLINE, Scopus, ISI Web of Knowledge, LILACS e SciELO e resumos de conferências. Foram incluídos estudos transversais ou de coorte, publicados até 1 de janeiro de 2020, que compararam mulheres com cancro da mama com infeção por HIV (WLWH) *vs* mulheres com cancro da mama HIV-negativas em relação às características no momento do diagnóstico do cancro (estádio e/ou subtipos) e/ou à sobrevivência global. Calculámos estimativas sumárias dos odds ratios (pOR) e dos hazard ratios ajustados (pHR) por meta-análise de efeitos aleatórios, globalmente e por subgrupos definidos de acordo com a região do mundo.

Foram incluídos 18 estudos (quatro da América do Norte, 14 da África Subsaariana – incluindo a coorte Moza-BC), com 3174 WLWH e 2 394 598 mulheres HIV-negativas. Nas WLWH da América do Norte e da África Subsaariana foi mais frequente o cancro da mama em estádio III/IV em comparação com as mulheres HIV-negativas – pOR 1,76 (IC 95%, 1,58-1,95) e 1,23 (1,06-1,42), respetivamente. As WLWH da África Subsaariana foram diagnosticadas menos frequentemente com tumores HR-positivos/HER2-negativo (pOR 0,81 [0,66-0,99]). As WLWH apresentam uma pior sobrevivência global em comparação com as mulheres HIV-negativas, tanto na América do Norte (pHR 2,45 [1,11-5,41]) como na África Subsaariana (pHR 1,43 [1,06-1,92]).

Em conclusão, esta tese demonstrou como a heterogeneidade do cancro da mama, a vários níveis, tem influência na etiologia, gestão clínica e prognóstico. Mostrámos pela primeira vez a heterogeneidade etiológica do cancro da mama de acordo com os subtipos tumorais numa população africana. Além disso, demonstrámos que a proporção de TNBC é maior entre as doentes de Moçambique e que, apesar do mau prognóstico global neste contexto de baixo rendimento, a presença dum tumor TNBC determina uma sobrevivência ainda pior. Vimos também que o subtipo tumoral é o principal determinante da utilização de recursos de saúde e do custo dos cuidados em Portugal, embora não tenha praticamente impacto na adequação do tratamento em Moçambique – que apresenta um subfinanciamento do tratamento do cancro da mama.

Verificámos que o facto de se ter cancro da mama como segunda neoplasia maligna primária não levou, de forma global, a alterações *major* nos cuidados prestados e que não teve um impacto significativo na sobrevivência livre de recidiva em mulheres portuguesas. Vimos também que a implementação de um uma consulta de grupo multidisciplinar aumentou a sobrevivência das mulheres com cancro da mama precoce em Moçambique e demonstrámos, pela primeira vez, que esta é uma intervenção custo-efetiva neste contexto de baixo rendimento. Finalmente, observámos que a infeção pelo HIV está associada a um estádio mais avançado ao diagnóstico de cancro da mama e a uma pior sobrevivência global, tanto em países de alto como de baixo rendimento.

Desta forma, os decisores em saúde a nível global devem mudar a sua visão do cancro da mama como uma doença única e compreender que as mulheres com neoplasias da mama constituem um grupo muito heterogéneo, com diferentes fatores de risco e necessidades diversas em termos de diagnóstico, tratamento e seguimento. Esta compreensão mais profunda da complexidade, tanto ao nível molecular quanto ao nível da doente e do país, pode contribuir para o desenvolvimento de politicas de alocação de recursos e de organização de cuidados de saúde mais adequados, traduzindo-se numa prestação mais eficaz de cuidados às doentes com cancro da mama e, em última análise, em melhores resultados clínicos.

INTRODUCTION

Breast cancer is the most frequent cancer among women in the world, with an estimated incidence rate of 46.3/100 000 women in 2018, accounting for nearly one-quarter of all diagnosed cancer cases.[1] The highest figures for age-standardized (world standard population) incidence rates are observed in Oceania, and in Western and Northern Europe (>85.0/100 000 women). The lowest are in South-Central Asia, and in Eastern and Middle Africa (<30.0/100 000 women) – **Figure 1**.

Breast cancer is also the leading cause of cancer death worldwide among women (15% of the total number of cancer deaths) and 71% of these deaths occur in low/middle-income countries. Thus, age-standardized (world standard population) mortality rates are higher in low/middle-income than in high-income countries (14.9 *vs* 11.6 per 100 000 women, respectively).[1]

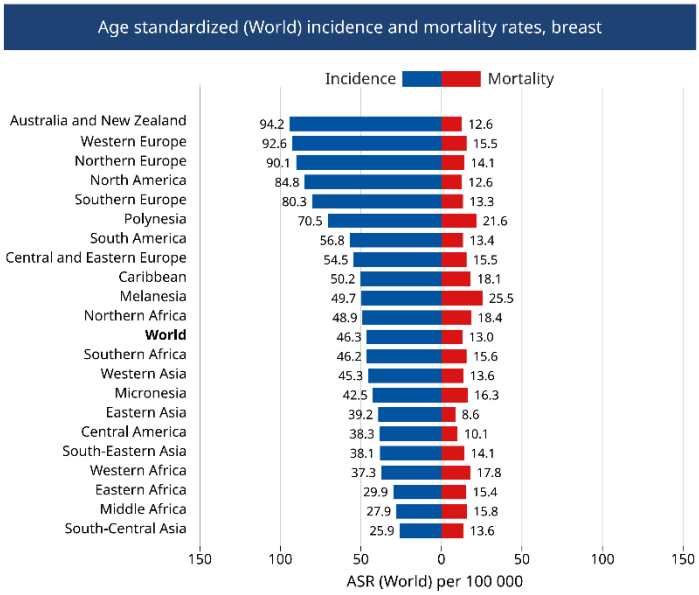


Figure 1: *Estimated age-standardized (world standard population) incidence rates and mortality rates, per 100 000 women (source: GLOBOCAN 2018 [International Agency for Research on Cancer])* [1]

In the most developed countries, there has been a decrease in mortality rates in the last decades, along with a growing number of cancer cases being diagnosed. The increasing incidence may be explained by a more frequent exposure to factors that contribute to a greater risk of breast cancer (*e.g.* aging of the population, delayed childbearing, lower parity, and increasing obesity and alcohol consumption), coupled with the widespread use of mammographic screening, which further contributed to these upward trends in incidence.[2,3] Yet, since the beginning of the millennium, a slight downward trend in incidence began to be observed among post-menopausal women (aged ≥50 years) in some high-income countries, such as the United States of America (US) or Israel.[4,5] The decline in the use of hormone-replacement therapy, which is more frequent in these settings than in the rest of the world and/or a decreased use of

mammographic screening have been presented as potential explanations for this decline in incidence.[6] Nonetheless, an increasing incidence of breast cancer among pre-menopausal women is occurring in high-income countries, which may be related to changes in reproductive behaviors (*e.g.* decreased parity), lifestyle (*e.g.* increased alcohol consumption) and/or anticipation of mammographic screening (*i.e.* before the age of 50).[5] Contrarily, the overall drop in mortality in high-income countries has been attributed to both an increasing frequency of early diagnosis, and access to more effective and targeted systemic treatments, besides improved loco-regional care (*i.e.* radiotherapy and surgery).[3,7]

In developing countries, breast cancer incidence has been increasing among both pre- and post-menopausal women.[5] This may be due to the ageing population, and changes in reproductive factors and lifestyles linked to socio-economic development (*e.g.* delayed childbearing and having fewer children, higher levels of physical inactivity and obesity, and increases in breast cancer screening and awareness).[1] As previously mentioned, mortality rates are higher in developing regions compared to developed countries. In Africa specifically, the age-standardized mortality rate was 17.2/100 000 women in 2018 and, at that time, around 12% of the global deaths from breast cancer occurred in this continent. Survival is much lower here than in more affluent regions of the world and a pooled analysis from several Sub-Saharan African cancer registries[8] showed an overall three-year relative survival of only 66% – in stark contrast with the five-year relative survival rate of 90% in the US.[9] This can be attributed to the long delays between symptoms and diagnosis,[10] leading to a high proportion of patients presenting with locally advanced/metastatic disease,[11] and to the limited access to diagnosis and treatment, such as surgery, radiation, systemic therapy and/or supportive treatment.[12] A detailed assessment of the burden, risk factors, screening, diagnosis and treatment of cancer in Sub-Saharan Africa is provided in **Supplementary Paper I** (chapter "*Cancer in Sub-Saharan Africa*").

These global trends in incidence and mortality lead to the worldwide increase in the absolute number of women living with breast cancer, some of whom have a degree of disability linked to the disease. Hence, breast cancer burden may also be measured by "disability-adjusted life-years" (DALY), which express the number of years lost due to ill-health, disability or early death.[13] A recent report, based on data from the Global Burden of Disease Study 2017, showed that from 1990 to 2017, the worldwide amount of DALY attributed to breast cancer increased by almost 70%.[13] There was a greater increase for "years lived with disability" than for "years of life lost" (124% *vs* 66% increase), which reflects global improvements in survival.

Additionally, the cost of each patient's cancer care has also been rising, due to new treatments and to the development of novel technologies, such as imaging (*e.g.* breast MRI, PET-CT scans) or radiotherapy modalities (*e.g.* three-dimensional conformal radiotherapy, intensity modulated radiotherapy, volumetric modulated arc therapy).[14] In the European Union, in 2009, breast cancer represented the second highest economic cost (€15.0 billion, 12% of overall cancer costs) and accounted for the highest cancer-related

healthcare costs (€6.7 billion; 13% of all cancer-related healthcare costs).[15] In the US, estimates of lifetime per-patient costs of breast cancer in 2009 ranged from $US20 000 to $US100 000,[16] corresponding to 43%-212% of the US gross domestic product (GDP) per capita at that time. Data from developing countries are scarcer, but some studies have also shown an increased cost of breast cancer care with rising stage.[17–20] These costs will tend to rise in the future in both developed and developing countries, both because the number of patients is growing and due to the increasing costs of new treatment options.

1. Breast cancer burden according to tumor subtypes

Definition and determination of each subtype

Breast cancer comprises four molecular subtypes (luminal A, luminal B, HER2-enriched and basal), with different risk factors, clinical courses, prognosis and treatment.[2,21] As this molecular determination is not yet widely available, a surrogate classification based on immunohistochemistry and *in situ* hybridization (ISH) assessments of hormone receptors (HR; which comprise the estrogen receptor [ER] and the progesterone receptor [PR]), HER2 and Ki67 biomarkers was proposed at the St. Gallen conference:[22]

- Luminal A-*like*: ER-positive (expression in ≥1% of cells), HER2-negative, low Ki67 (cut-off to be determined locally), high PR (expression in ≥20% of cells);
- Luminal B-*like*:
  - Luminal B-*like* (HER2-negative): ER-positive, HER2-negative and either high Ki67 or low PR (<20%);
  - Luminal B-*like* (HER2-positive): ER-positive, HER2-positive, any Ki67, any PR;
- HER2-enriched: HER2-positive, and ER and PR negative (expression <1%);
- Basal-*like*: ER and PR negative, and HER2-negative.

Nonetheless, in clinical practice and trials, breast tumors are still classified according to the "classic" definition of:

- HR-positive/HER2-negative (also named as ER-positive/HER2-negative);
- HER2-positive: HR-positive/HER2-positive and HR-negative/HER2-positive;
- Triple-negative breast cancer (TNBC): ER and PR negative, HER2-negative;

***Note*:** the majority of tumors classified as TNBC show the intrinsic basal-*like* subtype (*i.e.* molecularly defined), therefore these two classifications overlap in about 80% of cases and are often used interchangeably in clinical practice; nonetheless, the designation "TNBC" also includes luminal A, luminal B or HER2-enriched intrinsic subtypes and some special histological types, such as adenoid cystic carcinoma.[22,23]

Expression of ER, PR and HER2 should be assessed as recommended by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines.[24,25] The evaluation of Ki67 should be performed according to the International Ki67 in Breast Cancer Working Group.[26]

Geographical distribution

The distribution of tumor subtypes is heterogeneous around the world and, in Sub-Saharan Africa particularly, there is a higher proportion of patients with tumors classified as TNBC (around 21% in a pooled analysis of prospective studies)[27] and a lower proportion with ER-positive/HER2-negative tumors (around 52%) compared to Western countries (around 10-15% and 70-75% of each subtype, respectively).[28] Nonetheless, there is a wide variation in these proportions among different African studies[27] – as an example, reports from West Africa showed a TNBC prevalence ranging from 16% to 46%.[29–31]

One of the potential reasons for the difference between African and Western populations regarding the prevalence of ER and PR is the frequently long "delay to formalin fixation" or "cold ischemia time", *i.e.* the delay between surgical resection and formalin fixation, which may lead to increased antigen degradation.[32] Another reason is antigen degradation in archived tissue that is proportional to the duration of storage,[30] which could explain why there is a low expression of ER and PR on the samples of tumors from African women assessed retrospectively. However, several studies using adequately stored material show that breast cancers from African-American patients have a lower likelihood of being HR-positive and are associated with higher histological grade and greater Ki67 expression, compared to those from White American patients.[33]

Risk factors

The understanding of risk factors for the development of breast cancer has evolved over the past two decades, along with the discovery of the different breast cancer subtypes.[2,21] Multiple studies have shown that the effect of reproductive behaviors (*e.g.* parity, age at first birth, breastfeeding) and age at menarche/menopause may vary among breast cancer subtypes, being more strongly associated with the occurrence of ER-positive/HER2-negative tumors.[34,35] On the other hand, some reports suggested that parous women who never breastfed have a higher risk of developing HR-negative tumors or TNBC compared to nulliparous women.[36–38]

The effects of body parameters on breast cancer risk have also been explored and appear to be modified by menopausal status. While a higher weight or body mass index (BMI) are associated with reduced risk

among pre-menopausal women, they are a risk factor among post-menopausal women.[39] However, their effect according to subtype is still debatable. One Latin American study suggested that a higher BMI was protective for the development of HR-negative tumors among pre-menopausal women,[40] while a study from the US demonstrated that it was a risk factor for HR-negative tumors among pre-menopausal women.[41] Compared to the observed in Western countries, African patients have different levels of exposure to risk factors, such as higher parity and breastfeeding or reduced obesity, that could partly explain differences in the distribution of breast cancer subtypes. Yet, to the best of our knowledge, there are no studies addressing the association between known breast cancer risk factors and each of its subtypes in African countries.

Financial burden

Due to the increasing number of available systemic therapy options for each breast cancer subtype, there is a growing concern regarding the "financial toxicity" of treating breast cancer due to the escalation of costs. This concern exists not only in the metastatic setting, but increasingly in the early disease setting as well.[42,43] The European Society for Medical Oncology (ESMO) developed the "ESMO-Magnitude of Clinical Benefit Scale", which aims to objectively evaluate the clinical benefit of each approved systemic drug, taking into account the drug's survival benefit, associated toxicity and impact on quality-of-life.[44] These evaluations, coupled with real-life cost assessments, may help to inform stakeholders on reimbursement options for the many drugs that are currently being tested as well as regarding other cancer care policies.

Survival

Survival also differs according to tumor subtype – in a large analysis using data from women diagnosed during 2010 to 2013 and included in the Surveillance, Epidemiology, and End Results (SEER) database from the US, the best survival pattern was observed among women with ER-positive/HER2-negative subtype (four-year survival of 92.5%), followed by ER-positive/HER2-positive (90.3%), ER-negative/HER2-positive (82.7%) and TNBC (77.0%).[45] TNBC's dismal prognosis is attributed to its high proliferative capacity as well as to the lack of targeted therapies. Nonetheless, in Sub-Saharan Africa, where the proportion of TNBC is especially high,[27] there is very scarce data regarding the prognosis of each breast cancer subtype.[46]

2. Overview of breast cancer diagnosis and treatment

The management of breast cancer includes the treatment of local disease with surgery, radiation therapy, or both and the treatment of systemic disease with cytotoxic chemotherapy, endocrine therapy, targeted

therapy, immunotherapy or combinations of these.[2] The choice of treatment strategy is based on the tumor extent/location and biology, as well as on the age and general health status of the patient, personal preferences and locally available resources.[2] As stressed at the 2019 St. Gallen Conference, "*Shared clinical decision making is essential when caring for individual breast cancer patients*."[47]

2.1 Diagnosis and staging

The diagnosis of breast cancer includes clinical examination as well as imaging and is confirmed by pathological assessment. ESMO recommends that pathological diagnosis be based on a core needle biopsy (or, if not possible, at least a fine needle aspiration cytology [FNAC] indicating carcinoma), performed before starting treatment.[2] However, in developing countries, the choice of tissue sampling procedure largely depends on the equipment and expertise locally available. In low-resource settings, 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.[48] Additionally, studies from high-income countries have demonstrated that biomarker assessment can be performed on cell blocks taken from FNAC, with concordances around 96-98% for ER and 96% for HER2 status when compared to histological samples.[49,50]

Staging should be evaluated according to the AJCC TNM staging system.[51] ESMO only recommends a comprehensive radiological staging for higher-risk patients, such as those with high tumor burden (clinically positive axillary nodes and/or tumors >5 cm), aggressive biology (*e.g.* TNBC) and with symptoms, signs or laboratory values suggestive of metastases.[2] In those cases, a chest CT scan, abdominal imaging and a bone scan may be considered. Nonetheless, in developing countries, staging options may be limited to chest X-ray and abdominal ultrasound, which can lead to a suboptimal staging assessment.[12]

2.2 Surgery

Surgical treatment of primary breast cancer can consist of breast-conservation surgery or modified radical mastectomy, with or without axillary clearance. In developed countries, the use of (neo)adjuvant chemotherapy, targeted agents and radiotherapy, as well as the development of techniques such as the sentinel-lymph node biopsy (SLNB) led to considerable improvements of breast tumor surgery over the last decades. Therefore, breast surgery may now be much less aggressive (*i.e.* breast-conservation surgery with or without SLNB), with decreased morbidity and a benefit in patients' quality-of-life. In Western Europe, around 60-80% of newly diagnosed cancers are amenable to a breast conservation approach[52] and, in the cases in which that it is not possible as an upfront strategy, there is the option of using systemic therapy to downstage the tumor.[2]

Nonetheless, in developing countries, mastectomy is still the most common intervention for breast cancer.[12,53] In Sub-Saharan Africa, this is partly due to the large proportion of patients with stage III/IV disease at presentation, which was around 75% in a large pooled analysis.[11] On the other hand, radiotherapy is not available in many Sub-Saharan African countries, which limits de use of breast-conserving surgery. Thus, it is not surprising that, in Africa, around 85% of the patients undergoing breast surgery receive a mastectomy.[54] Still, breast surgery, even as a mastectomy, may not be an option when the disease is already very locally advanced or metastatic – in a report from Nigeria, only 35% of patients were operated, as many of them presented with inoperable local or metastatic tumors at diagnosis.[55] Similarly, in Rwanda, among 145 patients with breast cancer, only 48% underwent surgery.[56]

2.3 Radiotherapy

Radiation therapy is an important component of cancer control programs. In breast cancer, it can be used as an adjuvant therapy (*e.g.* after breast-conservation surgery), in isolated locoregional recurrence or in the palliative setting, for symptom control. Yet, its availability varies widely across the globe. In Africa, according to a survey conducted by the International Atomic Energy Agency (IAEA) in 2013, from the 52 countries included, only 23 were known to have external beam radiotherapy, and these facilities were concentrated in the southern and northern countries of the continent.[57] Adding to that, even in countries with radiation therapy facilities, access can be difficult. A study from Nigeria reported a long waiting list and undue pressure on the centers; consequently, a number of patients referred for radiation therapy ended up not receiving the treatment while many others were lost to follow-up from the time of referral.[31] Similarly, a series from Ghana showed that only 15% of patients with breast cancer were able to receive radiotherapy.[58] Fortunately, IAEA and other collaborators are now partnering with many African countries, in order to establish new radiotherapy centers or to modernize existing ones, through the acquisition of new equipment and the training of technical staff.

2.4 Systemic therapy

Systemic therapy given before or after local treatment of early breast cancer substantially improves survival.[59] It consists of chemotherapy, endocrine therapy, targeted agents, immunotherapy and/or bone-modifying agents. The most commonly prescribed chemotherapy regimens are based on the combination of anthracyclines with taxanes, although cyclophosphamide, fluoropyrimidines, platinum salts and other chemotherapy drugs may also be used.[2,60] In terms of endocrine therapy, tamoxifen, a selective estrogen receptor modulator, was one of the first targeted drugs to be used in the treatment of cancer.[61]

Afterwards, aromatase inhibitors and the selective estrogen receptor degrader fulvestrant were also developed for HR-positive breast cancer.[62,63]

Regarding targeted therapies, there are now multiple agents available, targeting the HER2 receptor, the mTOR/PI3K pathway, the CDK4/6 pathway or the poly-ADP ribose polymerase (PARP) enzyme, among others.[2,14,60] More recently, immune checkpoint inhibitors have been shown to be effective for the treatment of early-stage and metastatic breast cancer, in combination with chemotherapy.[64–66] Bone-modifying agents include bisphosphonate drugs as well as denosumab, a monoclonal antibody that works as a RANK ligand inhibitor.[67]

To assess the need and type of treatment, some variables are considered: tumor stage, HR status, HER2 status, measures of tumor growth rate (*e.g.* Ki67), response to neoadjuvant therapy and, when available, the result of gene expression signatures.[2] However, one of the most important determinants of systemic therapy is the tumor subtype. Thus, the tailoring of systemic therapy according to each breast cancer subtype is further developed below (**section 3.1**).

2.5 Multidisciplinary care

Given the heterogeneity of breast cancer and the multiple clinical scenarios and treatment options, its management is an increasingly complex task. Therefore, in order to provide coordinated, evidence-based therapy, most international oncology societies consider multidisciplinarity an essential part of breast cancer care, proposing that all patients be presented in a multidisciplinary tumor board (MTB) before initiating treatment.[2] Nonetheless, there is a wide gap between this universal recommendation and its effective implementation, even in developed countries. A French multicentric study showed that, although all breast cancer cases were presented in an MTB, only 64% were discussed before surgery, with significant differences between academic *vs* non-academic hospitals.[68] In a report using data from the US SEER-Medicare database, only 70% of patients had access to multidisciplinary care, and this proportion was even lower among those living in a rural area.[69] In China, less than 2% of patients operated for breast cancer from 2006 to 2016 in a large cancer center were discussed in an MTB.[70]

In Sub-Saharan Africa, many patients are independently evaluated by the surgeon, medical oncologist and/or radiation oncologist, without a comprehensive discussion in an MTB. This adds delays to the patient care pathway, increases the chance of discontinuing care and impairs the achievement of the best possible treatment for the individual patient, all of which compromise survival. In a recent survey conducted among members of the African Organisation for Research and Training in Cancer, 80% of the respondents reported they were involved in a multidisciplinary breast cancer team, but a generalization of this finding

to the entire continent is debatable, as there were only 37 respondents.[71] Additionally, there are no data regarding MTB effectiveness for breast cancer care in Africa.

3. Breast cancer heterogeneity: implications for clinical management, health resource use and survival

Breast cancer patients are a heterogeneous group: due to socioeconomic factors, clinical characteristics and tumor biology – all of which influence their healthcare use and prognosis.

3.1 Tumor subtypes

There are many systemic therapy options available according to each tumor subtype, both in the early and in the advanced setting. The most relevant studies evaluating (neo)adjuvant treatments for each breast cancer subtype, which reported results or updates between 2017 and early 2019, were discussed at the St. Gallen Oncology Conference of 2019 and are addressed in **Supplementary Paper II** ("*Systemic treatment of patients with early breast cancer: recent updates and state of the art*").

ER-positive/HER2-negative subtype

It is increasingly recognized that the most important systemic treatment option for ER-positive/HER2-negative tumors, both in the early and advanced settings, is endocrine therapy.[2,60] Moreover, chemotherapy and targeted agents are also used for the treatment of this subtype. Nonetheless, in low-resource countries, the absence of modern pathology services to determine a tumor's ER status hinders appropriate decision making for endocrine treatment.[72]

Among women with early-stage ER-positive/HER2-negative disease, there has been a trend over the past decade to de-escalate the use of adjuvant chemotherapy. The 2019 St. Gallen Conference recommended the use of genomic assays, such as OncotypeDx® or Mammaprint™, in patients with T1/T2N0, T3N0 and TxN1 tumors, to help with the decision about the use of chemotherapy.[2,47] A comprehensive description of the development and application of Mammaprint™ and other gene-expression signatures is provided in **Supplementary Paper III** ("*Mammaprint™: a comprehensive review*").

For pre-menopausal women, the options in terms of endocrine therapy are now tamoxifen alone (for low-risk tumors) or tamoxifen/aromatase inhibitor plus ovarian function suppression.[73] Among post-menopausal women, tamoxifen and/or an aromatase inhibitor for a total of five to ten years are recommended, depending on the baseline risk of recurrence and on tolerance to treatment. Escalating the

intensity and duration of endocrine therapy brings survival benefits, but also additional direct costs and potential impairments in patients' long-term quality-of-life. Therefore, similarly to what has been done regarding chemotherapy, there are now genomic signatures trying to predict which patients benefit the most from extended endocrine therapy, but none of them have been applied in clinical practice yet. A discussion of potential tools to help with the decision of extending endocrine therapy or not is given in **Supplementary Paper IV** ("*Patient-reported outcomes and genomic signatures: tools to tailor adjuvant endocrine treatment?*").

Regarding the treatment of patients with metastatic disease, there are now multiple options in terms of targeted therapies, to be used in conjugation with endocrine therapy. The most notable drug class is CDK4/6 inhibitors, which are now considered the best option for the first-line treatment of advanced breast cancer, when added to endocrine therapy.[60] Still, there are other targeted options available after first-line treatment, such as mTOR inhibitors (*e.g.* everolimus) or PI3K inhibitors (*e.g.* alpelisib). An ample comparison of these agents is provided in **Supplementary Paper V** ("*Endocrine therapy-based treatments in hormone receptor-positive/HER2-negative advanced breast cancer: systematic review and network meta-analysis*").

These targeted agents are now also being tested in patients with early-stage disease. The monarchE trial randomized patients with high-risk ER-positive/HER2-negative tumors to standard-of-care adjuvant endocrine therapy (at least five years) with or without abemaciclib (a CDK4/6 inhibitor) for two years; the recently presented results showed that the addition of abemaciclib improved invasive disease-free survival (IDFS), with two-year IDFS of 92.2% with placebo *vs* 88.7% with abemaciclib.[74] If this drug is approved for this indication, it may significantly increase the cost of treatment for this subgroup of patients. The total cost of two years of therapy with abemaciclib is nearly 100 000€/patient in Europe, only considering the cost of the drug and not taking into account all the other direct (*e.g.* laboratory tests, medical appointments) and indirect expenses (*e.g.* patient's loss of productivity, travel expenses) associated with the treatment – which raises concerns regarding its potential "financial toxicity".[43]

Furthermore, postmenopausal women with HR-positive early breast cancer may also benefit from adjuvant treatment with bisphosphonates, which were shown to decrease breast cancer recurrence and mortality.[75] A debate regarding the use of bisphosphonates and the newer bone-modifying agent denosumab is provided in **Supplementary Paper VI** ("*Denosumab in early-stage breast cancer*").

HER2-positive subtype

After the publication of trials including patients with metastatic disease,[76] and then of the landmark HERA trial,[77] the anti-HER2 antibody trastuzumab was established as an essential component of the treatment of HER2-positive breast cancer, being part of the World Health Organization (WHO) list of essential

medicines for cancer.[78] Since then, many other agents, including pertuzumab, neratinib, T-DM1, lapatinib, tucatinib and trastuzumab deruxtecan have been approved for the treatment of early and/or advanced HER2-positive disease.[14,79,80] Recently, the KATHERINE trial has shown that among patients with residual invasive disease following neoadjuvant treatment with chemotherapy and trastuzumab (with or without pertuzumab), the use of adjuvant T-DM1 resulted in an improvement in IDFS compared to standard trastuzumab treatment.[81] These results led to a definitive shift in the clinical algorithm for HER2-positive early disease, as it has now been endorsed by ESMO that HER2-positive tumors >2 cm and/or with clinically positive axillary lymph nodes (cT2-T4N_{any} or cT_{any}N1-3) should receive neoadjuvant chemotherapy plus anti-HER2 therapy, regardless of the feasibility of optimal surgery.[2] A detailed view on the benefits of neoadjuvant treatment for HER2-positive and TNBC is given in **Supplementary Paper VII** ("*Neoadjuvant treatment for intermediate/high-risk HER2-positive and triple-negative breast cancers: no longer an 'option' but an ethical obligation*").

HER2-positive breast cancer is also a diverse entity, including all the intrinsic tumor subtypes.[82,83] So far, the only biomarker used for the selection of HER2-targeted therapy is HER2 status itself. Nonetheless, ER status is emerging as a robust predictive marker within HER2-positive disease, which could be used for treatment tailoring. The data supporting the potential role of ER status on the prognosis and treatment of HER2-positive early disease are described in **Supplementary Paper VIII** ("*What Is the Real Impact of Estrogen Receptor Status on the Prognosis and Treatment of HER2-Positive Early Breast Cancer?"*).

Triple-negative breast cancer

For many years, the only effective therapy for the treatment of early or advanced TNBC was chemotherapy. Of note, this is the only subtype in which the use of platinum salts (*e.g.* carboplatin) is recommended, as it is effective in the metastatic setting and increases pathological complete response rates when given as a neoadjuvant treatment.[84] Recently, PARP inhibitors were approved for the treatment of metastatic disease in patients carrying germline *BRCA1* or *BRCA2* mutations.[14,85] Additionally, immunotherapy agents have shown efficacy in early and advanced TNBC.[64–66] Therefore, similar to the other subtypes, treatment costs of early TNBC may significantly escalate in the future due to the possible introduction of new immunotherapy drugs[65,66] and PARP inhibitors (being tested as an adjuvant therapy in the OlympiA trial [ClinicalTrials.gov identifier NCT02032823]).

3.2 Previous cancer history

Cancer patients have a greater risk of developing second primary cancers, which may be related with treatments for the first primary cancer, environmental exposures (*e.g.,* tobacco smoking, alcohol drinking)

or individual susceptibility (*e.g.*, age, sex, genetic background).[86] Therefore, as the number of cancer survivors increases worldwide, a higher number of individuals are diagnosed with subsequent primary cancers.[87] This increasing incidence of second primary cancers has been seen especially in the US and Australia, where standardized incidence ratios ranged from 1.14 for a first cancer diagnosis in the early 1980s to 1.21–1.46 in the late 1990s.[87] Moreover, in nine SEER registries of the US, multiple primary malignancies already constituted 19% of all cancer diagnoses between 2005 and 2009.[88] However, data regarding the incidence of second primary malignancies in developing countries is virtually absent, which reflects the lower number of cancer survivors in developing countries compared to high-income regions.

Regarding breast cancer in particular, not only are women with a first breast cancer at substantially greater risk of a contralateral breast cancer compared to the general population,[89] but survivors of other cancer sites, namely endometrial, ovarian, colon, thyroid and Hodgkin lymphoma, also have elevated risks of breast cancer as a subsequent malignancy.[90]

These patients diagnosed with a subsequent cancer pose greater challenges to healthcare professionals, as prior treatments and their toxicities must be taken into consideration when determining the therapeutic plan for that specific patient.[90] Nevertheless, there is a lack of information about the impact of a previous cancer diagnosis on the clinical management of patients with breast cancer and their use of healthcare resources.

3.3 HIV infection

Another factor that may contribute to breast cancer heterogeneity in terms of clinical management and prognosis between developed countries and Sub-Saharan countries is the high prevalence of HIV infection among women living in the latter. A recent report estimated the global burden of breast cancer among women living with HIV (WLWH) for the first time: worldwide, they constituted <1% of the breast cancer clinical burden.[91] However, in Middle, Western and Eastern Africa they comprised 4-6% of patients <50 years, and in Southern Africa they represented 26% of breast cancer patients diagnosed under the age of 50.[91] Similarly, reports from the US[92] and from Sub-Saharan Africa[93,94] show that WLWH with breast cancer tend to be younger than patients without HIV infection. Nonetheless, epidemiological data are conflicting regarding tumor stage at diagnosis: some series report that WLWH tend to present with later stage disease as compared to HIV-negative patients,[95,96] while others do not describe such a difference.[94,97] Likewise, data on the distribution of breast cancer subtypes between WLWH *vs* HIV-negative patients are still scarce. The same applies to mortality: while some reports show that WLWH with breast cancer have worse survival compared to HIV-negative patients, even after adjusting for stage and age,[94] other studies do not demonstrate a significant worse outcome among WLWH.[83] These apparent contradictions may be due to the fact that most series have a low number of HIV-positive breast cancer patients and/or follow-up time

and, therefore, may not have enough power to demonstrate differences. Another reason may be due to a low accessibility to diagnosis and treatment in some parts of the world, leading to a high proportion of late stage disease presentation and dismal long-term survival both in WLWH and HIV-negative patients. However, as the number of WLWH with breast cancer is increasing worldwide,[91] it is important to better understand the specific characteristics of their disease and, critically, to develop specific screening and treatment strategies.

4. Setting: Portugal and Mozambique

This thesis has been conducted in two different settings: in Portugal, a high-income country, and in Mozambique, a low-income country. Although the two countries present important differences in terms of demographics, availability of health resources and breast cancer burden, they also have multiple historical and current connections.

Mozambique was a colony of Portugal from the end of the XIX century until 1975, when it became an independent country. After independence, Mozambique adopted Portuguese as its official language.

Interestingly, the first Cancer Survey in a Portuguese territory was conducted in the city of Maputo (at that time, called "Lourenço Marques") between 1956 and 1961 by the Department of Pathology of the Maputo Central Hospital (known at the time as the "Miguel Bombarda Hospital").[98]

More than 50 years later, in 2014, the Maputo Central Hospital implemented a hospital-based cancer registry; it has the collaboration of the Institute of Public Health of the University of Porto (ISPUP) and received funding from the Calouste Gulbenkian Foundation, under the Project "*Atenção Integrada ao Doente Oncológico*", both from Portugal. The creation and first results of the registry are presented in **Supplementary Paper IX** ("*Cancer incidence in Mozambique in 2015– 2016: data from the Maputo Central Hospital Cancer Registry*"). Additionally, there is a population-based cancer registry that covers the city of Maputo, which predominantly includes cases reported by the cancer registry of the Maputo Central Hospital, and another in the city of Beira.[99]

In metropolitan Portugal, the first population-based cancer registry was created in 1976, followed by the creation of different Regional Cancer Registries, which covered most of the Portuguese territory for more than 40 years.[100] An unified National Cancer Registry, for the entire Portuguese territory, began operating in January 2018.[101]

In terms of breast cancer burden, in 2018, the age-standardized breast cancer incidence was estimated to be almost five-fold higher in Portugal (70.7/100 000 women) compared to Mozambique (14.5/100 000 women).[102] Breast cancer is the most incident cancer among women in Portugal, while it is the second

most incident cancer in Maputo City.[103] However, age-standardized mortality is much closer, being 11.3/100 000 women in Portugal *vs* 8.5/100 000 women in Mozambique.[102]

4.1 Demographics

The demographic characteristics of Portugal and Mozambique are quite distinct. Mozambique is in stage two of demographic transition, with a very high birth rate and a high, but decreasing death rate; while Portugal is in stage four, with a low birth rate and a low death rate – **Figure 2 and Table 1**.[104,105]

In Portugal, noncommunicable diseases are the main cause of death, with cardiovascular diseases accounting for an estimated 29% of deaths in 2018, while cancer was responsible for 24%.[106] In Mozambique, communicable, maternal, neonatal and nutritional diseases are still the main causes of death, with HIV/AIDS being the first cause, followed by neonatal disorders, tuberculosis and malaria.[107,108] Remarkably, the prevalence of HIV infection among adults aged 15-49 years in Mozambique is 13.2%,[109] while it is around 0.5% in Portugal.[110] Nonetheless, Mozambique is now undergoing a surge of non-communicable diseases, such as diabetes, cardiovascular disease and cancer.[111]

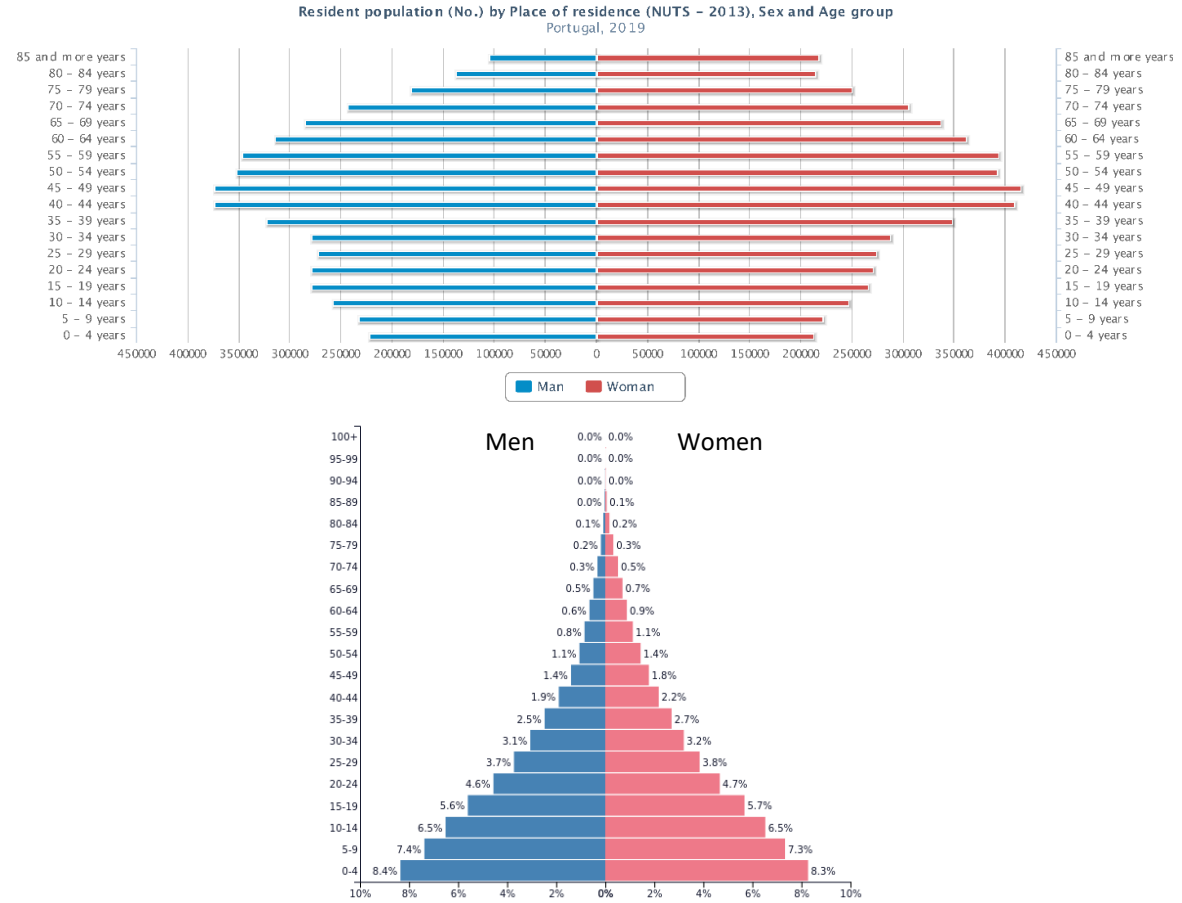


***Figure 2**: Demographic pyramids of Portugal (top panel) and Mozambique (bottom panel) – source: "Instituto Nacional de Estatística" website (Portugal) and 2017 Population Census (Mozambique)*[104,105]

Table 1: *Basic demographic data of Portugal and Mozambique*

	Portugal	Mozambique
Population	10 million	28 million
Median age of the population	45.2 years	17.6 years
Median life expectancy at birth	81.5 years	53.7 years
Fertility rate	1.31 births/woman	4.75 births/woman

Source: "Instituto Nacional de Estatística" website (Portugal) and 2017 Population Census (Mozambique)[104,105]

4.2 Health resources for cancer care

Both Portugal and Mozambique have a public health system, usually free-of-charge, which is the largest health care provider in each country. However, the proportion of the National State Budget allocated to healthcare is higher in Portugal compared to Mozambique – **Table 2**.

In terms of health resources for the diagnosis and treatment of cancer, there is an enormous disparity between the two countries. Mozambique published its first Cancer Control Plan in 2019 (for 2019-2029),[112] while in Portugal the National Program for Oncological Diseases exists since 1990 (with successive updates).[113] Portugal has mammography screening programs since 1986, which now cover most of the country.[114] There is no mammography-based organized screening in Mozambique, but national guidelines for early breast cancer detection based on breast self-exam and clinical exam were implemented in 2010.[115]

There are Pathology Departments in most of the public Portuguese Hospitals, with different levels of specialization, which led to the organization of a reference network in 2016.[116] Most Departments analyze cytological and histological specimens, retrieved from core biopsies or surgeries, and perform routine immunohistochemistry and ISH assessments. Breast cancer diagnosis is usually performed by a core needle biopsy, as recommended by ESMO.[2]

There are currently four Pathology Departments in Mozambique, in the Maputo, Beira, Nampula and Quelimane Central Hospitals; the latter started its activity recently, in 2017.[117] Given the very low accessibility/absence of core biopsies in all of these Hospitals, the diagnosis of breast cancer is usually performed by FNAC.[117] The Pathology Department of the Maputo Central Hospital is the national reference department, also being responsible for the training of all Mozambican pathologists. Moreover, it has a dedicated FNAC clinic since 1996, carrying out daily exams in multiple organs – as an example, 1357 breast FNACs were done between 2009 and 2010. FNAC is usually performed by the pathologist using palpation, but sampling of smaller and/or deeper breast lesions is guided by ultrasound, once per week, with the support of a radiologist. The Pathology Department of Maputo Central Hospital centralizes the performance of immunohistochemistry on histological samples.

In terms of delivery of treatment, there are three dedicated cancer centers in Portugal (in Porto, Coimbra and Lisbon), and most of the public hospitals and many private hospitals have Surgical and Oncology Departments as well. In Mozambique, oncological surgery is performed in the four largest Hospitals (Maputo, Beira, Nampula and Quelimane), but only Nampula and Maputo Central Hospitals provide systemic therapy in an Oncology Department.

Mozambique had a cobalt-based radiotherapy unit, which was shutdown in 1998; but it opened a new unit, with modern linear accelerators, in August 2019. In Portugal, there are several radiotherapy units – **Table 2**.

Table 2: *Health resources in Portugal and Mozambique*

	Portugal	**Mozambique**
Gross domestic product (GDP) per capita	23 145 US$ (in 2019)[118]	492 US$ (in 2019)[119]
Healthcare budget (as % of the National State Budget)	12.3% (in 2020)[120]	8.7% (in 2018)[121]
Number of medical doctors per 1000 inhabitants	5.1 (in 2017)[122]	0.1 (in 2018)[123]
Number of pathologists (total)	253 (in 2011)[124]	17 (in 2020)
Number of medical oncologists (total)	193 (in 2016)	5 (in 2020)
Number of radiotherapy units per 1 million inhabitants	4.3 (in 2018)[125]	0.07 (in 2020)

OBJECTIVES

Differences in the risk factors, clinical management and survival of patients with breast cancer according to tumor subtypes in Western countries have been well described in the literature.[14] However, despite some investigations that have addressed the allocation of health resources to some specific breast cancer patient subgroups,[126] there are scarce data concerning their use according to tumor subtypes. In the era of personalized medicine, in which subtypes are used to guide the decision of (neo)adjuvant and palliative treatment, it is essential to know the determinants of health resource use, as well as the cost of cancer care by subtype.[127]

At a larger plan, there is a tremendous heterogeneity in the use of health resources across different geographic and economic settings – as an example, in Sub-Saharan African countries, the availability of diagnostic means and treatments is much lower than in more developed countries.[12] This creates a huge gap between the needs of patients with breast cancer and their access to care in these countries. Nonetheless, very few studies have assessed the clinical management of breast cancer in Sub-Saharan Africa according to subtypes and only one tried to quantify subtype-specific survival, but with some methodological flaws (*i.e.* large loss to follow-up, incomplete determination of HER2 status).[46] At the same time, in both developed and developing countries, there are differences in access to care and type of treatment received according to past history of malignancy, HIV infection and other clinical characteristics, as well as according to the organization of care. However, there are few data about the influence of these factors on healthcare use and prognosis among patients with breast cancer.

Therefore, with this thesis we aimed to understand the impact of breast cancer heterogeneity on etiology, clinical management, health resource use and survival in different settings: in a high-income, developed country (Portugal) and in a low-income, developing country (Mozambique).

The specific objectives were:

- Regarding breast cancer subtypes:
    - To assess the relation between risk factors and the occurrence of breast cancer, overall and by each tumor subtype in women from Mozambique (***Paper I***);
    - To assess the distribution of breast cancer subtypes, and its impact on patients' treatment and survival in Mozambique (***Paper II***);
    - To quantify the relation between breast cancer subtypes and clinical management and health resource use among women with early breast cancer from Portugal (***Paper III***);

- To compare health resource use and the cost of care of women with non-metastatic breast cancer in a high-income (Portugal) *vs* a low-income country (Mozambique), overall and according to stage and subtype (***Paper IV***);

- To quantify the association between a previous cancer diagnosis and the clinical management, healthcare use and survival of women with early breast cancer from a high-income setting (***Paper V***);
- To evaluate the impact on survival and the cost-effectiveness of a multidisciplinary tumor board for breast cancer in a low-resource setting (***Paper VI***);
- To measure the impact of HIV infection in the clinical presentation and prognosis of women diagnosed with breast cancer from both high- and low-income countries (***Paper VII***).

METHODS

The objectives of this thesis were accomplished through:

1. The analysis of two prospective cohorts of patients with newly diagnosed breast cancer:

The prospective "**NEON-BC – Neuro-oncological complications of breast cancer**" cohort was set up with the global aim of understanding the burden of neurological complications of breast cancer treatment and their potential role as mediators of the impact of cancer treatment in different dimensions of the patients' quality-of-life in Northern Portugal.[128]

Women admitted to the Breast Clinic of the P Instituto Português de Oncologia do Porto Francisco Gentil (IPO-Porto), during 2012, were invited to participate if they were aged 18 years or older, and proposed for surgery, either as primary treatment or after neo-adjuvant chemotherapy and, if applicable, expected to receive cancer treatments other than surgery at IPO-Porto.

Most participants were included at diagnosis, in 2012 (n=506), and the others were invited to participate at the third and fifth year after diagnosis. In total, 746 women were enrolled in this cohort.

Participants were followed until the fifth year after diagnosis, at regular intervals. At each evaluation, sociodemographic, lifestyle and quality-of-life data were collected through face-to-face interviews using structured questionnaires, whereas clinical and tumor characteristics were retrieved from clinical records. Additionally, data regarding treatments and other healthcare use were obtained from administrative and clinical records.

The NEON-BC cohort study was approved by the Ethics Committee of IPO-Porto (ref. CES 406/011 and CES 99/014) and by the Portuguese Data Protection Authority (ref. 9469/2012 and 8601/2014). All participants provided written informed consent.

The prospective "**Moza-BC – Mozambican Breast Cancer**" cohort study included consecutive incident cases of breast cancer, with a pathological diagnosis performed in one of the three Central Hospitals of Mozambique (Maputo, Beira and Nampula), from January/2015 to August/2017.

Data on sociodemographic, clinico-pathological characteristics, treatment and survival of patients followed in the Oncology Unit of the Maputo Central Hospital were prospectively collected until July/2018. Survival data were updated through the Maputo Central Hospital Cancer Registry, health records and telephone interviews in November/2019.

The National Health Bioethical Committee of Mozambique approved this study (ref. 226/CNBS/15) and all participants provided written informed consent.

In both cohorts, women were divided according to their tumor's biomarkers into the classic subtypes (HR-positive/HER2-negative, HER2-positive and TNBC) and the surrogate intrinsic subtypes (luminal A-*like*, luminal B-*like*, HER2-enriched and basal-*like*). In the NEON-BC cohort, data on tumor biomarkers were retrieved from the patient's clinical files, as they are routinely assessed for every patient. In Mozambique, biomarker determination is often not performed in routine clinical practice, due to: i) lack of histological specimens, as many diagnoses are performed by cytological assessment (by FNAC); ii) frequent lack of reagents, impairing this determination in histological specimens. Therefore, in order to have this information for patients included in the Moza-BC cohort, we: i) tested for the first time in Mozambique the use of a cell block created from FNAC to perform the determination of biomarkers and ii) applied for a grant, in order to acquire the reagents needed for this assessment. Hence, tumor biomarker evaluation was available for most of the patients enrolled in the Moza-BC cohort.

The selection of participants of each cohort to be used in each analysis depended on the specific objectives of the investigations and is described in detail in the methods sections of the papers.

2. A case-control analysis with cases from Moza-BC and controls from the general population, who had been evaluated as part of the population-based "World Health Organization STEPwise approach to Surveillance Noncommunicable Disease Risk Factor Survey" (WHO-STEPS). This survey was conducted in Mozambique between 2014 and 2015, and assessed a representative sample of the Mozambican population aged 15-64 years (n=3119) for risk factors for noncommunicable diseases.

3. A systematic review and meta-analysis, also including data from the Moza-BC cohort. MEDLINE, Scopus, ISI Web of Knowledge, LILACS, SciELO and conference abstracts were searched up to 1st January 2020. Cross-sectional and cohort studies comparing baseline characteristics (stage and/or subtypes) and/or overall survival of WLWH *vs* HIV-negative women with breast cancer were included.

MANUSCRIPTS

Paper I: *Risk factors for breast cancer, overall and by tumor subtype, among women from Mozambique, Sub-Saharan Africa*

Risk Factors for Breast Cancer, Overall and by Tumor Subtype, among Women from Mozambique, Sub-Saharan Africa

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ABSTRACT

Background: Breast cancer incidence is rising in Africa, but there are scare data regarding risk factors in this region. We assessed the relation between risk factors and the occurrence of breast cancer, overall and by tumor subtype in women from Mozambique.

Methods: The associations between education, number of births, height, weight, body mass index (BMI), and breast cancer risk among 138 cases (participants from the Moza-BC cohort) and 638 controls from the general population (from a World Health Organization stepwise approach to surveillance survey), recruited during 2014 to 2017, were investigated. Adjusted ORs (aOR) and 95% confidence intervals (CI) were estimated using multivariable logistic regression.

Results: Multiparity (≥6 vs. 0–1 live births) was a protective factor for the development of hormone receptor (HR)–positive (aOR = 0.22; 95% CI, 0.08–0.64) and HR-positive/HER2-negative tumors (aOR = 0.20; 95% CI, 0.06–0.68), whereas a higher educational level (≥8 vs. 0 schooling years) increased breast cancer risk across all subtypes (overall aOR = 1.98; 95% CI, 1.04–3.80). Higher weight and BMI were associated with a higher breast cancer risk among postmenopausal women (per 1-kg increase: aOR = 1.05; 95% CI, 1.02–1.08; per 1-kg/m^2 increase: aOR = 1.11; 95% CI, 1.04–1.18, respectively), but were protective in premenopausal women (aOR = 0.98; 95% CI, 0.96–0.99; aOR = 0.95; 95% CI, 0.91–0.99, respectively), regardless of subtype. Higher height increased the risk of HR-negative tumors in postmenopause (per 10-cm increase: aOR = 2.81; 95% CI, 1.41–6.03).

Conclusion: These results demonstrate the etiological heterogeneity of breast cancer among native African women, namely regarding the differential effect of multiparity, education, and body parameters in breast cancer risk.

Impact: As the prevalence of obesity grows, these findings are important to inform public health policies on cancer prevention, by highlighting obesity as a modifiable risk factor for breast cancer among African women.

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Introduction

The understanding of risk factors for the development of breast cancer has evolved over the past two decades, along with the discovery of the different breast cancer subtypes (1, 2). The effect of reproductive behaviors (e.g., parity, age at first birth, breastfeeding) and age at menarche/menopause may vary among these subtypes, being more strongly associated with hormone receptor (HR)–positive tumors (3, 4). The effects of body parameters on breast cancer risk may be modified by menopausal status, with higher weight and body mass index (BMI) appearing to be associated with a lower risk among premenopausal women and with an increased risk after menopause (5). Yet, their effect across breast cancer subtypes is still debated.

There are important differences in the overall incidence of breast cancer between Western and Sub-Saharan African countries (age-standardized incidence rate of >80.0 vs. <50.0/100,000 women, respectively; ref. 6) and in the distribution of tumor subtypes [e.g., 10%–15% vs. >20% triple-negative breast cancer (TNBC), respectively; refs. 7, 8]. Therefore, as the incidence and mortality from breast cancer are rising in Africa (6, 9), understanding the risk factors for the overall development of breast cancer in this region is of particularly importance.

Compared with Western countries, African patients have a different expression of risk factors, such as higher parity, longer breastfeeding patterns, or a lower prevalence of obesity, which could partly explain these differences (10). Multiple Sub-Saharan African reports have shown that multiparity protects from breast cancer, overall, but without an assessment by subtype (10–13). Some studies demonstrated that a higher educational level increased breast cancer risk (11, 13),

whereas others suggested it to be a protective factor (12, 14). Data are also contradictory regarding body parameters: A report found that a higher BMI was a risk factor among postmenopausal women (14), whereas in others, it appeared to have no effect (15, 16). The specificities of Sub-Saharan African populations in terms of the distribution of potential risk factors and subtypes may allow for a better understanding of the etiology of each breast cancer subtype; however, this has never been explored, probably due to the difficulties in routinely determining breast cancer subtypes in this region (17). This case–control study aimed to assess the relation between factors, such as education, parity, BMI, height and weight, and the overall development of breast cancer as well as of each tumor subtype in African women from Mozambique.

Mozambique is a low-income country located in Southeast Sub-Saharan Africa, with a population of 30 million people (18) and a fertility rate close to five births/woman (19). Education is mandatory until the ninth grade, but even nowadays, only 45% of students complete the first four years of basic education (20). One in eight adults ages 15 to 49 years is infected by HIV (21), and noncommunicable diseases are also on the rise (22, 23). For instance, between 2005 and 2014/2015, the prevalence of overweight/obesity increased from 18.3% to 30.5% among adult women (24). Breast cancer is now the most common non-AIDS defining malignancy among Mozambican women, with an incidence rate of 8.6/100,000 women in Maputo City (9, 25). National guidelines for early breast cancer detection based on breast self-exam and clinical exam were implemented in 2010 (26), but there are no organized mammography screening programs, and women are usually diagnosed with advanced disease (stage III/IV; ref. 27).

Materials and Methods

This case–control analysis included patients with breast cancer recruited for the Moza-BC cohort and controls from the general population selected among the participants of the "World Health Organization stepwise approach to surveillance of noncommunicable disease risk factor" (WHO-STEPS) Survey.

Source of breast cancer cases: Moza-BC cohort

Cases were selected among women with breast cancer enrolled in the prospective Moza-BC cohort, previously described elsewhere (27). Briefly, it included a consecutive series of women with newly diagnosed primary breast cancer, with a pathologic diagnosis performed at one of the three Central Hospitals in Mozambique, from January 2015 to August 2017, which were the only hospitals with a Pathology Department at the time. Breast tissue samples were collected by fine-needle aspiration cytology (FNAC), surgical biopsy, breast surgery, or core-needle biopsy. Aspirated material from FNAC was used to create cell blocks, and histologic samples were handled according to the recommendations of the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP; ref. 28). Immunostaining of histologic samples and cell blocks for estrogen receptor (ER), progesterone receptor (PR), and HER2 was carried and assessed as described in the literature (28, 29). If the IHC score was 0 or 1+, the tumor was considered to be "HER2-negative"; if it was 3+, it was classified as "HER2-positive." In the case of an HER2 IHC score of 2+ (HER2-equivocal), samples were submitted to *in situ* hybridization.

Tumors were grouped according to their HR status as HR-positive (ER-positive or PR-positive, defined as an expression of ER or PR ≥ 1%; ref. 28) or HR-negative (ER-negative and PR-negative), regardless of the tumor's HER2 status; and then according to the classic tumor subtypes classified into HR-positive/HER2-negative, HER2-positive (HR-positive/HER2-positive and HR-negative/HER2-positive), and TNBC (HR-negative/HER2-negative).

Patients subsequently followed at the Maputo Central Hospital were interviewed regarding their place of residence, education, age of menarche/menopause and menopausal status, age at first live birth, number of live births, and family history of breast cancer. Study investigators assessed height and weight before the start of chemotherapy, to the nearest 0.1 kg or 0.1 cm, respectively, with participants in a standing position and wearing light clothing and no footwear.

Source of controls: WHO-STEPS survey

Controls were selected from among participants of the WHO-STEPS survey, conducted in Mozambique between December 2014 and February 2015, as previously described (23, 24, 30). This population-based survey assessed a representative sample of the Mozambican population ages 15 to 64 years ($n = 3119$) for risk factors for noncommunicable diseases. Participants were evaluated using WHO-STEPS–standardized methods. Data regarding place of residence, age, education, menopausal status, and number of live births were collected by face-to-face interviews. Anthropometric measurements were obtained by investigators, with the participants wearing light clothing and no footwear. Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm in the standing position.

Case–control design and statistical analysis

Sociodemographic and clinical characteristics of the participants with breast cancer were described for a total of 205 cases. From these, 138 were cases younger than 65 years (the WHO-STEPS survey only included potential controls younger than 65 years) who were dwellers in Maputo (City or Province) or in the south and center provinces closest to Maputo that had at least five cases (Gaza, Inhambane, and Sofala), and thus were included in the main case–control analyses. A total of 118 of these cases also had information on the breast cancer subtype and were therefore considered for the analyses by subtype.

A total of 638 participants in the WHO-STEPS survey were selected as controls, corresponding to all nonpregnant women who lived in the same provinces of the cases considered for the case–control analyses (**Fig. 1**).

Education, number of live births, height, weight, and BMI were selected as the potential risk factors to be analyzed in this study, given their availability in both the Moza-BC cohort and the WHO-STEPS survey. Height and weight were used to compute BMI, and participants were classified as obese if their BMI was ≥30 kg/m^2. Cases were compared across tumor subtype subgroups, namely HR status and classic subtypes, using ANOVA for continuous variables, and χ^2 or Fisher exact tests for categorical variables. Case–control analyses were conducted for all cases and according to subtype. Adjusted ORs (aOR) and corresponding 95% confidence intervals (CI) were estimated using multivariable logistic regression. Interactions were tested by including the interaction terms between menopausal status and body parameters in the logistic regression models, and stratum-specific estimates were obtained through the linear combination of coefficients. All tests were two-sided, and a P value of <0.05 was considered significant. Analyses were carried out using STATA version 15 (StataCorp). This study is reported according to the STROBE statement (31).

Ethics

The National Health Bioethical Committee of Mozambique approved the Moza-BC cohort study (226/CNBS/15) and the WHO-STEPS survey (98/CNBS/14). All participants provided written

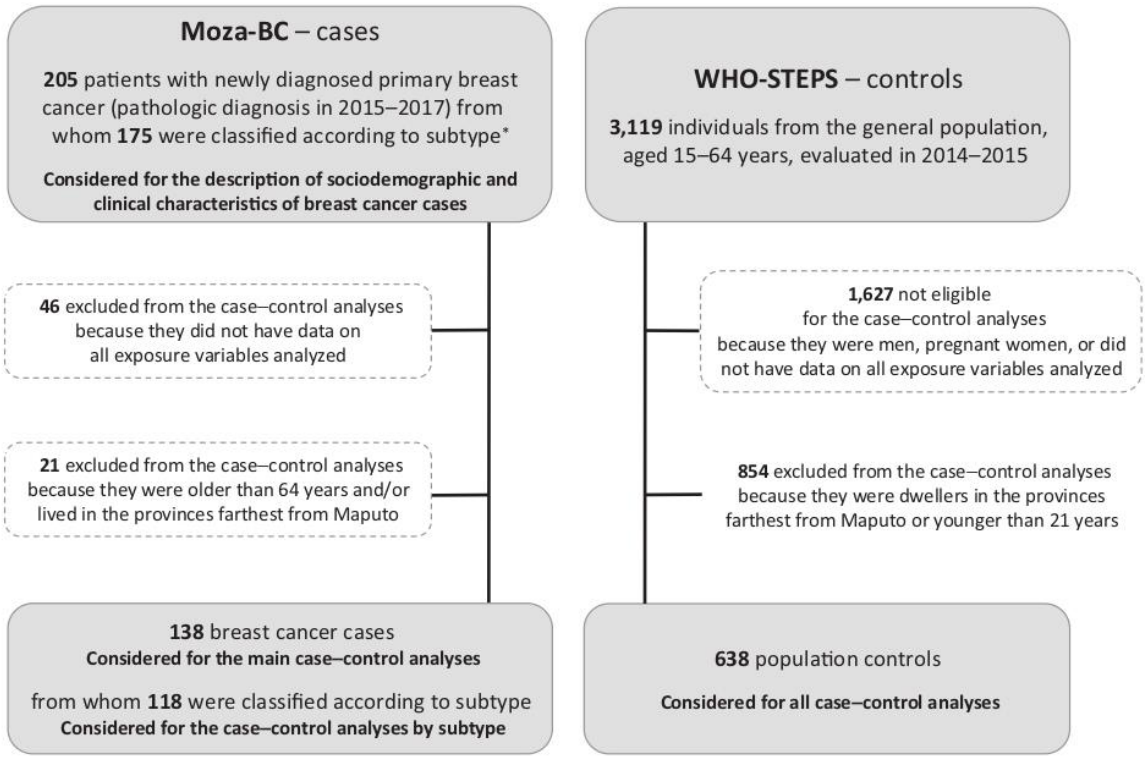


Figure 1.

Flowchart of patients selected for the current case–control study, from the Moza-BC cohort and the WHO-STEPS survey. * Biomarker determination was unknown due to: (i) carcinoma *in situ* or pathologic complete response (i.e., no residual invasive tumor in the surgical specimen): $n = 8$ patients; (ii) inadequate tissue fixation: $n = 2$ patients; and (iii) unavailable samples (i.e., there were no cell blocks from fine-needle aspiration biopsies, core-needle biopsies, or surgical specimens available to perform subtype determination): $n = 20$ patients. This last group included 13 patients diagnosed by a fine-needle aspiration biopsy, but for whom a cell block was not performed due to logistical reasons; and 7 patients for whom a cell block was performed, but there was insufficient material (<100 cells) to perform biomarker determination.

informed consent, and the study was conducted according to the Declaration of Helsinki recommendations.

Results

Cases

Overall, 64% of cases had HR-positive tumors; regarding classic subtypes, 52% had HR-positive/HER2-negative tumors, 25% HER2-positive, and 23% TNBC. Women with breast cancer had a mean age of 49.3 years (range, 21–87) and were mostly premenopausal (53.7%), parous (93.6%), black (98.0%), and dwellers in the South of the country (90.7%). Approximately a third of the cases were obese, and about a quarter had no formal education (**Table 1**).

Risk factors for breast cancer, overall

Women with 8 or more years of education had a higher risk of developing breast cancer compared with women without formal education (aOR 1.98; 95% CI, 1.04–3.80; **Table 2**). Women reporting six or more live births were less likely to develop breast cancer (aOR 0.41; 95% CI, 0.18–0.91) compared with women with none or one live births—who were combined to define the reference category, given the very low number of nulliparous women.

Higher weight and BMI were associated with an increased risk of breast cancer among postmenopausal women, whereas they were protective among premenopausal women (P for interaction < 0.001). When analyzing height separately from BMI, there was an 87% overall increase in the risk of breast cancer for each 10-cm increase in height among postmenopausal women (aOR 1.87; 95% CI, 1.13–3.10).

Risk factors for breast cancer, by subtype

Higher education (≥8 years) was a risk factor for breast cancer across all tumor subtypes, but this association was stronger for TNBC (**Table 3**). Higher parity was a protective factor, but only for HR-positive tumors overall (≥6 vs. 0–1 live births: aOR 0.22; 95% CI, 0.08–0.64; P for trend = 0.003) and, more specifically, for HR-positive/HER2-negative tumors (aOR 0.20; 95% CI, 0.06–0.68; P for trend = 0.007); whereas no association with the development of HR-negative tumors overall (aOR 0.91; 95% CI, 0.24–3.51; P for trend = 0.543) or TNBC (aOR 0.58; 95% CI, 0.12–2.79; P for trend = 0.287) was observed. Among postmenopausal women, the influence of height was stronger for HR-negative tumors (aOR 2.81 per 10-cm increase; 95% CI, 1.41–6.03). The risk of breast cancer increased with higher weight and BMI among postmenopausal women in all subtypes. Their protective effect was apparent in premenopausal women in all subtypes, with a significant association being observed for HR-positive tumors and the HR-positive/HER2-negative subtype.

Table 1. Sociodemographic and clinicopathologic characteristics of the cases with breast cancer ($n = 205$), overall and according to subtypes ($n = 175$).

		HR status		Classic tumor subtypes		
	All cases ($n = 205$) *n* (%)[a]	HR^- ($n = 63$) *n* (%)[a]	HR^+ ($n = 112$) *n* (%)[a]	$HR^+/HER2^-$ ($n = 91$) *n* (%)[a]	$HER2^+$ ($n = 44$) *n* (%)[a]	TNBC ($n = 40$) *n* (%)[a]
Age (years)						
21–39	54 (26.3)	16 (25.4)	28 (25.0)	21 (23.1)	12 (27.3)	11 (27.5)
40–49	57 (27.8)	15 (23.8)	34 (30.4)	28 (30.8)	11 (25.0)	10 (25.0)
50–64	66 (32.2)	25 (39.7)	35 (32.2)	28 (30.8)	17 (38.6)	15 (37.5)
65–87	28 (13.7)	7 (11.1)	15 (13.4)	14 (15.4)	4 (9.1)	4 (10.0)
Race (black)	201 (98.0)	201 (100)	109 (97.3)	89 (97.8)	43 (97.7)	40 (100)
Place of residence						
Maputo (city and province)	153 (74.6)	51 (81.0)	80 (71.4)	68 (74.7)	30 (68.2)	33 (82.5)
Provinces closest to Maputo[b]	33 (16.1)	6 (9.5)	23 (20.5)	15 (16.5)	10 (22.7)	4 (10.0)
Provinces farthest from Maputo[c]	19 (9.3)	6 (9.5)	9 (8.0)	8 (8.8)	4 (9.1)	3 (7.5)
Education, complete school years						
0	41 (23.7)	8 (14.6)	24 (26.4)	20 (27.8)	7 (18.0)	5 (14.3)
1–7	65 (37.6)	23 (41.8)	29 (31.9)	24 (33.3)	11 (28.2)	17 (48.6)
≥8	67 (38.7)	24 (43.6)	38 (41.8)	28 (38.9)	21 (53.8)	13 (37.1)
Missing	32	8	21	19	5	5
Family history of breast cancer (yes)	8 (3.9)	1 (1.6)	5 (4.5)	4 (4.4)	2 (4.5)	0
Number of live births						
0	13 (6.4)	2 (3.3)	7 (6.3)	7 (7.7)	1 (2.4)	1 (2.6)
1	16 (7.9)	3 (4.9)	11 (9.9)	8 (8.8)	3 (7.1)	3 (7.7)
2–3	73 (36.1)	23 (37.7)	45 (40.5)	35 (38.5)	19 (45.2)	14 (35.9)
4–5	53 (26.2)	14 (23.0)	27 (24.3)	24 (26.4)	8 (19.0)	9 (23.1)
≥6	47 (23.3)	19 (31.2)	21 (18.9)	17 (18.7)	11 (26.2)	12 (30.8)
Missing	3	2	1	0	2	1
Age at first live birth, years[d]	21.5 (4.6)	21.1 (4.7)	21.8 (4.6)	21.8 (4.9)	21.2 (3.5)	21.5 (5.2)
Not applicable	13	2	7	7	1	1
Missing	42	14	17	13	10	8
Age at menarche, years[d]	15.1 (2.2)	14.8 (2.1)	15.1 (2.3)	14.9 (2.1)	14.9 (2.8)	15.3 (1.7)
Missing	53	18	29	25	10	12
Menopause (yes)	95 (46.3)	32 (50.8)	52 (46.4)	44 (48.4)	20 (45.4)	20 (50.0)
Age at menopause, years[d]	49.3 (4.5)	48.7 (5.0)	49.9 (4.1)	49.8 (4.4)	49.9 (4.2)	48.1 (5.0)
Not applicable	110	31	60	47	24	20
Missing	22	6	13	12	3	4
Weight, kg[d]	70.1 (17.7)	73.8 (16.1)	69.4 (19.0)	68.3 (18.2)	74.0 (19.1)	65.5 (14.7)
Missing	17	6	9	7	5	3
Height, cm[d]	158.0 (13.8)	158.4 (10.0)	158.6 (6.5)	158.0 (6.6)	161.0 (7.0)	157.3 (10.7)
Missing	16	5	9	7	4	3
BMI, kg/m^2 [d]	27.8 (6.8)	29.5 (6.5)	27.5 (7.1)	27.3 (6.9)	28.5 (7.1)	29.9 (6.8)
Missing	17	6	9	7	5	3
Stage at diagnosis						
0–II[e]	53 (25.9)	22 (34.9)	22 (19.6)	15 (16.5)	16 (36.4)	13 (32.5)
III	110 (53.7)	30 (47.6)	69 (61.6)	58 (63.7)	23 (52.3)	18 (45.0)
IV	42 (20.5)	11 (17.5)	21 (18.8)	18 (19.8)	5 (11.4)	9 (22.5)

[a]*N* (%), except if otherwise specified.
[b]Includes the south and center provinces of Inhambane, Gaza, and Sofala.
[c]Includes Manica, Tete, Zambézia, Nampula, Niassa, and Cabo Delgado.
[d]Mean (SD).
[e]Among cases within the category 0–II stage, one patient had stage 0 and four patients had stage I.

Discussion

With this case–control study, we showed for the first time that multiparity is a protective factor for the development of HR-positive and HR-positive/HER2-negative tumors among native Sub-Saharan African women. A higher educational level was associated with an increased risk of breast cancer across all tumor subtypes, whereas being taller was associated with HR-negative tumors among postmenopausal women. In addition, higher weight and BMI were risk factors among postmenopausal women regardless of the subtype, whereas they were protective for premenopausal women.

Education is a proxy for socioeconomic status and is usually related to reproductive behaviors (e.g., delayed age at first childbirth, reduced parity; ref. 32) and access to healthcare (e.g., cancer screening; ref. 33). Multiple studies, including from Africa, have shown a positive association between a higher educational level and breast cancer risk (11, 13, 32, 34), even after adjustment for parity or age at first birth. However, none of them evaluated this association by subtype. In

Table 2. Case–control analysis including cases with complete information for the variables analyzed, including cases ($n = 138$) and general population controls ($n = 638$).

	Controls ($n = 638$) n (%)[a]	Cases ($n = 138$) n (%)[a]	Adjusted OR	(95% CI)	P[b]
Age (years)[c]					
21–39	343 (53.8)	41 (29.7)	1	(ref.)	NA
40–49	126 (19.8)	46 (33.3)	3.49	(2.12–5.72)	
50–64	169 (26.5)	51 (37.0)	2.83	(1.76–4.54)	
Education (complete school years)[d]					
0	164 (25.7)	28 (20.3)	1	(ref.)	NA
1–7	305 (47.8)	54 (39.1)	1.04	(0.58–1.85)	
≥8	169 (26.5)	56 (40.6)	1.98	(1.04–3.80)	
P for trend			*0.008*		
Parity[e]					
Nulliparous (0 live births)	50 (7.8)	10 (7.2)	1	(ref.)	NA
Parous (≥1 live births)	588 (92.2)	128 (92.8)	0.68	(0.30–1.51)	
Number of live births[e]					
0–1	128 (20.1)	22 (15.9)	1	(ref.)	NA
2–3	207 (32.4)	55 (39.9)	0.90	(0.48–1.70)	
4–5	158 (24.8)	36 (26.1)	0.66	(0.32–1.35)	
≥6	145 (22.7)	25 (18.1)	0.41	(0.18–0.91)	
P for trend			*0.015*		
Height (per 10-cm increase)[f]—overall[g]	159.0 (7.4)	159.5 (7.0)	1.33	(0.99–1.79)	NA
Among premenopausal women	158.4 (7.4)	159.5 (6.8)	1.11	(0.76–1.60)	0.101
Among postmenopausal women	157.1 (7.4)	159.6 (7.5)	1.87	(1.13–3.10)	
Weight (per 1-kg increase)[f]—overall[h]	64.6 (15.4)	71.9 (18.2)	1.00	(0.99–1.02)	NA
Among premenopausal women	64.4 (15.5)	67.9 (17.8)	0.98	(0.96–0.99)	<0.001
Among postmenopausal women	65.0 (15.0)	78.5 (17.2)	1.05	(1.02–1.08)	
BMI (per 1-kg/m^2 increase)[f]—overall[g]	25.8 (5.8)	28.2 (6.8)	1.00	(0.97–1.03)	NA
Among premenopausal women	25.7 (5.9)	26.6 (6.4)	0.95	(0.91–0.99)	<0.001
Among postmenopausal women	26.3 (5.6)	30.0 (6.7)	1.11	(1.04–1.18)	
Obesity (BMI ≥ 30 kg/m^2)—overall[g]	137 (21.5)	51 (37.0)	1.00	(0.63–1.61)	NA
Among premenopausal women	89 (18.6)	25 (29.1)	0.69	(0.37–1.28)	0.055
Among postmenopausal women	48 (30.0)	26 (50.0)	1.73	(0.84–3.60)	

Abbreviation: NA, not applicable.
[a]*N* (%), except if otherwise specified.
[b]*P* for interaction.
[c]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala).
[d]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), and number of live births (0–1, 2–3, 4–5, and ≥6 years).
[e]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), education (0, 1–5, 6–7, 8–10, 11–12, and ≥13 years), BMI (continuous), menopausal status (no/yes), and interaction term BMI*menopausal status.
[h]Mean (SD).
[g]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), number of live births (0–1, 2–3, 4–5, and ≥6 years), and education (0, 1–5, 6–7, 8–10, 11–12, and ≥13 years).
[h]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), number of live births (0–1, 2–3, 4–5, and ≥6), education (0, 1–5, 6–7, 8–10, 11–12, and ≥13 years), and height (continuous).

Western countries, there are some case–case studies assessing the risk of each tumor subtype according to region/neighborhood socioeconomic status. One large study observed higher incidence rates of HR-positive breast cancer among white and black women residing in areas with higher versus lower socioeconomic status, but not of HR-negative tumors (35). Yet, as individual-participant data were not available, these estimates were not adjusted for parity or breastfeeding. Our case–control study, with individual-subject data of native African women from all socioeconomic strata, shows that a higher educational level was a risk factor for all breast cancer subtypes, even following adjustment, and especially for HR-negative tumors and TNBC, which was intriguing. We would expect this effect to be more evident for HR-positive tumors, as they are more biologically indolent and frequently diagnosed by mammographic opportunistic screening, which is more accessible to highly educated women in this low-resource setting. However, it can also be the case that there is an underdiagnosis of breast cancer among women with a lower educational level, who may be treated by traditional healers and/or cannot reach the health system and thus were not included in this study. This may be especially true for women with HR-negative tumors and particularly for those with TNBC, who frequently have faster and more aggressive clinical courses.

Previous African studies have found that multiparity protects from breast cancer (10–13). Nonetheless, we have observed, for the first time in African women, that the protection given by multiparity is restricted to HR-positive tumors. A large meta-analysis also demonstrated that the protective effect of parity (parous vs. nulliparous) was limited to HR-positive tumors (3) and had no effect on the development of HER2-positive/HR-negative or TNBC. Yet, some studies have suggested that parous women who have never breastfed have a higher risk of developing HR-negative or TNBC compared with nulliparous women (36–38). The mammary gland reaches its full development

Table 3. Case–control analysis including cases of different subtypes with complete information for the variables analyzed ($n = 118$) and general population controls ($n = 638$).

	HR status				Classic tumor subtypes					
	HR-negative (46 cases)		HR-positive (72 cases)		$HR^+/HER2^-$ (55 cases)		$HER2^+$ (32 cases)		TNBC (31 cases)	
	Adjusted OR (95% CI)	P[a]	Adjusted OR (95% CI)	P[a]	Adjusted OR (95% CI)	P[a]	Adjusted OR (95% CI)	P[a]	Adjusted OR (95% CI)	P[a]
Age (years)[b]										
21–39	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA
40–49	3.14 (1.36–7.26)		3.80 (2.03–7.08)		4.09 (2.02–8.28)		3.39 (1.27–9.08)		3.05 (1.18–7.88)	
50–64	3.72 (1.78–7.77)		2.58 (1.37–4.84)		2.59 (1.26–5.30)		4.10 (1.69–9.96)		2.78 (1.16–6.70)	
Education (complete school years)[c]										
0	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA
1–7	1.83 (0.66–5.15)		0.81 (0.38–1.71)		0.75 (0.32–1.72)		0.93 (0.31–2.83)		2.96 (0.74–11.85)	
≥8	4.16 (1.34–12.91)		1.82 (0.79–4.18)		1.48 (0.58–3.78)		3.19 (0.98–10.39)		5.16 (1.17–22.70)	
P for trend	*0.007*		*0.032*		*0.154*		*0.009*		*0.037*	
Parity[d]										
Nulliparous (0 live births)	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA
Parous (≥1 live births)	1.03 (0.22–4.87)		0.65 (0.23–1.88)		0.49 (0.16–1.47)		1.33 (0.16–11.18)		1.70 (0.21–13.93)	
Number of live births[d]										
0–1	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.)	NA	1 (ref.]	NA
2–3	1.44 (0.47–4.37)		0.75 (0.35–1.63)		0.67 (0.28–1.63)		1.38 (0.40–0.74)		1.26 (0.36–4.42)	
4–5	0.76 (0.21–2.80)		0.46 (0.19–1.13)		0.46 (0.17–1.27)		0.69 (0.16–2.97)		0.64 (0.14–2.84)	
≥6	0.91 (0.24–3.51)		0.22 (0.08–0.64)		0.20 (0.06–0.68)		0.81 (0.17–3.79)		0.58 (0.12–2.79)	
P for trend	*0.543*		*0.003*		*0.007*		*0.441*		*0.287*	
Height (per 10-cm increase)—overall[e]	1.43 (0.90–2.26)	NA	1.15 (0.79–1.68)	NA	1.03 (0.67–1.58)	NA	1.86 (1.07–3.23)	NA	1.30 (0.74–2.28)	NA
Among premenopausal women	0.88 (0.48–1.63)	0.021	1.00 (0.62–1.60)	0.287	0.90 (0.53–1.52)	0.328	1.35 (0.67–2.72)	0.158	0.83 (0.41–1.70)	0.047
Among postmenopausal women	2.81 (1.41–6.03)		1.54 (0.80–2.95)		1.39 (0.68–2.83)		3.18 (1.22–8.25)		2.83 (1.09–7.36)	
Weight (per 1-kg increase)—overall[f]	1.01 (0.99–1.03)	NA	1.00 (0.98–1.02)	NA	0.99 (0.97–1.01)	NA	1.02 (0.99–1.04)	NA	1.01 (0.98–1.03)	NA
Among premenopausal women	0.99 (0.96–1.01)	0.004	0.97 (0.95–0.99)	<0.001	0.96 (0.94–0.99)	<0.001	1.00 (0.97–1.03)	0.013	0.98 (0.95–1.02)	0.003
Among postmenopausal women	1.06 (1.02–1.10)		1.05 (1.02–1.09)		1.05 (1.01–1.08)		1.06 (1.02–1.11)		1.07 (1.02–1.13)	
BMI (per 1-kg/m^2 increase)—overall[e]	1.02 (0.96–1.07)	NA	1.00 (0.95–1.04)	NA	0.98 (0.93–1.02)	NA	1.03 (0.97–1.09)	NA	1.02 (0.96–1.08)	NA
Among premenopausal women	0.98 (0.91–1.04)	0.045	0.93 (0.88–0.99)	<0.001	0.90 (0.85–0.97)	<0.001	0.99 (0.92–1.07)	0.061	0.97 (0.90–1.05)	0.019
Among postmenopausal women	1.10 (0.99–1.21)		1.14 (1.05–1.23)		1.12 (1.03–1.22)		1.12 (1.01–1.25)		1.14 (1.02–1.29)	
Obesity (BMI ≥ 30 kg/m^2)—overall[e]	1.10 (0.54–2.24)	NA	0.86 (0.47–1.59)	NA	0.82 (0.41–1.61)	NA	0.69 (0.28–1.68)	NA	1.57 (0.67–3.65)	NA
Among premenopausal women	0.78 (0.28–2.11)	0.315	0.44 (0.19–1.03)	0.012	0.34 (0.12–0.90)	0.005	0.67 (0.20–2.21)	0.914	0.90 (0.28–2.83)	0.142
Among postmenopausal women	1.61 (0.57–4.57)		2.27 (0.87–5.97)		2.74 (0.92–8.18)		0.74 (0.20–2.74)		3.36 (0.87–13.01)	

Abbreviation: NA, not applicable.

[a]*P* for interaction.

[b]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala).

[c]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), and number of live births (0–1, 2–3, 4–5, and ≥6).

[d]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), education (0, 1–5, 6–7, 8–10, 11–12, and ≥13 years), BMI (continuous), menopausal status (no/yes), and interaction term BMI*menopausal status.

[e]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), number of live births (0–1, 2–3, 4–5, and ≥6), and education (0, 1–5, 6–7, 8–10, 11–12, and ≥13 years).

[f]OR adjusted for province (Maputo City/Province, Gaza, Inhambane, Sofala), age (continuous), number of live births (0–1, 2–3, 4–5, and ≥6), education (0, 1–5, 6–7, 8–10, 11–12, and ≥13 years), and height (continuous).

during pregnancy and lactation, and after its cessation, the breast undergoes involution (39). A lower degree of terminal duct lobular units involution has been associated with higher breast cancer risk (40), especially of basal-like breast cancers (41). Thus, it has been proposed that long-term breastfeeding could reduce the risk of TNBC/basal-like breast cancers by promoting involution. However, this has yet to be proven. Although we did not collect data on breastfeeding, this is a common practice in Mozambique: most children are still breastfed at 1 year of age (94% in 2003, 86% in 2013; ref. 42), and the mean duration of breastfeeding ranges between 11 and 17 months (43). Moreover, given the high fertility rate, the lifetime duration of lactation among our participants should be high. This generalized breastfeeding adherence may explain why, in our study, there was no association between multiparity and HR-negative tumors nor with TNBC.

The prevalence of obesity is increasing in most Sub-Saharan African countries (44), currently being around 10% among adult Mozambican women (24). However, most studies assessing the impact of high BMI on breast cancer risk have been performed among white women in the United States or Europe, and show that obesity is a risk factor among postmenopausal women, whereas it is protective among premenopausal women (5, 45, 46). This applies mostly to HR-positive tumors, as its effect in HR-negative or TNBC is less well-defined (47). However, data in Africa are scarce and without information on tumor subtypes. One large study from Ghana indicated that a "heavier" body size (classified by pictograms) conferred a higher risk of breast cancer (aOR 1.50), but without stratification for menopausal status (11). In a Nigerian study, higher BMI had a protective effect among premenopausal women (per 5-kg/m^2 increase: aOR 0.89), whereas it appeared to have no effect in postmenopausal women (16). However, they showed that a higher waist–hip ratio was a risk factor both in pre- and postmenopausal women (highest vs. lowest quartile category: aOR 2.15; ref. 48). Interestingly, this effect persisted in normal-weight women (BMI < 25 mg/kg^2), indicating that increased central adiposity was also a risk factor *per se* (48). These findings somehow differ from the large AMBER Consortium report, which included over 3,000 Afro-American women (49). Among postmenopausal women, obesity was a risk factor for HR-positive tumors, but it was protective for TNBC (aOR 0.60). However, women with central obesity have been found to have a higher risk of TNBC, which may be related to components of metabolic syndrome (e.g., insulin resistance) that are related with carcinogenesis (50). Curiously, BMI appeared to have no effect on the risk of breast cancer among premenopausal women, regardless of the subtype.

Nevertheless, these somehow contradictory findings are not so surprising. Although BMI is a standardized body measurement, it does not capture other aspects of body composition and metabolism, such as the proportion of central/subcutaneous adiposity, muscle mass, and insulin sensitivity (51, 52). Afro-American women tend to have more lean mass and lower fat mass than non-Hispanic white women with a similar BMI do (53), but tend to be more insulin-resistant (54). In addition, Africa is a vast region, with large genetic, cultural, and socioeconomic diversity (55); therefore, we should be cautious when generalizing findings from Afro-American women to the African context or even across African regions. Similarly, the African women lifestyle may be very different from Afro-American women, namely in terms of diet, physical activity, and reproductive behaviors. Our finding that higher weight and BMI were risk factors for both HR-positive and HR-negative tumors (including TNBC) among postmenopausal women, whereas these were protective in premenopausal women, should thus be further analyzed. It would be interesting to integrate data on waist–hip ratio, diet, alcohol consumption (56), and physical activity (57) to explore this relationship in future studies.

Most studies assessing the association between height and breast cancer risk were conducted in Western countries, where attained height is not conditioned by nutritional status during childhood. Currently, the prevalence of stunting in Mozambique is still around 43% among children under 5 years of age (58). Therefore, it is likely that many of our participants had an insufficient energy intake during childhood/adolescence, which may explain their shorter stature compared with those in developed countries (59, 60). Still, we have seen that higher height is a risk factor for HR-negative tumors in postmenopausal women, even after adjusting for education (a proxy for socioeconomic status), age, and number of live births. Data from Nigeria mirrored this finding, but without information by subtype (16). However, in the US-based Black Women's Health Study, being tall was a risk factor for breast cancer, but for HR-positive tumors among Afro-American women, most of whom were premenopausal (59). The hypothesis that a higher attained height is associated with increased sex hormones and insulin-like growth factors during the pubertal growth spurt, a period in which the breast is also developing (60, 61), would be more in line with a higher risk for HR-positive tumors. Yet our findings go in the opposite direction, deserving further clarification in future studies.

This study has several strengths. Both the WHO-STEPS survey and the Moza-BC cohort collected data on weight/height that was measured by the investigators, instead of self-reported (11). The classification of tumor subtypes was done prospectively and according to the ASCO/CAP guidelines (28, 29), allowing a reliable assessment of the HR and HER2 status, which was not the case in some previous reports (e.g., that did not use *in situ* hybridization to determine HER2 status; refs. 62, 63). Moreover, the controls were selected from a population-based study, unlike other African studies that used hospital-based controls (14). In addition, we analyzed an ethnically homogeneous population, as almost all participants were black (98%, among both cases and controls), African women born in Mozambique.

Nevertheless, besides the limitations already discussed, others should be mentioned. Although we analyzed the effect of parity, we did not have data on age at first child, age at menarche, or age at menopause among controls, which impaired its analysis. Likewise, we have no information on breast cancer family history among the controls, but as the proportion of cases with a positive family history was very low (4%), this may be of little relevance within this population. Around 25% of the cases were HIV-positive (27), but this information was not available for controls. Yet, although some U.S. studies have reported a lower incidence of breast cancer among women with HIV infection compared with the general population (64), African studies show no effect of HIV on breast cancer risk (65, 66).

Three quarters of the cases had advanced breast cancer (stage III/IV) at the time of diagnosis, which might raise concerns regarding the reliability of their weight at diagnosis as a proxy to their usual weight. Nevertheless, in the Moza-BC cohort, the majority of women were overweight or obese at enrolment (23% and 36%, respectively), and only 4% of cases were underweight. Furthermore, unlike what is seen in head and neck cancer, where patients frequently present with critical weight loss at the time of cancer diagnosis, this is rarely seen among women with breast cancer, even among those with advanced disease (67). Hence, we expect our weight and BMI measurements to be a reliable proxy to the patient's usual body parameters.

Our sample size is much smaller compared with larger studies from the United States and other developed countries. However, we could detect significant effects for many of the tested factors and also when

stratifying the analysis by menopausal status or subtype. To do so, we combined data from two studies, conducted during different time periods, but as these were close (2014–2015 vs. 2015–2017), we do not expect differences in the distribution of risk factors between cases and controls to be due to the timing of data collection. We did not have information regarding previous cancer history among controls, thus we could not exclude women with a previous diagnosis of breast cancer; yet, the likelihood of having controls with a previous breast cancer is very low, thus it should not influence the validity of our estimates. As the cases were followed at the Maputo Central Hospital, most of them lived in the South of the country, which partially impairs the generalization of these findings to the entire country.

As this is the first study exploring the risk effects of different factors according to breast cancer subtypes among native African women, it has implications for the management of breast cancer in Sub-Saharan Africa. We confirm that multiparity protects from HR-positive breast cancer, but unlike what was suggested by other studies, for HR-negative tumors, the point estimates suggest a much weaker association, and there was no evidence of a linear trend. Still, an absence of effect in HR-negative tumors could be explained by the high adherence to breastfeeding among Mozambican women, which reinforces the need to promote this behavior—especially since the proportion of women with HR-negative and TNBC is already high in Mozambique (33% and 25%, respectively; ref. 27).

We have also confirmed that higher weight and BMI are risk factors for breast cancer among postmenopausal women, regardless of the subtype. As the prevalences of overweight/obesity are rapidly rising throughout Africa (44), this may contribute to an increasing breast cancer incidence. Besides, obesity seems to be associated with worse prognosis after the diagnosis of breast cancer regardless of menopausal status (68–70), making its control crucial. Therefore, it is essential that awareness campaigns be conducted in this setting to promote healthier diets and lifestyles.

In conclusion, these results highlight the etiological heterogeneity of breast cancer among native African women, namely in terms of the differential effect of multiparity, education, and body parameters in breast cancer risk. As the prevalence of obesity is increasing in Africa, these findings are important to inform public health policies regarding cancer prevention, by highlighting that obesity is a modifiable risk factor for breast cancer among African women.

Authors' Disclosures

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Ethics Committee Approval

The Moza-BC cohort study (reference 226/CNBS/15) and the WHO-STEPS Survey (reference 98/CNBS/14) were approved by the National Health Bioethical Committee of Mozambique.

Authors' Contributions

M. Brandão: Conceptualization, data curation, formal analysis, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing. **A. Guisseve:** Data curation, investigation, project administration, writing–review and editing. **A. Damasceno:** Conceptualization, resources, supervision, funding acquisition, methodology, project administration, writing–review and editing. **G. Bata:** Resources, data curation, writing–review and editing. **C. Silva-Matos:** Conceptualization, resources, supervision, funding acquisition, methodology, project administration, writing–review and editing. **M. Alberto:** Investigation, writing–review and editing. **J. Ferro:** Resources, investigation, writing–review and editing. **C. Garcia:** Resources, investigation, writing–review and editing. **C. Zaqueu:** Resources, investigation, writing–review and editing. **C. Lorenzoni:** Resources, investigation, writing–review and editing. **D. Leitão:** Investigation, writing–review and editing. **O. Soares:** Resources, data curation, writing–review and editing. **A. Gudo-Morais:** Resources, data curation, writing–review and editing. **F. Schmitt:** Supervision, investigation, methodology, writing–review and editing. **S. Morais:** Investigation, methodology, writing–review and editing. **S. Tulsidás:** Resources, data curation, writing–review and editing. **C. Carrilho:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **N. Lunet:** Conceptualization, formal analysis, supervision, funding acquisition, methodology, project administration, writing–review and editing.

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

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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.

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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

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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

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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

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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 448cells/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%),

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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	

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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.

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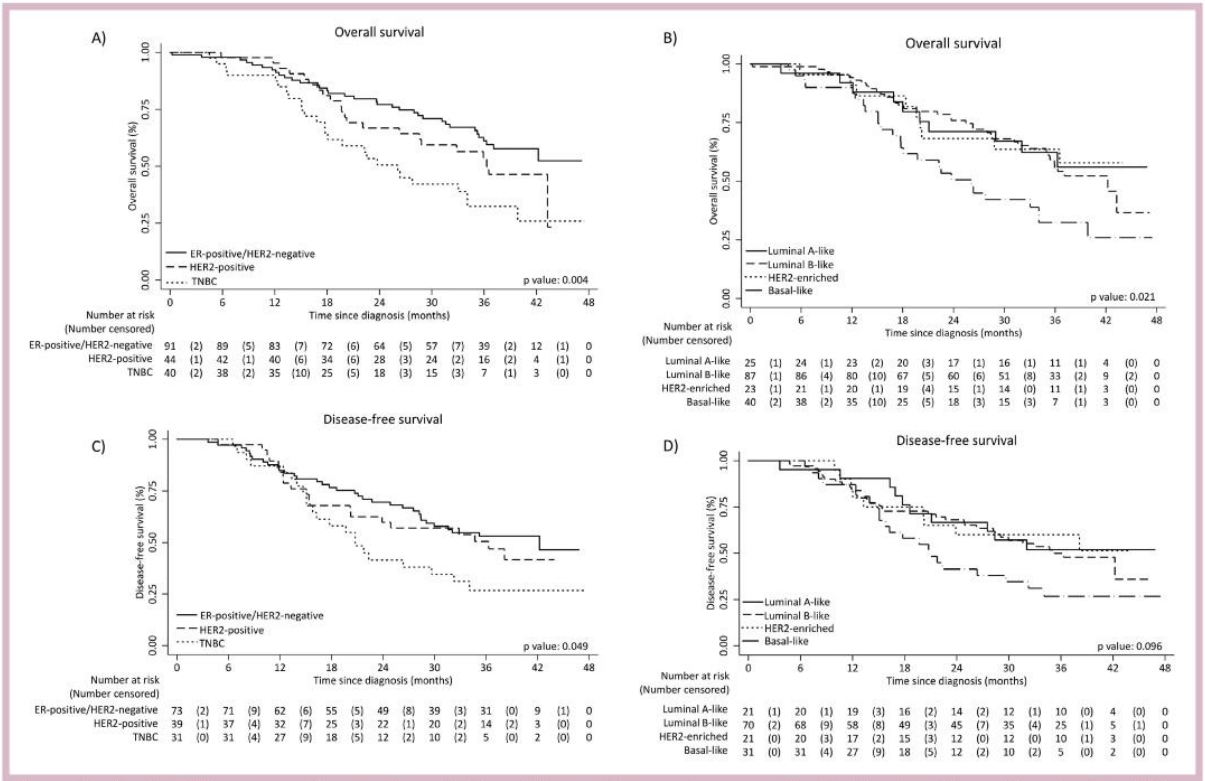


Figure 1 Kaplan-Meier curves for overall survival and disease-free survival according to classic subtypes (panels A and C) and surrogate intrinsic subtypes (panels B and D). ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

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

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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 3years 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

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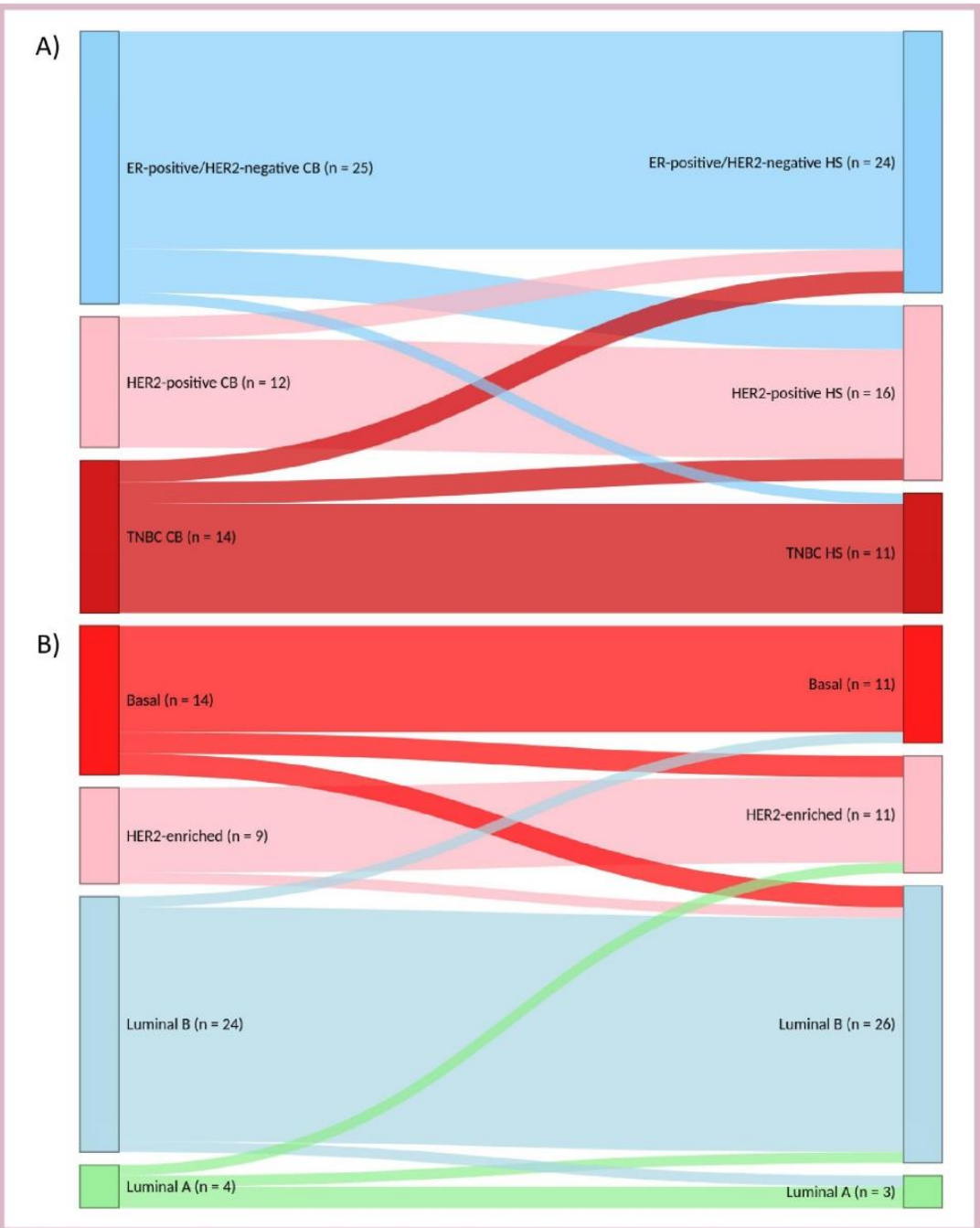


Figure 2 Sankey diagrams showing the reclassification of classic subtypes (panel A) and surrogate intrinsic subtypes (panel B) between CBs versus paired histological specimens (n=51). CB, cell blocks; ER: oestrogen receptor; HER2, human epidermal growth factor receptor 2; HS, histological samples; TNBC,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

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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.

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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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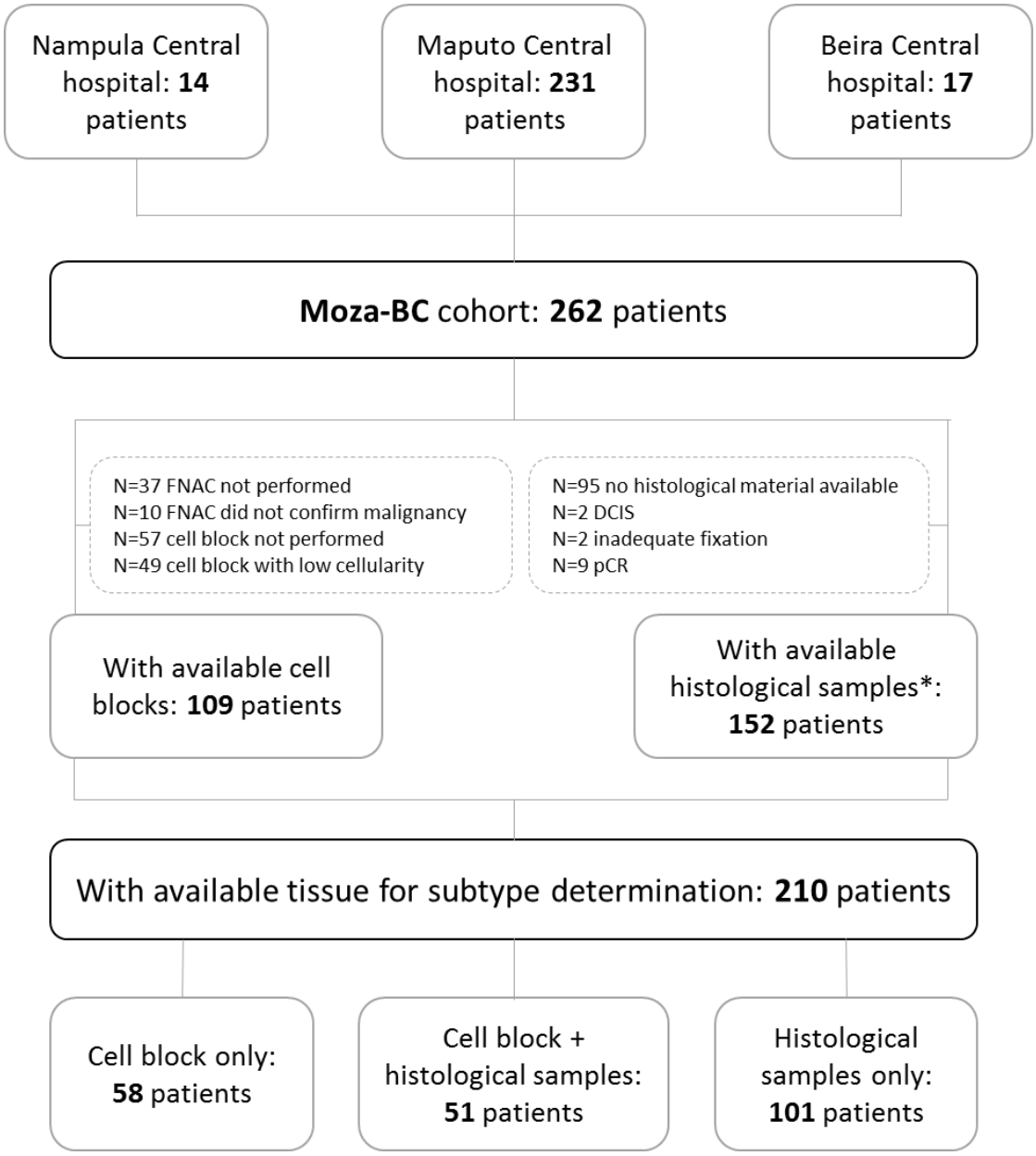
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Supplementary material

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

Supplementary figure 1 – Participants of the Moza-BC cohort, according to the hospital from which they were included and according to tissue availability for the assessment of breast cancer subtypes



* 152 patients, with a total of three core needle biopsies, 13 incisional surgical biopsies, 22 excisional surgical biopsies, four tumorectomies and 128 mastectomies.

Supplementary table 1 – Comparison of baseline characteristics of patients included in the Moza-BC cohort with data regarding breast cancer subtypes who were treated at the Oncology Unit of the Maputo Central Hospital (MCH; n=175) *vs* those who were not treated at the Oncology Unit of the MCH (n=35)

	Followed at the MCH, n=175	Not followed at the MCH, n=35	P
Age in years (n, %)			.150
<40	44 (25.1)	8 (25.0)	
40-49	49 (28.0)	7 (21.9)	
50-59	42 (24.0)	4 (12.5)	
≥60	40 (22.9)	13 (40.6)	
Missing	0	3	
Race (n, %)			.585
Black	172 (98.3)	34 (100)	
Other[1]	3 (1.7)	0	
Missing	0	1	
Place of residence (n, %)			**<.001**
South (including Maputo)	150 (90.9)	20 (64.5)	
Center/North	15 (9.1)	11 (35.5)	
Missing	10	4	
Classic subtypes (n, %)			.303
ER-positive/HER2-negative	91 (52.0)	17 (48.6)	
HER2-positive	44 (25.1)	6 (17.1)	
TNBC	40 (22.9)	12 (34.3)	
Surrogate intrinsic subtypes (n, %)[2]			.491
Luminal A-like	25 (14.3)	3 (8.6)	
Luminal B-like	87 (49.7)	16 (45.7)	
HER2-enriched	23 (13.1)	4 (11.4)	
Basal-like	40 (22.9)	12 (34.3)	
Tumor characteristics (clinical staging) (n, %)			.293
cT1	6 (3.5)	1 (2.9)	
cT2	43 (24.9)	13 (37.1)	
cT3	52 (30.1)	12 (34.3)	
cT4	72 (41.6)	9 (25.7)	
Missing	2	0	
Lymph node status (clinical staging) (n, %)			.068
cN0	43 (28.1)	16 (45.7)	
cN+	110 (71.9)	19 (54.3)	
Missing	22	0	

[1] Includes: mixed and Indian race.

[2] Tumors were classified into surrogate intrinsic subtypes according to the St. Gallen classification as: Luminal A-like: estrogen receptor (ER)-positive, progesterone receptor (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.

cN+: lymph node positive; ER: estrogen receptor; TNBC: triple-negative breast cancer.

Supplementary table 2 – Patients' baseline characteristics according to each breast cancer surrogate intrinsic subtype (St. Gallen classification)

	Luminal A-like N=28	Luminal B-like N=103	HER2-enriched n=27	Basal-like n=52	P
Age in years (n, %)					.769
<40	6 (22.2)	27 (26.7)	5 (18.5)	14 (26.9)	
40-49	7 (25.9)	31 (30.7)	6 (22.2)	12 (23.1)	
50-59	6 (22.2)	23 (22.8)	8 (29.6)	9 (17.3)	
≥60	8 (29.6)	20 (19.8)	8 (29.6)	17 (32.7)	
Missing	1	2	0	0	
Race (n, %)					**.044**
Black	25 (92.6)	102 (99.0)	27 (100)	52 (100)	
Other[1]	2 (7.4)	1 (1.0)	0	0	
Missing	1	0	0	0	
Education (n, %)					.532
0 years	7 (33.3)	17 (24.3)	3 (15.0)	5 (14.3)	
1-4 years	2 (9.5)	8 (11.4)	2 (10.0)	7 (20.0)	
>4 years	12 (57.1)	45 (64.3)	15 (75.0)	23 (65.7)	
Missing	7	33	7	17	
Place of residence (n, %)					.243
South (including Maputo)	22 (84.6)	89 (90.8)	22 (88.0)	37 (78.7)	
Center/North	4 (15.4)	9 (9.2)	3 (12.0)	10 (21.3)	
Missing	2	5	2	5	
Menopausal status (n, %)					.907
Pre-menopausal	13 (52.0)	48 (55.2)	11 (47.8)	20 (50.0)	
Post-menopausal	12 (48.0)	39 (44.8)	12 (52.2)	20 (50.0)	
Missing	3	16	4	12	
Body mass index (n, %)					.250
Under/normal weight (<25 kg/m^2)	9 (39.1)	36 (45.0)	6 (30.0)	10 (27.0)	
Overweight/obese (≥25 kg/m^2)	14 (60.9)	44 (55.0)	14 (70.0)	27 (73.0)	
Missing	5	23	7	15	
HIV status (n, %)[2]					.064
Negative/unknown	17 (68.0)	67 (77.0)	21 (91.3)	25 (62.5)	
Positive	8 (32.0)	20 (23.0)	2 (8.7)	15 (37.5)	
Missing	3	16	4	12	
Tumor characteristics (clinical staging) (n, %)					.065
cT1	3 (10.7)	1 (1.0)	2 (7.7)	1 (1.9)	
cT2	6 (21.4)	27 (26.5)	9 (34.6)	14 (26.9)	
cT3	6 (21.4)	29 (28.4)	11 (42.3)	18 (34.6)	
cT4	13 (46.4)	45 (44.1)	4 (15.4)	19 (36.5)	
Missing	0	1	1	0	
Lymph node status (clinical staging) (n, %)					.572
cN0	9 (36.0)	28 (29.8)	10 (41.7)	12 (26.7)	
cN+	16 (64.0)	66 (70.2)	14 (58.3)	33 (73.3)	
Missing	3	9	3	7	
Tumor characteristics (pathological staging) (n, %)					.101
(y)pT0/Tis	0	5 (6.5)	0	2 (5.9)	
(y)pT1	7 (33.3)	10 (13.0)	6 (27.3)	7 (20.6)	
(y)pT2	8 (38.1)	31 (40.3)	13 (59.1)	10 (29.4)	
(y)pT3	2 (9.5)	17 (22.1)	3 (13.6)	11 (32.4)	
(y)pT4	4 (19.0)	14 (18.2)	0	4 (11.8)	
Missing	7	26	5	8	
Lymph node status (pathological staging) (n, %)					.916
(y)pN0	4 (28.6)	15 (24.2)	5 (23.8)	9 (31.0)	
(y)pN1	7 (50.0)	22 (35.5)	7 (33.3)	11 (37.9)	
(y)pN2	2 (14.3)	16 (25.8)	6 (28.6)	4 (13.8)	
(y)pN3	1 (7.1)	9 (14.5)	3 (14.3)	5 (17.2)	
Missing	14	41	6	23	
Median tumor size at surgery in mm(median, range)	35 (12-120)	40 (0-180)	40 (2.5-80)	45 (0-180)	**.046**
Missing	8	26	5	18	

Multifocal tumors at surgery (n, %)					.933
No	15 (88.2)	66 (90.4)	18 (85.7)	28 (90.3)	
Yes	2 (11.8)	7 (9.6)	3 (14.3)	3 (9.7)	
Missing	11	30	6	21	
Lympho-vascular invasion at surgery (n, %)					.791
No	5 (35.7)	16 (24.2)	5 (23.8)	9 (30.0)	
Yes	9 (64.3)	50 (75.8)	16 (76.2)	21 (70.0)	
Missing	12	37	6	22	
Perineural invasion at surgery (n, %)					.803
No	11 (78.6)	49 (74.2)	16 (76.2)	25 (83.3)	
Yes	3 (21.4)	17 (25.8)	5 (23.8)	5 (16.7)	
Missing	12	37	6	22	
Histological grade at surgery (n, %)					**.038**
1	4 (20.0)	18 (25.0)	8 (38.1)	5 (16.1)	
2	11 (55.0)	37 (51.4)	7 (33.3)	9 (29.0)	
3	5 (25.0)	17 (23.6)	6 (28.6)	17 (54.8)	
Missing	8	31	6	21	
Histological subtype at surgery (n, %)					.661
No residual tumor/*in situ* carcinoma	0	5 (6.4)	0	2 (5.7)	
Invasive ductal carcinoma (NST)	18 (90.9)	63 (80.8)	19 (90.5)	31 (88.6)	
Other invasive subtypes[3]	2 (10.0)	10 (12.8)	2 (9.5)	2 (5.7)	
Missing	8	25	6	17	
Stage at diagnosis (n, %)					.285
I	0	1 (1.1)	1 (4.3)	1 (2.5)	
II	8 (32.0)	13 (14.9)	8 (34.8)	12 (30.0)	
III	13 (52.0)	56 (64.4)	12 (52.2)	18 (45.0)	
IV	4 (16.0)	17 (19.5)	2 (8.7)	9 (22.5)	
Missing[4]	3	16	4	12	

[1] Includes: mixed and Indian race.

[2] 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 anti-retroviral treatment (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+ cells count was 448 cells/µL (range 43-1104) and 39 (87%) patients had a CD4+ cells count > 200/µL.

[3] Includes: lobular, mixed, papillary, squamous cell carcinoma, metaplastic and mucinous breast cancer.

[4] 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.

NST: no special type.

Supplementary table 3 – Breast cancer clinical management according to each surrogate intrinsic subtype (St. Gallen classification)

	Luminal A-like N=28	Luminal B-like N=103	HER2-enriched N=27	Basal-like n=52	P
Time from first symptom to diagnosis (n, %)					.409
<180 days	5 (35.7)	31 (53.4)	7 (50.0)	13 (65.0)	
≥180 days	9 (64.3)	27 (46.6)	7 (50.0)	7 (35.0)	
Missing	14	45	13	32	
Timing of diagnosis (n, %)					.127
Pre-MTB implementation	10 (40.0)	50 (57.5)	8 (34.8)	23 (57.5)	
Post-MTB implementation	15 (60.0)	37 (42.5)	15 (65.2)	17 (42.5)	
Not applicable[1]	3	16	4	12	
Type of first treatment received (n, %)					.225
No treatment received	0	1 (1.2)	0	2 (5.0)	
Surgery	5 (20.0)	18 (20.9)	8 (34.8)	14 (35.0)	
Chemotherapy/Endocrine therapy[2]	20 (80.0)	67 (77.9)	15 (65.2)	24 (60.0)	
Missing	3	17	4	12	
Time from diagnosis until first treatment (n, %)					**.007**
<45 days	11 (44.0)	33 (38.8)	18 (78.3)	21 (55.3)	
≥45 days	14 (56.0)	52 (61.2)	5 (21.7)	17 (44.7)	
Missing	3	18	4	14	
Surgery (ever) (n, %)					.094
No	7 (26.9)	10 (11.4)	1 (4.0)	6 (15.0)	
Yes	19 (73.1)	78 (88.6)	24 (96.0)	34 (85.0)	
Missing	2	15	2	12	
Surgical intent (n, %)[3]					.562
Diagnostic	0	3 (3.8)	1 (4.2)	1 (2.9)	
Curative	17 (89.5)	61 (78.2)	20 (83.3)	28 (82.4)	
Palliative	0	11 (14.1)	1 (4.2)	2 (5.9)	
Unknown	2 (10.5)	3 (3.8)	2 (8.3)	3 (8.8)	
Type of breast surgery (n, %)[4]					.503
Total mastectomy	17 (89.5)	73 (93.6)	21 (87.5)	33 (97.1)	
Tumorectomy	2 (10.5)	5 (6.4)	3 (12.5)	1 (2.9)	
Status of surgical margins (n, %)					.521
Clean	16 (94.1)	64 (90.1)	20 (95.2)	26 (83.9)	
Positive	1 (5.9)	7 (9.9)	1 (4.8)	5 (16.1)	
Missing	2	7	3	3	
Axillary surgery – type (n, %)[5]					.799
Axillary dissection	16 (100)	68 (98.6)	22 (100)	31 (100)	
Sentinel lymph node biopsy	0	1 (1.4)	0	0	
Not done/missing	3	9	2	3	
Axillary surgery – completeness (n, %)[6]					.577
Not done/no isolated lymph nodes	3 (15.8)	9 (11.7)	2 (9.1)	3 (9.1)	
Incomplete	4 (21.1)	22 (28.6)	2 (9.1)	9 (27.3)	
Complete	12 (63.2)	46 (59.7)	18 (81.8)	21 (63.6)	
Missing	0	1	2	1	
Chemotherapy (ever received) (n, %)					.619
No	1 (4.0)	4 (4.6)	1 (4.3)	4 (10.0)	
Yes	24 (96.0)	83 (95.4)	22 (95.7)	36 (90.0)	
Missing	1	16	4	12	
Intent of first-line CT (n, %)[7]					.170
Neoadjuvant only	7 (29.2)	9 (10.8)	2 (9.1)	2 (5.6)	
Neoadjuvant + adjuvant	10 (41.7)	45 (54.2)	12 (54.5)	16 (44.4)	
Adjuvant only	3 (12.5)	13 (15.7)	6 (27.3)	10 (27.8)	
Palliative	4 (16.7)	16 (19.3)	2 (9.1)	8 (22.2)	
Neoadjuvant CT – outcome (n, %)					.174
Same stage	4 (23.5)	17 (31.5)	6 (42.9)	10 (55.6)	
Upstaging	1 (5.9)	10 (18.5)	2 (14.3)	1 (5.6)	
Downstaging	7 (41.2)	21 (38.9)	6 (42.9)	6 (33.3)	
Unknown[8]	1 (5.9)	6 (11.1)	0	1 (5.6)	
pCR rate after neoadjuvant CT (n, %)					.762
No pCR	17 (100)	50 (90.9)	14 (100)	16 (88.9)	
pCR only in the breast (ypT0/is)	0	3 (5.5)	0	1 (5.6)	

pCR in the breast and lymph nodes (ypT0/is, ypN0)	0	2 (3.6)	0	1 (5.6)	
Type of first-line CT regimen (n, %)[7]					.066
Anthracycline-based only	14 (58.3)	38 (45.8)	4 (18.2)	20 (55.6)	
Anthracyclines + taxanes based	10 (41.7)	41 (49.4)	17 (77.3)	15 (41.7)	
Other[9]	0	4 (4.8)	1 (4.5)	1 (2.8)	
First-line CT dose-intensity (n, %)[7]					.204
<85%	16 (69.6)	41 (51.9)	10 (50.0)	24 (68.6)	
≥85%	7 (30.4)	38 (48.1)	10 (50.0)	11 (31.4)	
Missing	1	4	2	1	
Cumulative dose of doxorubicin in mg/m² (median, range)	240 (180-360)	240 (60-420)	240 (180-360)	240 (120-360)	.588
Endocrine therapy (ever) (n, %)					**<.001**
No	9 (36.0)	28 (32.6)	12 (52.2)	31 (79.5)	
Yes	16 (64.0)	58 (67.4)	11 (47.8)	8 (20.5)	
Missing	1	17	4	13	
Radiotherapy (ever) (n, %)					.222
No	23 (92.0)	83 (96.5)	20 (87.0)	39 (97.5)	
Yes	2 (8.0)	3 (3.5)	3 (13.0)	1 (2.5)	
Missing	1	17	4	12	

[1] Not applicable as patients were not treated/followed at the Maputo Central Hospital and, therefore, were not discussed by the multidisciplinary tumor board.

[2] One patient received endocrine therapy as first treatment, who had a Luminal B-like tumor.

[3] Patients submitted to a surgical biopsy with diagnostic intent followed by a tumorectomy or a mastectomy with curative intent were included in the "Curative" intent group.

[4] Patients submitted to a tumorectomy followed by a mastectomy were included in the "Mastectomy" group.

[5] One patient received a sentinel lymph node biopsy followed by an axillary dissection and was, therefore, included in the “axillary dissection” group.

[6] 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).

[7] First line of chemotherapy that the patient received includes neoadjuvant, adjuvant or palliative treatment. If the patient received part of chemotherapy as neoadjuvant (*e.g.* AC regimen), and another part as adjuvant chemotherapy (*e.g*. paclitaxel), the type of regimen, dose-intensity and abandonment refers to the entire scheme (neoadjuvant plus adjuvant).

[8] Cases in whom there was missing data regarding clinical staging or patient abandoned treatment.

[9] 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 three weeks) and the preferred taxane used was paclitaxel (175 mg/m^2 every three weeks); dose-dense regimens were not used due to the unpredictable availability of granulocyte-colony stimulating factors.

CT: chemotherapy; ER: estrogen receptor; MTB: multidisciplinary tumor board; pCR: pathological complete response.

Supplementary Table 4 – Overall survival and disease-free survival estimates and multivariable analysis separately for stages I-II and III-IV, according to classic and surrogate intrinsic subtypes

	N	3-year overall survival % (95% CI)	P[1]	Adjusted HR (95% CI)[2]
Stages I-II				
Classic subtypes				
ER-positive/HER2-negative	15	77.0 (43.9-92.0)	0.228	1
HER2-positive	16	92.9 (59.1-99.0)		0.27 (0.03-2.65)
TNBC	13	57.0 (24.3-80.0)		1.13 (0.25-5.20)
Surrogate intrinsic subtypes				
Luminal A-like	8	75.0 (31.5-93.1)	0.201	1
Luminal B-like	14	84.6 (51.2-95.9)		0.66 (0.10-4.33)
HER2-Enriched	9	100		NC
Basal-like	13	57.0 (24.3-80.0)		1.00 (0.21-4.82)
Stages III-IV				
Classic subtypes				
ER-positive/HER2-negative	76	57.9 (45.1-68.7)	**<0.001**	1
HER2-positive	28	32.1 (14.9-50.8)		**2.16 (1.23-3.80)**
TNBC	27	21.0 (7.8-38.5)		**3.39 (1.91-6.01)**
Surrogate intrinsic subtypes				
Luminal A-like	17	57.0 (30.3-76.8)	**0.004**	1
Luminal B-like	73	51.7 (38.8-63.1)		0.82 (0.35-1.87)
HER2-Enriched	14	42.9 (17.7-66.0)		1.47 (0.52-4.12)
Basal-like	27	21.0 (7.8-38.5)		2.38 (0.99-5.68)
	N	**3-year disease-free survival % (95% CI)**	**P[1]**	**Adjusted HR (95% CI)[2]**
Stages I-II				
Classic subtypes				
ER-positive/HER2-negative	15	64.7 (34.4-83.7)	0.155	1
HER2-positive	16	92.9 (59.1-99.9)		0.21 (0.02-1.94)
TNBC	13	57.0 (24.3-80.0)		0.92 (0.22-3.90)
Surrogate intrinsic subtypes				
Luminal A-like	8	62.5 (22.9-86.1)	0.298	1
Luminal B-like	14	77.4 (4.9-92.1)		0.78 (0.15-4.17)
HER2-Enriched	9	100%		NC
Basal-like	13	57.0 (24.3-80.0)		0.91 (0.19-4.42)
Stage III				
Classic subtypes				
ER-positive/HER2-negative	58	50.0 (36.3-62.3)	**<0.001**	1
HER2-positive	23	23.2 (8.1-42.8)		**2.08 (1.15-3.79)**
TNBC	18	5.6 (0.4-22.4)		**3.52 (1.88-6.60)**
Surrogate intrinsic subtypes				
Luminal A-like	13	46.2 (19.2-69.6)	**0.005**	1
Luminal B-like	56	44.1 (30.5-57.0)		0.99 (0.42-2.31)
HER2-Enriched	12	33.3 (10.3-58.8)		1.90 (0.65-5.59)
Basal-like	18	5.6 (0.4-22.4)		**3.11 (1.25-7.70)**

[1] p value for the univariable survival analysis.

[2] Adjusted for age (<40 *vs* 40-49 *vs* 50-59 *vs* ≥60 years), HIV status (negative/unknown *vs* positive) and date of diagnosis (pre- *vs* post-multidisciplinary tumor board implementation).

ER: estrogen receptor; NC: non-calculable.

Supplementary table 5 – Concordance between cell blocks and histological samples for the estrogen receptor, the progesterone receptor, HER2 and Ki67 status in patients with both samples available (n=51)[1]

		Histological samples		Concordance (%)	κ
		ER-negative	ER-positive		
Cell blocks	ER-negative	20	3	88.2%	0.762
	ER-positive	3	25		
		PR-negative	PR-positive		
	PR-negative	28	3	80.4%	0.574
	PR-positive	7	13		
		HER2-negative	HER2-positive		
	HER2-negative	31	6	83.7%[2]	0.603
	HER2-positive	2	10		
		Ki67 low	Ki67 high		
	Ki67 low	31	6	76.1%[3]	0.271
	Ki67 high	5	4		

[1] Positive estrogen receptor (ER) and progesterone receptor (PR) status were defined with a ≥1% cut-off, and high Ki67 was defined as >29%. HER2-positivity was defined as an immunohistochemistry score of 3+ and/or a score of 2+ with an amplified silver *in situ* hybridization (SISH) test.

[2] Regarding HER2 evaluation, there were two surgical samples for which the immunohistochemistry score was 2+ (equivocal), but for which the SISH test was not successful.

[3] Regarding Ki67 evaluation, there were five samples (two cell blocks and three surgical samples) in which Ki67 was not tested.

Supplementary table 6 – Concordance for the estrogen receptor, the progesterone receptor, HER2 and Ki67 status in the 15 cell blocks submitted to re-staining and re-evaluation in Portugal by a third pathologist (quality control)[1]

		Portugal			Concordance (%)	κ
		ER-negative		ER-positive		
Mozambique	ER-negative	4		1	93.3%	0.842
	ER-positive	0		10		
		PR-		PR+		
	PR-negative	5		1	93.3%	0.857
	PR-positive	0		9		
		HER2 0/1+	2+	3+		
	HER2 0/1+	10	0	0	80.0%	0.541
	HER2 2+	1	1	0		
	HER2 3+	1	1	1		
		Ki67 low		Ki67 high		
	Ki67 low	6		4	50.0%[2]	0.248
	Ki67 high	3		1		

[1] Positive estrogen receptor (ER) and progesterone receptor (PR) status defined with a ≥1% cut-off and high Ki67 was defined as >29%.

[2] Regarding Ki67 evaluation, there was one sample in which Ki67 was not tested.

Paper III: *Healthcare use and costs in early breast cancer: a patient-level data analysis according to stage and breast cancer subtype*

ESMO Open Cancer Horizons

Healthcare use and costs in early breast cancer: a patient-level data analysis according to stage and breast cancer subtype

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ABSTRACT

Background The cost of breast cancer care rises with higher stage at diagnosis; however, there are no real-world data regarding the cost of care according to breast cancer subtypes. This study aimed to estimate direct medical costs for early breast cancer care in the first 3 years after diagnosis according to subtype and stage, using patient-level data.

Methods Women with newly diagnosed stage I–III breast cancer, admitted in 2012 to a Portuguese cancer centre were prospectively followed within the NEON-BC cohort. The use of health resources was obtained from each patient's clinical and administrative records and costs were computed. Tumours were classified into the classic subtypes (hormone receptor-positive (HR+)/HER2–; HER2-positive (HER2+); triple-negative breast cancer (TNBC)) and surrogate intrinsic subtypes (luminal A-like; luminal B-like; HER2 enriched; basal like).

Results A total of 703 patients were included: 48.9% had stage I, 35.8% stage II and 15.2% stage III breast cancer; 76.4% had HR+/HER2–, 15.9% HER2+ and 7.7% TNBC. Median cost of care was €9215/patient in stage I, €13 019/patient in stage II and €15 011/patient in stage III and €10 540/patient in HR+/HER2–, €11 224/patient in TNBC and €41 513/patient in HER2+ breast cancer. Systemic therapy accounted for 69.2% of the cost of care among patients with HER2+, 12.0% among HR+/HER2– and 7.5% among TNBC patients. Similar differences were observed across surrogate intrinsic subtypes.

Conclusions The cost of early breast cancer care was mainly driven by the tumour subtype and, to a lesser extent, by stage. The median cost of care was fourfold higher among patients with HER2+ tumours compared with those with HR+/HER2– and TNBC. These data provide information for the economic evaluation of innovative treatments for early breast cancer and highlight the weight that targeted systemic therapy might have in the overall cost of care among patients with early breast cancer.

Key questions

What is already known about this subject?

► Breast cancer comprises different tumour subtypes, associated with specific treatment strategies, yet there are no real-world data regarding cost of care according to each of these subtypes. This knowledge is paramount to the economic evaluation of innovative therapies, to provide evidence for reimbursement schemes and to substantiate healthcare policies.

What does this study add?

► We estimated direct medical costs for early breast cancer care in the first 3 years after diagnosis according to subtype and stage, using patient-level data. We have seen that the median cost of care was fourfold higher among patients with HER2+ tumours compared with those with hormone receptor-positive/HER2-negative and triple-negative breast cancer. This difference was mainly attributed to adjuvant treatment with trastuzumab.

How might this impact on clinical practice?

► We demonstrate that the cost of early breast cancer care was mainly driven by tumour subtype. Clinicians and stakeholders must be aware of the weight that targeted systemic therapies may have in the overall cost of care and, given the recent approval of many drugs for the treatment of early breast cancer, that this predominance will probably increase in the future.

INTRODUCTION

In developed countries, breast cancer is the most incident cancer and its standardised mortality rates have been decreasing over the last decades.[1] This decline has been attributed to both an increasing frequency of early diagnosis and access to more efficient and targeted systemic treatments, besides improved radiotherapy and surgery.[2] At the same time, the cost of each patient's cancer care increased, due to new treatments, but also to the development of new technologies, such as imaging.[3]

There are several studies estimating the cost of breast cancer care according to stage, showing that it increases from stages 0 to IV.[4] Nevertheless, it is widely recognised that

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breast cancer is a group of molecularly distinct neoplasms,[5] thus, drug regimens are now tailored not only to stage, but also to the tumour molecular subtype.[6] This may have also led to rising costs, due to an increased use of targeted drugs, such as anti-HER2 treatments (eg, trastuzumab).[7] Although health resource use may be expected to differ according to tumour subtype, there are other factors, such as number of affected lymph nodes, histological grade and medical history, that affect treatment decisions.[6] Hence, the individual cost of cancer care can differ between patients with tumours of the same subtype, even within the same hospital. Nonetheless, there are no real-world data regarding cost of early breast cancer treatment according to the different tumour subtypes. This information is paramount to economic evaluations of innovative therapies, to provide evidence for reimbursement schemes and to substantiate healthcare policies. This is particularly important as the sustainability of healthcare systems is now being debated, and especially of cancer care.[8]

In the European Union, in 2009, breast cancer represented the second highest economic cost (€15.0 billion, 12% of overall cancer costs) and accounted for the highest cancer-related healthcare costs (€6.7 billion; 13% of all cancer-related healthcare costs).[9] In the USA, estimates of lifetime per-patient costs of breast cancer in 2009 ranged from US$20 000 to US$100 000,[10] corresponding to 43%–212% of the US gross domestic product per capita at that time. Data from low-income and middle-income countries is scarcer, but some economic studies have also shown an increased cost of breast cancer care with rising stage.[11–14]

Therefore, the aim of the present study was to estimate all direct medical costs regarding diagnosis and treatment of patients with early breast cancer for the first 3 years after diagnosis according to tumour subtype and stage, using patient-level data.

METHODS

Setting

The Portuguese Institute of Oncology of Porto (IPO-Porto) is the largest cancer-dedicated hospital in Portugal, admitting patients from any part of the country, but especially from the Northern region.[15] IPO-Porto admits over 1000 new breast cancer cases/year in its Breast Clinic and covers the entire breast cancer continuum, from diagnosis to treatment (surgery, radiotherapy, systemic therapy and supportive care) and follow-up. All patients are discussed at a multidisciplinary tumour board and treatment is usually decided according to the European Society for Medical Oncology guidelines.[16] After concluding surgery, radiotherapy and/or chemotherapy, patients with early breast cancer enter a follow-up phase, in which they have a medical consultation every 3 months until the end of the third year of follow-up and then every 6 months.

Study design and participants

Patients with newly diagnosed breast cancer, admitted to the Breast Clinic of IPO-Porto in 2012 were included in the "NEON-BC - Neuro-oncological complications of breast cancer" prospective observational cohort.[17–20] Patients were eligible if they were 18 years or older, with histologically confirmed breast cancer, proposed for surgery and expected to be followed at IPO-Porto. In 2012, participants were excluded if they had a previous primary cancer treated with chemotherapy and/or axillary/thoracic radiotherapy; received breast surgery outside IPO-Porto; had a Montreal Cognitive Assessment score below 17 (or below 16 for women over 65 years old); were illiterate had a major psychiatric illness (eg, bipolar disorder, schizophrenia, among others); or due to other reasons (eg, previous breast surgery for benign conditions). However, at the third and fifth year of follow-up, 272 patients initially excluded from the cohort due to the above-mentioned reasons were invited to join the study and thus 240 additional patients were included (online supplemental figure 1). Women with stage 0 (*in situ* carcinoma) or stage IV breast cancer, and those without tumour biomarkers' assessment were excluded from the present analysis.

Data collection

Patients' sociodemographic characteristics were collected through face-to-face interviews using a structured questionnaire. Tumour characteristics, including stage, grade and biomarkers' assessment were retrieved from clinical records. The occurrence of a previous primary cancer was collected through face-to-face interviews and from clinical records.

Staging was defined by the AJCC TNM seventh edition classification.[21] Histological grade was classified from grade 1 (well differentiated) to grade 3 (poorly differentiated).[22] Expression of hormone receptors (HR; oestrogen receptor (ER) and progesterone receptor (PgR)), overexpression/amplification of HER2 and Ki67 score were assessed by immunohistochemistry on the primary tumour, as described in the literature.[23–25] When the immunohistochemistry score was 2+ (HER2-equivocal), fluorescence *in situ* hybridisation was performed. HRs were considered positive when their expression was ≥1%. Tumours were grouped according to the 'classic' classification into HR-positive/HER2-negative (HR+/HER2–); HER2-positive (HER2+); and triple-negative breast cancer (TNBC; HR-negative/HER2-negative). Tumours were also classified into surrogate intrinsic subtypes, using the 2011 St. Gallen Consensus guidelines: luminal A-like (ER-positive/PgR-positive/HER2-negative and Ki67 <15% (or grade 1, in cases in which Ki67 was not available)); luminal B-like (ER-positive, HER2-negative and either PgR positivity <20% or Ki67 ≥15% (or grade 3); or ER-positive and HER2-positive); HER2 enriched (HR-negative/HER2-positive); and basal like (HR-negative/HER2-negative).[26]

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Healthcare use during the first 3 years following breast cancer diagnosis was obtained from clinical and administrative records of each participant, namely: surgery (ie, breast and axillary); systemic treatment (ie, chemotherapy, hormone therapy and targeted therapy); radiation (ie, external radiotherapy and brachytherapy); appointments (ie, medical outpatient visits and nursing, psychology and social services appointments); hospitalisations; imaging; and laboratory and pathology examinations (including genetic testing). As IPO-Porto is a dedicated cancer centre, all patients' healthcare use within the hospital was considered to be related with their breast cancer diagnosis. This information was used to compute the cost of each component of treatment and follow-up for each patient. The unit cost of drugs were provided by the Management and Planning Service of IPO-Porto and all other costs were retrieved from the 2015 Price Tables of the Portuguese National Health Service (NHS).[27] When patients received breast cancer surgery, chemotherapy or targeted therapy before being admitted at IPO-Porto, the cost of those procedures was imputed based on the median cost of the same procedures at IPO-Porto. All costs were reported in euros at constant 2015 prices.

Information on relapse, diagnosis of a second primary cancer or death by any cause during the first 3 years after diagnosis was obtained from clinical records. Costs related to disease relapse or to a second primary cancer were integrated in this analysis.

Statistical analysis

Patients' characteristics are presented as counts and proportions for all categorical variables, and median and percentiles 25 and 75 (P25–P75) for continuous variables. For statistical analysis, patients were stratified by classic tumour subtype. Adjusted ORs (aORs) and 95% CIs were computed using multinomial logistic regression to quantify the association between tumour subtype and healthcare use and costs from diagnosis to the third year of follow-up (dependent variable). Models were adjusted for age (continuous variable), education (≤4, 5–9, ≥10 years) and stage (I, II, III). Additionally, analyses were performed according to the surrogate intrinsic subtypes. The median cost of overall cancer care per patient according to stage (I vs II vs III) was also assessed. All analyses were performed using STATA V.15 (StataCorp). All tests were two sided and a $p<0.05$ was considered significant.

RESULTS

Patients' characteristics

Among the 703 patients, 48.9% presented with stage I, 35.8% with stage II and 15.2% with stage III; 76.4% had a HR+/HER2- tumour, 15.9% presented with HER2+ and 7.7% with TNBC (online supplemental figure 1, table 1). Patients with TNBC were younger, and patients with HER2+ and TNBC tumours were more frequently diagnosed at a later stage — proportion of patients with stage III: 20.5% and 20.4% among HER2+ and TNBC, respectively, vs 13.6% among HR+/HER2–. Other sociodemographic characteristics were similar among the three subgroups, as well as history of a previous cancer diagnosis. During the first 3 years of follow-up, there were 18 patients (out of 617; 2.9%) with disease relapse, 12 (out of 656; 1.8%) with a second primary cancer and 12 (out of 703; 1.7%) patients had died.

Healthcare use

When adjusting for age, education and stage at diagnosis, there were no differences in the type of breast or axillary surgery across the three classic subtype subgroups (table 2). Patients with HER2+ (aOR 27.09; 95% CI 9.79 to 74.98) and TNBC (aOR 2.74; 95% CI 1.08 to 6.97) were more likely to receive chemotherapy, compared with patients with HR+/HER2– tumours. Almost all patients with HR+/HER2– tumours received hormone therapy (99.8%) and 93.8% of HER2+ patients received targeted systemic therapy (trastuzumab). Overall, 74.1% of patients received adjuvant radiotherapy, with no significant differences between subgroups.

Healthcare costs

The median (P25–P75) overall cost of care per patient during the first 3 years after diagnosis was €11 516 (€7890–€16 376). The median cost of care was €9215 (€7268–€13 117)/patient for stage I, €13 019 (€10 075–€19 194)/patient for stage II and €15 011 (€11 469–€22 530)/patient for stage III (figure 1).

The median (P25–P75) overall cost was €10 540 (€7480–€13 611)/patient among HR+/HER2€, €11 224 (€9158–€14 645)/patient among TNBC and €41 513 (€36 011–€47 015)/patient among patients with HER2+ tumours (figure 1, table 3). The discrepancy between patients with HER2+ vs HR+/HER2– and TNBC was mainly driven by the cost of trastuzumab (median €25 006/patient). There was also a higher cost associated with chemotherapy and day hospital use among patients with HER2+ (median of €386 and €2164/patient, respectively) and TNBC (€398 and €590/patient) compared with those with HR+/HER2– tumours (€98 and €394/patient). HER2+ patients also had a higher cost associated with surgeries, appointments, imaging and other medical expenses than the HR+/HER2– subgroup. Patients with TNBC had a significantly higher cost associated with other medical expenses than HR+/HER2– patients. Overall, expenses associated with systemic therapy constituted 69.2% of the median cost of care of HER2+ patients, while they contributed to 12.0% and 7.5% among HR+/HER2– and TNBC patients, respectively (figure 2). In the HR+/HER2– and TNBC subgroups, surgery accounted for around 25% of the total cost, similar to radiotherapy (20% of total cost).

The cost of care by year after cancer diagnosis dropped abruptly from the first to the second and third years for patients with HR+/HER2– and TNBC (figure 1). However, among patients with HER2+ tumours, the cost was €9347/patient during the second year due to adjuvant trastuzumab treatment.

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Table 1 Sociodemographic and clinical characteristics of patients at baseline, according to classic breast cancer subtypes*

	HR+/HER2− (N=537)	HER2+ (N=112)		TNBC (N=54)	
	N (%)	N (%)	P value†	N (%)	P value‡
Age, years					
<50	165 (30.7)	43 (38.4)		26 (48.1)	
50–64	228 (42.5)	46 (41.1)		19 (35.2)	
≥65	144 (26.8)	23 (20.5)	0.206	9 (16.7)	**0.028**
Education, years					
≤4	243 (48.3)	39 (37.9)		23 (46.0)	
5–9	124 (24.7)	32 (31.1)		16 (32.0)	
≥10	136 (27.0)	32 (31.1)	0.144	11 (22.0)	0.483
Net monthly income before diagnosis, euros					
≤500	282 (57.1)	49 (49.0)		27 (54.0)	
>500	212 (42.9)	51 (51.0)	0.138	23 (46.0)	0.675
Marital status					
Married or cohabitating	362 (72.0)	74 (72.8)		35 (70.0)	
Other§	141 (28.0)	28 (27.2)	0.861	15 (30.0)	0.768
Employment					
Employed	239 (47.5)	51 (49.5)		31 (62.0)	
Other¶	264 (52.5)	52 (50.5)	0.711	19 (38.0)	0.051
Place of residence					
Porto Metropolitan Area	181 (36.3)	39 (38.2)		22 (44.0)	
Outside Porto Metropolitan Area	318 (63.7)	63 (61.8)	0.708	28 (56.0)	0.281
Breast cancer stage					
I	283 (52.7)	43 (38.4)		18 (33.3)	
II	181 (33.7)	46 (41.1)		25 (46.3)	
III	73 (13.6)	23 (20.5)	**0.016**	11 (20.4)	**0.024**
Previous primary cancer diagnosis (including breast cancer)	48 (9.8)	5 (5.1)	0.134	3 (6.0)	0.384

The total may not add to 703 due to missing data. May not sum to 100% due to rounding.
*Classic subtypes were defined according to the assessment of the oestrogen receptor, the progesterone receptor and HER2 status on the surgical specimen (preferably) or on the biopsy core specimen (when the surgical specimen's pathology report was unavailable). HR+/HER2−: oestrogen receptor and/or progesterone receptor positive and HER2-negative (a score of 0 or 1+ in immunohistochemistry, or a score of 2+ with a non-amplified fluorescence *in situ* hybridizsation (FISH) test result); HER2+: HER2-positive (a score of 3+ in immunohistochemistry or a score of 2 with an amplified FISH test result), regardless of the oestrogen and progesterone receptor status; TNBC: oestrogen receptor negative, progesterone receptor negative and HER2-negative.
†HER2+ vs HR+/HER2−. Values in bold correspond to statisticallysignificant differences.
‡TNBC vs HR+/HER2−. Values in bold correspond to statisticallysignificant differences.
§Other marital status includes: single, widowed and divorced women.
¶Other employment includes: unpaid family workers, unemployed, housewives, retired and sick leave.
HR, hormone receptors; TNBC, triple negative breast cancer.

When combining the cost of care per subtype and stage, although patients with stage II and III had a higher median cost of care/patient versus those with stage I, these differences were small within each subtype—among HR+/HER2− patients, the cost ranged from €8498/patient (stage I) to €13 332/patient (stage III); in the HER2+ subgroup, it ranged from €38 216/patient (stage I) to €41 564/patient (stage III). Likewise, among patients with TNBC, the median cost was €9566/patient in stage I and €14 645/patient in stage III (online supplemental figure 2).

Surrogate intrinsic subtypes analyses

When categorising tumours according to the surrogate intrinsic subtypes classification, there were 87 patients with HR+/HER2− tumours, for whom it was not possible to discriminate between luminal A-like and luminal B-like, due to absence of information regarding PgR, Ki67 and/or histological grade. Among the 616 patients with available surrogate intrinsic subtype information, 33.1% had luminal A-like tumours, 52.9% had luminal B-like tumours (of which 246 were HER2− and 80 HER2+), 5.2% had HER2-enriched and 8.8%

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Table 2 Association between breast cancer classic subtype and healthcare use in the first 3 years following a breast cancer diagnosis in 2012

	HR+/HER2− (N=537)	HER2+ (N=112)			TNBC (N=54)		
	N (%)	N (%)	aOR (95% CI)*	aOR (95% CI)†	N (%)	aOR (95% CI)*	aOR (95% CI)†
Surgery							
Breast surgery‡							
Total mastectomy (vs breast-conserving surgery)	241 (51.2)	59 (62.1)	1.66 (1.04 to 2.67)	1.41 (0.85 to 2.35)	25 (51.0)	1.18 (0.64 to 2.18)	0.84 (0.43 to 1.64)
Axillary surgery§							
Axillary dissection (vs none or sentinel lymph node biopsy)	154 (33.3)	45 (48.4)	1.74 (1.08 to 2.78)	1.32 (0.67 to 2.61)	19 (39.6)	1.30 (0.69 to 2.44)	0.51 (0.21 to 1.24)
Other surgeries/interventions (≥4 vs <4)¶	181 (33.7)	85 (75.9)	6.40 (3.83 to 10.69)	6.60 (3.81 to 11.43)	34 (63.0)	3.01 (1.60 to 5.67)	2.43 (1.24 to 4.74)
Systemic therapy							
Any chemotherapy (yes vs no)	286 (53.3)	105 (93.8)	17.58 (6.87 to 44.97)	27.09 (9.79 to 74.98)	44 (81.5)	3.70 (1.64 to 8.36)	2.74 (1.08 to 6.97)
Timing of chemotherapy							
Adjuvant (vs neoadjuvant)	260 (91.9)	91 (89.2)	0.70 (0.32 to 1.56)	0.56 (0.22 to 1.41)	38 (86.4)	0.53 (0.20 to 1.41)	0.49 (0.16 to 1.52)
Chemotherapy scheme							
Anthracycline-based (yes vs no)**	283 (99.0)	99 (94.3)	0.21 (0.05 to 0.90)	0.18 (0.04 to 0.82)	43 (97.7)	0.48 (0.05 to 4.95)	0.50 (0.05 to 5.45)
Hormone therapy (yes vs no)††	536 (99.8)	80 (71.4)	--	--	0 (0.0)	--	--
Targeted therapy (yes vs no)‡‡	0 (0.0)	105 (93.8)	--	--	0 (0.0)	--	--
Other treatments							
Radiotherapy (yes vs no)	395 (73.6)	82 (73.2)	0.85 (0.52 to 1.40)	0.72 (0.43 to 1.22)	44 (81.5)	1.47 (0.68 to 3.16)	1.19 (0.54 to 2.65)
Brachytherapy (yes vs no)§§	110 (27.8)	14 (17.1)	0.58 (0.31 to 1.08)	0.71 (0.37 to 1.36)	8 (18.2)	0.52 (0.22 to 1.22)	0.67 (0.27 to 1.61)
Other healthcare use¶¶							
Appointments (≥55 vs <55)***	132 (24.6)	45 (40.2)	1.77 (1.12 to 2.80)	1.55 (0.96 to 2.52)	19 (35.2)	1.54 (0.81 to 2.90)	1.27 (0.65 to 2.46)
Hospitalisations (≥2 vs <2)†††	209 (38.9)	43 (38.4)	0.93 (0.60.1.45)	0.97 (0.62 to 1.51)	19 (35.2)	0.86 (0.46 to 1.57)	0.90 (0.49 to 1.66)
Genetic testing (yes vs no)‡‡‡	63 (11.7)	12 (10.7)	0.60 (0.29 to 1.27)	0.63 (0.27 to 1.34)	9 (16.7)	0.90 (0.37 to 2.23)	0.94 (0.38 to 2.34)

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Table 2 Continued

	HR+/HER2− (N=537)	HER2+ (N=112)		TNBC (N=54)		
	N (%)	N (%)	aOR (95% CI)*	aOR (95% CI)†	N (%)	aOR (95% CI)*	aOR (95% CI)†

The total may not add to 703 due to missing data. May not sum to 100% due to rounding.
*Adjusted for age (continuous) and education (≤4, 5–9, ≥10 years).
†Further adjusted for stage (I–III).
‡Patients who had breast-conserving surgery followed by total mastectomy are included in the total mastectomy group. Information regarding the type of breast surgery is not available for 84 patients who underwent surgery outside IPO-Porto.
§Patients who had sentinel lymph node biopsy followed by axillary lymph node dissection are included in the axillary dissection group. One patient did not receive any type of axillary surgery (included in the HR+/HER2− subgroup). Information regarding the type of axillary surgery is not available for 84 patients who underwent surgery outside IPO-Porto.
¶Other surgeries/interventions include breast reconstruction surgeries, central venous catheter placement/removal and other ambulatory surgeries/interventions. The cut-off used was determined as percentile 75 of the number of other surgeries/interventions among the HR+/HER2− subgroup.
**Anthracycline-based chemotherapy includes: AC regimen: four or six cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2); FEC regimen: six cycles of concomitant 5-FU (500 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2); AC-T regimen: four cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2), followed by four cycles of docetaxel (100 mg/m^2); AC-pacilitaxel regimen: four cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2), followed by four cycles of pacilitaxel (80 mg/m^2); FEC-D regimen: three cycles of concomitant 5-FU (500 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2), followed by three cycles of docetaxel (100 mg/m^2).
††Among HR+/HER2-: 71 patients (13.2%) only received tamoxifen; 116 (21.6%) only received aromatase inhibitors; 104 (19.4%) received both tamoxifen and aromatase inhibitors; 90 (16.8%) received tamoxifen and/or aromatase inhibitors, plus goserelin (for ovarian function suppression during adjuvant treatment) or fulvestrant (for relapsed disease); 155 (28.9%) unknown. Among HER2+: 9 (11.2%) only received tamoxifen; 24 (30.0%) only received aromatase inhibitors; 10 (12.5%) received both tamoxifen and aromatase inhibitors; 18 (22.5%) received tamoxifen and/or aromatase inhibitors, plus goserelin (for ovarian function suppression during adjuvant treatment) or fulvestrant (for relapsed disease); 19 (23.8%) unknown.
‡‡Targeted therapy includes trastuzumab. Seven patients with HER2+ breast cancer did not receive trastuzumab due to: age >80 years (n=2), cardiovascular disease (n=3), T1a tumour (n=1), refusal (n=1).
§§Among those who received radiotherapy (n=521).
¶¶Does not include the number of laboratory and pathology tests, and imaging examinations, for which only the total cost per patient was available (included in table 3).
***Appointments: number of outpatient visits (surgery, medical oncology and radiation oncology, among others), and nursing, psychology and social services appointments. The cut-off used was determined as percentile 75 of the number of appointments among the HR+/HER2− subgroup.
†††The cut-off used was determined as percentile 75 of the number of hospitalisations among the HR+/HER2− subgroup.
‡‡‡Genetic testing includes: *BRCA1* mutations and *BRCA2* mutations (hereditary breast and ovarian cancer syndrome); deletions in *MLH1* (hereditary nonpolyposis colon cancer); and *TP53* mutations (Li-Fraumeni syndrome).
aOR, adjusted OR; HR, hormone receptors; IPO-Porto, Portuguese Institute of Oncology of Porto; TNBC, triple negative breast cancer.

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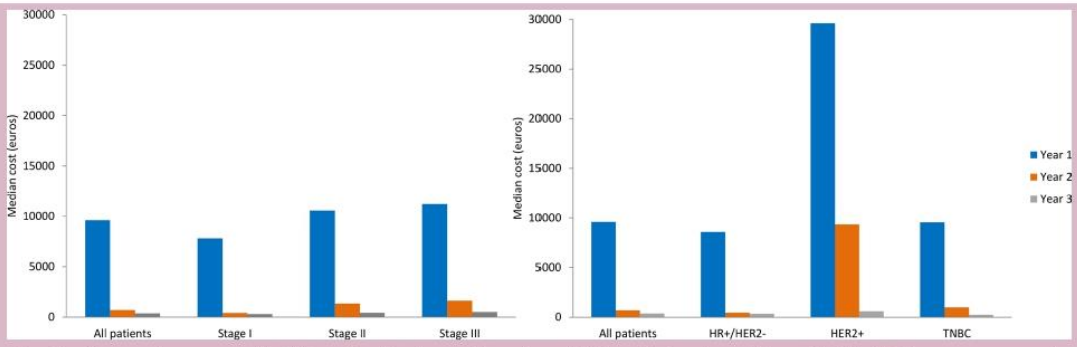


Figure 1 Median cost per year (1–3) for all patients and by stage (left) and classic subtype (right). HR: hormone receptor; TNBC: triple-negative breast cancer.

had basal-like tumours. Patients with luminal B-like, HER2-enriched and basal-like tumours were diagnosed at a more advanced stage compared with luminal A-like patients (online supplemental table 1). Only 16.2% of luminal A-like patients received chemotherapy vs 76.4% of luminal B-like, 100% of HER2-enriched and 81.5% of basal-like patients (online supplemental table 2). Patients with luminal A-like tumours also had a lower number of appointments than the other subgroups.

The median overall cost of care during the first 3 years after diagnosis was €8232 (€6391–€10 931)/patient among luminal A-like, €12 493 (€8723–€22 237)/patient among luminal B-like, €38 297 (€34 510–€42 529)/patient among HER2-enriched and €11 224 (€9158–€14 645)/patient among the basal-like subgroup (online supplemental table 3 and online supplemental figure 3). Similar to that described for classic subtypes above, the higher treatment cost among patients with HER2-enriched tumours was mainly driven by the cost of trastuzumab. Among luminal B-like patients, 73 (22.4%) also received trastuzumab (as they had HER2+ tumours), which also partly explains the higher median cost of care of luminal B-like vs luminal A-like patients. The other reason is the higher spending related to chemotherapy and day hospital use among luminal B-like (median of €255 and €590/patient) vs luminal A-like patients (median of €0/patient, as 84.0% did not receive chemotherapy). Overall, systemic therapy accounted for 9.0% of the total median cost of care of luminal A-like patients, while it accounted for 45.6% among luminal B-like and 69.6% among HER2-enriched patients (online supplemental figure 4). Among patients with HER2-enriched tumours, treatment cost was stable across the different stages at diagnosis, due to the fact that they all received trastuzumab. Conversely, there was an increase in treatment cost from stage I to stage III among patients with the other three subtypes, especially among the luminal A-like subgroup (online supplemental figure 5).

DISCUSSION

To the best of our knowledge, this is the first study to estimate all direct medical costs among patients with early breast cancer according to tumour subtype. We found that the median overall cost of care during the first 3 years after breast cancer diagnosis was fourfold higher among patients with HER2+ tumours than HR+/HER2− or TNBC patients, and that this was primarily driven by the cost of adjuvant trastuzumab treatment. This cost relates not only to the trastuzumab price itself, but also to all other expenditures related to its administration and toxicity monitoring, that is, daily hospital use, appointments and medical tests (including cardiac imaging).

We have also shown that, among patients with HR+/HER2− and TNBC, the cost of breast cancer care was mainly driven by surgery and radiotherapy rather than by systemic therapy (chemotherapy and/or hormone therapy). Similar results were observed when comparing cost of care according to patients' surrogate intrinsic subtype.

Additionally, we found that there is an increase in the total median cost of care from stage I (€9215/patient) to stage III (€15 011/patient). This is in line with a previous study from Portugal, conducted at IPO-Lisbon (a similar, dedicated cancer centre) in 2014, assessing costs within the first 2 years after breast cancer diagnosis. The authors estimated the mean cost to be €6094/patient in stage I, €9785/patient in stage II and €11 893/patient in stage III.[28] These figures are similar to those reported in other European countries with an NHS, such as Italy,[29] France[30] and the UK.[31]

Yet interestingly, the cost discrepancies by stage are negligible when compared with the differences observed according to breast cancer subtypes. This is primarily explained by the fact that, in breast cancer, the intensity and type of treatment is driven not only by stage, but mostly by biology: most patients with TNBC and HER2+ tumours have indication to receive chemotherapy and anti-HER2 therapy (in the case of HER2+). Likewise, all patients with HR+ tumours should receive hormone therapy, regardless of stage.[16] Concerning locoregional therapy, most

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Table 3 Association between breast cancer classic subtype and healthcare costs, in euros, in the first 3 years following a breast cancer diagnosis in 2012

	HR+/HER2− (N=537)		HER2+ (N=112)			TNBC (N=54)	
	N (%)	N (%)	aOR (95% CI)*	aOR (95% CI)†	N (%)	aOR (95% CI)*	aOR (95% CI)†
Surgery‡§							
Surgeries, euros							
Median (P25–P75)	2522.01 (1434.07–2880.94)	2684.49 (2511.01–3939.67)			2522.01 (2094.72–3654.94)		
≤1800¶	179 (33.3)	10 (8.9)			9 (16.7)		
1801–2700	208 (38.7)	58 (51.8)	6.31 (2.77 to 14.40)	5.88 (2.53 to 13.64)	25 (46.3)	2.20 (0.93 to 5.23)	1.65 (0.68 to 4.02)
>2700	150 (27.9)	44 (39.3)	6.28 (2.70 to 14.63)	6.02 (2.55 to 14.19)	20 (37.0)	2.63 (1.09 to 6.37)	2.09 (0.85 to 5.14)
Systemic therapy							
Chemotherapy§**, euros							
Median (P25–P75)	98.35 (0.00–389.44)	385.96 (265.92–412.82)			397.58 (269.14–533.39)		
None (0)¶	251 (46.7)	7 (6.3)			10 (18.5)		
≤400	164 (30.5)	65 (58.0)	18.74 (7.22 to 48.66)	27.65 (9.95 to 76.80)	17 (31.5)	2.66 (1.08 to 6.53)	2.16 (0.80 to 5.81)
>400	122 (22.7)	40 (35.7)	15.68 (5.84 to 42.06)	23.83 (8.06 to 70.52)	27 (50.0)	5.27 (2.20 to 12.64)	4.07 (1.47 to 11.29)
Hormone therapy§, euros							
Median (P25–P75)	101.71 (73.27–120.79)	78.41 (0.00–112.51)			0.00 (0.00–0.00)		
≤80¶	177 (33.0)	58 (51.8)			54 (100.00)		
81–110	161 (30.0)	22 (19.6)	0.43 (0.24 to 0.76)	0.41 (0.23 to 0.73)	0 (0.0)	--	--
>110	199 (37.1)	32 (28.6)	0.52 (0.32 to 0.85)	0.52 (0.32 to 0.87)	0 (0.0)	--	--
Targeted therapy§††, euros							
Median (P25–P75)	0.00 (0.00–0.00)	25005.56 (21353.67–29808.79)			0.00 (0.00–0.00)		
None (0)¶	537 (100.0)	7 (6.3)			54 (100.00)		
≤26000	0 (0.0)	53 (47.3)	--	--	0 (0.0)	--	--
>26000	0 (0.0)	52 (46.4)	--	--	0 (0.0)	--	--
Day hospital§‡‡, euros							

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Table 3 Continued

	HR+/HER2− (N=537)		HER2+ (N=112)			TNBC (N=54)	
	N (%)	N (%)	aOR (95% CI)*	aOR (95% CI)†	N (%)	aOR (95% CI)*	aOR (95% CI)†
Median (P25–P75)	393.52 (0.00–590.28)	2164.36 (2164.36–2262.74)			590.28 (590.28–590.28)		
None (0)¶	228 (42.5)	4 (3.6)			10 (18.5)		
≤600	193 (35.9)	2 (1.8)	0.49 (0.05 to 4.52)	0.72 (0.07 to 7.07)	35 (64.8)	3.70 (1.63 to 8.40)	2.60 (1.01 to 6.69)
>600	116 (21.6)	106 (94.6)	246.48 (69.64 to 872.35)	485.71 (123.60 to 1908.67)	9 (16.7)	0.85 (0.27 to 2.72)	0.56 (0.16 to 2.03)
Radiotherapy§§§, euros							
Median (P25–P75)	2627.35 (0.00–3240.43)	2615.60 (0.00–3273.33)			2649.30 (2569.82–3401.36)		
None (0)¶	149 (27.7)	30 (26.8)			10 (18.5)		
≤2675	192 (35.8)	44 (39.3)	1.01 (0.58 to 1.75)	0.77 (0.42 to 1.40)	22 (40.7)	1.62 (0.70 to 3.74)	1.11 (0.45 to 2.72)
>2675	196 (36.5)	38 (33.9)	0.81 (0.46 to 1.43)	0.76 (0.43 to 1.35)	22 (40.7)	1.53 (0.66 to 3.52)	1.37 (0.58 to 3.21)
Other healthcare use							
Appointments§, euros							
Median (P25–P75)	1139.00 (909.00–1495.00)	1370.00 (1127.00–1785.50)			1374.50 (1084.00–1676.00)		
≤1000¶	189 (35.2)	15 (13.4)			11 (20.4)		
1001–1350	174 (32.4)	39 (34.8)	2.63 (1.33 to 5.23)	2.53 (1.26 to 5.07)	15 (27.8)	1.48 (0.60 to 3.67)	1.24 (0.50 to 3.11)
>13 500	174 (32.4)	58 (51.8)	3.89 (1.98 to 7.63)	3.50 (1.74 to 7.06)	28 (51.9)	2.92 (1.25 to 6.83)	2.22 (0.92 to 5.34)
Hospitalisation§, euros							
Median (P25–P75)	1694.78 (1434.07–2346.70)	1694.78 (1434.07–3391.17)			1694.78 (1434.07–3000.90)		
≤1450¶	179 (33.3)	36 (32.1)			21 (38.9)		
1451–1800	194 (36.1)	30 (26.8)	0.82 (0.47 to 1.41)	0.65 (0.36 to 1.16)	17 (31.5)	0.78 (0.38 to 1.57)	0.54 (0.26 to 1.16)
>1800	164 (30.5)	46 (41.1)	1.36 (0.81 to 2.27)	1.14 (0.67 to 1.93)	16 (29.6)	0.82 (0.40 to 1.67)	0.62 (0.29 to 1.31)
Genetic testing§, euros							
Median (P25–P75)	0.00 (0.00–0.00)	0.00 (0.00–0.00)			0.00 (0.00–0.00)		

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Table 3 Continued

	HR+/HER2− (N=537)		HER2+ (N=112)			TNBC (N=54)	
	N (%)	N (%)	aOR (95% CI)*	aOR (95% CI)†	N (%)	aOR (95% CI)*	aOR (95% CI)†
None (0)¶	474 (88.3)	100 (89.3)			45 (83.3)		
≤550	19 (3.5)	4 (3.6)	0.58 (0.18 to 1.90)	0.58 (0.18 to 1.90)	2 (3.7)	0.64 (0.13 to 3.09)	0.63 (0.13 to 3.05)
>550	44 (8.2)	8 (7.1)	0.61 (0.26 to 1.43)	0.65 (0.27 to 1.56)	7 (13.0)	1.03 (0.38 to 2.78)	1.11 (0.40 to 3.04)
Other medical expenses§¶¶, euros							
Median (P25-P75)	623.84 (268.50–1119.00)	1121.48 (747.39–1806.18)			883.83 (579.64–1217.50)		
≤350¶	180 (33.5)	2 (1.8)			6 (11.1)		
351–900	176 (32.8)	40 (35.7)	37.46 (5.08 to 276.41)	43.10 (5.78 to 321.52)	21 (38.9)	3.54 (1.28 to 9.77)	2.93 (1.02 to 8.42)
>900	181 (33.7)	70 (62.5)	60.09 (8.23 to 438.59)	73.12 (9.74 to 549.08)	27 (50.0)	4.82 (1.80 to 12.89)	3.50 (1.18 to 10.37)
Imaging§, euros							
Median (P25-P75)	560.00 (347.80–874.22)	1348.91 (1043.57–1714.80)			821.91 (456.59–1099.03)		
≤450¶	192 (35.8)	5 (4.5)			12 (22.2)		
451–750	165 (30.7)	7 (6.3)	2.05 (0.59 to 7.20)	2.60 (0.73 to 9.29)	14 (25.9)	2.04 (0.82 to 5.10)	1.57 (0.60 to 4.07)
>750	180 (33.5)	100 (89.3)	24.94 (8.82 to 70.53)	36.32 (12.08 to 109.21)	28 (51.9)	3.25 (1.38 to 7.68)	2.16 (0.84 to 5.58)
Total cost§, euros							
Median (P25-P75)	10539.5 (7480.38–13610.61)	41513.47 (36010.75–47015.02)			11224.27 (9157.58–14644.81)		
≤8250¶	179 (33.3)	3 (2.7)			9 (16.7)		
8251–12250	179 (33.3)	2 (1.8)	1.18 (0.16 to 8.55)	1.63 (0.22 to 12.12)	25 (46.3)	3.13 (1.24 to 7.89)	2.34 (0.91 to 6.03)
>12250	179 (33.3)	107 (95.5)	63.70 (14.88 to 272.80)	103.91 (22.62 to 477.38)	20 (37.0)	2.35 (0.87 to 6.30)	1.41 (0.50 to 4.01)

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Table 3 Continued

	HR+/HER2− (N=537)		HER2+ (N=112)			TNBC (N=54)	
	N (%)	N (%)	aOR (95% CI)*	aOR (95% CI)†	N (%)	aOR (95% CI)*	aOR (95% CI)†

The total may not add to 703 due to missing data. May not sum to 100% due to rounding.
For items: surgeries, hormone therapy, appointments, hospitalisation, other medical expenses, imaging and total cost, the cut-offs used to define each category were determined as percentiles 33 and 66 of the respective cost among the HR+/HER2− subgroup. For items: chemotherapy, targeted therapy, day hospital, radiotherapy and genetic testing, categories were defined as none (0) and the median cost among the HR+/HER2− subgroup (among those who received the respective item) was used as the cut-off to define the other two categories.
*Adjusted for age (continuous) and education (≤4, 5–9, ≥10 years).
†Further adjusted for stage (I–III).
‡All surgeries/interventions, including breast surgeries and axillary surgeries. Imputed surgery cost for 87 patients who underwent surgery outside IPO-Porto.
§Dependent variable in the multinomial logistic regression model.
¶Category being used as the reference in the multinomial logistic regression model.
**Imputed chemotherapy cost for three patients who received chemotherapy outside IPO-Porto.
††Imputed targeted therapy cost for one patient who received targeted therapy outside IPO-Porto.
‡‡Day hospital sessions include the administration of intravenous, subcutaneous and/or intramuscular therapy (ie, chemotherapy, trastuzumab, goserelin and fulvestrant).
§§The cost of brachytherapy for the seven patients who only received brachytherapy (and no external radiotherapy) is included in hospitalisation costs and not in radiotherapy costs.
¶¶Other medical expenses include laboratory and pathology tests, and blood transfusions.
aOR, adjusted OR; HR, hormone receptors; IPO-Porto, Portuguese Institute of Oncology of Porto; TNBC, triple negative breast cancer.

patients with smaller tumours receive breast-conserving surgery, and subsequently, radiotherapy; while patients with more advanced tumours may undergo mastectomy, but likely receive radiotherapy afterwards as well. Therefore, the cost of locoregional therapy may not change dramatically according to stage. Similarly, as there are no guidelines defending a differential timing of follow-up by stage, we did not expect to see differences in follow-up intensity according to stage.[16] Conversely, breast cancer subtype determines the type of adjuvant systemic therapy that the patient receives, which may lead to an increased number of appointments, in order to monitor toxicity to chemotherapy, trastuzumab and/or hormone therapy.

This study has the advantage of having used the micro-costing method, in which resources and associated unit costs were directly measured at the patient level, making estimates much more reliable. Additionally, unlike previous studies,[28–31] we have also reported the quantities of resources used separately from costs, allowing for a more detailed assessment of healthcare use.

Many studies assessing the cost of breast cancer care have compared costs of breast cancer cases to control groups (without breast cancer), in order to calculate breast cancer-attributable treatment costs, as a way to avoid overestimations.[4] Since IPO-Porto is a dedicated cancer centre, we did not need to perform these estimations, as healthcare use among patients in this study can all be virtually attributed to their breast cancer care, making our estimates much more accurate. Moreover, we also included the costs associated with the treatment of patients who have a disease relapse, increasing the generalisation of our findings to the real-world clinical setting.

We only assessed direct medical costs attributable to IPO-Porto and the Portuguese NHS, and did not take into account out-of-pocket expenses paid by patients. However, in Portugal, as in other European NHS,[28–31] almost all direct medical costs related to cancer care are covered by the public health system, thus out-of-pocket expenses are small. Yet, some direct costs related to breast cancer care such as physiotherapy (usually received in the community setting) were also not included in the analysis. Indirect costs, such as loss of productivity, have been shown to account for more than half of the total costs associated with breast cancer;[9] however, these were not included in this analysis, which may have underestimated the cost associated with some procedures, such as surgery or chemotherapy.

This study also has the limitation of being a single-centre study, which may impair the generalisation of the results. Nonetheless, IPO-Porto is a large hospital, receiving patients from any part of the country, with different sociodemographic backgrounds and it includes patients presenting with the entire spectrum of breast cancer in terms of stage and subtypes. Moreover, this also presents advantages: there was no lost to follow-up and we were able to obtain detailed patient-level data, which allowed for understanding the main factors behind the cost of care.

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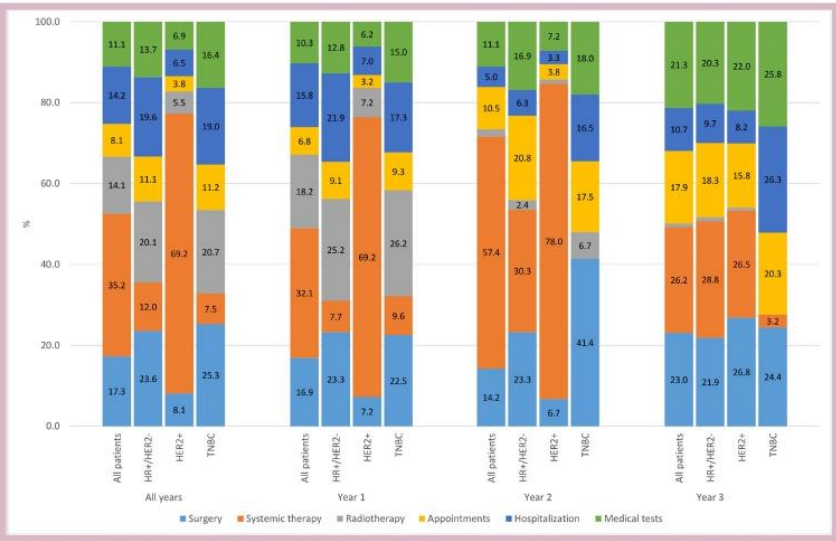


Figure 2 Proportion of the median cost attributed to surgery, systemic therapy, radiotherapy, appointments, Hospitalisation and medical tests among all patients, by year after breast cancer diagnosis (all years; years 1–3) and by classic subtype. 'Systemic therapy' includes chemotherapy, targeted therapy, hormone therapy and day hospital use. 'Medical tests' includes laboratory and pathology tests, blood transfusions, imaging and genetic testing. When the proportion of the median cost of a certain item was ≤2% of the overall cost, the respective label was deleted from the figure. HR: hormone receptor; TNBC: triple-negative breast cancer.

From 2012 until now, there have been major advances in loco-regional and in systemic therapy for early breast cancer.[3] The cost of radiotherapy may have decreased due to the adoption of hypofractionated regimens and of accelerated partial-breast irradiation (to selected patients), which decreases the number of radiotherapy sessions needed and, therefore, reduces both direct and indirect costs.[32 33]

Among patients with HER2+ breast cancer, the introduction of trastuzumab biosimilar drugs and of a subcutaneous trastuzumab formulation lowered the price of this adjuvant therapy.[34 35] Additionally, the Persephone trial showed that 6-month therapy with trastuzumab is non-inferior to the standard 12-month regimen.[36] Nonetheless, the 6-month regimen has not been widely adopted, due to the negative results of other non-inferiority trials and due to concerns regarding its efficacy among higher-risk groups.[37] Hence, 12 months of adjuvant trastuzumab continues to be considered the standard-of-care in developed countries.[16] On the other hand, since 2013, three more anti-HER2 drugs were approved in the (neo)adjuvant setting: pertuzumab, neratinib and T-DM1.[16] As an example, in our cohort, 60% of patients with HER2+ tumours had stage II/III and would currently be proposed to neoadjuvant chemotherapy plus trastuzumab/pertuzumab. As four cycles of pertuzumab have a direct cost of €13 978,[38] this means that the overall median cost per patient could have increased by 20% among this subgroup. However, these drugs also decrease the likelihood of disease relapse, thus potentially reducing expenditures related to the treatment of breast cancer recurrence.

On the other hand, around half of the patients with HR+/HER2– tumours in our cohort received chemotherapy. Nowadays, a part of these patients could have their tumours tested with a genomic signature (eg, PAM50, oncotype, Mammaprint)[16] in order to avoid chemotherapy, which would decrease both direct and indirect costs. However, after the publication of the SOFT/TEXT trials,[39] many premenopausal women with HR+ tumours started receiving adjuvant tamoxifen or exemestane plus ovarian suppression for 5 years, which have potentially increased the cost of their treatment. Therefore, patients' menopausal status should be taken into account in future analyses of the cost of breast cancer care. In addition, there are large ongoing randomised clinical trials, testing two to 3 years of adjuvant treatment with CDK4/6 inhibitors in patients with HR+/HER2– breast cancer (monarchE; NATALEE, NCT03701334), which may significantly increase the cost of treatment for this subgroup of patients. For example, the total cost of 2 years of therapy with abemaciclib (tested in the monarchE trial[40]) is almost €100 000/patient, only considering the cost of the drug and not taking into account all the other direct (laboratory tests, medical appointments, etc) and indirect expenses associated with the treatment. Likewise, in early TNBC, treatment costs may significantly escalate in the future due to the possible introduction of new immunotherapy drugs[41] or poly-ADP ribose polymerase (PARP) inhibitors (OlympiA trial, NCT02032823).

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Therefore, due to the increase in the number of available systemic therapy options, namely of targeted drugs, there is a growing concern regarding the 'financial toxicity' of treating breast cancer due to the escalation of costs. This concern exists not only in the metastatic setting, but increasingly in the early setting as well.[42 43] The European Society for Medical Oncology developed the 'Magnitude of Clinical Benefit Scale', which aims to objectively evaluate the clinical benefit of each approved systemic drug, taking into account the drug's survival benefit, associated toxicity and impact on quality of life.[44] These evaluations, coupled with real-life cost assessments such as the one presented in this study, may help inform stakeholders on reimbursement options for the many drugs that are currently being tested and regarding other cancer care policies.

CONCLUSION

In this patient-level data analysis, the cost of early breast cancer care within the first 3years following diagnosis was mainly driven by the breast cancer subtype and, to a lesser extent, by stage at diagnosis. Among patients with HER2+breast cancer, the median overall cost of care was fourfold higher compared with those with HR+/HER2– and TNBC, and this difference was mostly related to the cost of systemic therapy, including trastuzumab. These data provide important information for the design of future economic studies, including the evaluation of innovative drugs, as it accurately quantified the use and cost associated with each of the different components of early breast cancer care. Moreover, these findings highlight the preponderance that targeted systemic therapy might have in the overall cost of care for patients with early breast cancer.

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Competing interests MBr has received a travel grant and speaker honorarium, unrelated to this study, from Roche/GNE, and the Institut Jules Bordet has received research grants, unrelated to this study, from Roche/GNE, Radius, AstraZeneca, Lilly, MSD, GSJ/Novartis, Synthon, Servier and Pfizer. DP has participated in advisory boards, unrelated to this study, from Roche, AstraZeneca, Pfizer, Yansen and Novartis. MBo has received travel grants, unrelated to this study, from Roche and Janssen.

Patient consent for publication Not required.

Ethics approval All procedures performed in the present study were in accordance with the ethical standards of the institutional Ethics Committee of the Portuguese Institute of Oncology of Porto (ref. CES 406/011, CES 99/014 and CES 290/014) and the national research committee (Portuguese Data Protection Authority, ref. 9469/2012 and 8601/2014), and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The datasets generated and analysed during the current study are not publicly available given 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 patients were treated in the same institution and diagnosed in the same year, with some of the groups being small. Nonetheless, data are available from the corresponding author on reasonable request.

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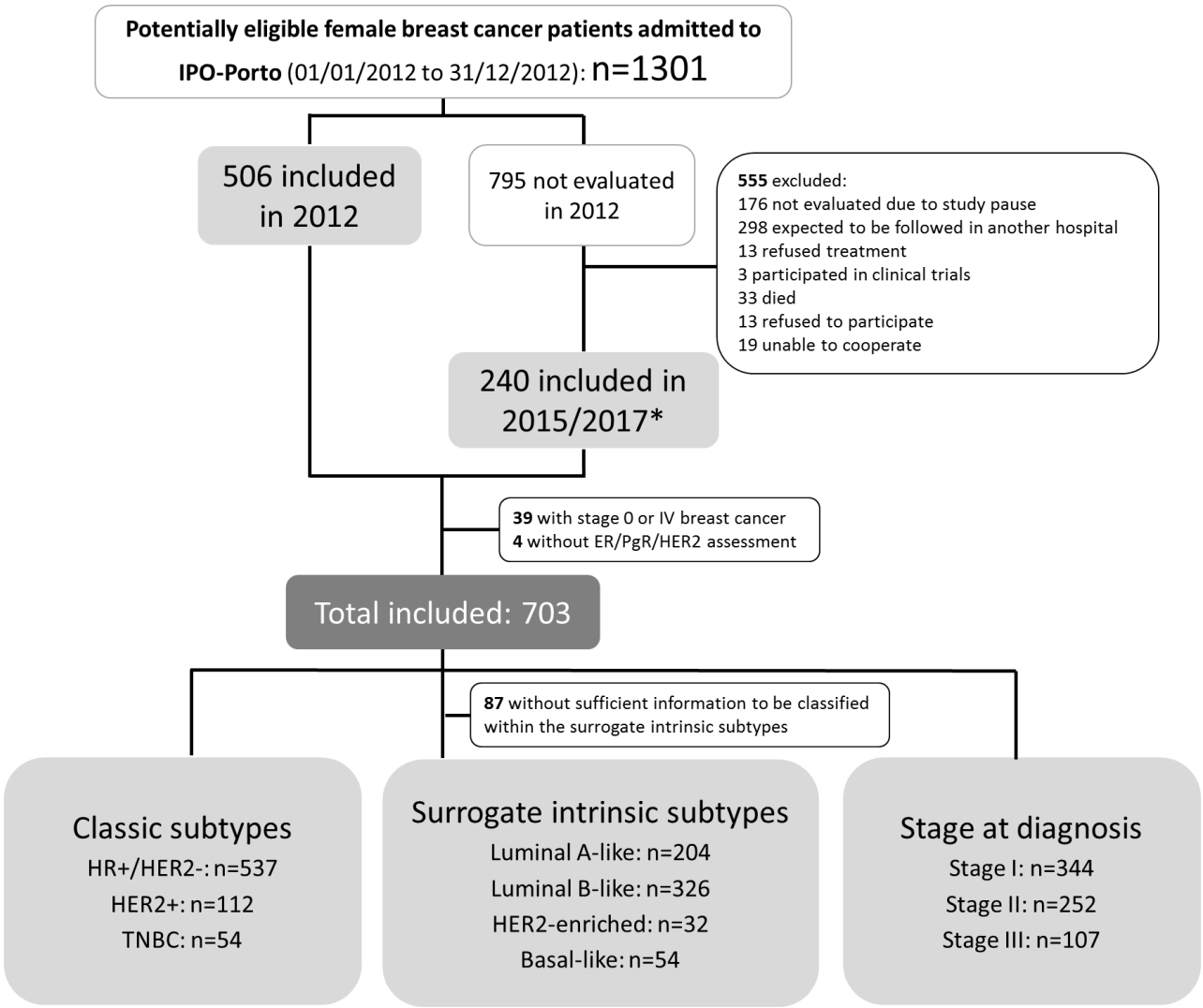
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Supplementary material

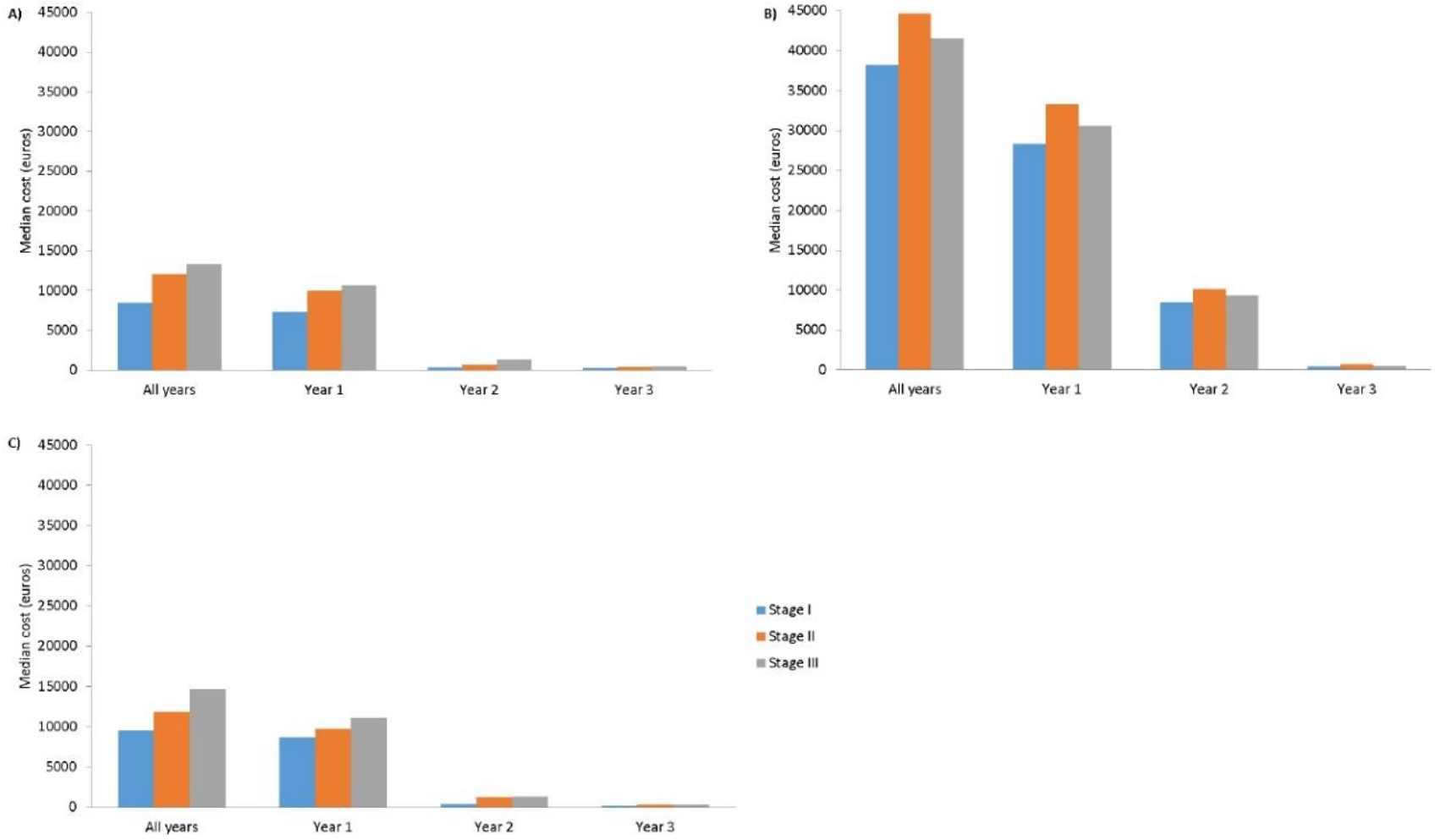
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Supplementary Figure 1 – Flowchart of patients included in the present study



*Participants not included at baseline (2012) due to: history of a previous primary cancer treated with chemotherapy and/or axillary/thoracic radiotherapy; submitted to breast surgery outside IPO-Porto; initial Montreal Cognitive Assessment score below 17 (or below 16 for women over 65 years old); being illiterate; presenting with a psychiatric illness (*e.g.,* bipolar disorder, schizophrenia, among others); other reasons (*e.g.,* previous breast surgery for benign conditions).
ER: estrogen receptor; HR: hormone receptor; IPO-Porto: Portuguese Institute of Oncology of Porto; PgR: progesterone receptor; TNBC: triple negative breast cancer

Supplementary Figure 2 – Median cost per year (all years; years one, two and three) and by stage (I, II and III) among patients with A) HR+/HER2-, B) HER2+ and C) TNBC



Median total cost of care (all years) among HR+/HER2- was: 8498€/patient for stage I, 12074€/patient for stage II and 13332€/patient for stage III. Among HER2+ patients: 38216€/patient for stage I, 44670€/patient for stage II and 41564€/patient for stage III. Among TNBC patients: 9566€/patient for stage I, 11824€/patient for stage II and 14645€/patient for stage III.
HR: hormone receptor; TNBC: triple-negative breast cancer

Supplementary Table 1 – Sociodemographic and clinical characteristics of breast cancer patients at baseline, according to the surrogate intrinsic subtypes' classification[a]

	Luminal A-like (N=204)	Luminal B-like (N=326)		HER2-enriched (N=32)		Basal-like (N=54)	
	N (%)	N (%)	p-value[b]	N (%)	p-value[c]	N (%)	p-value[d]
Age, years							
<50	52 (25.5)	115 (35.3)		6 (18.8)		26 (48.1)	
50-64	78 (38.2)	140 (42.9)		19 (59.4)		19 (35.2)	
≥65	74 (36.3)	71 (21.8)	**<0.001**	7 (21.9)	0.075	9 (16.7)	**0.002**
Education, years							
≤4	95 (51.6)	137 (44.6)		13 (44.8)		23 (46.0)	
5-9	43 (23.4)	80 (26.1)		9 (31.0)		16 (32.0)	
≥10	46 (25.0)	90 (29.3)	0.317	7 (24.1)	0.657	11 (22.0)	0.460
Net monthly income before diagnosis, euros							
≤500	96 (53.6)	167 (55.3)		17 (60.7)		27 (54.0)	
>500	83 (46.3)	135 (44.7)	0.723	11 (39.3)	0.484	23 (46.0)	0.963
Marital status							
Married or cohabitating	131 (71.2)	219 (71.3)		23 (79.3)		35 (70.0)	
Other[e]	53 (28.8)	88 (28.7)	0.974	6 (20.7)	0.364	15 (30.0)	0.869
Employment							
Employed	79 (42.9)	148 (48.2)		12 (41.4)		31 (62.0)	
Other[f]	105 (57.1)	159 (51.8)	0.257	17 (58.6)	0.875	19 (38.0)	0.017
Place of residence							
Porto Metropolitan Area	67 (36.4)	111 (36.5)		10 (35.7)		22 (44.0)	
Outside Porto Metropolitan Area	117 (63.6)	193 (63.5)	0.982	18 (64.3)	0.943	28 (56.0)	0.327
Breast cancer stage							
I	146 (71.6)	158 (48.5)		12 (37.5)		18 (33.3)	
II	48 (23.5)	114 (35.0)		11 (34.4)		25 (46.3)	
III	10 (4.9)	54 (16.6)	**<0.001**	9 (28.1)	**<0.001**	11 (20.4)	**<0.001**
Previous primary cancer diagnosis (including breast cancer)	17 (9.3)	26 (8.8)	0.836	3 (10.7)	0.818	3 (6.0)	0.456

The total may not add to 616 due to missing data. May not sum to 100.0% due to rounding.

[a] Surrogate intrinsic subtypes' classification: Luminal A-like (estrogen receptor [ER]-positive/progesterone receptor [PgR]-positive/HER2-negative and Ki67<15% or grade 1); Luminal B-like (ER-positive, HER2-negative and either PgR positivity <20% or Ki67≥15% or grade 3; or hormone receptors[HR]-positive and HER2-positive); HER2-enriched (HR-negative/HER2-positive); and Basal-like (HR-negative/HER2-negative)

[b] Luminal B-like *vs.* Luminal A-like.

[c] HER2-enriched *vs.* Luminal A-like.

[d] Basal-like *vs.* Luminal A-like.

[e] Other marital status includes: single, widowed and divorced women.

[f] Other employment includes: unpaid family workers, unemployed, housewives, retired and sick leave.

Supplementary Table 2 – Association between surrogate intrinsic subtype and healthcare use in the first three years following a breast cancer diagnosis in 2012

	Luminal A-like (N=204)	Luminal B-like (N=326)		HER2-enriched (N=32)			Basal-like (N=54)		
	N (%)	N (%)	aOR (95% CI)[a]	aOR (95% CI)[b]	N (%)	aOR (95% CI)[a]	aOR (95% CI)[b]	N (%)	aOR (95% CI)[a]	aOR (95% CI)[b]
Surgery										
Breast surgery[c]										
Total mastectomy (*vs.* Breast-conserving surgery)	76 (42.7)	154 (55.4)	1.83 (1.22-2.76)	1.30 (0.84-2.01)	17 (65.4)	2.59 (1.07-6.25)	1.63 (0.63-4.20)	25 (51.0)	1.82 (0.93-3.57)	0.96 (0.46-2.01)
Axillary surgery[d]										
Axillary dissection (*vs.* None or Sentinel lymph node biopsy)	23 (13.5)	108 (39.1)	4.61 (2.64-8.03)	2.53 (1.20-5.35)	12 (48.0)	6.79 (2.68-17.23)	3.14 (0.83-11.92)	19 (39.6)	4.90 (2.25-10.66)	1.12 (0.38-3.34)
Other surgeries/interventions (≥3 *vs.* <3)[e]	38 (18.6)	189 (58.0)	5.52 (3.53-8.63)	4.19 (2.60-6.75)	28 (87.5)	37.50 (10.55-133.21)	29.15 (7.84-108.44)	41 (75.9)	13.68 (6.17-30.31)	8.36 (3.61-19.38)
Systemic therapy										
Any chemotherapy (Yes *vs.* No)	33 (16.2)	249 (76.4)	22.56 (13.05-39.01)	25.57 (13.33-49.02)	32 (100.0)	--	--	44 (81.5)	28.33 (11.18-71.79)	19.12 (6.70-54.60)
Timing of chemotherapy										
Adjuvant (*vs.* Neoadjuvant)	30 (96.8)	223 (90.7)	0.37 (0.05-2.85)	0.27 (0.03-2.42)	27 (87.1)	0.29 (0.03-2.97)	0.23 (0.02-2.97)	38 (86.4)	0.23 (0.03-2.06)	0.19 (0.02-2.02)
Chemotherapy scheme										
Anthracycline-based (Yes *vs.* No)[f]	33 (100.0)	245 (98.4)	1.42 (0.15-13.57)	1.28 (0.13-12.45)	28 (87.5)	0.25 (0.02-2.60)	0.22 (0.02-2.42)	43 (97.7)	--	--
Hormone therapy (Yes *vs.* No)[g]	204 (100.0)	325 (99.7)	--	--	0 (0.0)	--	--	0 (0.0)	--	--
Targeted therapy (Yes *vs.* No)[h]	0 (0.0)	73 (22.4)	--	--	32 (100.0)	--	--	0 (0.0)	--	--
Other treatments										
Radiotherapy (Yes *vs.* No)	136 (66.7)	244 (74.8)	1.25 (0.82-1.90)	0.99 (0.64-1.53)	23 (71.9)	1.11 (0.45-2.71)	0.77 (0.30-1.98)	44 (81.5)	1.86 (0.83-4.18)	1.30 (0.55-3.03)
Brachytherapy (Yes *vs.* No)[i]	57 (41.9)	51 (20.9)	0.35 (0.22-0.57)	0.52 (0.31-0.85)	3 (13.0)	0.21 (0.06-0.76)	0.38 (0.10-1.45)	8 (18.2)	0.27 (0.11-0.66)	0.47 (0.18-1.22)
Healthcare use[j]										
Appointments (≥45 *vs.* <45)[k]	56 (27.5)	174 (53.1)	2.55 (1.70-3.84)	2.05 (1.34-3.14)	20 (62.5)	5.43 (2.25-13.07)	4.03 (1.61-10.07)	33 (61.1)	4.26 (2.11-8.61)	3.09 (1.48-6.43)
Hospitalizations (≥1 *vs.* <1)[l]	80 (39.2)	120 (36.8)	0.86 (0.59-1.27)	0.96 (0.64-1.42)	11 (34.4)	0.94 (0.42-2.12)	1.09 (0.48-2.49)	19 (35.2)	0.85 (0.44-1.63)	1.01 (0.51-2.00)
Genetic testing (Yes *vs.* No)[m]	16 (7.8)	42 (12.9)	1.14 (0.58-2.22)	1.27 (0.63-2.53)	3 (9.4)	0.89 (0.20-3.97)	1.05 (0.23-4.88)	9 (16.7)	1.15 (0.41-3.20)	1.34 (0.47-3.87)

The total may not add to 616 due to missing data. May not sum to 100.0% due to rounding. aOR: adjusted odds ratio; CI: confidence interval.

[a] Adjusted for age (continuous) and education (≤4, 5-9, ≥10 years).

[b] Further adjusted for stage (I, II, III).

[c] Patients who had breast-conserving surgery followed by mastectomy are included in the mastectomy group. Information regarding the type of breast surgery is not available for 81 patients who underwent surgery outside IPO-Porto.

[d] Patients who had sentinel lymph node biopsy followed by axillary lymph node dissection are included in the axillary dissection group. One patient did not receive any type of axillary surgery (included in the Luminal A-like subgroup). Information regarding the type of axillary surgery is not available for 81 patients who underwent surgery outside IPO-Porto.

[e] Other surgeries/interventions include breast reconstruction surgeries, central venous catheter placement/removal and other ambulatory surgeries/interventions. The cut-off used was determined as percentile 75 of the number of other surgeries/interventions among the Luminal A-like subgroup.

[f] Anthracycline-based chemotherapy includes: AC regimen: four or six cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2); FEC regimen: six cycles of concomitant 5-FU (500 mg/m^2), epirubicin (100 mg/m^2), and cyclophosphamide (500 mg/m^2); AC-T regimen: four cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by four cycles of docetaxel (100 mg/m^2); AC-paclitaxel regimen: four cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by four cycles of paclitaxel (80 mg/m^2); FEC-D regimen: three cycles of concomitant 5-FU (500 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2) followed by three cycles of docetaxel (100 mg/m^2).

[g] Among Luminal A-like: 27 (13.2%) only received tamoxifen; 42 (20.6%) only received aromatase inhibitors; 45 (22.1%) received both tamoxifen and aromatase inhibitors; 26 (12.7%) received tamoxifen and/or aromatase inhibitors, plus goserelin (for ovarian function suppression during adjuvant treatment) or fulvestrant (for relapsed disease); 64 (31.4%) unknown. Among Luminal B-like: 45 (13.9%) only received tamoxifen; 80 (24.6%) only received aromatase inhibitors; 46 (14.2%) received both tamoxifen and aromatase inhibitors; 66 (20.3%) received tamoxifen and/or aromatase inhibitors, plus goserelin (for ovarian function suppression during adjuvant treatment) or fulvestrant (for relapsed disease); 88 (27.1%) unknown.

[h] Targeted therapy includes trastuzumab.

[i] Among those who received radiotherapy (n=447).

[j] Does not include the number of laboratory and pathology tests, and imaging exams, for which only the total cost per patient was available (included in **Online Resource 4**).

[k] Appointments: number of outpatient visits (surgery, medical oncology, and radiation oncology, among others), and nursing, psychology and social services appointments. The cut-off used was determined as percentile 75 of the number of appointments among the Luminal A-like subgroup.

[l] The cut-off used was determined as percentile 75 of the number of hospitalizations among the Luminal A-like subgroup.

[m] Genetic testing includes: *BRCA* 1 mutations; *BRCA* 2 mutations (hereditary breast and ovarian cancer syndrome); deletions in *MLH1* (hereditary nonpolyposis colon cancer); *TP53* mutations (Li-Fraumeni syndrome).

Supplementary Table 3 – Association between surrogate intrinsic subtype and healthcare costs, in euros, in the first three years following a breast cancer diagnosis in 2012

	Luminal A-like (N=204) N (%)	Luminal B-like (N=326) N (%)	aOR (95% CI)[a]	aOR (95% CI)[b]	HER2-enriched (N=32) N (%)	aOR (95% CI)[a]	aOR (95% CI)[b]	Basal-like (N=54) N (%)	aOR (95% CI)[a]	aOR (95% CI)[b]
Surgery										
Surgeries[c,d], euros										
Median (P25-P75)	2054.55 (1249.58-2647.32)	2522.01 (2094.72-3411.29)			2684.49 (2522.01-3591.86)			2522.01 (2094.72-3654.94)		
≤1450[e]	96 (47.1)	67 (20.6)			2 (6.3)			8 (14.8)		
1451-2525	53 (26.0)	110 (33.7)	2.56 (1.58-4.16)	1.96 (1.18-3.24)	12 (37.5)	16.84 (2.07-136.77)	12.27 (1.48-101.68)	20 (37.0)	3.12 (1.21-8.03)	1.81 (0.67-4.84)
>2525	55 (27.0)	149 (45.7)	3.25 (2.03-5.18)	2.51 (1.55-4.07)	18 (56.3)	27.88 (3.59-216.75)	20.52 (2.58-162.87)	26 (48.1)	4.55 (1.87-11.07)	2.74 (1.08-6.92)
Systemic therapy										
Chemotherapy[d,f], euros										
Median (P25-P75)	0.00 (0.00-0.00)	254.98 (89.01-416.51)			383.53 (265.92-403.43)			397.58 (269.14-533.39)		
None (0)[e]	171 (83.8)	77 (23.6)			0 (0.0)			10 (18.5)		
≤350	15 (7.4)	97 (29.8)	17.98 (9.16-35.28)	20.33 (9.74-42.42)	10 (31.3)	--	--	7 (13.0)	9.84 (2.95-32.84)	7.64 (2.13-27.42)
>350	18 (8.8)	152 (46.6)	27.11 (13.98-52.58)	32.31 (14.78-70.63)	22 (68.8)	--	--	37 (68.5)	46.40 (16.94-127.06)	33.82 (10.67-107.02)
Hormone therapy[d,g], euros										
Median (P25-P75)	101.71 (76.92-121.58)	101.71 (72.53-120.79)			0.00 (0.00-0.00)			0.00 (0.00-0.00)		
≤80[e]	63 (30.9)	110 (33.7)			32 (100.0)			54 (100.0)		
81-120	84 (41.2)	133 (40.8)	1.27 (0.79-2.02)	1.20 (0.74-1.95)	0 (0.0)	--	--	0 (0.0)	--	--
>120	57 (27.9)	83 (25.5)	0.76 (0.47-1.24)	0.77 (0.46-1.28)	0 (0.0)	--	--	0 (0.0)	--	--
Targeted therapy[d], euros										
Median (P25-P75)	0.00 (0.00-0.00)	0.00 (0.00-0.00)			24007.14 (20580.12-29287.09)			0.00 (0.00-0.00)		
None (0)[e]	204 (100.0)	253 (77.6)			0 (0.0)			54 (100.0)		
≤26000	0 (0.0)	32 (9.8)	--	--	21 (65.6)	--	--	0 (0.0)	--	--
>26000	0 (0.0)	41 (12.6)	--	--	11 (34.4)	--	--	0 (0.0)	--	--
Day hospital[d,h], euros										
Median (P25-P75)	0.00 (0.00-344.33)	590.28 (393.52-2164.36)			2164.36 (2115.17-2164.36)			590.28 (590.28-590.28)		

None (0)[e]	151 (74.0)	71 (21.8)			0 (0.0)			10 (18.5)		
≤900	27 (13.2)	136 (41.7)	14.24 (7.97-25.46)	12.33 (6.57-23.13)	1 (3.1)	--	--	42 (77.8)	24.06 (9.54-60.68)	13.11 (4.71-36.52)
>900	26 (12.7)	119 (36.5)	13.64 (7.07-26.30)	12.49 (6.19-25.18)	31 (96.9)	--	--	2 (3.7)	0.74 (0.13-4.12)	0.45 (0.08-2.68)
Radiotherapy[d,i], euros										
Median (P25-P75)	2613.25 (0.00-3185.25)	2632.05 (0.00-3278.03)			2613.25 (0.00-2973.84)			2649.30 (2569.82-3401.36)		
None (0)[e]	72 (35.3)	85 (26.1)			9 (28.1)			10 (18.5)		
≤2900	66 (32.4)	130 (39.9)	1.37 (0.86-2.19)	0.88 (0.53-1.45)	15 (46.9)	1.60 (0.61-4.19)	0.87 (0.30-2.50)	24 (44.4)	2.25 (0.94-5.40)	1.15 (0.45-2.97)
>2900	66 (32.4)	111 (34.0)	1.22 (0.76-1.95)	1.13 (0.70-1.83)	8 (25.0)	0.82 (0.27-2.45)	0.74 (0.24-2.28)	20 (37.0)	1.81 (0.74-4.45)	1.58 (0.63-4.01)
Healthcare use										
Appointments[d], euros										
Median (P25-P75)	1000.50 (769.50-1224.00)	1248.50 (983.00-1626.00)			1415.50 (1050.00-1789.00)			1374.50 (1084.00-1676.00)		
≤850[e]	67 (32.8)	43 (13.2)			3 (9.4)			8 (14.8)		
851-1125	70 (34.3)	79 (24.2)	1.43 (0.82-2.49)	1.37 (0.78-2.40)	8 (25.0)	2.27 (0.43-11.91)	2.46 (0.46-13.06)	8 (14.8)	0.76 (0.22-2.60)	0.67 (0.19-2.32)
>1125	67 (32.8)	204 (62.6)	3.87 (2.24-6.66)	2.90 (1.65-5.08)	21 (65.6)	8.59 (1.85-39.95)	6.15 (1.29-29.43)	38 (70.4)	4.96 (1.79-13.76)	2.92 (1.02-8.39)
Hospitalization[d], euros										
Median (P25-P75)	1694.78 (1434.07-1786.64)	1694.78 (1434.07-2566.51)			1694.78 (1434.07-3351.98)			1694.78 (1434.07-3000.90)		
None (0)[e]	81 (39.7)	104 (31.9)			12 (37.5)			21 (38.9)		
≤1700	75 (36.8)	110 (33.7)	1.27 (0.82-1.97)	0.90 (0.57-1.43)	8 (25.0)	0.78 (0.28-2.17)	0.45 (0.15-1.33)	17 (31.5)	0.97 (0.45-2.05)	0.52 (0.23-1.19)
>1700	48 (23.5)	112 (34.4)	1.81 (1.13-2.91)	1.48 (0.91-2.40)	12 (37.5)	2.04 (0.81-5.12)	1.43 (0.55-3.74)	16 (29.6)	1.31 (0.60-2.87)	0.90 (0.39-2.04)
Genetic testing[d], euros										
Median (P25-P75)	0.00 (0.00-0.00)	0.00 (0.00-0.00)			0.00 (0.00-0.00)			0.00 (0.00-0.00)		
None (0)[e]	188 (92.2)	284 (87.1)			29 (90.6)			45 (83.3)		
≤550	3 (1.5)	14 (4.3)	1.97 (0.53-7.32)	1.88 (0.49-7.27)	0 (0.0)	--	--	2 (3.7)	1.40 (0.21-9.48)	1.39 (0.20-9.84)
>550	13 (6.4)	28 (8.6)	0.94 (0.45-1.98)	1.11 (0.52-2.38)	3 (9.4)	1.12 (0.25-5.00)	1.51 (0.31-7.29)	7 (13.0)	1.10 (0.36-3.36)	1.37 (0.43-4.40)
Other medical expenses[d,i], euros										
Median (P25-P75)	357.27 (181.91-763.00)	830.99 (423.89-1405.74)			908.02 (747.39-1723.02)			883.83 (579.64-1217.50)		
≤250[e]	76 (37.3)	41 (12.6)			1 (3.1)			4 (7.4)		
251-600	61 (29.9)	74 (22.7)	2.32 (1.36-3.96)	2.23 (1.30-3.81)	2 (6.3)	--	--	12 (22.2)	4.11 (1.07-15.74)	3.62 (0.94-14.04)

>600	67 (32.8)	211 (64.7)	6.09 (3.70-10.03)	4.45 (2.61-7.57)	29 (90.6)	--	--	38 (70.4)	13.41 (3.91-46.05)	6.80 (1.87-24.66)
Imaging[d], euros										
Median (P25-P75)	392.35 (217.70-566.35)	771.74 (480.60-1194.73)			1264.75 (952.65-1677.03)			821.91 (456.59-1099.03)		
≤300[e]	72 (35.3)	34 (10.4)			1 (3.1)			5 (9.3)		
301-525	62 (30.4)	57 (17.5)	1.90 (1.07-3.35)	1.85 (1.05-3.28)	2 (6.3)	--	--	9 (16.7)	1.65 (0.44-6.18)	1.44 (0.38-5.47)
>525	70 (34.3)	235 (72.1)	6.54 (3.89-10.99)	4.96 (2.87-8.56)	29 (90.6)	--	--	40 (74.1)	9.10 (3.00-27.55)	4.66 (1.46-14.91)
Total cost[d], euros										
Median (P25-P75)	8232.22 (6390.76-10930.53)	12492.95 (8722.68-22236.64)			38296.99 (34509.61-42529.39)			11224.27 (9157.58-14644.81)		
≤7150[e]	69 (33.8)	45 (13.8)			0 (0.0)			4 (7.4)		
7151-10250	67 (32.8)	55 (16.9)	1.19 (0.68-2.09)	1.19 (0.68-2.10)	0 (0.0)	--	--	18 (33.3)	5.19 (1.38.19.54)	5.17 (1.34-19.93)
>10250	68 (33.3)	226 (69.3)	4.74 (2.76-8.12)	3.43 (1.96-6.00)	32 (100.0)	--	--	32 (59.3)	9.18 (2.44-34.57)	4.28 (1.09-16.77)

The total may not add to 616 due to missing data. May not sum to 100.0% due to rounding. aOR: adjusted odds ratio; CI: confidence interval.
For items: Surgeries, Hormone therapy, Appointments, Hospitalization, Other medical expenses, Imaging and Total cost, the cut-offs used to define each category were determined as percentiles 33 and 66 of the respective cost among the Luminal A-like subgroup. For items: Chemotherapy, Targeted therapy, Day hospital, Radiotherapy and Genetic testing, categories were defined as none (0) and the median cost among the Luminal A-like subgroup (among those who received the respective item) was used as the cut-off to define the other two categories.

[a] Adjusted for age (continuous) and education (≤4, 5-9, ≥10 years).

[b] Further adjusted for stage (0/I, II, III).

[c] All surgeries/interventions, including breast surgeries and axillary surgeries. Imputed surgery cost for 81 patients who underwent surgery outside IPO-Porto.

[d] Dependent variable in the multinomial logistic regression model.

[e] Category being used as reference in the multinomial logistic regression model.

[f] Imputed chemotherapy cost for three patients who received chemotherapy outside IPO-Porto.

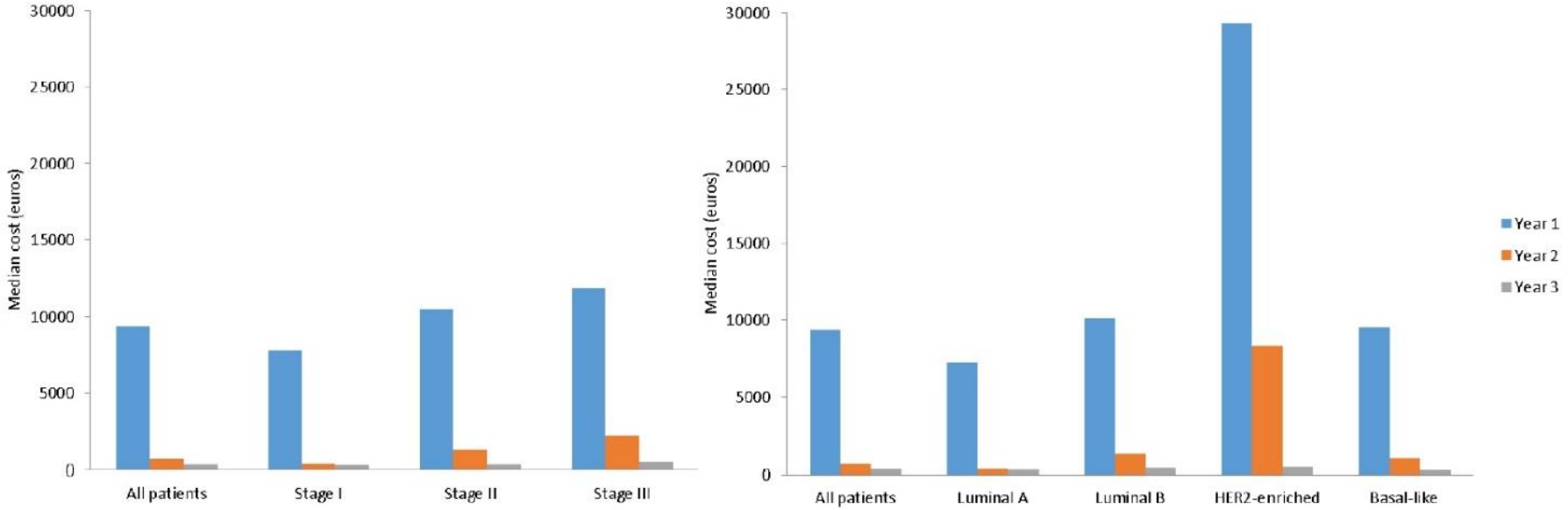
[g] Imputed targeted therapy cost for one patient who received targeted therapy outside IPO-Porto.

[h] Day hospital sessions include the administration of intravenous, subcutaneous and/or intramuscular therapy (*i.e.* chemotherapy, trastuzumab, goserelin and fulvestrant).

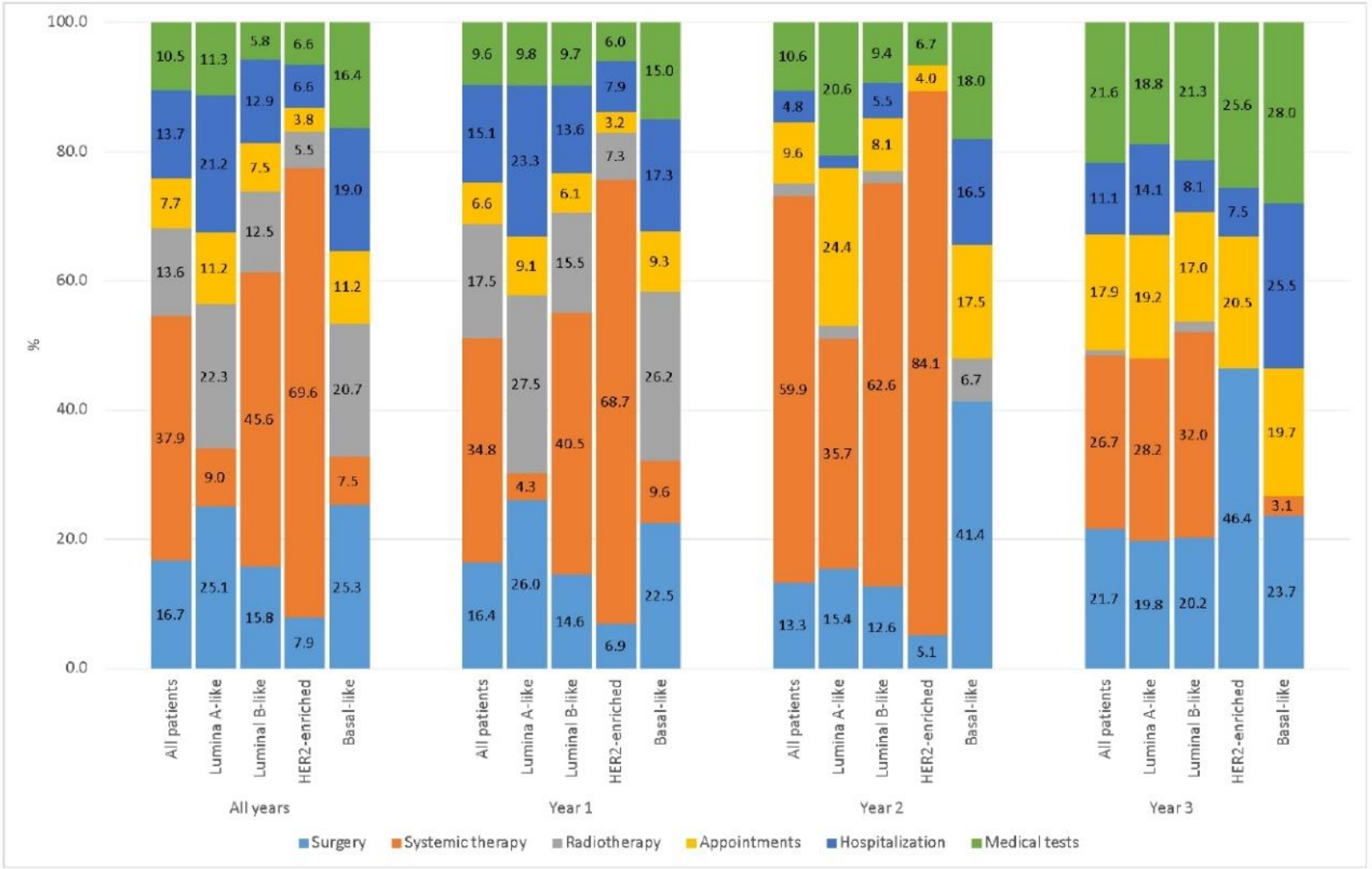
[i] The cost of brachytherapy for the seven patients who only received brachytherapy (and not external radiotherapy) is included in Hospitalization costs and not in Radiotherapy costs.

[j] Other medical expenses include laboratory and pathology tests, and blood transfusions.

Supplementary Figure 3 – Median cost per year (one, two and three) for all patients and by stage (left) and intrinsic surrogate subtype (right)

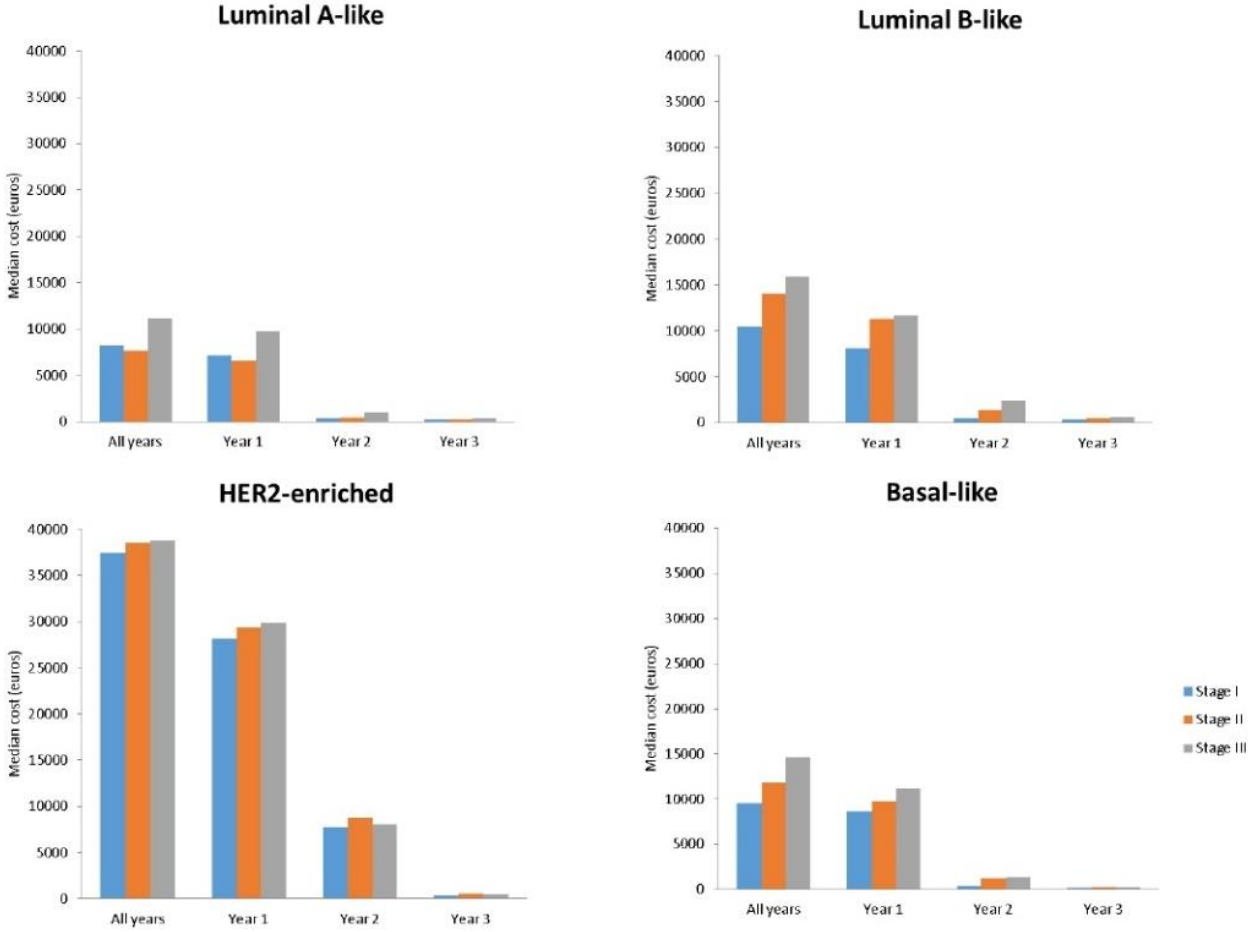


Supplementary Figure 4 – Proportion of the median cost attributed to Surgery, Systemic therapy, Radiotherapy, Appointments, Hospitalization and Medical tests among all patients, by year after breast cancer diagnosis (all years; years one, two and three) and by surrogate intrinsic subtype



"Systemic therapy" includes chemotherapy, targeted therapy, hormone therapy and day hospital use. "Medical tests" includes laboratory and pathology tests, blood transfusions, imaging and genetic testing. When the proportion of the median cost of a certain item was ≤2% of the overall cost, the respective label was deleted from the figure.

Supplementary Figure 5 – Median cost per year (all years; years one, two and three) and by stage (I, II and III) among patients with Luminal-A like, Luminal B-like, HER2-enriched and Basal-like breast cancer



Median total cost of care (all years) among Luminal A-like was: 8,232€/patient for stage I, 7,683€/patient for stage II and 11,183€/patient for stage III. Among Luminal B-like: 10,491€/patient for stage I, 14,068€/patient for stage II and 15,892€/patient for stage III. Among HER2-enriched: 37,465€/patient for stage I, 38,475€/patient for stage II and 38,742€/patient for stage III. Among Basal-like: 9,566€/patient for stage I, 11,824€/patient for stage II and 14,645€/patient for stage III.

Paper IV: *Comparing the cost of non-metastatic breast cancer care in a low-income vs a high-income country: a plea for an optimal allocation of health resources in Sub-Saharan Africa*

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Short communication

Comparing the cost of non-metastatic breast cancer care in a low-income *vs* a high-income country: A plea for an optimal allocation of health resources in Sub-Saharan Africa

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Costs and cost analysis
Cancer staging

ABSTRACT

Breast cancer incidence is rising in low-income countries, but there is limited information regarding health resource allocation for its care. We assessed the cost of care during the first three years after diagnosis in a low-income country (Mozambique; n = 162 women) and compared it with a high-income country (Portugal, n = 703 women). Local currency prices were converted to 2019 international dollars (Int$). In Mozambique, the median cost was lower than in Portugal (2888 *vs* 18,533 Int$, respectively) and did not vary across stage or tumor subtype. These findings may help improving resource allocation for breast cancer care in Sub-Saharan Africa, despite reflecting an underfunding of treatment in this setting.
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1. Introduction

Globally, the number of women with breast cancer is increasing, especially in low/middle-income countries [1]. Moreover, the cost of each patient's cancer care has also been rising, due to the availability of new treatments and technologies [2]. In the European Union, breast cancer accounted for the highest cancer-related healthcare costs in 2009 [3] and limited data from developing countries show a rising cost with higher cancer stage [4]. As the case number and the costs of new treatment options for breast cancer increase globally [5], its financial burden will grow worldwide. Hence, estimating this cost is important, particularly in low/middle-income settings, where it is ill-defined, to design public policies on resource allocation.

Therefore, we assessed the cost of care of women with non-metastatic breast cancer during the first three years after diagnosis in a low-income country (Mozambique, Eastern Sub-Saharan Africa), and compared it with a high-income country (Portugal, Southern Europe).

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2. Methods

We used data from women with newly diagnosed non-metastatic breast cancer (stage I-III), enrolled in two prospective cohort studies, the Moza-BC (n = 162 participants) and the NEON-BC (n = 703) cohorts [6–8]. Briefly, the Moza-BC cohort included women diagnosed during 2015–2017 in the three largest hospitals in Mozambique (Maputo, Beira and Nampula Central Hospitals) and followed at the Maputo Central Hospital. The NEON-BC cohort enrolled women in 2012 at the Portuguese Institute of Oncology of Porto, the largest cancer-dedicated hospital in Portugal [9]. Both cohorts collected patients' baseline sociodemographic and clinico-pathological data, diagnostic procedures, and treatments received for at least three years after diagnosis. Treatment decisions were usually based on the European Society for Medical Oncology guidelines [10], though adapted to each country's resources. In both countries, chemotherapy usually consisted of a combined anthracycline-taxane based regimen. Nonetheless, there were occasional treatment interruptions/adjustments in Mozambique due to drug supply shortages. Radiotherapy, trastuzumab and aromatase inhibitors were not available at the Maputo Central Hospital, but some Mozambican patients (n = 9 [6%]) received radiotherapy outside the country.

Staging was defined by the AJCC TNM 7th edition classification [11] and tumors were classified into the classic subtype definition of hormone receptor (HR)-positive/HER2-negative, HER2-positive and triple-negative (HR-negative/HER2-negative) [12,13].

In the Portuguese cohort, healthcare costs were provided in Euros, as previously reported [7]. In Mozambique, healthcare costs were given by the Maputo Central Hospital and/or retrieved from the World Health Organization-CHOICE estimates [14].

In both cohorts, costs of care during the first three years after diagnosis were computed for each individual patient. To improve the comparability between these two countries, with different economic backgrounds and discrepancies in the cost of healthcare components, local currency prices were then converted to 2019 international dollars (Int$) using purchasing power parity exchange rates for household consumption [15]. This indicator, retrieved from the World Bank, measures the amount of local currency units required to buy the same amounts of goods and services in the domestic market as US dollars would buy in the United States.

2.1. Statistical analysis

Baseline clinico-pathological characteristics and healthcare costs were compared between the two cohorts using the chi-square test and the Wilcoxon rank-sum test, respectively. All tests were two-sided and a p-value of <0.05 was considered significant.

3. Results and discussion

Patients from Mozambique were younger and presented with a higher stage at diagnosis compared to Portuguese women (68% *vs* 15% with stage III, respectively) – Supplemental Table 1.

The median cost of care was 18,533 Int$ *vs* 2888 Int$ among Portuguese and Mozambican patients, respectively – Fig. 1. Among Portuguese women, the median cost of care rose with increasing stage (from 14,831 Int$ in stage I to 24,159 Int$ in stage III), but was stable in Mozambique. There was a great disparity in the cost of care in Portugal according to tumor subtypes, with a median value of 66,811 Int$/patient with a HER2-positive tumor, which was mostly due to the use of trastuzumab. The cost of treatment did not vary across subtypes in Mozambique, as trastuzumab is unavailable.

Regarding the proportion of resources allocated to each component of care, nearly half of Mozambican resources were used in medical tests and only 22% in systemic therapy – Fig. 2. In Portugal, systemic therapy accounted for 35%, while medical tests for 11% of the healthcare cost, with variations across subtypes. Overall, surgery had a similar "weight" in Portugal and Mozambique (17% and 18% of the total cost, respectively). Additional data regarding healthcare costs according to subtypes and stage is available in Supplemental Tables 2 and 3.

This comparison brought three major findings. First, the median cost of care is six-fold higher in Portugal compared to Mozambique, even though both countries have universal health coverage and prices were normalized by using Int$. Second, stage and subtypes led to significant cost differences in the high-income country (as expected) [4,7], but they had virtually no impact in health resource allocation in the low-income country. Third, there was a disproportionate weight of medical tests among the resources spent in Mozambique, which may be due to the high cost of reagents and other lab materials in Africa [16].

These findings reflect the difficulties in the regular assessment of tumor subtypes and the low access to radiotherapy and targeted agents in Mozambique, which impair the adequacy of care according to the patient's stage and breast tumor subtype. Ultimately, they reflect the underfunding of breast cancer care on the "treatment side" in this low-income setting. The likely cost impact of stage migration (stage III to stage IV), due to under-staging in Mozambique, is probably balanced by the limited treatment options for metastatic breast cancer, which do not meaningfully

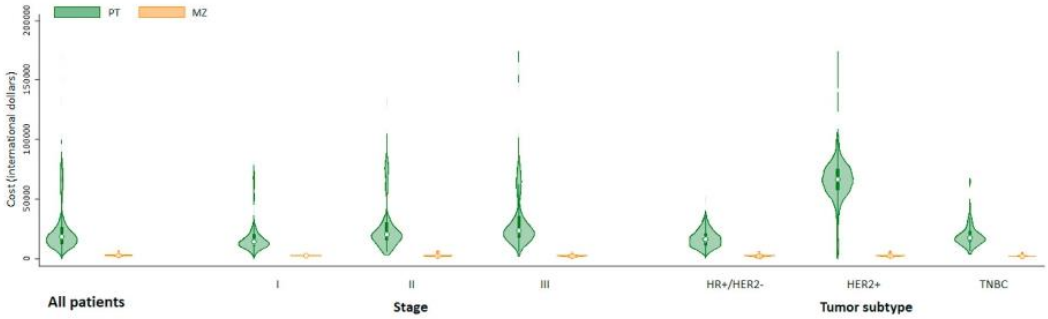


Fig. 1. Distribution of the total cost of non-metastatic breast cancer care during the first three years after diagnosis in 2019 international dollars for all patients, by stage and by tumor subtypes, among patients from Portugal (PT) and Mozambique (MZ).

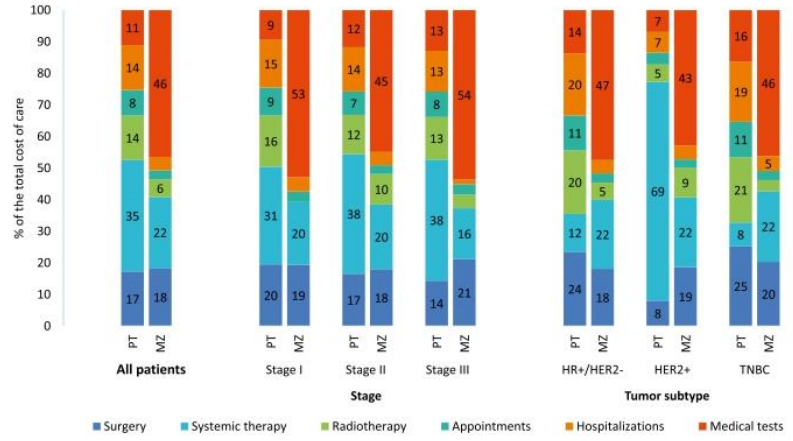


Fig. 2. Proportion (%) of the total cost attributed to each component of care among all patients, by stage and by tumors subtypes, among patients from Portugal (PT) and from Mozambique (MZ).

influence the overall cost of care.

To the best of our knowledge, this is the first comprehensive assessment of health resources use and cost of care of patients with non-metastatic breast cancer in a Sub-Saharan African country. Despite the still small share of cancer in the global disease burden in Sub-Saharan Africa and the existence of competing priorities from communicable diseases (now also including COVID-19), we should keep in mind that breast cancer incidence is rapidly rising in the continent [17]. Thus, we hope that these real-world data can be used for economic evaluations of new treatment options and to improve resource allocation for breast cancer care in this region. This is especially relevant given that allocating the adequate resources for an affordable and effective treatment of non-metastatic breast cancer is key to improve the survival outcomes of these women.

Ethics Committee approval

The NEON-BC cohort study was approved by the Ethics Committee of the Portuguese Institute of Oncology of Porto (IPO-Porto; ref. CES 406/011 and CES 99/014) and by the Portuguese Data Protection Authority (ref. 9469/2012 and 8601/2014). The Moza-BC cohort study was approved by the National Health Bioethical Committee of Mozambique (ref. 226/CNBS/15). In both studies, all participants provided written informed consent.

Funding sources

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Data statement

The datasets generated and analysed during the current study are not publicly available given that the included patients did not specifically provide their consent for public sharing of their data. Additionally, even if possible, anonymization is partially impaired by the fact that patients in each cohort were treated in the same institution and diagnosed within a restricted period of time, with some of the groups being small. Nonetheless, data are available from the corresponding author on reasonable request.

Authors' contributions

Conceptualization: MB, AG, SP, CC, NL. Data curation: MB, AG, CC. Formal analysis: SM. Investigation and methodology: all authors. Supervision: NL. Writing – original draft: MB. Writing – review & editing: all authors.

Declaration of competing interest

MB: travel grant and speaker honorarium from Roche/GNE; all other authors: no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2021.02.010.

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Supplementary Tables

"Comparing the cost of non-metastatic breast cancer care in a low-income *vs* a high-income country: a plea for an optimal allocation of health resources in Sub-Saharan Africa"

Supplemental Table 1. Sociodemographic and clinical characteristics at baseline of patients diagnosed in Portugal or Mozambique, according to classic breast cancer subtypes.[a]

	ALL			HR+/HER2-			HER2+			TNBC		
	PT (N=703) N (%)	MZ (N=162) N (%)	p-value[b]	PT (N=537) N (%)	MZ (N=73) N (%)	p-value[b]	PT (N=112) N (%)	MZ (N=39) N (%)	p-value[b]	PT (N=54) N (%)	MZ (N=31) N (%)	p-value[b]
Age, years												
<50	234 (33.3)	83 (51.2)		165 (30.7)	36 (49.3)		43 (38.4)	20 (51.3)		26 (48.1)	17 (54.8)	
50-64	293 (41.7)	54 (33.3)		228 (42.5)	25 (34.2)		46 (41.1)	15 (38.5)		19 (35.2)	10 (32.3)	
≥65	176 (25.0)	25 (15.4)	**<0.001**	144 (26.8)	12 (16.4)	**0.005**	23 (20.5)	4 (10.3)	0.233	9 (16.7)	4 (12.9)	0.816
Education, years												
≤4	305 (46.5)	52 (37.4)		243 (48.3)	23 (39.0)		39 (37.9)	10 (28.6)		23 (46.0)	8 (28.6)	
5-9	172 (26.2)	51 (36.7)		124 (24.6)	21 (35.6)		32 (31.1)	14 (40.0)		16 (32.0)	13 (46.4)	
≥10	179 (27.3)	36 (25.9)	**0.001**	136 (27.0)	15 (25.4)	0.176	32 (31.1)	11 (31.4)	0.532	11 (22.0)	7 (25.0)	0.294
Missing	*47*	*23*		*34*	*14*		*9*	*4*		*4*	*3*	
Place of residence[c]												
Metropolitan Area	242 (37.2)	111 (72.5)		181 (36.3)	50 (72.5)		39 (38.2)	25 (67.6)		22 (44.0)	23 (82.1)	
Outside Metropolitan Area	409 (62.8)	42 (27.4)	**<0.001**	318 (63.6)	19 (27.5)	**<0.001**	63 (61.8)	12 (32.4)	**0.002**	28 (56.0)	5 (17.9)	**0.001**
Missing	*52*	*9*		*38*	*4*		*10*	*2*		*4*	*3*	
Breast cancer stage												
I	344 (48.9)	4 (2.5)		283 (52.7)	1 (1.4)		43 (38.4)	1 (2.6)		18 (33.3)	1 (3.2)	
II	252 (35.8)	48 (29.6)		181 (33.7)	14 (19.2)		46 (41.1)	15 (38.5)		25 (46.3)	12 (38.7)	
III	107 (15.2)	110 (67.9)	**<0.001**	73 (13.6)	58 (79.5)	**<0.001**	23 (20.5)	23 (59.0)	**<0.001**	11 (20.4)	18 (58.1)	**<0.001**

May not sum to 100.0% due to rounding. HR: hormone receptors; MZ: Mozambique; PT: Portugal; TNBC: triple negative breast cancer.

[a] Classic subtypes were defined according to the assessment of the estrogen receptor, the progesterone receptor and HER2 status on the surgical specimen (preferably) or on the biopsy core specimen (when the surgical specimen's pathology report was unavailable). Hormone receptor (HR)+/HER2-: estrogen receptor and/or progesterone receptor positive (expression in ≥1% of cells) and HER2-negative (a score of 0 or 1+ in immunohistochemistry, or a score of 2+ with a non-amplified FISH test result); HER2+: HER2-positive (a score of 3+ in immunohistochemistry or a score of 2 with an amplified FISH test result), regardless of the estrogen and progesterone receptor status; triple negative breast cancer: estrogen receptor negative, progesterone receptor negative and HER2-negative. Breast cancer subtype information available for 703 Portuguese women and 143 Mozambican women.

[b] The chi-square test was used to compare categories across groups. Missing values not included in comparisons.

[c] In Portugal: Metropolitan Area – Porto Metropolitan Area, Outside Metropolitan Area – Outside the Porto Metropolitan Area; in Mozambique: Metropolitan Area – Maputo City, Outside Metropolitan Area – South, Centre and North.

Supplemental Table 2. Median healthcare costs, in 2019 international dollars, in the first three years following a breast cancer diagnosis in Portuguese and Mozambican women, according to stage.

	STAGE I			STAGE II			STAGE III		
	PT (N=344) Median (P25-P75)	**MZ** (N=4) Median (P25-P75)	**p-value**[a]	**PT** (N=252) Median (P25-P75)	**MZ** (N=48) Median (P25-P75)	**p-value**[a]	**PT** (N=107) Median (P25-P75)	**MZ** (N=110) Median (P25-P75)	**p-value**[a]
Surgery[b]	3869.77 (2040.33-4621.61)	615.45 (615.45-615.45)	**<0.001**	4058.86 (3632.67-5882.52)	615.45 (615.45-615.45)	**<0.001**	4320.35 (4058.86-4581.65)	615.45 (615.45-615.45)	**<0.001**
Systemic therapy									
Chemotherapy[c]	0.00 (0.00-189.94)	330.10 (236.41-366.85)	0.051	605.29 (243.37-694.64)	284.18 (173.38-376.12)	**<0.001**	656.87 (401.23-710.74)	312.36 (199.82-414.46)	**<0.001**
Hormone therapy	144.75 (115.96-189.29)	55.86 (27.89-113.77)	0.050	153.47 (106.11-190.19)	0.00 (0.00-144.64)	**<0.001**	138.12 (102.58-175.71)	35.46 (0.00-122.44)	**<0.001**
Targeted therapy (trastuzumab)[d]	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.470	0.00 (0.00-0.00)	0.00 (0.00-0.00)	**0.002**	0.00 (0.00-0.00)	0.00 (0.00-0.00)	**<0.001**
Day hospital[e]	0.00 (0.00-949.98)	260.53 (244.25-260.53)	0.661	949.98 (870.82-3483.27)	260.53 (195.40-260.53)	**<0.001**	1266.64 (949.98-3483.27)	260.53 (195.40-293.10)	**<0.001**
Radiotherapy[f]	4235.96 (0.00-5286.93)	0.00 (0.00-0.00)	**0.021**	4213.26 (0.00-5168.77)	0.00 (0.00-0.00)	**<0.001**	4235.96 (4166.96-4326.72)	0.00 (0.00-0.00)	**<0.001**
Healthcare use									
Appointments	1686.62 (1384.87-2170.24)	108.33 (94.44-119.44)	**<0.001**	2076.09 (1652.02-2594.31)	88.88 (72.22-105.55)	**<0.001**	2446.25 (2050.34-3057.81)	88.88 (66.66-105.55)	**<0.001**
Hospitalization	2727.54 (1620.29-3240.56)	142.08 (142.08-142.08)	**<0.001**	2727.54 (2307.96-4863.40)	142.08 (142.08-142.08)	**<0.001**	2727.54 (2727.54-5798.56)	142.08 (142.08-142.08)	**<0.001**
Genetic testing[g]	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.483	0.00 (0.00-0.00)	0.00 (0.00-0.00)	**0.005**	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.001
Other medical expenses[h]	629.39 (332.01-1293.58)	1266.52 (1118.90-1386.40)	0.099	1530.83 (988.43-2417.53)	1054.51 (842.52-1252.11)	**<0.001**	2070.94 (1345.15-3054.16)	1012.28 (801.44-1227.69)	**<0.001**
Imaging	746.44 (455.32-1200.39)	419.64 (382.78-456.50)	0.055	1358.43 (897.44-1874.04)	382.78 (309.06-456.50)	**<0.001**	1546.30 (1063.83-2504.22)	382.78 (309.06-456.50)	**<0.001**
Total cost	14830.86 (11697.01-21110.88)	3183.44 (2878.48-3444.74)	**<0.001**	20952.95 (16214.62-30889.68)	2901.64 (2560.55-3323.91)	**<0.001**	24158.90 (18459.46-36259.22)	2864.28 (2353.43-3326.06)	**<0.001**

MZ: Mozambique; PT: Portugal. P25: 25th percentile. P75: 75th percentile.

[a] The Wilcoxon rank-sum test was used to compare groups.

[b] All surgeries/interventions, including breast surgeries and axillary surgeries. Imputed surgery cost for 87 patients who underwent surgery outside IPO-Porto.

[c] Imputed chemotherapy cost for three patients who received chemotherapy outside IPO-Porto and three patients who received chemotherapy outside the Maputo Central Hospital.

[d] Targeted therapy was only available in Portugal (IPO-Porto). Imputed targeted therapy cost for one patient who received targeted therapy outside IPO-Porto. Number of women from Portugal receiving trastuzumab: 40 (11.6%) women in Stage I – median (P25-P75) cost: 39160.52 (33121.13-45144.91); 43 (17.1%) women in Stage II – median (P25-P75) cost: 45265.17 (38216.68-54140.31); and 22 (20.6%) women in Stage III - median (P25-P75) cost: 42096.26 (36363.76-48176.18).

[e] Day hospital sessions include the administration of intravenous, subcutaneous and/or intramuscular therapy (*i.e.* chemotherapy, trastuzumab, goserelin and fulvestrant).

[f] The cost of brachytherapy for the seven patients in PT who only received brachytherapy (and no external radiotherapy) is included in Hospitalization costs and not in Radiotherapy costs.

[g] Genetic testing was only available in Portugal (IPO-Porto). Number of women from Portugal receiving genetic testing: 38 (11.0%) women in Stage I – median (P25-P75) cost: 886.12 (886.12-886.12); 36 (14.3%) women in Stage II – median (P25-P75) cost: 886.12 (886.12-886.12); and 10 (9.3%) women in Stage III – median (P25-P75) cost: 781.51 (386.25-886.12).

[h] Other medical expenses include laboratory and pathology tests, and blood transfusions.

Supplemental Table 3. Median healthcare costs, in 2019 international dollars, in the first three years following a breast cancer diagnosis in Portuguese and Mozambican women, according to classic breast cancer subtypes.[a]

	HR+/HER2-			HER2+			TNBC		
	PT (N=537) Median (P25-P75)	**MZ** (N=73) Median (P25-P75)	**p-value**[b]	**PT** (N=112) Median (P25-P75)	**MZ** (N=39) Median (P25-P75)	**p-value**[b]	**PT** (N=54) Median (P25-P75)	**MZ** (N=31) Median (P25-P75)	**p-value**[b]
Surgery[c]	4058.86 (2307.95-4636.51)	615.45 (615.45-615.45)	**<0.001**	4320.35 (4041.15-6340.40)	615.45 (615.45-615.45)	**<0.001**	4058.86 (3371.18-5882.16)	615.45 (615.45-615.45)	**<0.001**
Systemic therapy									
Chemotherapy[d]	158.28 (0.00-626.75)	227.53 (160.65-403.58)	**0.011**	621.15 (427.97-664.38)	356.11 (250.24-402.87)	**<0.001**	639.86 (433.15-858.42)	279.61 (223.34-405.88)	**<0.001**
Hormone therapy	163.69 (117.92-194.40)	95.06 (0.00-144.55)	**<0.001**	126.19 (0.00-181.08)	37.24 (0.00-126.18)	**<0.001**	0.00 (0.00-0.00)	0.00 (0.00-0.00)	--
Targeted therapy (trastuzumab)[e]	0.00 (0.00-0.00)	0.00 (0.00-0.00)	--	40243.33 (34366.06-47973.52)	0.00 (0.00-0.00)	**<0.001**	0.00 (0.00-0.00)	0.00 (0.00-0.00)	--
Day hospital[f]	633.32 (0.00-949.98)	227.96 (195.40-293.10)	**0.018**	3483.27 (3483.27-3641.60)	260.53 (227.96-260.53)	**<0.001**	949.98 (949.98-949.98)	260.53 (195.40-260.53)	**<0.001**
Radiotherapy[g]	4228.39 (0.00-5215.07)	0.00 (0.00-0.00)	**<0.001**	4209.48 (0.00-5268.01)	0.00 (0.00-0.00)	**<0.001**	4263.72 (4135.80-5474.06)	0.00 (0.00-0.00)	**<0.001**
Healthcare use									
Appointments	1833.08 (1462.92-2406.02)	88.88 (66.66-111.11)	**<0.001**	2204.84 (1813.77-2873.54)	88.88 (66.66-111.11)	**<0.001**	2212.09 (1744.56-2697.31)	83.33 (72.22-94.44)	**<0.001**
Hospitalization	2727.54 (2307.96-3776.72)	142.08 (142.08-142.08)	**<0.001**	2727.54 (2307.96-5457.66)	142.08 (142.08-142.08)	**<0.001**	2727.54 (2307.96-4829.57)	142.08 (142.08-142.08)	**<0.001**
Genetic testing[h]	0.00 (0.00-0.00)	0.00 (0.00-0.00)	**0.002**	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.034	0.00 (0.00-0.00)	0.00 (0.00-0.00)	**0.017**
Other medical expenses[i]	1003.99 (432.12-1800.89)	1070.10 (825.87-1274.29)	0.913	1804.88 (1202.83-2906.83)	1032.32 (825.87-1351.98)	**<0.001**	1422.41 (932.86-1959.41)	941.31 (825.87-1081.18)	**0.001**
Imaging	901.25 (559.74-1406.95)	382.78 (309.06-456.50)	**<0.001**	2170.91 (1679.49-2759.76)	382.78 (309.05-456.50)	**<0.001**	1322.76 (734.82-1768.75)	309.06 (309.06-382.78)	**<0.001**
Total cost	16962.14 (12038.73-21904.57)	3015.93 (2440.67-3344.74)	**<0.001**	66810.75 (57954.80-75664.80)	3103.48 (2578.82-3337.74)	**<0.001**	18064.06 (14737.99-23568.99)	2793.35 (2503.28-2934.02)	**<0.001**

HR: hormone receptors; MZ: Mozambique; PT: Portugal; TNBC: triple negative breast cancer.

[a] Classic subtypes were defined according to the assessment of the estrogen receptor, the progesterone receptor and HER2 status on the surgical specimen (preferably) or on the biopsy core specimen (when the surgical specimen's pathology report was unavailable). Hormone receptor (HR)+/HER2-: estrogen receptor and/or progesterone receptor positive and HER2-negative (a score of 0 or 1+ in immunohistochemistry, or a score of 2+ with a non-amplified FISH test result); HER2+: HER2-positive (a score of 3+ in immunohistochemistry or a score of 2 with an amplified FISH test result), regardless of the estrogen and progesterone receptor status; triple negative breast cancer: estrogen receptor negative, progesterone receptor negative and HER2-negative.

[b] The Wilcoxon rank-sum test was used to compare groups.

[c] All surgeries/interventions, including breast surgeries and axillary surgeries. Imputed surgery cost for 87 patients who underwent surgery outside IPO-Porto.

[d] Imputed chemotherapy cost for three patients who received chemotherapy outside IPO-Porto and three patients who received chemotherapy outside the Maputo Central Hospital.

[e] Targeted therapy was only available in Portugal (IPO-Porto) and was only given to women with the HER2+ subtype. Imputed targeted therapy cost for one patient who received targeted therapy outside IPO-Porto.

[f] Day hospital sessions include the administration of intravenous, subcutaneous and/or intramuscular therapy (*i.e.* chemotherapy, trastuzumab, goserelin and fulvestrant).

[g] The cost of brachytherapy for the seven patients in PT who only received brachytherapy (and no external radiotherapy) is included in Hospitalization costs and not in Radiotherapy costs.

[h] Genetic testing was only available in Portugal (IPO-Porto). Number of women from Portugal receiving genetic testing: 63 (11.7%) women with HR+/HER2- subtype – median (P25-P75) cost: 886.12 (676.90-886.12); 12 (10.7%) women with HER2+ subtype – median (P25-P75) cost: 886.12 (386.25-886.12); and 9 (16.7%) women with TNBC – median (P25-P75) cost: 886.12 (886.12-1329.18).

[i] Other medical expenses include laboratory and pathology tests, and blood transfusions.

Paper V: *Treatment and other healthcare use of breast cancer patients with a previous cancer diagnosis*

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Treatment and Other Healthcare Use of Breast Cancer Patients With a Previous Cancer Diagnosis

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Abstract. *Background/Aim: To quantify the association between a previous cancer diagnosis and healthcare use among breast cancer (BC) patients, and estimate five-year recurrence-free survival (RFS). Patients and Methods: Women with BC were classified according to a previous cancer diagnosis (BC or other). Healthcare use during the first year and five-year RFS were obtained through clinical and administrative records. Adjusted odds ratios and hazard ratios (HR) were estimated. Results: Among 681 BC patients, 21 had a previous BC and 32 a previous non-BC. The latter were less likely to receive anthracycline-based combination chemotherapy. The former had higher odds of mastectomy and genetic testing. Five-year RFS HRs (95% confidence interval) were 2.75 (0.79-9.52) and 0.52 (0.07-3.89) for previous BC and non-BC, respectively. Conclusion: Previous cancer was associated with less anthracycline-based combination chemotherapy, and patients were more likely to undergo mastectomy and genetic testing. These findings highlight the need for assessment of previous treatments, personal genetic risk and current BC characteristics.*

The number of cancer survivors is growing, reflecting increases in the overall number of cancer cases and improvements in prognosis, due to earlier detection and improved treatment (1). In 2018, there was an estimated worldwide incidence of over 18 million cancer cases, and estimates are expected to reach nearly 30 million by 2040 (2). This has led to a growing burden associated with cancer survivorship as a higher number of individuals are diagnosed with subsequent primary cancers (3). In particular, not only are women with a first breast cancer at substantially greater risk of a contralateral breast cancer compared to the general population (4-6), but survivors of other cancer sites, namely endometrial, ovarian, colon, thyroid and Hodgkin lymphoma, also have elevated risks of breast cancer as a subsequent malignancy (7-9).

The development of multiple primary cancers has been studied for many cancers and types of treatment (10). In general, cancer survivors may be susceptible to develop a subsequent cancer due to a variety of factors, including increased genetic susceptibility, shared risk factors, effects of previous oncological treatments and a greater diagnostic surveillance (11). Moreover, patients diagnosed with a subsequent cancer pose greater challenges to medical professionals, as prior treatments and their toxicities must be taken into account when deciding the therapeutic plan for that specific patient (8).

However, there is a lack of information about the impact of a previous cancer diagnosis on the clinical management of cancer patients and their use of healthcare resources. In fact, no studies have examined this among women with breast cancer, which accounts for almost one-quarter of all cancers in women (2). Therefore, the aim of this study was to quantify the association between a previous cancer diagnosis and the treatment and other healthcare use among a cohort of breast cancer patients, and to estimate five-year recurrence-free survival (RFS).

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Key Words: Breast neoplasm, healthcare use, second primary neoplasms, treatment.

Patients and Methods

Study design and participants. This study included women newly diagnosed with in situ or invasive breast cancer admitted to the Breast Clinic of the Portuguese Institute of Oncology of Porto (IPO-Porto) in 2012 and is based on a previously described prospective cohort study (12). For the present study, participants were eligible if they were aged 18 years or older, with histologically confirmed breast cancer, proposed for surgery, either as primary treatment or after neoadjuvant chemotherapy and, if applicable, expected to receive cancer treatments other than surgery at IPO-Porto. Those who could understand the purposes of the study and were willing to collaborate were included. Further, women with stage IV breast cancer were excluded (n=12) (Figure 1).

Data collection. Sociodemographic data were collected through face-to-face interviews using a structured questionnaire.

Information on stage and immunohistochemistry (IHC) assessment of the breast cancer diagnosed in 2012, and the existence of a previous or subsequent primary cancer was collected from clinical records. Cancer stage was classified according to the seventh American Joint Committee on Cancer staging manual (13). As per the American Society of Clinical Oncology/College of American Pathologists guidelines, tumours were considered to be hormone receptor-positive (HR+) if the expression of estrogen receptor and/or progesterone receptor was ≥1% and HER2-positive (HER2+) if they had an IHC score ≥3 or, alternatively, a score ≥2 with a FISH-amplified result (14). Breast tumours were categorized according to the following IHC-based subtypes: HR+/HER2-negative (HR+/HER2–); HER2+; triple negative (HR–/HER2–). Other primary cancers were considered when a malignancy was diagnosed in the contralateral breast or in another site or tissue, which were not a loco-regional recurrence or metastasis. Further, as per the guidelines proposed by the International Association of Cancer Registries and the International Agency for Research on Cancer, different morphologies (even with the same topography) or dissimilar topographies were regarded as multiple primary cancers, regardless of the time between diagnoses (15).

Data regarding treatment and other healthcare use during the first year following breast cancer diagnosis in 2012 were obtained from clinical and administrative records. Information on treatment included surgery (*i.e.*, breast and axillary), systemic (*i.e.*, chemotherapy, hormone therapy and targeted therapy) and radiation (*i.e.*, external radiotherapy and brachytherapy). Data on healthcare use were obtained regarding the number of appointments [*i.e.*, outpatient visits (medical and surgical oncology, and radiation therapy), and nursing, psychology and social services appointments], and hospitalizations, which were defined taking into account percentile 75 of the distribution among those with no previous cancer (≥36 and ≥2, respectively). Finally, genetic testing was considered as having any relevant test carried out [*i.e.*, *BRCA1*/*BRCA2* mutations (hereditary breast and ovarian cancer syndrome); deletions of *MLH1* (hereditary nonpolyposis colon cancer); *TP53* mutation (Li-Fraumeni syndrome)].

Information on recurrence or death by any cause following the diagnosis of breast cancer in 2012 up to five years post diagnosis was also obtained from clinical records.

Statistical analysis. Patients' characteristics are presented as counts and proportions for all categorical variables, and median with percentiles 25 and 75 (P25-P75) for quantitative variables.

For statistical analysis, women with a breast cancer diagnosed in 2012 were divided according to the existence of a previous primary cancer: 1) no previous primary cancer (first breast cancer in 2012); 2) previous breast cancer diagnosis (breast cancer in 2012 + previous breast cancer); 3) previous non-breast cancer diagnosis (breast cancer in 2012 + previous non-breast cancer). Women with a subsequent primary cancer diagnosed in 2012 or 2013 were excluded from the analysis (n=6).

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were computed using multinomial logistic regression to quantify the association between a previous cancer diagnosis and treatment and other healthcare use in the first year following a breast cancer diagnosis in 2012. Models were adjusted for age (continuous variable), education (≤4, 5-9, ≥10), stage (0/I, II, III) and IHC-based subtypes (HR+/HER2–, HER2+, triple negative).

RFS was defined as the time between the date of breast cancer diagnosis in 2012 and the date of breast cancer recurrence or death by any cause, whichever occurred first (16). RFS was calculated using the Kaplan–Meier estimator (17). Patients who remained without an event by the end of the five-year follow-up period and those lost to follow-up were censored, considering the date referring to the last follow-up registered.

Cox proportional hazards regression analyses were used to compute hazard ratios (HR) for recurrence or death by any cause adjusted for age (continuous variable), education (≤4, 5-9, ≥10) and stage (0/I, II, III) with the corresponding 95%CI. The proportional hazards assumption was evaluated using Schoenfeld residuals.

Ethics. The study received ethical approval from the Ethics Committee of the Portuguese Institute of Oncology of Porto (ref. CES 406/011, CES 99/014 and CES 290/014) and the national research committee [Portuguese Data Protection Authority (ref. 9469/2012 and 8601/2014)]. Informed consent was obtained from all individual participants included in the study.

Results

Participants' sociodemographic and clinical characteristics according to previous cancer history are shown in Table I. Among 681 women with a breast cancer diagnosed in 2012, 21 (3.1%) had a prior primary breast cancer diagnosis and 32 (4.7%) had a previous non-breast cancer. In 2012, over two-thirds of those with a previous breast cancer were 65 or older (66.7%), while 43.6% and 43.7% of women with a first breast cancer and a previous non-breast cancer were aged between 50 and 64 years old, respectively. More than half of the women had successfully completed more than four years of education (53.3%), while close to two-thirds lived outside the Porto Metropolitan Area (63.3%). Regarding clinical characteristics, half of the patients were diagnosed with stage 0/I breast cancer in 2012 (51.5%) and over two thirds presented with HR+/HER2– tumours (76.4%). Most previous breast cancers were diagnosed more than 10 years before the current breast cancer diagnosis (71.4%), while 43.8% of previous non-breast cancers were diagnosed between five and 10 years before the present breast cancer diagnosis. The most common previous cancer sites were female genital

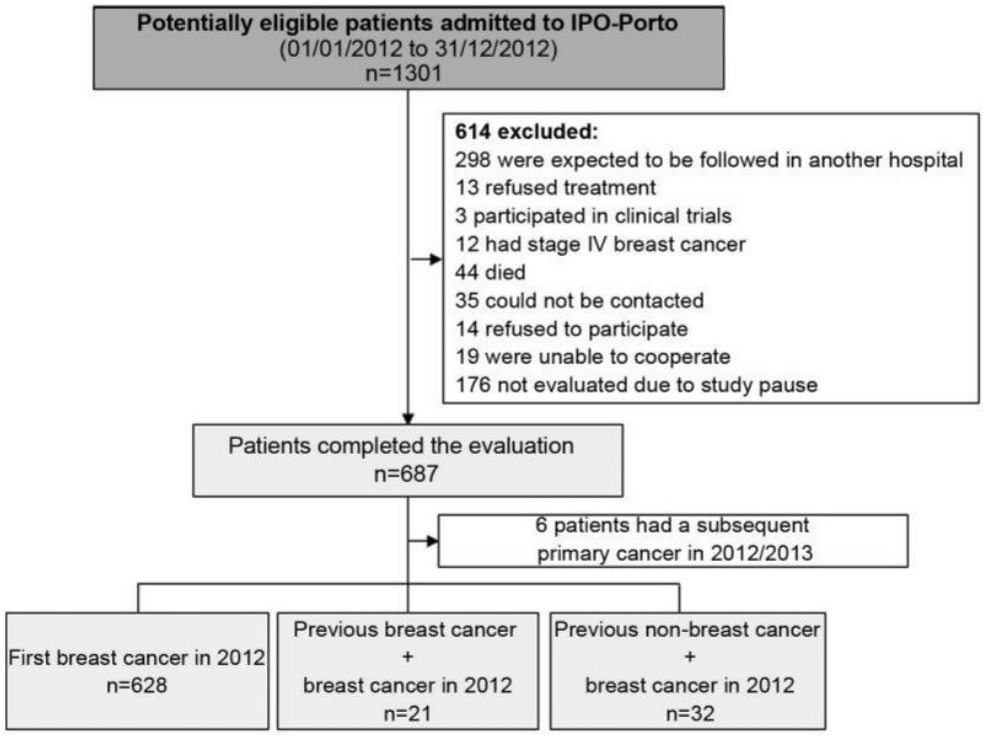


Figure 1. *Flowchart of patient inclusion in the study.*

organs (21.9%), thyroid and other endocrine glands (18.7%), digestive organs (15.6%), and melanoma and other malignant neoplasms of skin (15.6%).

Table II shows the association between a previous cancer diagnosis and treatment and other healthcare use in the first year following a breast cancer diagnosis in 2012. After adjusting for age and education, women with a previous breast cancer had higher odds of undergoing mastectomy versus breast-conserving surgery (OR=4.17; 95%CI=1.36-12.72). Regarding chemotherapy, women with a previous non-breast cancer diagnosis were significantly less likely to receive anthracycline-based combination chemotherapy (OR=0.03; 95%CI=0.01-0.20). This association was also observed among women with a previous breast cancer though was not statistically significant (OR=0.23; 95%CI=0.02-2.57). Finally, women with a previous breast cancer had significantly higher odds of undergoing genetic testing (OR=14.82; 95%CI=3.60-61.01); this was also seen for women with a previous non-breast cancer though was not statistically significant (OR=2.05; 95%CI=0.65-6.44). Estimates remained essentially the same when breast cancer stage and IHC-based subtype were added to the model (Table II).

Five-years following breast cancer diagnosis in 2012, 23 events (recurrence or death by any cause) had occurred among women with a first breast cancer in 2012, three events (recurrence or death by any cause) in women with a previous breast cancer, while no events (recurrence or death by any cause) were observed for women with a previous non-breast cancer (Figure 2). Compared to women with a first breast cancer in 2012, the age and education adjusted HRs (95%CI) were 3.43 (1.01-11.59) for women with a previous breast cancer and 0.57 (0.08-4.26) for women with a previous non-breast cancer. The corresponding age, education and stage adjusted estimates were 2.75 (95%CI=0.79-9.52) and 0.52 (95%CI=0.07-3.89).

Discussion

The present study shows that in the first year following a breast cancer diagnosis, women with a previous cancer were less likely to receive any anthracycline-based combination chemotherapy. Additionally, women with a previous cancer had higher odds of undergoing mastectomy and genetic testing.

In general, cancer treatment has evolved extensively over the past few decades shifting to less aggressive local treatment and to an increase in the use of systemic treatment (18). Regardless, patients with a previous primary cancer are an added challenge as prior treatments must be taken into

Table I. *Sociodemographic and clinical characteristics of breast cancer patients in 2012.*

Characteristics	First breast cancer in 2012 (N=628)	Breast cancer in 2012+ previous breast cancer (N=21)		Breast cancer in 2012+ previous non-breast cancer (N=32)	
	N (%)	N (%)	*p*-Value[a]	N (%)	*p*-Value[b]
Age (years)					
<50	214 (34.1)	2 (9.5)		10 (31.3)	
50-64	274 (43.6)	5 (23.8)		14 (43.7)	
≥65	140 (22.3)	14 (66.7)	<0.001	8 (25.0)	0.918
Education (years)					
≤4	289 (46.0)	13 (61.9)		16 (50.0)	
5-9	162 (25.8)	3 (14.3)		8 (25.0)	
≥10	177 (28.2)	5 (23.8)	0.317	8 (25.0)	0.895
Place of residence					
Porto Metropolitan Area	221 (35.4)	11 (52.4)		16 (51.6)	
Outside the Porto Metropolitan Area	403 (64.6)	10 (47.6)	0.111	15 (48.4)	0.067
Breast cancer stage					
0/I	327 (52.1)	7 (35.0)		16 (50.0)	
II	216 (34.4)	9 (45.0)		8 (25.0)	
III	85 (13.5)	4 (20.0)	0.315	8 (25.0)	0.161
Immunohistochemistry-based subtypes[c]					
HR+/HER2–	454 (75.8)	17 (81.0)		27 (84.4)	
HER2+	98 (16.4)	2 (9.5)		4 (12.5)	
Triple negative	47 (7.8)	2 (9.5)	0.694	1 (3.1)	0.481
Previous cancer diagnosis (years)					
≤2001	--	15 (71.4)		8 (25.0)	
2002-2006	--	4 (19.1)		10 (31.2)	
≥2007	--	2 (9.5)	--	14 (43.8)	--
Previous cancer site					
Digestive organs	--	--		5 (15.6)	
Melanoma and other malignant neoplasms of skin	--	--		5 (15.6)	
Female genital organs	--	--		7 (21.9)	
Urinary tract	--	--		3 (9.4)	
Thyroid and other endocrine glands	--	--		6 (18.7)	
Other[d]	--	--	--	6 (18.7)	--

The total may not add to 681 due to missing data. May not sum to 100.0% due to rounding. [a]Breast cancer in 2012 + previous breast cancer *versus* first breast cancer in 2012. [b]Breast cancer in 2012 + previous non-breast cancer *versus* first breast cancer in 2012. [c]Immunochemistry-based subtypes were defined according to the assessment of estrogen receptor, progesterone receptor and HER2 on the surgical specimen. HR+/HER2–: estrogen receptor and/or progesterone receptor positive and HER2-negative (score <2 in immunohistochemistry and/or a score ≥2 with a non-amplified FISH test result); HER2+: HER2-positive (score ≥3 in immunohistochemistry and/or a score ≥2 with an amplified FISH test result), regardless of the estrogen and progesterone receptors status; triple negative: estrogen receptor negative, progesterone receptor negative and HER2-negative. [d]Other previous sites include: mesothelial and soft tissue, osteosarcoma, Hodgkin lymphoma and not specified.

account. We found that patients with a previous cancer had lower odds of receiving anthracycline-based combination chemotherapy, which is still part of the (neo)adjuvant chemotherapy backbone in breast cancer (19). Further, these patients also had lower odds of being submitted to radiotherapy. These findings may be a result of the prior cancer treatment received by these patients, namely radiation and chemotherapy, as there are lifetime dose limits as well as residual toxicity (8, 20, 21). For radiation in particular, patients often cannot receive radiation therapy to a specific or an adjacent area a second time as a result of dose-limiting toxicities (8, 20).

Women with a previous breast cancer diagnosis were more likely to undergo mastectomy versus breast-conserving surgery. This result remained statistically significant following adjustment for stage and IHC-based subtype, which are intermediate steps in this relation. It is possible that a more aggressive approach may be used among patients with a previous breast cancer, as previous studies have found that contralateral breast cancers are more difficult to treat than the

Table II. *Association between a previous cancer diagnosis and the treatment and other healthcare use in the first year following a breast cancer diagnosis in 2012.*

	First breast cancer in 2012	Breast cancer in 2012+ previous breast cancer			Breast cancer in 2012+ previous non-breast cancer		
	(N=628)	(N=21)			(N=32)		
	N (%)	N (%)	OR (95%CI)[a]	OR (95%CI)[b]	N (%)	OR (95%CI)[a]	OR (95%CI)[b]
Surgery							
Breast surgery[c]							
Breast-conserving	342 (54.5)	4 (19.0)	1	1	16 (50.0)	1	1
Mastectomy	286 (45.5)	17 (80.9)	4.17 (1.36-12.72)	3.30 (1.02-10.72)	16 (50.0)	1.00 (0.49-2.07)	0.93 (0.42-2.04)
Axillary surgery[d]							
None or sentinel lymph node biopsy	437 (69.6)	13 (61.9)	1	1	22 (68.7)	1	1
Lymph node dissection	191 (34.4)	8 (38.1)	1.88 (0.74-4.74)	1.11 (0.32-3.81)	10 (32.3)	1.06 (0.49-2.30)	0.60 (0.19-1.95)
Chemotherapy							
Any chemotherapy (Yes)	376 (59.9)	10 (47.6)	1.38 (0.53-3.62)	1.17 (0.37-3.73)	18 (56.2)	0.92 (0.42-2.03)	0.73 (0.29-1.82)
Timing of chemotherapy							
Neoadjuvant	35 (9.4)	0 (0.0)	--	--	2 (11.1)	1	1
Adjuvant	336 (90.6)	10 (100.0)	--	--	16 (88.9)	0.89 (0.19-4.07)	1.08 (0.20-5.73)
Chemotherapy scheme							
Anthracycline-based (Yes)[e]	371 (98.7)	9 (90.0)	0.23 (0.02-2.57)	0.05 (0.00-0.54)	15 (83.3)	0.03 (0.01-0.20)	0.01 (0.00-0.10)
Other treatments							
Radiotherapy (Yes)	469 (74.7)	12 (57.1)	0.65 (0.26-1.63)	0.54 (0.19-1.50)	21 (65.6)	0.68 (0.31-1.47)	0.51 (0.22-1.18)
Brachytherapy (Yes)[f]	127 (27.1)	2 (16.7)	0.44 (0.09-2.12)	0.79 (0.16-3.99)	5 (23.8)	0.82 (0.29-2.29)	0.87 (0.29-2.63)
Hormone therapy (Yes)	534 (85.0)	18 (85.7)	0.91 (0.26-3.21)	0.41 (0.03-5.85)	29 (90.6)	1.69 (0.50-5.66)	0.34 (0.05-2.50)
Targeted therapy (Yes)[g]	92 (14.6)	2 (9.5)	0.86 (0.19-3.85)	--	3 (9.4)	0.61 (0.18-2.06)	--
Healthcare use							
Appointments (≥36)[h]	171 (27.2)	4 (19.0)	1.13 (0.36-3.61)	0.86 (0.25-2.94)	8 (25.0)	0.91 (0.39-2.14)	0.79 (0.32-1.99)
Hospitalization (≥2)	191 (30.4)	4 (19.0)	0.57 (0.19-1.73)	0.66 (0.21-2.06)	12 (37.5)	1.38 (0.66-2.87)	1.40 (0.66-2.95)
Genetic testing (Yes)[i]	55 (8.8)	4 (19.0)	14.82 (3.60-61.01)	21.81 (5.10-93.30)	5 (15.6)	2.05 (0.65-6.44)	1.99 (0.62-6.40)

The total may not add to 681 due to missing data. May not sum to 100.0% due to rounding. 5-FU: 5-fluorouracil. [a]Adjusted for age (continuous) and education (≤4, 5-9, ≥10). [b]Further adjusted for stage (0/I, II, III) and immunohistochemistry-based subtypes (HR+/HER2–, HER2+, triple negative). [c]Patients who had breast-conserving surgery followed by mastectomy are reported as mastectomy. [d]Patients who had sentinel lymph node biopsy followed by lymph node dissection are reported as lymph node dissection. [e]Anthracycline-based chemotherapy includes: AC regimen: four or six cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2); FEC regimen: six cycles of concomitant 5-FU (500 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2); AC-T regimen: four cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by four cycles of docetaxel (100 mg/m^2); AC-paclitaxel regimen: four cycles of concomitant doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by four cycles of paclitaxel (80 mg/m^2); FEC-D regimen: three cycles of concomitant 5-FU (500 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2) followed by three cycles of docetaxel (100 mg/m^2). [f]Among those who received radiotherapy (n=502). [g]Targeted therapy includes trastuzumab. [h]Appointments: number of outpatient visits (medical oncology and radiation therapy), and nursing, psychology and social services appointments. [i]Genetic testing includes: *BRCA1* mutations; *BRCA2* mutations (hereditary breast and ovarian cancer syndrome); deletions in *MLH1* (hereditary nonpolyposis colon cancer); *TP53* mutations (Li-Fraumeni syndrome).

original cancer due to a more resistant biology and the inability to reuse previous effective therapy, such as anthracycline-based chemotherapy (5, 22). Nonetheless, among the 17 patients with a previous breast cancer who were submitted to mastectomy, five presented with stage IA and five with stage IIA, and of those who underwent genetic testing in the first year, none presented a *BRCA1/2* mutation, suggesting that they may have been over-treated. Likewise, among the 16 patients with a previous non-breast cancer who were submitted to mastectomy, six presented with stage IA/IB.

Several studies have found that hereditary cancer syndromes account for an estimated 5 to 10% of all cancers due to heightened susceptibility of specific cancers (23); as such, patients with multiple primary cancers are expectedly more likely to undergo genetic testing, as observed in our study. In particular, individuals with inherited mutations in *BRCA1/2,*

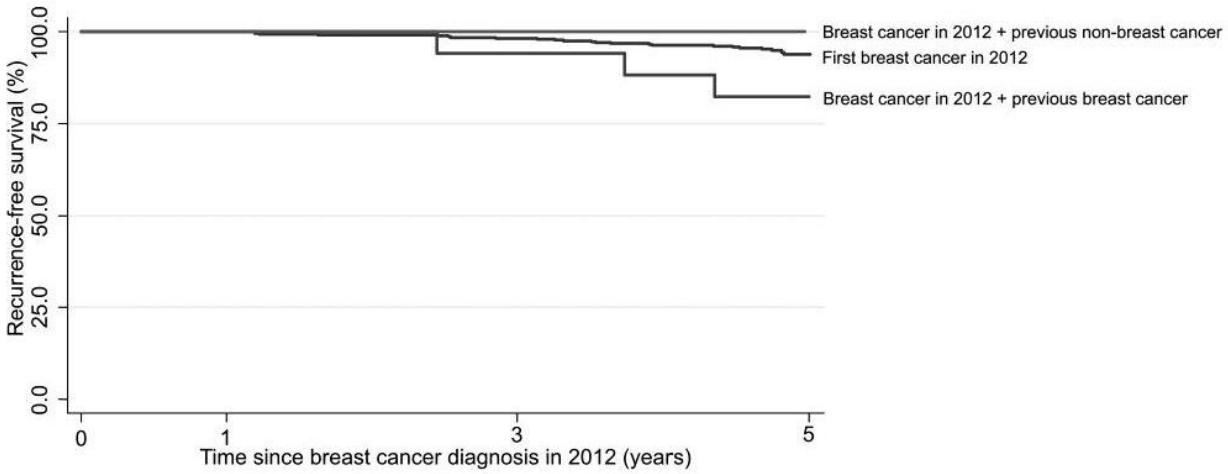


Figure 2. *Recurrence-free survival [calculated using the Kaplan–Meier estimator (17)] of breast cancer patients diagnosed in 2012 according to previous cancer diagnosis.*

TP53 (Li-Fraumeni syndrome) or *MLH1* (hereditary nonpolyposis colon cancer or Lynch syndrome) have an increased risk of developing subsequent cancers, namely of the colon and rectum, breast, uterine body and cervix, thyroid as well as connective tissues (23, 24). In fact, the most common sites of previous cancers in our study were contralateral breast, female genital organs including the cervix, thyroid and digestive organs such as the colon. Nevertheless, only 19.0% of patients with a previous breast cancer and 15.6% of patients with a previous non-breast cancer received genetic testing during the first year following breast cancer diagnosis in 2012. However, when considering younger ages at diagnosis and triple negative subtypes of breast cancer, which are important indicators for hereditary diagnostic testing (25), a larger number of women with a previous cancer diagnosis had genetic tests completed.

We also found that patients with a previous breast cancer had a trend for worse five-year RFS, which is in line with the literature: a contralateral breast cancer diagnosis is negatively associated with survival (26-28). This may not only be related with a more aggressive breast cancer biology, but also with the fact that patients with a previous breast cancer were generally older and as such may have more co-morbidities. Additionally, the cumulative toxicity of treatments for both breast cancers (*i.e.*, chemotherapy, radiotherapy, endocrine therapy) may also impair survival. On the other hand, after adjusting for age, education and stage, patients with a previous non-breast cancer did not have a significantly worse five-year RFS, albeit the lower use of anthracycline-based chemotherapy, which is generally associated with better outcomes (29, 30). However, this analysis is essentially exploratory since the number of events (recurrence or death by any cause) was very small, and a poorer survival of patients with a previous cancer cannot be excluded.

Strengths and limitations. The systematic and thorough evaluation of the treatment and other healthcare use during the first year following a breast cancer diagnosis is a methodological strength of the present study. Data were collected from clinical records by health professionals and other healthcare use data were obtained from administrative records, which allowed us to have high quality data with a low level of missing information.

Our study evaluated essentially women with early breast cancer, which limits generalizability to those with more advanced disease; and women with a subsequent cancer diagnosis in 2012 or 2013 were excluded as this would have influenced the outcome under study. Furthermore, all patients were selected and treated in the same institution; however, IPO-Porto is the largest hospital providing care to cancer patients in Northern Portugal and receives patients referred from a wide geographical area.

The uptake of genetic testing among patients in our study was low, even among those with a previous breast cancer (19.0%), but this may be related to the timing of the analysis: back in 2012/2013, access to genetic testing was lower than what it is nowadays (31), therefore the values presented may no longer reflect current clinical practice. However, it is also possible that women underwent genetic testing outside of IPO-Porto or the time period considered in the current analyses (*i.e.*, before or more than one year following the breast cancer diagnosis in 2012), and this information may not have been available to us for all patients.

Although there were a small number of cases in some strata, the associations found were strong and therefore there was sufficient statistical power. However, as we have a large heterogeneity in the previous non-breast cancer sites, we were unable to perform subgroup analysis, which may have yielded

more consistent results. Similarly, it may have been interesting to evaluate previous cancer treatments received in order to examine possible toxicity levels. However, considering the sample size, these analyses would likely not have yielded meaningful results. Regardless, this is one of the first studies evaluating the association between a previous cancer diagnosis and the treatment and other healthcare use among a cohort of breast cancer patients.

Conclusion

The increasing number of breast cancer patients being diagnosed who have a previous cancer diagnosis requires a specific investigation to understand its impact on the clinical management of these patients and their use of healthcare resources. Our study provides evidence that a previous cancer diagnosis is associated with a lower use of anthracycline-based combination chemotherapy and that these patients are more likely to undergo genetic testing. Furthermore, patients with a previous breast cancer had a higher chance of undergoing a mastectomy, even if they presented with a low-stage disease, which suggests that these patients may have been locally over-treated. Five-year RFS also tended to be worse among patients with a previous breast cancer. As such, these findings highlight that in patients with a previous cancer diagnosis, there is a need for a careful assessment of previous treatments and toxicities, of personal genetic risk and of the present breast cancer's characteristics, in order to avoid over- or under-treatment. A comprehensive and personalized approach is paramount, in order to improve survival and reduce morbidity among patients with multiple cancers.

Conflicts of Interest

Mariana Brandão has received a travel grant, unrelated to this study, from Roche/GNE, and the Institut Jules Bordet has received research grants, unrelated to this study, from Roche/GNE, Radius, AstraZeneca, Lilly, MSD, GSJ/Novartis, Synthon, Servier and Pfizer. Marina Borges has received travel grants, unrelated to this study, from Roche and Janssen. All other Authors declare that they have no conflict of interest (Samantha Morais, Ana Rute Costa, Luisa Lopes-Conceição, Natália Araújo, Teresa Dias, Filipa Fontes, Susana Pereira and Nuno Lunet).

Authors' Contributions

SM performed the statistical analysis and interpreted the data, drafted and revised the manuscript. LC, MB, MB, NA and FF collected the data. ARC, LC, NA, MB, MB, FF, TD and SP critically reviewed the manuscript and made important intellectual contributions. NL supervised the analysis and interpretation of data and reviewed the manuscript. SP and NL conceived, designed and implemented the cohort. NL defined the study hypothesis. All Authors contributed to the discussion of the results. All Authors read and approved the final version of the manuscript.

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Paper VI: *Survival impact and cost-effectiveness of a multidisciplinary tumor board for breast cancer in Mozambique, Sub-Saharan Africa*

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Global Health and Cancer

Survival Impact and Cost-Effectiveness of a Multidisciplinary Tumor Board for Breast Cancer in Mozambique, Sub-Saharan Africa

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Breast neoplasms • Sub-Saharan Africa • Developing countries • Survival analysis • Cost-effectiveness analysis

Abstract

Background. Despite the international endorsement of multidisciplinary tumor boards (MTBs) for breast cancer care, implementation is suboptimal worldwide, and evidence regarding their effectiveness in developing countries is lacking. We assessed the impact on survival and the cost-effectiveness of implementing an MTB in Mozambique, sub-Saharan Africa.

Materials and Methods. This prospective cohort study included 205 patients with breast cancer diagnosed between January 2015 and August 2017 (98 before and 107 after MTB implementation), followed to November 2019. Pre- and post-MTB implementation subcohorts were compared for clinical characteristics, treatments, and overall survival. We used hazard ratios and 95% confidence intervals (CI), computed by Cox proportional hazards regression. The impact of MTB implementation on the cost per quality-adjusted life year (QALY) was estimated from the provider perspective.

Results. We found no significant differences between pre- and post-MTB subcohorts regarding clinical characteristics or treatments received. Among patients with early breast cancer (stage 0–III; n = 163), the 3-year overall survival was 48.0% (95% CI, 35.9–59.1) in the pre-MTB and 73.0% (95% CI, 61.3–81.6) in the post-MTB subcohort; adjusted hazard ratio, 0.47 (95% CI, 0.27–0.81). The absolute 3-year mean cost increase was $119.83 per patient, and the incremental cost-effectiveness ratio was $802.96 per QALY, corresponding to 1.6 times the gross domestic product of Mozambique.

Conclusion. The implementation of a MTB in Mozambique led to a 53% mortality decrease among patients with early breast cancer, and it was cost-effective. These findings highlight the feasibility of implementing this strategy and the need for scaling-up MTBs in developing countries, as a way to improve patient outcomes. ***The Oncologist*** 2021;26:1–13

Implications for Practice: Currently, more than half of the deaths from breast cancer in the world occur in developing countries. Strategies that optimize care and that are adjusted for available resources are needed to improve the outcomes of patients with breast cancer in these regions. The discussion of cases at multidisciplinary tumor boards (MTBs) may improve survival outcomes, but implementation is suboptimal worldwide, and evidence regarding their effectiveness in developing countries is lacking. This study evaluated the impact of implementing an MTB on the care and survival of patients with breast cancer in Mozambique, sub-Saharan Africa and its cost-effectiveness in this low-income setting.

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Introduction

Breast cancer is the most frequent cancer among women in the world, and its incidence rates have been increasing over the last decades, especially in developing countries [1]. Specifically in Mozambique, it is currently the second most incident cancer in Maputo City, with an age-standardized rate of 15.5 per 100,000 women [2].

Breast cancer is still the most frequent cause of cancer death among women in developing regions, and age-standardized mortality rates are higher in low- and medium-income than in high-income countries (14.9 vs. 11.6 per 100,000 women, respectively) [1]. In sub-Saharan Africa, this is partly due to the large proportion of patients with stage III/IV disease at presentation, which was around 75% in a large pooled analysis [3]. Additionally, the percentage of women diagnosed with stage IV disease may also be high, reaching 70% in some series [3]. Moreover, the high prevalence of triple-negative breast cancer (TNBC) [4] and the limited access to adequate diagnosis and care, namely pathology services, surgical care, radiotherapy, and systemic treatment, also contribute to patients' dismal prognosis [5].

The discussion of cases at multidisciplinary tumor boards (MTBs) may be one of the strategies to improve outcomes in breast cancer. Most international oncology societies consider multidisciplinarity an essential part of breast cancer care, proposing that all patients should be presented in a MTB before initiating treatment [6]. Nonetheless, there is a wide gap between this universal recommendation and its effective implementation, even in developed countries. A French multicentric study showed that, although all breast cancer cases were presented in an MTB, only 64% were discussed before surgery, with significant differences between academic vs. nonacademic hospitals [7]. In a report using data from the U.S. SEER-Medicare database, only 70% of patients had access to multidisciplinary care, and this proportion was even lower among those living in a rural area [8]. In an Australian study, only 60% of breast cancer cases diagnosed during 2010–2015 were discussed in MTBs [9]. In China, less than 2% of patients operated for breast cancer during 2006–2016 in a large cancer center were discussed in an MTB [10], and, in Taiwan, only 28% were presented in a multidisciplinary meeting [11]. In a recent survey conducted among members of the African Organisation for Research and Training in Cancer, 80% of the respondents reported they were involved in a multidisciplinary breast cancer team, but this is likely to be an overestimate, as there were only 37 respondents [12]. A total of 72% of the participants in a survey conducted in Arab countries reported having an MTB at their institution, although only 49% of these MTBs met on a weekly basis [13].

Data regarding the impact of breast cancer MTBs on survival is even scarcer. Although a large study from the U.K. and another from Germany demonstrated that multidisciplinary care improved survival among patients with breast cancer [14, 15], a population-based study from Germany did not present such a survival benefit [16]. Studies from China and Taiwan showed a survival advantage with the implementation of multidisciplinary care for breast cancer [10, 11], but there are no data from other parts of the world. A study from the U.K. revealed that MTBs can be expensive resources [17], and they may be burdensome to some clinical departments, raising the question of their added value [18]. However, we could not find any study assessing the cost-effectiveness of breast cancer MTBs.

Currently, more than half of the deaths from breast cancer in the world occur in developing countries. Therefore, it is paramount to develop strategies adjusted for the available resources that optimize care, translating into improved breast cancer survival. With this study, we aimed to assess the impact of implementing an MTB on the care and survival of patients with breast cancer in Mozambique. Additionally, its cost-effectiveness was evaluated in this low-resource setting.

Subjects, Materials, and Methods

Setting

Mozambique is a low-income country from Eastern sub-Saharan Africa with 30 million inhabitants. In 2018, the gross domestic product (GDP) was $499.00 per capita [19]. The average life expectancy is approximately 59 years [20], and 13.2% of adults aged 15–49 years are infected with HIV [21]. The country is now undergoing a surge of non-communicable diseases, such as diabetes, cardiovascular disease, and cancer [22]. The density of physicians was 0.1/1000 inhabitants in 2017 [23], and the public health system, which is free-of-charge, is the largest health care provider. A total of 8.7% of the 2018 Mozambican state budget was allocated to the health sector, which is similar to other low-income countries. It also relies on external resources (e.g., foreign donations, among others), which constituted 14% of the 2018 health budget [24]. The country has scarce resources for cancer care, namely in terms of facilities and health professionals. In 2015, there were only three pathology departments, two oncology units, and one surgery department with specialized surgical oncologists. Patients had free access to anthracyclines, cyclophosphamide, taxanes, methotrexate, and tamoxifen, although there were occasional interruptions in their supply. Trastuzumab and aromatase inhibitors were not provided by the public health system, and the first radiotherapy unit only opened in August 2019. Since 2013, the Calouste Gulbenkian Foundation has had a cancer care program for Mozambican health practitioners, in which they are trained in Portugal for short periods of time. Additionally, there are Cancer Registries in the Beira and Maputo Cities and in the Maputo Central Hospital [2, 25].

Study Design and Participants

We conducted the prospective Moza-BC cohort study, which included consecutive patients with newly diagnosed breast cancer with a pathological diagnosis between January 2015 and August 2017 (supplemental online Fig. 1) [26]. Data on socio-demographic characteristics, risk factors, HIV status, clinical and tumor characteristics, treatment, and survival of patients treated in the oncology unit of the

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Maputo Central Hospital were prospectively collected until July 2018. Survival data were updated through the Hospital's Cancer Registry, health records, and telephone interviews in November 2019.

Breast tissue samples were collected by surgical biopsy, breast surgery, and fine-needle aspiration cytology and infrequently by core-needle biopsies. Handling of breast specimens was standardized according to the American Society of Clinical Oncology/College of American Pathologists recommendations [27]. Expression of the estrogen receptor (ER), the progesterone receptor, and the overexpression and amplification of HER2 was assessed by immunohistochemistry on the primary tumor, as described in the literature [27, 28]. In cases in which the immunohistochemistry score of HER2 was 2+ (HER2-equivocal), silver *in situ* hybridization was performed at the Centro Hospitalar São João, Portugal. Tumors were grouped according to the classic subtypes into ER-positive/HER2-negative, HER2-positive (ER-positive/HER2-positive and ER-negative/HER2-positive), and TNBC (ER-negative/ progesterone receptor-negative/ HER2-negative).

Staging was defined by the AJCC TNM 7th edition classification [29]. Clinical staging was performed using clinical examination, mammography or breast ultrasound, chest x-ray, and abdominal ultrasound. Computed tomography scans were occasionally performed, but bone scans and breast magnetic resonance imaging were not available.

Multidisciplinary Tumor Board

The above-mentioned training program in cancer care, along with the assembling and follow-up of this cohort, raised awareness among Maputo Central Hospital physicians regarding the need for a better coordination of breast cancer care. Therefore, in March 2016, conditions were set to start an MTB for breast cancer, which was the first one to ever occur in Mozambique. The MTB meetings were performed on a weekly basis and lasted 1 hour, with the participation of at least one member from the surgery, oncology, pathology, and radiology departments and with trainees from these different specialties. Although radiotherapy was not yet available in Mozambique at the time, its implementation process was already ongoing, and there were Mozambican physicians being trained abroad in radiation oncology. Thus, a radiation oncologist who had recently completed his training was also present at the MTB and would indicate if the patient needed radiotherapy or not, even if only a small fraction of patients could have access to it abroad. A secretary recorded all decisions. With the creation of the MTB, patients' navigation inside the Maputo Central Hospital changed from erratic to organized (Fig. 1). Every new breast cancer case is now presented in the MTB, and patients with relapse are often discussed as well. The list of cases is sent to the team a few days in advance and the medical oncologist usually does the presentation. Moreover, an effort has been done by the oncology unit team to ensure the adherence of patients to systemic therapy and follow-up care, which led to an increase in the number of follow-up visits from three to four visits per year during the first 3 years after diagnosis.

Ethics

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

Statistical Analysis

Two subcohorts were defined according to the date of the MTB implementation (March 2016). Baseline patient, tumor, and treatment characteristics were compared between the two subcohorts using χ^2 test and Fisher's exact test for categorical variables and through adjusted odds ratios and 95% confidence intervals (CIs), computed using binomial logistic regression.

Overall survival was defined as time from diagnosis (date of pathological confirmation of breast cancer) until death by any cause. Among patients with early stage breast cancer (stage 0–III), disease-free survival was defined as time from diagnosis until loco-regional or distant relapse, second primary malignancy or death by any cause.

The median follow-up duration was 41.0 months (maximum, 47 months) among patients on the pre-MTB subcohort and 37.8 months (maximum, 43 months) among patients in the post-MTB subcohort; thus, survival analyses were censored at 36 months and performed using the Kaplan-Meier estimator. Comparisons of survival between the two subcohorts were accomplished through log-rank tests and crude and adjusted hazard ratios (HRs) and corresponding 95% CIs, computed using Cox proportional hazards regression. Analyses were carried out in STATA version 15 (StataCorp, College Station, TX). All tests were two-sided and a p value of <.05 was considered significant.

To estimate the impact of implementing a MTB on the cost per quality-adjusted life year (QALY), measured over a 3-year time horizon, a decision tree model was developed that integrated patient-level data from this cohort study, including survival estimates (supplemental online Methods). All cost calculations assumed 2019 as the reference year. Costs determined in previous years were corrected for inflation using the GDP deflator, as proposed by the World Health Organization [30]; correction factors were extracted from the World Bank [31]. All costs determined in U.S. dollars (USD) or Euros were converted to Mozambique Meticais (MZN), using the exchange rates \$1 = 63.8292 MZN and €1 = 70.5682 MZN, respectively; final results were converted back to USD, and are presented in both U.S. dollars and MZN. A detailed description of the cost-effectiveness analysis methods can be found in the supplemental online Methods. The model was implemented using TreeAgePro 2018 R2.1 (Williamstown, MA).

Results

Patients' median age was 48.0 years, and most were Black (98.0%), premenopausal (53.7%), and overweight or obese (54.1%) and presented with stage III/IV disease (74.1%). The prevalence of HIV infection was 25.4% (Table 1).

A total of 98 patients were included in the pre-MTB subcohort and 107 patients were included in the post-MTB subcohort. Patients in the post-MTB subcohort had a higher chance of having an unknown breast cancer subtype and of

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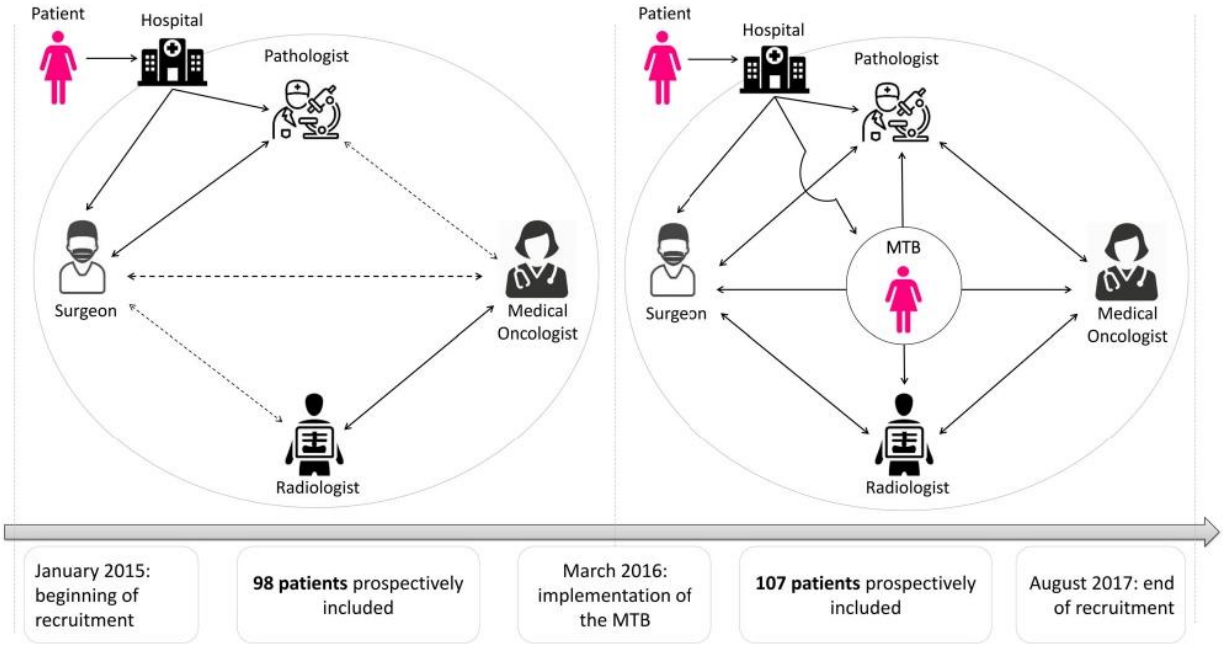


Figure 1. Implementation of the MTB at the Maputo Central Hospital. Before March 2016 (left panel), upon suspicion of a breast tumor, the patient was referred to the surgical department of the Maputo Central Hospital (MCH), and the surgeon usually asked for a breast fine-needle aspiration cytology biopsy to be performed at the pathology department. Alternatively, the patient had a biopsy performed outside the MCH or the surgeon performed a surgical biopsy at the MCH, but the tumor sample was analyzed and diagnosed at the pathology department. In those cases, the pathologist referred the patient to the surgical department. The surgeon then decided if the patient was to be operated on first or if she needed neoadjuvant chemotherapy. In the latter situation, the surgeon referred the patient to the oncology unit and the medical oncologist asked for staging exams to be performed at the radiology department. Upon completion of neoadjuvant chemotherapy, the patient was referred back to the surgical department. Following surgery, the patient was sent to the oncology unit again, to decide if she should also undergo adjuvant therapy and to receive long-term follow-up. After March 2016 (right panel), the referral scheme of patients from outside the MCH was maintained. However, following the pathological diagnosis of breast cancer, the patient is now directly referred to the oncology unit. She is often seen on the same day by the "on-duty" oncologist, who asks for staging exams and schedules the MTB discussion within 1–2 weeks. Every new case of breast cancer is presented at the MTB regardless of stage, and patients with relapse are often discussed as well. The MTB team decides if the patient with early breast cancer is to receive neoadjuvant chemotherapy or surgery first. Following completion of neoadjuvant chemotherapy, the oncologist refers the patient to the surgical department, but also verbally informs the surgeon of this referral at the weekly MTB. After surgery, the patient's case is discussed again at the MTB to decide if she should also undergo adjuvant treatment and its type. Dashed arrows: infrequent interaction; full arrows: frequent interaction.
Abbreviation: MTB, multidisciplinary tumor board.

living in the South of Mozambique, but there were no significant differences regarding other clinico-pathological characteristics. Nearly half of the patients were diagnosed more than 6 months after the first symptoms, and half of them started treatment more than 45 days after diagnosis. The MTB implementation led to a slight increase in the use of chemotherapy (from 91.8% to 96.3%), the use of neoadjuvant chemotherapy only (from 8.9% to 19.4%), tumor downstaging with chemotherapy (from 40.0% to 48.3%), and the use of taxanes with anthracyclines (from 44.4% to 53.4%); however, these differences were not significant. There were also nonsignificant increases in the proportions of clean surgical margins (from 88.4% to 92.3%) and in complete axillary surgery (from 58.4% to 67.9%).

Overall Survival

A total of 23 women (11.2%) were lost to follow-up, as their last contact with the hospital was more than 12 months before the survival cut-off date, but they were included in the analysis. The 3-year overall survival was 44.8% (95% CI, 34.2–54.8) in the pre-MTB and 62.6% (95% CI, 51.8–71.6) in the post-MTB subcohort (Fig. 2A). When restricting the analysis to patients with early breast cancer (stage 0–III), 3-year overall survival was 48.0% (95% CI, 35.9–59.1) in the pre-MTB and 73.0% (95% CI, 61.3–81.6) in the post-MTB subcohort (Fig. 2B). Among patients with metastatic breast cancer, there was no survival benefit with the introduction of the MTB: median overall survival was 19.4 months (95% CI, 15.6–23.2) before and 13.6 months (95% CI, 9.2–17.9) after MTB implementation (Fig. 2C).

In the multivariable analysis, the adjusted HR for death in the post-MTB vs. the pre-MTB subcohort was 0.77 (95% CI, 0.49–1.19) in the overall population and 0.47 (95% CI, 0.27–0.81) among patients with early breast cancer (Fig. 3; supplemental online Tables 1–2). Factors significantly associated with overall survival in both the overall population and patients with early breast cancer were stage, tumor subtype, and type of first-line chemotherapy regimen.

There was a benefit in MTB implementation among most subgroups of patients with early breast cancer (Fig. 4).

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Table 1. Patients' characteristics and treatment according to date of diagnosis (pre- vs. post-MTB implementation)

Characteristics	Pre-MTB, *n* = 98	Post-MTB, *n* = 107	*p*[a]	aOR[b] (95% CI)
Age in years, *n* (%)			.945	
<40	26 (26.5)	28 (26.2)		1
40–49	29 (29.6)	28 (26.2)		0.81 (0.36–1.80)
50–59	21 (21.4)	25 (23.4)		1.10 (0.48–2.56)
≥60	22 (22.4)	26 (24.3)		1.07 (0.46–2.47)
Race, *n* (%)			.623	
Black	97 (99.0	104 (97.2)		1
Other[c]	1 (1.0)	3 (2.8)		2.34 (0.22–25.41)
Education in years, *n* (%)			.618	
0	18 (22.0)	23 (25.3)		1
1–4	14 (17.1)	11 (12.1)		0.63 (0.22–1.83)
>4	50 (61.0)	57 (62.6)		1.09 (0.45–2.67)
Missing	16	16		
Place of residence, *n* (%)			.089	
South (including Maputo)	80 (86.0)	96 (94.1)		1
Center/North	13 (14.0)	6 (5.9)		**0.30 (0.10–0.88)**
Missing	5	5		
Menopausal status, *n* (%)			.889	
Premenopausal	52 (53.1)	58 (54.2)		1
Postmenopausal	46 (46.9)	49 (45.8)		0.47 (0.13–1.70)
Body mass index, *n* (%)			.460	
Under/normal weight, <25 kg/m^2	33 (37.9)	44 (43.6)		1
Overweight/obese, ≥25 kg/m^2	54 (62.1)	57 (56.4)		0.81 (0.42–1.59)
Missing	11	6		
HIV status, *n* (%)[d]			.873	
Negative/unknown	74 (75.5)	79 (73.8)		1
Positive	24 (24.5)	28 (26.2)		1.28 (0.65–2.53)
Classic subtype, *n* (%)			**.028**	
ER+/HER2−	46 (46.9)	45 (42.1)		1
HER2+	22 (22.4)	22 (20.6)		0.98 (0.46–2.05)
TNBC	23 (23.5)	17 (15.9)		0.72 (0.33–1.56)
Unknown[e]	7 (7.1)	23 (21.5)		**3.66 (1.39–9.64)**
Stage at diagnosis, *n* (%)			.538	
0-II[f]	23 (23.5)	30 (28.0)		1
III	52 (53.1)	58 (54.2)		0.92 (0.45–1.86)
IV	23 (23.5)	19 (17.8)		0.53 (0.22–1.31)
Time from symptoms to diagnosis, *n* (%)			.598	
<180 days	33 (54.1)	32 (50.0)		1
≥180 days	28 (45.9)	33 (50.8)		1.23 (0.57–2.64)
Missing	37	42		
Time from diagnosis to treatment, *n* (%)			.324	
<45 days	42 (44.7)	55 (51.9)		1
≥45 days	52 (55.3)	51 (48.1)		0.69 (0.38–1.25)
Missing	4	1		
No treatment received: yes, *n* (%)	3 (3.1)	1 (0.9)	.351	0.32 (0.03–3.52)
Type of first treatment received, *n* (%)			.999	
Surgery	24 (25.5)	26 (24.5)		1
Chemotherapy/endocrine therapy	70 (74.5)	80 (75.5)		0.94 (0.48–1.86)
Missing	1	0		

(continued)

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Table 1. (continued)

Characteristics	Pre-MTB, *n* = 98	Post-MTB, *n* = 107	*p*[a]	aOR[b] (95% CI)
Surgery (ever): yes, *n* (%)[g]	79 (80.6)	88 (82.2)	.858	1.44 (0.59–3.50)
Type of breast surgery, *n* (%)[h]			.257	
Total mastectomy	75 (94.9)	79 (89.8)		1
Tumorectomy	4 (5.1)	9 (10.2)		2.44 (0.65–9.23)
Status of surgical margins, *n* (%)			.575	
Clean	61 (88.4)	72 (92.3)		1
Positive	8 (11.6)	6 (7.7)		0.66 (0.20–2.19)
Missing[i]	10	10		
Histological grade at surgery, *n* (%)			.422	
1	16 (22.2)	20 (27.0)		1
2	35 (48.6)	28 (37.8)		0.62 (0.25–1.51)
3	21 (29.2)	26 (35.1)		1.22 (0.47–3.19)
Missing[i]	7	14		
Axillary surgery: type, *n* (%)[h]			1.0	
Sentinel lymph node biopsy	0	1 (1.3)		
Axillary dissection	70 (100)	77 (98.7)		
Axillary surgery: completeness, *n* (%)[j]			.423	
Not done	9 (11.7)	9 (10.7)		0.93 (0.30–2.89)
Incomplete	23 (29.9)	18 (21.4)		0.58 (0.27–1.24)
Complete	45 (58.4)	57 (67.9)		1
Missing	2	4		
Lymph node status, *n* (%)			.828	
(y)pN0	18 (27.7)	22 (32.4)		1
(y)pN1	23 (35.4)	25 (36.8)		0.97 (0.39–2.43)
(y)pN2	13 (20.0)	13 (19.1)		0.94 (0.30–2.93)
(y)pN3	11 (16.9)	8 (11.8)		0.58 (0.17–2.04)
Missing[i]	14	20		
Chemotherapy (ever): yes, *n* (%)	90 (91.8)	103 (96.3)	.237	2.36 (0.65–8.58)
Intent of first-line CT, *n* (%)[k]			.174	
Neoadjuvant only	8 (8.9)	20 (19.4)		1
Neoadjuvant + adjuvant	45 (50.0)	45 (43.7)		0.50 (0.19–1.31)
Adjuvant only	16 (17.8)	20 (19.4)		0.64 (0.21–1.96)
Palliative	21 (23.3)	18 (17.5)		
Neoadjuvant CT: outcome, *n* (%)			.682	
Same stage	19 (42.2)	22 (37.9)		1
Upstaging	8 (17.8)	8 (13.8)		0.72 (0.20–2.60)
Downstaging	18 (40.0)	28 (48.3)		1.35 (0.51–3.59)
Missing[l]	8	7		
pCR rate after neoadjuvant CT, *n* (%)			.494	
No pCR	48 (90.6)	56 (86.2)		1
pCR only in the breast (ypT0/is)	3 (5.7)	3 (4.6)		0.71 (0.11–4.75)
pCR in the breast and lymph nodes (ypT0/is, ypN0)	2 (3.8)	6 (9.2)		2.00 (0.34–11.96)
Type of first-line CT regimen, *n* (%)[k]			.393	
Anthracycline-based only	44 (48.9)	45 (43.7)		1
Anthracyclines + taxanes based	40 (44.4)	55 (53.4)		1.22 (0.61–2.43)
Other[m]	6 (6.7)	3 (2.9)		0.37 (0.07–1.80)

(continued)

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Table 1. (continued)

Characteristics	Pre-MTB, *n* = 98	Post-MTB, *n* = 107	*p*[a]	aOR[b] (95% CI)
First-line CT dose-intensity, *n* (%)[k]			.881	
<85%	48 (57.8)	60 (59.4)		1
≥85%	35 (42.2)	41 (40.6)		0.95 (0.50–1.79)
Endocrine therapy (ever): yes, *n* (%)	50 (51.0)	54 (50.5)	.888	0.87 (0.45–1.66)
Radiotherapy (ever): yes, *n* (%)	2 (2.0)	7 (6.5)	.175	3.88 (0.74–20.27)

[a]*p* value for the univariate comparison between the two subcohorts. Values in bold correspond to statistically significant differences.
[b]Adjusted for age (<40 vs. 40–49 vs. 50–59 vs. ≥60 years), HIV status (negative/unknown vs. positive), stage at diagnosis (0–II vs. III vs. IV), and classic subtypes (ER+/HER2− vs. HER2+ vs. TNBC vs. unknown). The reference is the "pre-MTB" implementation period. Values in bold correspond to statistically significant differences.
[c]Includes mixed and Asian race.
[d]Eight patients had unknown HIV status; among HIV-positive patients, 38 (74%) had been previously diagnosed with HIV infection; the median time since HIV diagnosis was 3.93 years (range, 0.1–12.2); 48 (98.1%) patients were under antiretroviral treatment (ART) when starting chemotherapy, mostly with the TDF + 3TC + EFV regimen (33 patients); the median time under ART was 2.00 years (range, 0.1–12.2); the median CD4+ cells count was 439 cells/μL (range, 43–1,104), and 46 (90%) patients had a CD4+ cell count >200/μL.
[e]Biomarker determination unknown due to carcinoma *in situ* or pathological complete response (i.e., no residual invasive tumor): *n* = 8 (*n* = 3 in the pre-MTB and *n* = 5 in the post-MTB subcohort); inadequate tissue fixation: *n* = 2 (0/2); unavailable samples: *n* = 20 (4/16).
[f]Among patients within the category 0–II stage, one patient had stage 0 and four patients had stage I.
[g]Includes 12 patients submitted to surgery outside the Maputo Central Hospital for whom complete pathological information regarding the surgical specimen is not available.
[h]Patients submitted to a tumorectomy followed by a mastectomy were included in the "Mastectomy" group and those receiving a sentinel lymph node biopsy followed by an axillary dissection were included in the "Axillary dissection" group.
[i]Cases with missing data for surgical margins, histological grade, or lymph node status include patients submitted to surgery outside the Maputo Central Hospital for whom complete pathological information is not available regarding the surgical specimen; patients with pathological complete response; patients for whom axillary surgery was not performed; and patients for whom these assessments at the Maputo Central Hospital are missing.
[j]Among patients receiving any type of breast surgery (*n* = 166). 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).
[k]First line of chemotherapy that the patient received includes neoadjuvant, adjuvant, or palliative treatment. If the patient received part of chemotherapy as neoadjuvant (e.g., doxorubicin plus cyclophosphamide [AC] regimen) and another part as adjuvant chemotherapy (e.g., paclitaxel), the type of regimen and dose-intensity refer to the entire scheme (neoadjuvant plus adjuvant).
[l]Cases with missing data regarding clinical staging or patient abandoned treatment.
[m]Includes taxane-based chemotherapy (three patients), nonanthracycline/nontaxane-based chemotherapy (five 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 three weeks); dose-dense regimens were not used because of the unpredictable availability of granulocyte-colony stimulating factors.
Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; CT, chemotherapy; ER, estrogen receptor; MTB, multidisciplinary tumor board; pCR, pathological complete response; TNBC, triple-negative breast cancer.

There were apparently no benefits among those with lower education, HER2-positive disease, grade 1 tumors, or receiving surgery as first treatment, but the low precision of the estimates precludes bold statements regarding stratum-specific results or potential interactions.

Disease-Free Survival

Among patients with early breast cancer, 48% had a disease-free survival event: 18% had loco-regional relapse, 8% had distant relapse, 6% had both loco-regional and distant relapse, 1% had a second primary cancer, and 16% died. The 3-year disease-free survival was 41.7% (95% CI, 30.2–52.8) in the pre-MTB and 56.8% (95% CI, 45.3–66.8) in the post-MTB subcohort (Fig. 2D). The proportion of patients with loco-regional relapses (with or without concomitant distant relapse) or death as the first disease-free survival event was numerically higher in the pre-MTB vs. the post-MTB subcohort (29% vs. 18%; and 23% vs. 10%, respectively), but these differences were not statistically significant (p = .07). In the multivariable analysis, the adjusted HR for relapse or death was 0.72 (95% CI, 0.46–1.13) in the post-MTB compared with the pre-MTB subcohort (Fig. 3; supplemental online Table 3). Tumor characteristics (subtype, lymph node status, and stage), quality of surgery (surgical margins status and axillary surgery completeness), and type of first-line chemotherapy regimen were significantly associated with disease-free survival.

Cost-Effectiveness Analysis

The absolute 3-year mean cost increase of implementing a MTB was $119.83 per patient (7648.42 MZN per patient). The incremental cost-effectiveness ratio was $802.96 per QALY (51252.57 MZN per QALY), corresponding to 1.6 times the GDP of Mozambique (Table 2). Assuming a willingness-to-pay threshold of three times the national GDP (1497.00 USD), MTB implementation is a cost-effective measure in this setting. Sensitivity analyses showed similar findings (supplemental online Table 4).

Discussion

To the best of our knowledge, this is the first study reporting the survival benefit and cost-effectiveness analysis of a MTB

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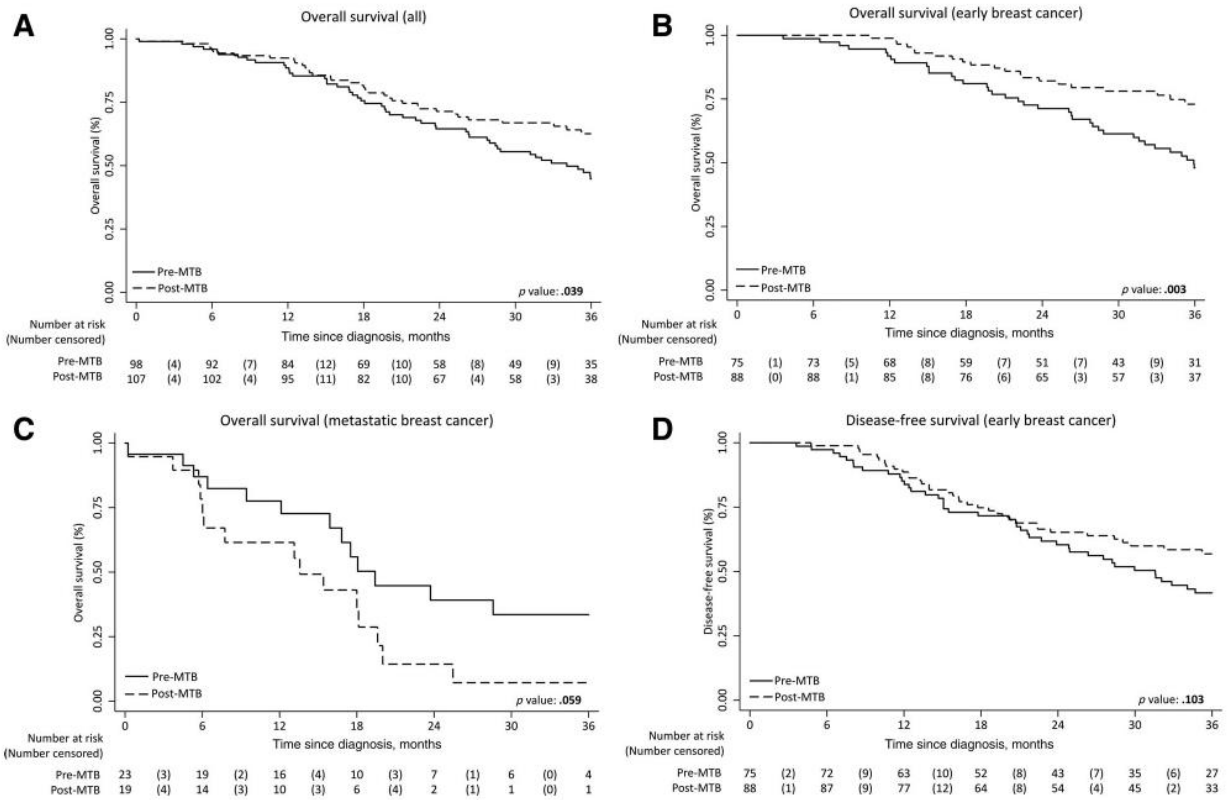


Figure 2. Kaplan-Meier curves for overall survival among all patients, patients with early breast cancer and patients with metastatic breast cancer; and for disease-free survival among patients with early breast cancer. **(A):** Overall survival in the total population: 3-year overall survival was 44.8% (95% confidence interval [CI], 34.2–54.8) in the pre-MTB subcohort versus 62.6% (95% CI, 51.8–71.6) in the post-MTB subcohort. **(B):** Overall survival among patients with early breast cancer (stage 0–III): 3-year overall survival was 48.0% (95% CI, 35.9–59.1) in the pre-MTB subcohort versus 73.0% (95% CI, 61.3–81.6) in the post-MTB subcohort. **(C):** Overall survival among patients with metastatic breast cancer (stage IV): median overall survival was 19.4 months (95% CI, 15.6–23.2) in the pre-MTB subcohort versus 13.6 months (95% CI, 9.2–17.9) in the post-MTB subcohort. **(D):** Disease-free survival among patients with early breast cancer (stage 0–III): 3-year disease-free survival was 41.7% (95% CI, 30.2–52.8) in the pre-MTB subcohort versus 56.8% (95% CI, 45.3–66.8) in the post-MTB subcohort.
Abbreviation: MTB, multidisciplinary tumor board.

for breast cancer in a low-income country and in Africa. Our prospective cohort study showed that the implementation of a MTB, after the training of a few key health professionals, led to a significant reduction in mortality among patients with early breast cancer. This benefit remained significant after adjusting for other prognostic factors. Given the baseline poor prognosis of these patients, the absolute improvement in 3-year overall survival among patients with early breast cancer was 25.0% (from 48.0% to 73.0%). Moreover, we have demonstrated that this is a cost-effective intervention.

Interestingly, time from diagnosis until treatment or the proportion of each treatment received were similar in both the pre-MTB and post-MTB subcohorts. Although not statistically significant, there were improvements in the quality of care (i.e., clean surgical margins, use of neoadjuvant chemotherapy, among others). Thus, the survival benefit brought by the MTB might be from the added effect of each of these small improvements and by the truly integrated multidisciplinary care of patients, generating high-value clinical practices and better patient navigation within the system. This is suggested by the fact that patients receiving neoadjuvant chemotherapy benefited more from MTB implementation than those receiving surgery first, probably because those patients would have had a more difficult navigation in the pre-MTB era, potentially leading to delays between treatments and a worse outcome.

In contrast, the absence of survival benefit among patients with stage IV breast cancer may be related to their baseline poor prognosis and the low availability of effective systemic therapies. This highlights the need to diagnose breast tumors at an earlier stage and to provide more effective treatment strategies to women with *de novo* metastatic disease in low-resource settings.

Studies assessing the composition, functioning, and impact of MTBs in different cancer types in high-income countries have heterogeneous results in terms of survival impact and cost-effectiveness [14–16, 32, 33]. These discrepancies may be due to different study designs, patient population, or MTB models. For instance, differences between two large studies in the U.K. [14] and Germany [16] in terms of inclusion periods (1990–2000 vs. 2004–2010, respectively), median follow-up time (7.0 vs. 3.3 years), and diverse

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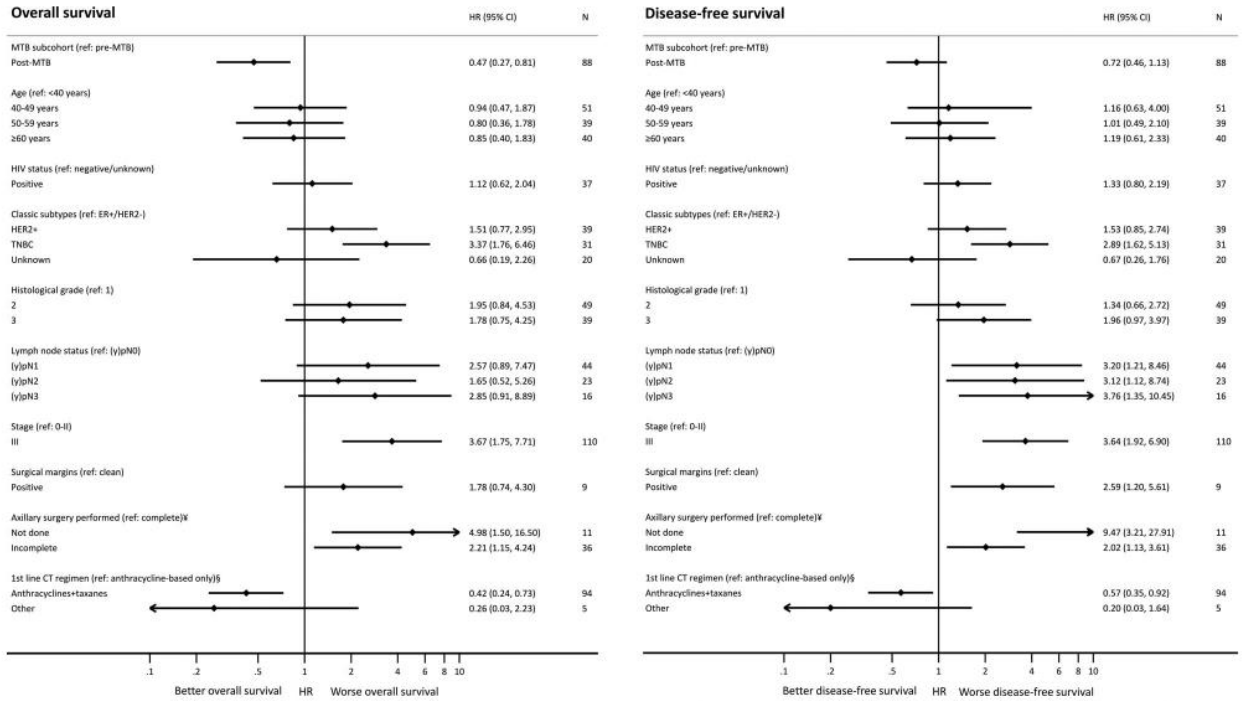


Figure 3. Multivariable analyses of prognostic factors for overall survival (left panel) and disease-free survival (right panel) among patients with early breast cancer (stage 0–III). All models included the following variables: MTB implementation period (pre vs. post), age (<40 vs. 40–49 vs. 50–59 vs. ≥60 years), HIV status (negative/unknown vs. positive), stage at diagnosis (0–II vs. III), and tumor subtype (ER+/HER2− vs. HER2+ vs. TNBC vs. unknown). ¥Among patients receiving any type of breast surgery. 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 and/or adjuvant treatment. If the patient received part of chemotherapy as neoadjuvant (e.g., doxorubicin plus cyclophosphamide [AC] regimen) and another part as adjuvant chemotherapy (e.g., paclitaxel), the type of regimen refers to the entire scheme (neoadjuvant plus adjuvant).
Abbreviations: CI, confidence interval; CT, chemotherapy; ER, estrogen receptor; HR, hazard ratio; MTB, multidisciplinary tumor board; TNBC, triple-negative breast cancer.

multidisciplinary care models may explain why the former showed a survival advantage of this intervention and the latter did not. In developing countries, data regarding MTBs are much scarcer, especially in sub-Saharan Africa. A study from Botswana showed a reduction in the time from biopsy to start of radiation therapy among patients with cervical cancer [34]. After the implementation of gastro-intestinal MTBs in Saudi Arabia and Ethiopia, adherence to evidence-based guidelines improved [35, 36]. Unfortunately, these studies did not provide survival data.

One of the major strengths of the present analysis is being based in the prospective follow-up of a cohort, unlike most breast cancer studies in sub-Saharan African, which are usually retrospective [5, 37–39]. We collected information regarding the entire spectrum of cancer care, from the presentation of symptoms to diagnosis, comorbidities, treatment, and survival outcomes. There were also fewer losses to follow-up (11.2%) than in previous larger retrospective studies (losses of 22%–53%) [38–40]. This reflects the efforts by the oncology unit staff to improve follow-up completeness, by recording multiple telephone numbers per patient and actively contacting those who missed appointments.

During the study, there was no improvement in the access to certain drugs (e.g., trastuzumab remained unavailable) or to other treatments (e.g., radiotherapy), thus not confounding the effect estimated for the introduction of the MTB. There were also no significant differences between patients regarding prognostic factors, such as age, stage, HIV status, or proportion of tumor subtypes.

However, our study has limitations that should be acknowledged. We compared patients diagnosed during the 15 months preceding the MTB implementation to patients diagnosed in the 18 months that followed it. Given the time proximity between the two subcohorts, there are patients in the pre-MTB subcohort who may have benefited from changes in cancer care brought by the training of professionals and MTB implementation and who may have been discussed in the MTB upon relapse. However, this might have contributed to underestimate the effectiveness of MTB implementation, and thus, this study provides conservative estimates of its effect.

There were no differences in disease-free survival between the two subcohorts, but this might be related to the timing of relapse diagnosis. Patients in the pre-MTB were not followed as closely as those in the post-MTB

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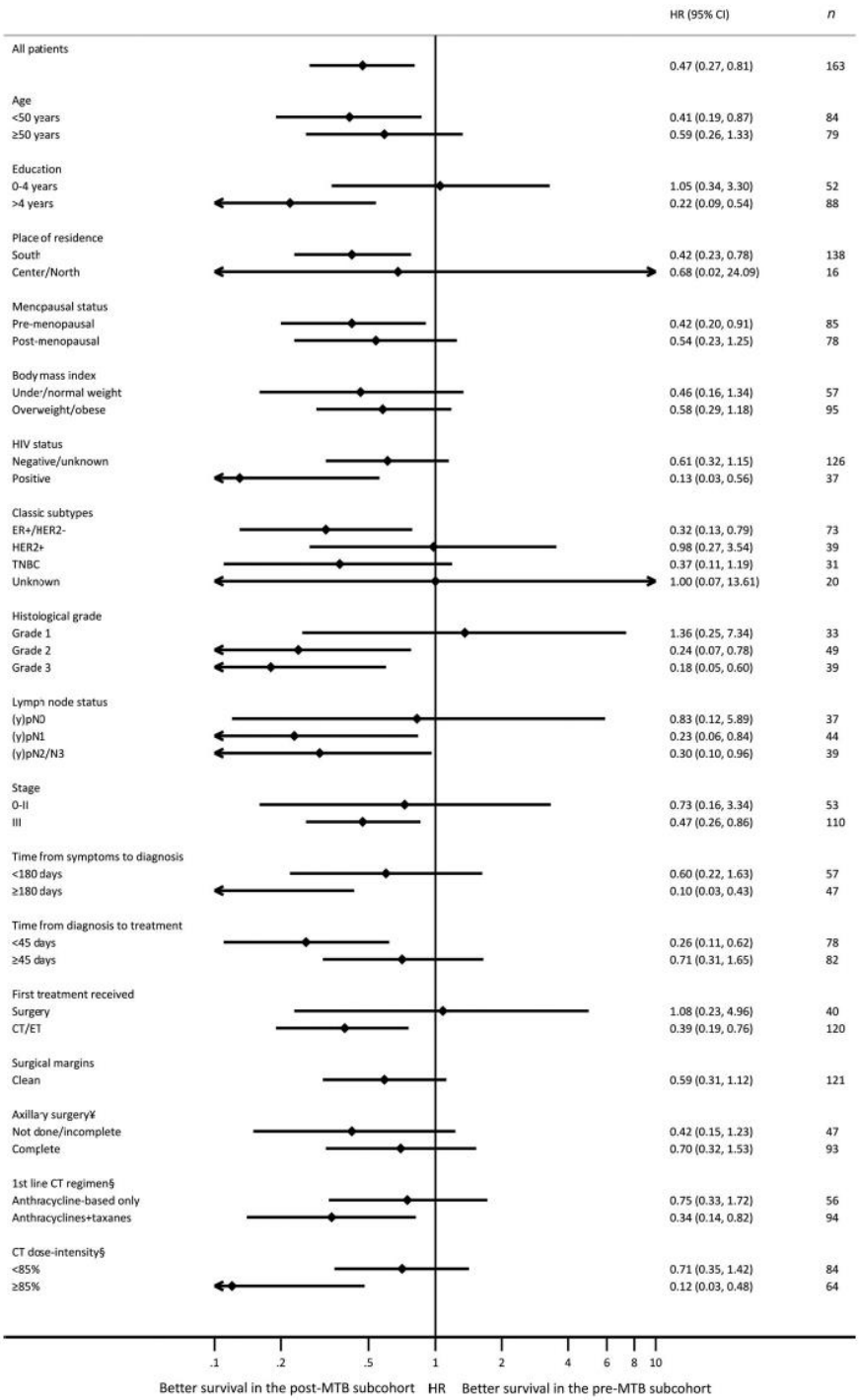


Figure 4. Multivariable analysis of overall survival in the post-MTB versus pre-MTB implementation periods within each subgroup of patients with early breast cancer (stage 0–III). All models included the following variables: MTB implementation period (pre vs. post), age (<40 vs. 40–49 vs. 50–59 vs. ≥60 years), HIV status (negative/unknown vs. positive), stage at diagnosis (0–II vs. III), and tumor subtype (ER+/HER2− vs. HER2+ vs. TNBC vs. unknown). ¥Among patients receiving any type of breast surgery. Not done/incomplete: 0–5 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 and/or adjuvant treatment. If the patient received part of chemotherapy as neoadjuvant (e.g., doxorubicin plus cyclophosphamide [AC] regimen) and another part as adjuvant chemotherapy (e.g., paclitaxel), the type of regimen and dose-intensity refer to the entire scheme (neoadjuvant plus adjuvant).

Abbreviations: CI, confidence interval; CT, chemotherapy; ER, estrogen receptor; ET, endocrine therapy; HR, hazard ratio; MTB, multidisciplinary tumor board; TNBC, triple-negative breast cancer.

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Table 2. Costs, benefits, costs per unit of benefit and incremental cost-effectiveness ratios, by strategy (no multidisciplinary tumor board [MTB] vs. MTB), in a provider perspective, over a 3-year time horizon

Strategy	Probability of being alive without relapse at 3 years	Probability of being alive with relapse at 3 years	Mean 3-year cost per patient treated[a]	Mean 3-year incremental cost per patient treated[a]	Mean QALYs per patient	ICER (cost/QALY)[b]
No MTB (usual care)	0.4166	0.0635	$993.1803; 63,393.9057 MZN		1.46225	
MTB	0.5681	0.1615	$1,113.0067; 71,042.3278 MZN	$119.8264; 7,648.4221 MZN	1.61148	$802.9646; 51,252.5772 MZN

[a]Future health benefits and costs were discounted at 3% annually, assuming a time-horizon of 3-years, based on the World Health Organization recommendations [30].
[b]The conversion between U.S. dollars (USD) and MZN was performed using the exchange rate 1 USD = 63.8292 MZN.
Abbreviations: ICER, incremental cost-effectiveness ratio; MTB, multidisciplinary tumor board; MZN, Mozambique Meticais; QALY, quality-adjusted life year.

subcohort, and, therefore, documentation of relapse may have occurred later compared with patients in the post-MTB subcohort. This is reinforced by the fact that most disease-free survival events consisted of loco-regional disease, a type of relapse that is easily recognized by both patients and doctors, when compared with distant metastases. Thus, a more frequent clinical examination can detect loco-regional relapse earlier, without the need for imaging exams. In addition, the proportion of patients with death as the first disease-free survival event was numerically higher in the pre-MTB vs. the post-MTB subcohort, suggesting that these patients probably had an earlier relapse that was not detected by the physician, possibly because of the less close follow-up in the pre-MTB subcohort. Even if there were no significant differences in disease-free survival, there was a significant benefit in overall survival, which is considered to be a more objective survival measure.

Surprisingly, there was a higher proportion of patients with tumors with unknown subtype in the post-MTB compared with the pre-MTB subcohort (21.5% vs. 7.1%, respectively). The most common reason was the unavailability of samples (i.e., there were no cell blocks from fine-needle aspiration biopsies, core-needle biopsies, or surgical specimens available to perform subtype determination). This could be because some patients were diagnosed and/or received surgery outside the Maputo Central Hospital and that there were fewer patients with stage IV disease operated to their breast tumor and a slightly higher number of patients with pathological complete response (i.e., without residual invasive tumor) in the post-MTB subcohort.

In this study, we only analyzed patients treated and followed at a single center. Nonetheless, our population had many of the characteristics demonstrated in other sub-Saharan African studies, such as a high proportion of stage III/IV at presentation [3], a delay of several months between first symptoms and diagnosis [41], a high proportion of TNBC [4], a high prevalence of HIV infection [40], and low survival [42]. This supports the possibility of generalizing our findings to other hospitals and settings in this region.

We showed that improving the continuum of breast cancer care leads to significant gains in survival among patients with early breast cancer, who are potentially curable. These results reflect (a) the training of key professionals in specialized breast cancer care; (b) a better coordination of care, through the implementation of a MTB; and (c) an active follow-up of patients, leading to an improved adherence to treatment. Some of these solutions do not need significant extra funding to be implemented, but just a better organizational model.

We demonstrated that beyond traditional clinicopathological factors, not only does the performance of surgery or chemotherapy matter, but its quality is also paramount to improve survival. This is another argument to adequately train surgeons and oncologists in the best cancer care practices, as it can improve survival without a large increase in cost. Additionally, strategies to increase access to taxanes and chemotherapy dose-intensity, such as an adequate supply of drugs and supportive treatment, should also be considered. These could lead to an increase in the proportion of patients with pathological complete response after neoadjuvant chemotherapy, which we have previously shown to be low across all tumor subtypes [26].

The success of this first MTB in Mozambique has recently led to the formal endorsement of multidisciplinarity as an essential part of cancer care, both in the Mozambican Breast Cancer Guidelines and in the Mozambican Cancer Control Plan 2019–2029 [43, 44]. Moreover, there are now separate MTBs for thoracic conditions, gynecological cancer, head and neck cancer, and esophageal cancer at the Maputo Central Hospital.

We will continue to update the survival follow-up of this cohort using data from the Hospital's Cancer Registry. Yet, given the early and wide separation of the overall survival curves among patients with early breast cancer, it is expected that the survival benefit brought by the MTB will be maintained in the future.

We acknowledge the wide variations in available resources for cancer care among sub-Saharan African countries and regions. Nonetheless, our proposed MTB model is simple, easy to implement, and cost-effective, so we believe that it could be scaled up to other countries and settings, in which MTB implementation remains suboptimal.

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Future research should also focus on ways to improve the effectiveness of MTBs in low-resource settings, by identifying the processes most associated with survival gain. Moreover, the selection of patients that benefit the most from multidisciplinarity is also warranted. This future knowledge could also be used to improve the effectiveness of MTBs in high-income countries.

Conclusion

Within this prospective cohort study in Mozambique, we showed that the implementation of an MTB led to a 53% mortality decrease among patients with early breast cancer. This intervention led to an absolute gain in 3-year overall survival of 25.0% and was cost-effective. These findings highlight the feasibility of implementing this strategy and the need for expanding the use of MTBs in developing countries as a way to improve patient outcomes. Given the high proportion of women with stage IV disease in our cohort, we also point to the need of diagnosing breast cancer at an earlier stage, to optimize patient outcomes.

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This study was approved by the National Health Bioethical Committee of Mozambique (reference number 226/CNBS/15). All participants provided written informed consent. This study was performed in accordance with the Declaration of Helsinki.

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Disclosures

Mariana Brandão: Radius, AstraZeneca, Eli Lilly & Co, Merck Sharpe & Dohme, GlaxoSmithKline/Novartis, Roche/GNE, Synthon, Servier, Pfizer (RF [Institution]). Roche/GNE (Other-travel grant, H); **Martine Piccart-Gebhart:** AstraZeneca, Camel-IDS, Crescendo Biologics, Debiopharm, G1 Therapeutics, Genentech, Huya, Immunomedics, Eli Lilly & Co, Menarini, Merck Sharpe & Dohme, Novartis, Odonate, Periphagen, Pfizer, Roche, Seattle Genetics (C/A, H), Oncolytics (SAB), IJB from Radius, AstraZeneca, Lilly, Merck Sharp & Dohme, GlaxoSmithKline/Novartis, Roche/GNE, Synthon, Servier, Pfizer (RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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Supplemental Appendix for:
Survival impact and cost-effectiveness of a multidisciplinary tumor board for breast cancer in Mozambique, Sub-Saharan Africa
Carla Carrilho et al.

Supplementary Methods

Detailed methods for cost-effectiveness analysis

The decision tree model is depicted in Figure M1. The parameters considered in the decision model (transition parameters, cost parameters and health benefits) are summarized in Table M1 and their calculation is detailed in Table M2.

Costs were calculated from the provider perspective, which includes the costs incurred by a health institution when providing a health service.

All future costs were discounted at a rate of 3% annually.[1] Infrastructure costs were assumed to be the same between the two strategies (no multidisciplinary tumor board [MTB] *vs* MTB) and were therefore not considered in data analysis.

In the sensitivity analyses, cost parameters and health benefits varied in ±20%.

The incremental cost-effectiveness ratio (ICER) was calculated as the additional costs divided by the additional health benefits of the "MTB" strategy compared to the "No MTB" strategy. The ICER was compared to an external willingness-to-pay threshold of three times the gross domestic product per capita of Mozambique to determine if the implementation of a "MTB" was a cost-effective strategy.

Figure M1 – Decision tree model overview for the cost-effectiveness analysis.

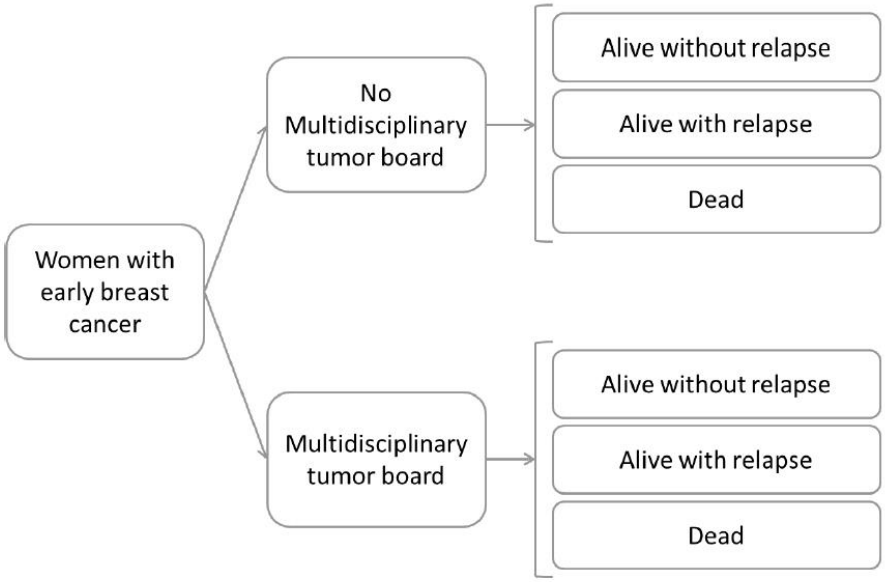


Table M1 – Summary of decision tree model parameters.

Description of the transition parameters	Base case (p)	Data source[a]
Proportion of women diagnosed before the implementation of a MTB, who were alive without relapse 3 years after diagnosis	0.417	a)
Proportion of women diagnosed before the implementation of a MTB, who were alive with relapse 3 years after diagnosis	0.064	a)
Median time from diagnosis until relapse, among women diagnosed before the implementation of a MTB who were alive with relapse 3 years after diagnosis	27.53 months	a)
Proportion of women diagnosed before the implementation of a MTB, who died 3 years after diagnosis	0.520	a)
Median time from diagnosis until relapse, among women diagnosed before the implementation of a MTB who died 3 years after diagnosis	15.08 months	a)
Median time from diagnosis until death, among women diagnosed before the implementation of a MTB	22.11 months	a)
Proportion of women diagnosed after the implementation of a MTB, who were alive without relapse 3 years after diagnosis	0.568	a)
Proportion of women diagnosed after the implementation of a MTB, who were alive with relapse 3 years after diagnosis	0.160	a)
Median time from diagnosis until relapse, among women diagnosed after the implementation of a MTB who were alive with relapse 3 years after diagnosis	13.27 months	a)
Proportion of women diagnosed after the implementation of a MTB, who died 3 years after diagnosis	0.272	a)
Median time from diagnosis until relapse, among women diagnosed after the implementation of a MTB who died 3 years after diagnosis	16.92 months	a)
Median time from diagnosis until death, among women diagnosed after the implementation of a MTB	20.24 months	a)

Description of cost parameters[b]	Mean cost per patient (USD)	Range (USD)	Mean cost per patient (MZN)	Range (MZN)	Data source[a]
Pathology exams	43.32	34.66-51.98	2765.43	2212.34-3318.52	b)
Staging	133.77	107.02-160.52	7694.45	6155.56-9233.34	b)
Breast surgery	239.23	191.38-287.08	15269.58	12215.66-18323.50	b), c)
Systemic therapy	352.63	282.10-423.16	22508.48	18006.78-27010.18	a-d)
Radiotherapy – among pre-MTB patients	27.94	22.35-33.53	1783.70	1426.96-2140.44	b)
Radiotherapy – among post-MTB patients	83.36	66.69-100.03	5320.71	4256.57-6384.85	b)
Follow-up among pre-MTB patients	209.5	167.60-251.40	13372.27	10697.82-16046.72	b)
Follow-up among post-MTB patients	261.99	209.59-314.39	16723.02	13378.42-20067.62	b), c)
MTB – among post-MTB patients	11.91	9.53-14.30	760.46	608.37-912.55	e), f)

Description of health state	Base case (QALY/year)	Mean total QALYs per patient (no MTB)[c]	Range	Mean total QALYs per patient (MTB)[c]	Range	Data source[a]
Patients who survived without relapse	0.617 QALY/year without relapse	1.814	1.451-2.177	1.814	1.451-2.177	a), g), h)
Patients who survived with relapse	0.567 QALY/year with relapse	1.816	1.453-2.179	1.756	1.405-2.108	a), g), h)
Patients who died	0 QALY/year after death	1.108	0.886-1.329	1.027	0.821-1.232	a), g), h)

MTB=multidisciplinary tumor board; MZN=Mozambique Meticais; QALY=quality-adjusted life years; USD=United States dollars (with 2019 as reference year).

[a]Data sources:
a) Moza-BC cohort study;
b) Maputo Central Hospital price tables for "Special Service";
c) WHO-CHOICE estimates (2010);[2]
d) Maputo Central Hospital Pharmacy price tables;
e) Amount spent by the Calouste Gulbenkian Foundation on the training of health professionals who were later part of the MTB;
f) Maputo Central Hospital Human Resources Department;
g) Peasgood et al (2010);[3]
h) Clarke et al (2017);[4]

[b]Values corrected for inflation and currency; for the detailed calculations, please refer to Supplementary Table 2.

[c]Total benefit over a three-year time horizon.

Table M2 – Cost calculation details for breast cancer diagnosis, treatment and follow-up.

Description of cost parameters[a]	Cost (USD) per unit	Average cost (USD) per patient	Cost (MZN) per unit	Average cost (MZN) per patient	Data source[b]
Pathology					
Pathology analysis of fine-needle aspiration biopsy (without immunohistochemistry)	12.53	9.67	800.00	617.28	b)
Pathology analysis of surgical specimen (with immunohistochemistry)	37.60	33.65	2400.00	2148.15	b)
Staging					
Staging – imaging	83.03	83.03	5300.00	5300.00	b)
Staging – lab tests	24.28	37.51	1550.00	1550.00	b)
Staging – HIV testing	12.53	10.37	800.00	661.73	b)
Staging – CD4+ cell count	12.53	2.86	800.00	182.72	b)
Breast surgery					
Breast surgery procedure (including axillary surgery)	217.14	194.36	13860.00	12405.56	b)
Inpatient stay due to surgery[c]	7.16	44.87	457.11	2864.02	c)
Systemic therapy					
Outpatient consultations during the first year after diagnosis (treatment phase)[d]	1.96	17.83	125.34	1138.07	c)
Chemotherapy treatment – drugs (overall)	-	105.27	-	6719.3	a), d)
Doxorubicin (price per mg)	*0.06760*	-	*4.31485*	-	d)
Cyclophosphamide (price per mg)	*0.00476*	-	*0.30383*	-	d)
Paclitaxel (price per mg)	*0.06767*	-	*4.31913*	-	d)
Methotrexate (price per mg)	*0.04320*	-	*2.75742*	-	d)
5-FU (price per mg)	*0.00076*	-	*0.04851*	-	d)
Carboplatin (price per mg)	*0.03858*	-	*2.46240*	-	d)
Chemotherapy treatment – day hospital use[e]	11.49	83.75	733.33	5345.98	b)
Chemotherapy treatment – lab tests[e]	16.45	119.92	1050.00	7654.50	b)
Hormone therapy (considering 24 months of treatment)[f]					
Tamoxifen	1.80	23.73	114.89	1514.67	d)
Letrozole	7.20	2.13	459.57	135.96	d)
Radiotherapy (25 sessions)					
Radiotherapy – among pre-MTB patients	1047.95	27.94	66890.00	1783.70	b)
Radiotherapy – among post-MTB patients	1047.95	83.36	66890.00	5320.71	b)
Follow-up (considering 2 years of follow-up after finishing breast surgery and chemotherapy)					
Outpatient follow-up consultations – among pre-MTB patients[d]	1.96	11.79	125.34	752.27	c)
Outpatient follow-up consultations – among post-MTB patients[d]	1.96	15.71	125.34	1003.02	c)
Lab tests during follow-up – among pre-MTB patients[a]	24.28	145.70	1550.00	9300.00	b)
Lab tests during follow-up – among post-MTB patients[d]	24.28	194.27	1550.00	12400.00	b)
Follow-up mammography (once a year)	26.01	52.01	1660.00	3320.00	b)
MTB					
Training of professionals for the MTB[g]	5749.00	4.02	366955.00	256.43	e)
Regular work of the MTB[h]					
Resident salary (cost per hour x 2 hours)	4.86	2.21	310.42	141.1	f)
Consultant salaries (cost per hour x 4 specialists per MTB)	22.93	5.21	1463.62	332.64	f)
Secretary salary (cost per hour x 2 hours per MTB)	2.08	0.47	133.27	30.29	f)

MTB=multidisciplinary tumor board; MZN=Mozambique Meticais; USD=United States dollars (with 2019 as reference year).
[a]Values corrected for inflation and currency.
[b]Data sources:
a) Moza-BC cohort study;
b) Maputo Central Hospital price tables for "Special Service";
c) WHO-CHOICE estimates (2010);[2]
d) Maputo Central Hospital Pharmacy price tables;
e) Amount spent by the Calouste Gulbenkian Foundation on the training of health professionals who were later part of the MTB;
f) Maputo Central Hospital Human Resources Department.
[c]Value per day of inpatient stay.
[d]Value per consultation.
[e]Value per cycle of chemotherapy.
[f]Value per one month of therapy.
[g]In total, covering the training of a surgeon, a radiologist and a pathologist technician, who were trained abroad specifically in breast cancer care within the Calouste Gulbenkian Foundation training program.
[h]Considering that a resident and a secretary usually spend two hours of work per MTB (preparation plus meeting itself) and that there are four specialists attending the MTB (medical oncologist, pathologist, surgeon and radiologist).

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Supplemental Tables for:
Survival impact and cost-effectiveness of a multidisciplinary tumor board for breast cancer in Mozambique, Sub-Saharan Africa
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Supplementary Table S1 – Multivariable analysis of prognostic factors for overall survival among the total population

	N	3-year OS, % (95% CI)	P[a]	Adjusted HR (95% CI)[b]
Date of diagnosis				
Pre-MTB implementation	98	44.8 (34.2-54.8)	0.039	1
Post-MTB implementation	107	62.6 (51.8-71.6)		0.77 (0.49-1.19)
Age in years				
<40	54	43.7 (29.1-57.3)	0.256	1
40-49	57	53.2 (38.5-65.9)		0.94 (0.53-1.70)
50-59	46	61.6 (44.5-74.8)		0.85 (0.45-1.60)
≥60	48	56.1 (39.8-69.6)		0.69 (0.38-1.26)
Education in years				
0	41	54.6 (37.3-69.0)	0.407	1
1-4	25	45.5 (24.9-64.0)		0.92 (0.42-2.01)
>4	107	55.1 (44.3-64.7)		0.63 (0.32-1.27)
Place of residence				
South (including Maputo)	176	52.0 (43.9-59.6)	0.479	1
Center/North	19	62.5 (34.1-81.4)		0.72 (0.30-1.70)
Menopausal status				
Pre-menopausal	110	49.3 (38.9-58.9)	0.294	1
Post-menopausal	95	58.1 (46.7-67.9)		1.09 (0.37-3.18)
Body mass index				
Under/normal weight (<25 kg/m^2)	77	57.9 (44.8-69.0)	0.640	1
Overweight/obese (≥25 kg/m^2)	111	50.2 (40.0-59.6)		1.14 (0.68-1.92)
HIV status				
Negative/unknown	153	56.2 (47.4-64.1)	0.230	1
Positive	52	44.5 (29.1-58.7)		1.02 (0.62-1.68)
Classic subtypes				
ER+/HER2-	91	61.1 (49.5-70.9)	0.014	1
HER2+	44	53.1 (36.5-67.3)		1.84 (1.03-3.29)
TNBC	40	32.4 (17.8-47.9)		2.96 (1.71-5.14)
Unknown	30	61.6 (40.0-77.4)		1.87 (0.90-3.89)
Histological grade at surgery				
1	36	63.5 (44.5-77.5)	0.163	1
2	63	50.4 (36.6-62.7)		1.59 (0.77-3.31)
3	47	45.8 (30.2-60.1)		1.31 (0.62-2.79)
Lymph node status at surgery				
(y)pN0	41	76.5 (59.6-87.1)	0.005	1
(y)pN1	48	53.6 (36.0-68.3)		2.33 (0.94-5.78)
(y)pN2	26	48.8 (28.5-66.4)		1.95 (0.73-5.22)
(y)pN3	19	30.7 (10.9-53.4)		2.83 (1.06-7.53)
Stage at diagnosis				
0-II	53	80.2 (65.1-89.2)	<0.001	1
III	110	51.8 (41.4-61.2)		3.52 (1.70-7.30)
IV	42	21.9 (9.8-37.0)		10.26 (4.62-22.81)
Time from symptoms to diagnosis				
<180 days	65	57.5 (43.6-69.1)	0.339	1
≥180 days	61	53.1 (38.9-65.4)		1.33 (0.71-2.49)
Time from diagnosis to treatment				
<45 days	97	52.0 (41.0-62.0)	0.205	1
≥45 days	103	57.6 (46.5-67.2)		0.58 (0.36-0.94)

Type of first treatment received				
Surgery	50	54.7 (39.3-67.7)	0.791	1
CT/endocrine therapy	150	54.6 (45.5-62.9)		1.20 (0.70-2.06)
Status of the surgical margins				
Clean	133	60.2 (50.7-68.5)	0.001	1
Positive	14	20.5 (3.9-46.3)		2.29 (1.11-4.71)
Axillary surgery performed[c]				
Complete	102	62.7 (51.8-71.7)	0.247	1
Incomplete	41	48.9 (31.9-63.9)		1.80 (0.99-3.29)
Not done	18	51.8 (26.2-72.4)		2.59 (0.99-6.76)
Type of first-line CT regimen[d]				
Anthracycline-based only	89	36.6 (25.8-47.4)	<0.001	1
Anthracyclines + taxanes based	95	68.3 (57.1-77.2)		0.48 (0.28-0.84)
Other	9	66.7 (28.2-87.8)		0.77 (0.24-2.57)
First-line CT dose-intensity[d]				
≥85%	76	63.9 (51.0-74.2)	0.032	1
<85%	108	46.7 (36.2-56.4)		1.53 (0.92-2.54)

[a]p value for the univariable survival analysis.
[b]Adjusted for: age (<40 *vs* 40-49 *vs* 50-59 *vs* ≥60 years), HIV status (negative/unknown *vs* positive), stage at diagnosis (0-II *vs* III *vs* IV), and classic subtypes (ER+/HER2- *vs* HER2+ *vs* TNBC *vs* unknown).
[c]Among patients receiving any type of breast surgery. 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).
[d]First-line of chemotherapy that the patient received includes neoadjuvant, adjuvant or palliative treatment. If the patient received part of chemotherapy as neoadjuvant (e.g. AC regimen) and another part as adjuvant chemotherapy (e.g. paclitaxel), the type of regimen and dose intensity refer to the entire scheme (neoadjuvant plus adjuvant).
aHR=adjusted hazard ratio; CI=confidence interval; CT=chemotherapy; ER=estrogen receptor; MTB=multidisciplinary tumor board; TNBC=triple-negative breast cancer.

Supplementary Table S2 – Multivariable analysis of prognostic factors for overall survival among patients with early stage breast cancer (stage 0-III)

	N (%)	3-year OS, % (95% CI)	P[a]	Adjusted HR[b] (95% CI)
Date of diagnosis				
Pre-MTB implementation	75	48.0 (35.9-59.1)	**0.003**	1
Post-MTB implementation	88	73.0 (61.3-81.6)		**0.47 (0.27-0.81)**
Age in years				
<40	33	54.7 (35.4-70.4)	0.568	1
40-49	51	56.4 (40.7-69.5)		0.94 (0.47-1.87)
50-59	39	68.4 (49.6-81.3)		0.80 (0.36-1.78)
≥60	40	63.3 (44.9-77.1)		0.85 (0.40-1.83)
Education in years				
0	33	63.5 (43.6-77.9)	0.414	1
1-4	19	48.8 (24.2-69.5)		1.13 (0.43-2.99)
>4	88	61.6 (49.5-71.6)		0.76 (0.32-1.81)
Place of residence				
South (including Maputo)	138	60.0 (50.6-68.1)	0.697	1
Center/North	16	65.8 (35.8-84.3)		0.54 (0.21-1.39)
Menopausal status				
Pre-menopausal	85	55.9 (43.9-66.3)	0.284	1
Post-menopausal	78	65.6 (53.0-75.7)		0.64 (0.19-2.13)
Body mass index				
Under/normal weight (<25 kg/m^2)	57	65.2 (49.6-77.0)	0.422	1
Overweight/obese (≥25 kg/m^2)	95	55.9 (44.7-65.7)		1.18 (0.62-2.23)
HIV status				
Negative/unknown	126	63.0 (53.2-71.3)	0.355	1
Positive	37	52.0 (33.4-67.6)		1.12 (0.62-2.04)
Classic subtypes				
ER+/HER2-	73	65.6 (52.6-75.8)	0.005	1
HER2+	39	60.5 (42.2-74.6)		1.51 (0.77-2.95)
TNBC	31	34.4 (17.0-52.5)		3.37 (1.76-6.46)
Unknown	20	81.9 (53.8-93.8)		0.66 (0.19-2.26)
Histological grade at surgery				
1	33	69.6 (49.5-83.0)	0.183	1
2	49	55.5 (39.7-68.7)		1.95 (0.84-4.53)
3	39	49.9 (32.1-65.4)		1.78 (0.75-4.25)
Lymph node status at surgery				
(y)pN0	37	79.3 (61.2-89.6)	0.010	1
(y)pN1	44	55.5 (37.2-70.5)		2.57 (0.89-7.47)
(y)pN2	23	55.2 (32.5-73.0)		1.65 (0.52-5.26)
(y)pN3	16	33.9 (11.8-57.7)		2.85 (0.91-8.89)
Stage at diagnosis				
0-II	53	80.2 (65.1-89.2)	0.002	1
III	110	51.8 (41.4-61.2)		3.67 (1.75-7.71)
Time from symptoms to diagnosis				
<180 days	57	60.5 (45.9-72.4)	0.868	1
≥180 days	47	62.5 (46.0-75.3)		1.53 (0.75-3.08)
Time from diagnosis to treatment				
<45 days	78	62.2 (49.6-72.4)	0.858	1
≥45 days	82	61.5 (49.0-71.7)		0.81 (0.47-1.42)
Type of first treatment received				
Surgery	40	62.3 (44.8-75.7)	0.754	1
CT/endocrine therapy	120	61.1 (50.8-69.9)		0.99 (0.52-1.87)
Status of the surgical margins				
Clean	121	62.4 (52.3-70.9)	0.036	1
Positive	9	29.6 (5.2-60.7)		1.78 (0.74-4.30)
Axillary surgery performed[c]				

Complete	93	67.1 (55.8-76.1)	0.242	1
Incomplete	36	49.0 (31.1-64.7)		2.21 (1.15-4.24)
Not done	11	61.4 (26.6-83.5)		4.98 (1.50-16.50)
Type of first-line CT regimen[d]				
Anthracycline-based only	56	44.7 (30.4-58.0)	0.005	1
Anthracyclines + taxanes based	94	69.1 (57.8-77.9)		0.42 (0.24-0.73)
Other	5	80.0 (20.4-96.9)		0.26 (0.03-2.23)
First-line CT dose-intensity[d]				
≥85%	64	68.8 (54.8-79.3)	0.065	1
<85%	84	53.6% (41.6-64.3)		1.61 (0.87-2.97)

[a]p value for the univariable survival analysis.
[b]Adjusted for: age (<40 *vs* 40-49 *vs* 50-59 *vs* ≥60 years), HIV status (negative/unknown *vs* positive), stage at diagnosis (0-II *vs* III *vs* IV), and classic subtypes (ER+/HER2- *vs* HER2+ *vs* TNBC *vs* unknown).
[c]Among patients receiving any type of breast surgery. 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).
[d]First-line of chemotherapy that the patient received includes neoadjuvant and/or adjuvant treatment. If the patient received part of chemotherapy as neoadjuvant (e.g. AC regimen) and another part as adjuvant chemotherapy (e.g. paclitaxel), the type of regimen and dose intensity refer to the entire scheme (neoadjuvant plus adjuvant).
aHR=adjusted hazard ratio; CI=confidence interval; CT=chemotherapy; ER=estrogen receptor; MTB=multidisciplinary tumor board; TNBC=triple-negative breast cancer.

Supplementary Table S3 – Multivariable analysis of prognostic factors for disease-free survival among patients with early stage breast cancer (stage 0-III)

	N (%)	3-year DFS, % (95% CI)	P[a]	Adjusted HR[b] (95% CI)
Date of diagnosis				
Pre-MTB implementation	75	41.7 (30.2-52.8)	0.103	1
Post-MTB implementation	88	56.8 (45.3-66.8)		0.72 (0.46-1.13)
Age in years				
<40	33	45.9 (27.9-62.2)	0.431	1
40-49	51	43.0 (28.9-56.3)		1.16 (0.63-4.00)
50-59	39	63.2 (45.7-76.4)		1.01 (0.49-2.10)
≥60	40	47.8 (30.6-63.1)		1.19 (0.61-2.33)
Education in years				
0	33	49.0 (30.7-65.0)	0.710	1
1-4	19	37.3 (15.7-59.1)		0.99 (0.43-2.24)
>4	88	51.9 (40.7-62.0)		0.76 (0.36-1.59)
Place of residence				
South (including Maputo)	138	48.5 (39.6-56.8)	0.659	1
Center/North	16	53.3 (25.9-74.6)		0.65 (0.29-1.46)
Menopausal status				
Pre-menopausal	85	44.1 (33.2-55.0)	0.311	1
Post-menopausal	78	55.3 (43.1-65.9)		0.63 (0.25-1.62)
Body mass index				
Under/normal weight (<25 kg/m^2)	57	51.3 (36.4-64.3)	0.662	1
Overweight/obese (≥25 kg/m^2)	95	46.2 (35.7-56.0)		1.36 (0.82-2.24)
HIV status				
Negative/unknown	126	54.4 (44.8-63.0)	0.059	1
Positive	37	34.8 (20.1-50.0)		1.33 (0.80-2.19)
Classic subtypes				
ER+/HER2-	73	53.1 (40.7-64.0)	0.012	1
HER2+	39	50.5 (33.2-65.5)		1.53 (0.85-2.74)
TNBC	31	26.7 (12.2-43.6)		2.89 (1.62-5.13)
Unknown	20	71.1 (43.4-87.0)		0.67 (0.26-1.76)
Histological grade at surgery				
1	33	58.1 (38.8-73.2)	0.015	1
2	49	48.8 (33.8-62.2)		1.34 (0.66-2.72)
3	39	29.1 (15.5-44.1)		1.96 (0.97-3.97)
Lymph node status at surgery				
(y)pN0	37	79.7 (61.8-89.8)	<0.001	1
(y)pN1	44	49.8 (33.2-64.4)		3.20 (1.21-8.46)
(y)pN2	23	30.4 (13.5-49.3)		3.12 (1.12-8.74)
(y)pN3	16	12.5 (2.1-32.8)		3.76 (1.35-10.45)
Stage at diagnosis				
0-II	53	72.7 (57.4-83.3)	<0.001	1
III	110	39.2 (29.8-48.4)		3.64 (1.92-6.90)
Time from symptoms to diagnosis				
<180 days	57	53.3 (39.4-65.4)	0.971	1
≥180 days	47	58.3 (42.6-71.1)		1.44 (0.75-2.79)
Time from diagnosis to treatment				
<45 days	78	51.9 (40.0-62.6)	0.853	1
≥45 days	82	48.8 (37.0-59.6)		0.85 (0.53-1.38)
Type of first treatment received				
Surgery	40	60.0 (42.6-73.7)	0.286	1
CT/endocrine therapy	120	47.2 (37.6-56.2)		1.46 (0.81-2.62)
Status of the surgical margins				
Clean	121	51.0 (41.3-59.8)	<0.001	1
Positive	9	11.1 (0.6-38.8)		2.59 (1.20-5.61)

Axillary surgery performed[c]				
Complete	93	54.1 (43.1-63.8)	0.330	1
Incomplete	36	41.0 (24.3-57.1)		2.02 (1.13-3.61)
Not done	11	45.5 (16.7-70.7)		9.47 (3.21-27.91)
Type of first-line CT regimen[c]				
Anthracycline-based only	56	39.2 (26.1-52.1)	0.040	1
Anthracyclines + taxanes based	94	55.4 (44.5-65.1)		0.57 (0.35-0.92)
Other	5	80.0 (20.4-96.9)		0.20 (0.03-1.64)
First-line CT dose-intensity[d]				
≥85%	64	54.5 (41.1-66.1)	0.404	1
<85%	84	44.6 (33.4-55.3)		1.02 (0.61-1.72)

[a]p value for the univariable survival analysis.
[b]Adjusted for: age (<40 *vs* 40-49 *vs* 50-59 *vs* ≥60 years), HIV status (negative/unknown *vs* positive), stage at diagnosis (0-II *vs* III *vs* IV), and classic subtypes (ER+/HER2- *vs* HER2+ *vs* TNBC *vs* unknown).
[c]Among patients receiving any type of breast surgery. 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).
[d]First-line of chemotherapy that the patient received includes neoadjuvant and/or adjuvant treatment. If the patient received part of chemotherapy as neoadjuvant (e.g. AC regimen) and another part as adjuvant chemotherapy (e.g. paclitaxel), the type of regimen and dose intensity refer to the entire scheme (neoadjuvant plus adjuvant).
aHR=adjusted hazard ratio; CI=confidence interval; CT=chemotherapy; ER=estrogen receptor; MTB=multidisciplinary tumor board; TNBC=triple-negative breast cancer.

Supplementary Table S4 – Cost-effectiveness sensitivity analyses[a]

Category	Parameter varied in the one-way sensitivity analyses		Parameter Range (USD)	Parameter Range (MZN)	Strategy	ICER (cost/QALY) in USD[b]	ICER (cost/QALY) In MZN[b]
Pathology costs	Pathology analysis of fine-needle aspiration biopsy (without IHC)	Minimum	7.74	493.80	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	11.60	740.70	No MTB	-	-
					MTB	802.96	51252.57
Pathology costs	Pathology analysis of surgical specimen (with IHC)	Minimum	26.92	1718.50	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	40.39	2577.80	No MTB	-	-
					MTB	802.96	51252.57
Staging costs	Imaging	Minimum	66.43	4240.00	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	99.64	6360.00	No MTB	-	-
					MTB	802.96	51252.57
Staging costs	Lab tests	Minimum	19.43	1240.00	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	29.14	1860.00	No MTB	-	-
					MTB	802.96	51252.57
Staging costs	HIV testing	Minimum	8.29	529.40	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	12.44	794.10	No MTB	-	-
					MTB	802.96	51252.57
Staging costs	CD4+ cell count	Minimum	2.29	146.20	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	3.44	219.30	No MTB	-	-
					MTB	802.96	51252.57
Surgery costs	Breast surgery procedure	Minimum	155.48	9924.40	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	233.23	14886.70	No MTB	-	-
					MTB	802.96	51252.57
Surgery costs	Inpatient stay due to surgery	Minimum	35.90	2291.20	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	53.84	3436.80	No MTB	-	-
					MTB	802.96	51252.57
Systemic therapy costs	Outpatient consultations during the first year after diagnosis (treatment phase)	Minimum	14.14	910.50	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	21.40	1365.70	No MTB	-	-
					MTB	802.96	51252.57
Systemic therapy costs	Chemotherapy treatment – drugs (overall)	Minimum	84.22	5375.40	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	126.32	8063.20	No MTB	-	-
					MTB	802.96	51252.57
Systemic therapy costs	Chemotherapy treatment – day hospital use	Minimum	67.00	4276.80	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	100.51	6415.20	No MTB	-	-
					MTB	802.96	51252.57
Systemic therapy costs	Chemotherapy treatment – lab tests	Minimum	95.94	6123.60	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	143.91	9185.40	No MTB	-	-
					MTB	802.96	51252.57
Systemic therapy costs	Hormone therapy – tamoxifen	Minimum	18.98	1211.70	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	28.48	1817.60	No MTB	-	-
					MTB	802.96	51252.57

Systemic therapy costs	Hormone therapy – letrozole	Minimum	1.70	108.80	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	2.56	163.20	No MTB	-	-
					MTB	802.96	51252.57
Radio-therapy costs	Radiotherapy among pre-MTB patients	Minimum	22.36	1427.00	No MTB	-	-
					MTB	840.41	53642.91
		Maximum	33.53	2140.40	No MTB	-	-
					MTB	765.52	48862.24
Radio-therapy costs	Radiotherapy among post-MTB patients	Minimum	66.69	4256.57	No MTB	-	-
					MTB	691.24	44121.50
		Maximum	100.03	6384.85	No MTB	-	-
					MTB	914.69	58383.65
Follow-up costs	Outpatient follow-up consultations – among pre-MTB patients	Minimum	9.43	601.80	No MTB	-	-
					MTB	818.76	52260.91
		Maximum	14.14	902.70	No MTB	-	-
					MTB	787.17	50244.51
Follow-up costs	Outpatient follow-up consultations – among post-MTB patients	Minimum	12.57	802.40	No MTB	-	-
					MTB	781.90	49908.17
		Maximum	18.86	1203.60	No MTB	-	-
					MTB	824.02	52596.71
Follow-up costs	Lab tests during follow-up – among pre-MTB patients	Minimum	116.56	7440.00	No MTB	-	-
					MTB	998.24	63716.88
		Maximum	174.84	11160.00	No MTB	-	-
					MTB	607.69	38788.26
Follow-up costs	Lab tests during follow-up – among post-MTB patients	Minimum	155.41	9920.00	No MTB	-	-
					MTB	542.60	34633.49
		Maximum	233.12	14880.00	No MTB	-	-
					MTB	1063.33	67871.65
Follow-up costs	Follow-up mammography	Minimum	41.61	2656.00	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	62.42	3984.00	No MTB	-	-
					MTB	802.96	51252.57
MTB costs	Training of professionals	Minimum	3.21	205.14	No MTB	-	-
					MTB	797.58	50908.89
		Maximum	4.81	307.18	No MTB	-	-
					MTB	808.29	51592.64
MTB costs	Resident salary	Minimum	1.77	112.9	No MTB	-	-
					MTB	802.96	51252.57
		Maximum	2.65	169.3	No MTB	-	-
					MTB	802.96	51252.57
MTB costs	Consultant salaries	Minimum	4.17	266.10	No MTB	-	-
					MTB	795.98	50806.67
		Maximum	6.25	399.20	No MTB	-	-
					MTB	809.95	51698.60
MTB costs	Secretary salary	Minimum	0.38	24.2	No MTB	-	-
					MTB	802.33	51211.77
		Maximum	0.57	36.3	No MTB	-	-
					MTB	803.60	51292.86
Category	**Parameter varied in the two-way sensitivity analyses**		**Parameter Range (no MTB)**	**Parameter Range (MTB)**	**Strategy**	**ICER (cost/QALY) in USD[b]**	**ICER (cost/QALY) In MZN[b]**
QALY	Mean total QALYs per patient who died	Minimum	0.886	0.821	No MTB	-	-
					MTB	574.71	36683.26
		Maximum	1.329	1.232	No MTB	-	-
					MTB	1331.98	85019.24
QALY	Mean total QALYs per patient with disease relapse	Minimum	1.453	1.405	No MTB		
					MTB	1032.84	65925.16
		Maximum	2.179	2.108	No MTB	-	-
					MTB	656.79	41922.19

ICER=incremental cost-effectiveness ratio; IHC=immunohistochemistry; MTB=multidisciplinary tumor board; MZN=Mozambique Meticais; QALY=quality-adjusted life years; USD=United States dollars (with 2019 as reference year).

[a]Cost parameters and health benefits were varied in ±20% (corresponding to the minimum and maximum values of the parameters assumed in these sensitivity analyses).

[b]The conversion between USD and MZN was performed using the exchange rate 1 USD=63.8292 MZN.

Paper VII: *Impact of HIV infection on baseline characteristics and survival of women with breast cancer*

Impact of HIV infection on baseline characteristics and survival of women with breast cancer

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Background: As women living with HIV (WLWH) become older, their risk of developing breast cancer increases. Nonetheless, literature is conflicting regarding tumor stage, distribution of subtypes and overall survival among WLWH vs. HIV-negative women with breast cancer. We assessed differences in clinicopathological characteristics and overall survival between these two groups.

Methods: Systematic review and meta-analysis using MEDLINE, Scopus, ISI Web of Knowledge, LILACS, SciELO and conference abstracts up to 1 January 2020. Cross-sectional/cohort studies comparing baseline characteristics (stage and/or subtypes) and/or overall survival of WLWH vs. HIV-negative women with breast cancer were included. We performed random-effects meta-analyses to estimate summary statistics and subgroup analyses according to region of the world.

Results: Eighteen studies [4 from North America, 14 from sub-Saharan Africa (SSA)] were included, with 3174 WLWH and 2 394 598 HIV-negative women. WLWH from North America and SSA were more likely to present with stage III/IV disease compared with HIV-negative women – pooled odds ratio (pOR) 1.76 [95% confidence interval (CI):1.58–1.95] and pOR 1.23 (95% CI: 1.06–1.42), respectively. WLWH from SSA were also less likely to have estrogen receptor-positive/HER2-negative tumors (pOR 0.81; 95% CI: 0.66–0.99). After adjustment, WLWH had worse overall survival compared with HIV-negative women, both in North America [pooled adjusted hazard ratio (aHR) 2.45; 95% CI: 1.11–5.41] and SSA (aHR 1.43; 95% CI: 1.06–1.92).

Conclusion: Compared with HIV-negative women, WLWH are diagnosed with breast cancer at a more advanced stage and have a worse overall survival. These results should raise awareness regarding the detection and survival gap among WLWH with breast cancer and further studies are needed to decipher the reasons behind these disparities.

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Keywords: breast neoplasms, cancer staging, HIV, meta-analysis, survival, women's health

Introduction

The number of women living with HIV (WLWH) is increasing globally thanks to the use of effective antiretroviral treatment [1]. As these women become older, their risk of developing cancer increases, including non-AIDS-defining malignancies, such as breast cancer [2]. Breast cancer is still the leading cause of cancer-related death among women worldwide [3] and its burden is increasing among specific subgroups of women, such as those with HIV/AIDS [4]. Globally, WLWH constitute less than 1% of patients with breast cancer, but in high endemic regions, such as Southern Africa, WLWH represent 26% of breast cancers diagnosed among women less than 50 years. Indeed, multiple reports from the United States of America (USA) [5] and from sub-Saharan Africa [6,7] reveal that WLWH with breast cancer tend to be younger than HIV-negative patients, which is explained by differences in age distributions of the underlying HIV-positive and general populations [7,8]. Nonetheless, data are discordant concerning tumor stage at diagnosis, as some series showed that WLWH tend to present with later stage disease compared with HIV-negative women [9,10], whereas others did not show such a difference [7,11].

Breast cancer is a heterogeneous disease constituting various subtypes, with different prognosis and treatment approaches [12]. However, data on distribution of classic subtypes [estrogen receptor (ER)-positive/HER2-negative, triple-negative (ER-negative/HER2-negative) and HER2-positive] and of surrogate intrinsic subtypes (luminal A-like, luminal B-like, HER2-enriched and basal-like) [13] between WLWH and HIV-negative patients is scarce.

Additionally, although some reports showed that WLWH with breast cancer have an increased mortality compared with HIV-negative women, even after adjusting for stage and age [5,14], other studies did not demonstrate worse survival outcomes among WLWH [15–17]. These discrepancies may be because of the low number of WLWH with breast cancer and/or short follow-up time in most publications. Another reason may be the low accessibility to breast cancer diagnosis and treatment in some parts of the world, leading to a high proportion of late-stage disease and poor survival both in WLWH and HIV-negative patients.

As the number of WLWH with breast cancer is increasing worldwide [4], a comprehensive understanding of their characteristics at diagnosis and survival is paramount for better tailoring their cancer care. In this systematic review and meta-analysis, we aimed to compare clinicopathological characteristics at diagnosis and overall survival between WLWH and HIV-negative women with breast cancer.

Methods

This systematic review and meta-analysis was conducted and reported according to the MOOSE guidelines [18] and registered in the PROSPERO database (CRD42019141887).

Search strategy and selection criteria

We performed a systematic search of the literature without language restrictions from inception of each database up to 1 January 2020. We combined keywords like 'breast cancer', 'breast' and 'HIV' in the search strategy, which was run in MEDLINE, Scopus, ISI Web of Knowledge, LILACS and SciELO. In addition, abstracts from major conferences from 2016 to January 2020 were manually searched, in order to include unpublished studies (Supplementary Methods, http://links.lww.com/QAD/B974). We screened the reference lists of included studies and review articles to identify additional publications.

Cross-sectional or cohort studies were eligible if providing information on breast cancer patients' baseline characteristics (stage and/or subtypes) and/or on overall survival, separately for WLWH and HIV-negative women, or measures of association for their comparison. Case–control studies, reports only including WLWH and studies with less than 10 WLWH with breast cancer were excluded. When more than one publication reported the same endpoint for the same/potentially overlapping study population, we used data preferentially from the full publication (vs. abstract), from the publication with the largest sample size or data corresponding to the longest follow-up period, as applicable.

WLWH vs. HIV-negative women with breast cancer were compared regarding the likelihood of being diagnosed with: locally advanced/metastatic breast cancer (tumour, node, metastasis stage III/IV); each classic subtype; each surrogate intrinsic subtype; and regarding: overall survival, defined as time from breast cancer diagnosis until death by any cause. Triple-negative and basal-like subtypes were analyzed together, as these

subtypes are equivalent when assessed by immunohistochemistry/in-situ hybridization. The likelihood of being diagnosed with each stage at diagnosis (0/I, II, III and IV) and the ER status of the tumor (positive vs. negative) were also assessed. Subgroup analyses of each endpoint were performed according to region of the world, depending on data availability.

Data extraction

Two reviewers independently evaluated the screened titles/abstracts; in case of disagreement, a third author resolved it. After this initial screening, full articles were reviewed and data extracted by three authors, namely regarding: study characteristics (design, patient selection); race/ethnicity; $CD4^+$ cell count and antiretroviral treatment at diagnosis of breast cancer; type of anticancer treatment received; age at diagnosis of breast cancer; adjusted odds ratio (OR) for the likelihood of being diagnosed with stage III/IV or, when adjusted ORs were not available, the number of patients with the combined III/IV stages and with each stage (0/I, II, III and IV); adjusted ORs or the number of patients with each classic and surrogate subtypes and estrogen receptor status positivity; and comparison of overall survival between WLWH vs. HIV-negative women, provided as hazard ratios and confidence intervals (CI). Both adjusted and unadjusted hazard ratios were collected.

Quality assessment and publication bias

A quality assessment was performed based on study design, patient selection, definition of stage according to the TNM classification, type of estrogen receptor/HER2 status assessment (if according to ASCO/CAP guidelines) [19–21], median follow-up, proportion of loss to follow-up and adjustment of survival analysis to confounding factors. In each category, risk was classified from 'low' to 'high'. Egger's test was used for assessing small studies effects/publication bias [22].

Data synthesis and statistical analysis

Aggregated data from each study were used for the quantitative synthesis of results. For categorical variables (stage and subtypes), when adjusted ORs were not provided, ORs were computed from the frequencies provided in the manuscript and then summary risk estimates [pooled OR (pOR)] with 95% CI were calculated using random-effects models [23]. Overall survival data were pooled as hazard ratios and 95% CI and modeled using a random-effects model, separately for adjusted and unadjusted hazard ratios. All analyses were performed in logarithmic scale. Heterogeneity was assessed by visual inspection of forest plots and calculation of the I^2 statistic. Leave-one-out sensitivity analyses and sensitivity analyses excluding poor-quality studies (with 'high-risk') were performed for each endpoint, whenever applicable. All analyses were run on Stata/SE 13.1 (StataCorp LP, Texas, USA).

Results

Characteristics of included studies

The systematic search of literature provided 10 174 unique citations, of which 62 were reviewed as full text (Fig. 1). Among them, 20 publications reporting on 18 studies [including 3174 WLWH (0.13%) and 2 394 598 HIV-negative women (99.87%)] were included: 15 assessed the likelihood of stage III/IV [6,7,9–11,14,17,24–31], 10 described each stage at diagnosis [6,7,9,14,17,24,25,27,29,31], nine reported on classic subtypes and estrogen receptor status [7,9,14,17,25,26,29,31,32], four described surrogate subtypes [17,26,29,31] and seven assessed overall survival [14–17,27,33,34] (Table 1).

Four of the studies were from North America (all from the USA) [10,27,29,33,34] and 14 from sub-Saharan Africa [6,7,9,11,14–17,24–26,28,30,31]. Patient inclusion dates spanned from 1996 to 2018, reflecting the timing of introduction of highly active antiretroviral treatment. Still, there was a wide variation on $CD4^+$ cell counts and on the proportion of WLWH under antiretroviral treatment at the time of cancer diagnosis (17–95%).

Studies' quality assessment is provided in the appendix (Supplemental Table 1, http://links.lww.com/QAD/B974). Of note, two studies from sub-Saharan Africa had a loss to follow-up of at least 40% and were considered to be of poor-quality for the overall survival analysis [15,16]. There was a suggestion of publication bias for estrogen receptor status (Egger's test: $P = 0.047$), but not for the other endpoints (Supplemental Figures 1–11, http://links.lww.com/QAD/B974).

Baseline characteristics

WLWH had an increased odds of presenting with stage III/IV disease compared with HIV-negative patients both in studies from North America (pOR 1.76; 95% CI, 1.58–1.95; $I^2 = 0\%$) and from sub-Saharan Africa (pOR 1.23; 95% CI, 1.06–1.42; $I^2 = 3.1\%$) (Fig. 2). Analyses of the likelihood of presenting with each stage at diagnosis (0/I, II, III and IV) are provided in the Supplemental Figures 12–15 (http://links.lww.com/QAD/B974).

Regarding the distribution of classic subtypes, WLWH from sub-Saharan Africa had smaller odds of presenting with the estrogen receptor-positive/HER2-negative subtype (pOR 0.81; 95% CI, 0.66–0.99; $I^2 = 0\%$; Fig. 3). No differences regarding the distribution of the other classic subtypes, surrogate intrinsic subtypes or estrogen receptor-positive status were observed (Supplemental Figures 16–19, http://links.lww.com/QAD/B974). Leave-one-out sensitivity analyses showed similar findings for all of these endpoints (Supplemental Tables 2–15, http://links.lww.com/QAD/B974).

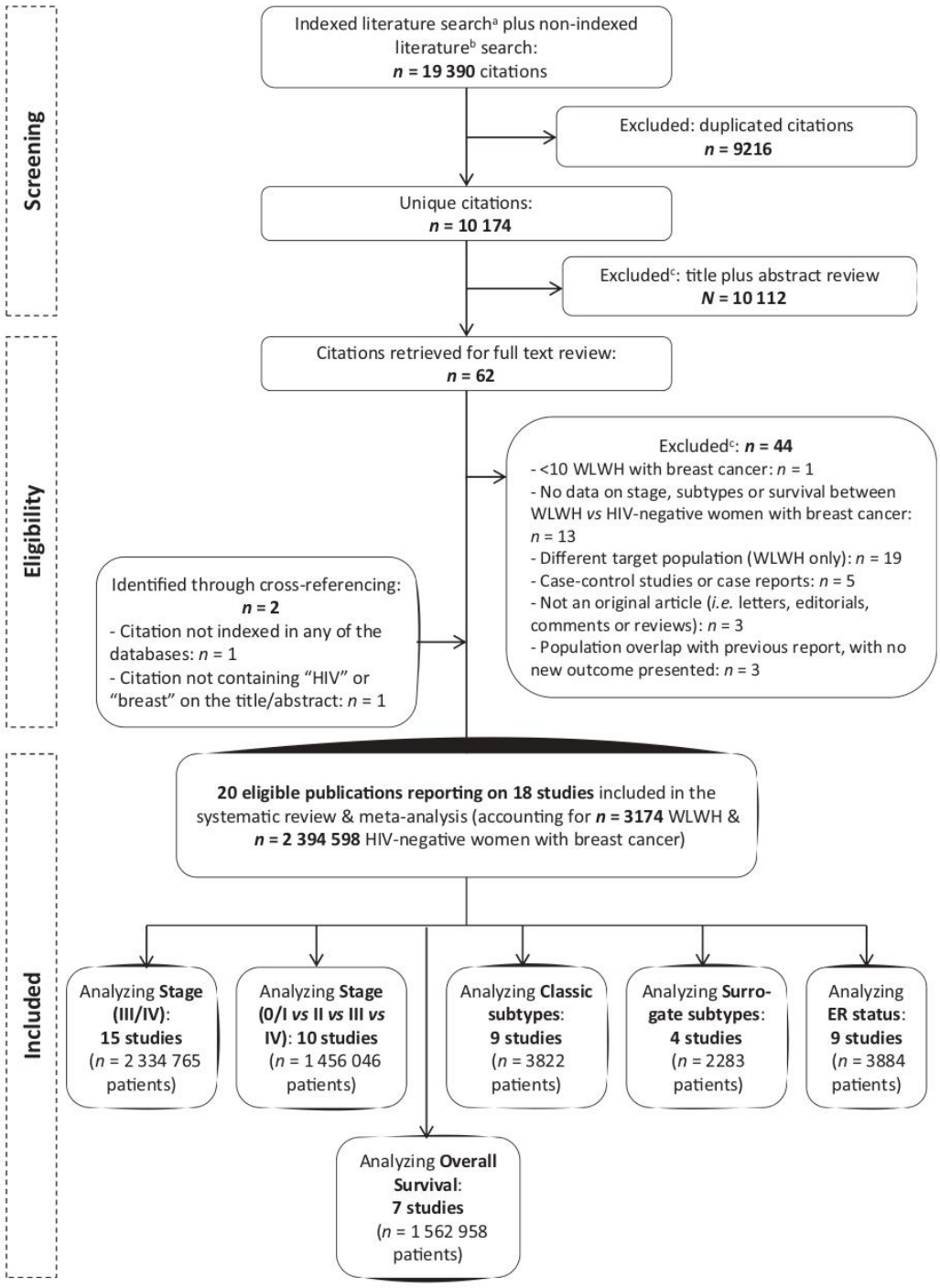


Fig. 1. Study-selection flow chart. [a]Indexed literature search (from each database inception until 1 January 2020): MEDLINE, Scopus, ISI Web of Knowledge, LILACS and SciELO. [b]Non-indexed literature search: Conference on Retroviruses and Opportunistic Infections, AORTIC's International Conference on Cancer in Africa, American Society of Clinical Oncology Annual Meeting, European Society for Medical Oncology (ESMO) Congress, ESMO Breast Cancer Congress, and San Antonio Breast Cancer Symposium, from 2016 to January 1, 2020. [c]Inclusion criteria: cross-sectional or cohort studies providing information on breast

Overall survival

WLWH from North America had an increased risk of dying compared with HIV-negative women, with a pooled adjusted hazard ratio for overall survival of 2.45 (95% CI, 1.11–5.41; $I^2 = 98.0\%$) (Fig. 4). Although all studies from North America presented a hazard ratio superior to 1.0, heterogeneity was high, possibly because of the high hazard ratio of 4.62 on the report by Coghill *et al.* [33] in 2015. When removing this study in the leave-one-out sensitivity analysis, the pooled adjusted hazard ratio was 1.77 (95% CI, 1.62–1.93), with no evidence of heterogeneity ($I^2 = 0\%$; Supplemental Table 16, http://links.lww.com/QAD/B974).

WLWH from sub-Saharan Africa presented a pooled adjusted hazard ratio for overall survival of 1.58 (95% CI, 1.25–1.98; $I^2 = 0$) compared with HIV-negative women (Fig. 4 and Supplemental Table 17, http://links.lww.com/QAD/B974). Studies reporting unadjusted hazard ratios for survival were all from sub-Saharan Africa and provided a pooled hazard ratio of 1.43 (95% CI, 1.06–1.92) (Supplemental Figure 20, http://links.lww.com/QAD/B974 and Table 18, http://links.lww.com/QAD/B974). In the sensitivity analysis, only including studies with 'low/medium-risk' for survival follow-up, the respective pooled hazard ratios were similar (Supplemental Table 19, http://links.lww.com/QAD/B974).

Discussion

This is the first meta-analysis to quantify the differences in baseline clinicopathological characteristics and survival outcomes between WLWH and HIV-negative women with breast cancer. Our findings suggest that WLWH are diagnosed with breast cancer at a more advanced stage and have a lower likelihood of presenting with the estrogen receptor-positive/HER2-negative subtype. In addition, even after adjusting for known prognostic factors, WLWH have worse overall survival compared with HIV-negative women, both in sub-Saharan Africa and in North America.

It is now understood that the classic risk factors (parity, age at first birth, breastfeeding and age at menarche/menopause) have different influences on the risk of developing each of the breast cancer subtypes, being mostly associated with the occurrence of estrogen receptor-positive/HER2-negative tumors [35,36]. However, no study has yet evaluated the association of HIV infection with the risk of developing each subtype. This could be interesting, as it is well known that they present distinct immunogenic profiles: estrogen receptor-positive/HER2-negative tumors have a lower proportion of tumor infiltrating lymphocytes and a lower tumor mutational burden compared with HER2-positive or triple-negative tumors [37,38]. Additionally, higher levels of tumor-infiltrating lymphocytes are associated with favorable outcomes among patients with early stage HER2-positive and triple-negative subtypes, but not with estrogen receptor-positive/HER2-negative tumors [37]. There was a wide variation on the proportion of WLWH under antiretroviral treatment at the time of cancer diagnosis in the included studies but it is known that even after antiretroviral treatment initiation, persisting immune alterations can affect local immune responses. Therefore, differences in the distribution of subtypes between WLWH vs. immunocompetent patients (HIV-negative) would be expected. Yet, the only difference observed was regarding the estrogen receptor-positive/HER2-negative subtype, which was less likely to be diagnosed among WLWH. Given that all included ORs were unadjusted to other clinicopathological characteristics, this difference could be partly explained by the younger age at diagnosis of WLWH (Supplemental Table 20, http://links.lww.com/QAD/B974) [5–7], as younger patients have a lower prevalence of estrogen receptor-positive/HER2-negative tumors compared with older patients [39]. On the other hand, WLWH under antiretroviral treatment may have lower blood estradiol levels and prolonged periods of amenorrhea [40,41] and this might also be a reason for their smaller likelihood of presenting with estrogen receptor-positive/HER2-negative tumors. This intriguing finding could also potentially justify why studies from the USA (in which estrogen receptor-positive/HER2-negative tumors represent more than 70% of cases [39]) report a lower incidence of breast cancer among WLWH compared with the general population [42], whereas studies from sub-Saharan Africa (in which the proportion of estrogen receptor-positive/HER2-negative tumors is much lower [43]) suggest that HIV infection does not increase nor decrease the likelihood of developing breast cancer [6,7]. Despite the fact that heterogeneity was low for the estrogen receptor-positive/HER2-negative meta-analysis, part of these studies did not follow ASCO/CAP guidelines on estrogen receptor/HER2 assessment [19–21], thus further data are needed in order to confirm these findings.

cancer patients' baseline characteristics (stage and/or subtypes) and/or on overall survival, separately for WLWH and HIV-negative women, or measures of association for their comparison. Exclusion criteria: case–control studies, reports only including WLWH and studies with less than 10 WLWH with breast cancer. When more than one publication reported the same endpoint for the same/potentially overlapping study population, we used data preferentially from the full publication (vs. abstract), from the publication with the largest sample size or data corresponding to the longest follow-up period, as applicable. ER, estrogen receptor; WLWH, women living with HIV.

Table 1. Characteristics of included studies.

Author (year) [reference no.]	Location	BC only?	Study design (study name)	Inclusion dates	WLWH (%)	WLWH: % ART[a]	WLWH: $CD4^+$ cell count[a]	WLWH: race/ethnicity	HIV-neg women (%)[b]	HIV-neg women: race/ethnicity	Variables provided
Biggar *et al.* (2005) [34]	USA/New York	No	Retrospective cohort	1996–2000	28 (0.18%)	NR	NR	NR	15 225 (99.8%)	NR	Survival
Shiels *et al.* (2015) [10]	USA	No	Retrospective cohort (HACM study)	1996–2010	429 (0.05%)	NR	NR	Overall[c]: 52% black; 47% white; 1% other	921 171 (99.95%)	Overall[c]: 11% black; 85% white; 4% other	Stage (III/IV)[d]
Coghill *et al.* (2015) [33]					314 (0.08%)			Overall[c]: 45% black; 39% WNH; Hispanic 15%	386 041 (99.92%)	Overall[c]: 12% black; 77% WNH; 8% Hispanic	Survival
Presti *et al.* (2017) [29]	USA/Baltimore	Yes	Retrospective, cross-sectional	2004–2014	43 (1.4%)	NR	NR	91% black, 9% other	3012 (98.6%)	81% black, 19% other	Stage (III/IV and detailed[e]), classic (TNBC only) and surrogate subtypes, ER status
Coghill *et al.* (2019) [27]	USA	No	Retrospective cohort (NCDB)	2004–2014	1197 (0.08%)	NR	NR	NR	1 448 757 (99.92%)	NR	Stage (III/IV and detailed[f]), survival
Sseggwanyi *et al.* (2011) [24]	Uganda/ Kampala	Yes	Prospective cohort	2011	22 (35.5%)	36%	<250 cells/μl: $n=3$ (13.6%)	100% black	40 (64.5%)	100% black	Stage (III/IV and detailed[f])
Coghill *et al.* (2013) [16]	Uganda/ Kyadondo County	No	Retrospective cohort	2003–2010	24 (10.9%)	NR	NR	NR	196 (89.1%)	NR	Survival
Cubasch *et al.* (2013) [7]	South Africa/ Johannesburg	Yes	Retrospective cohort	2006–2012	151 (19.7%)	17%[e]	Median count: 316 cells/μl (range: 7–1203; IQR 196–487); count <200 cells/μl: $n=37$ (26%)	100% black	614 (80.3%)	100% black	Stage (III/IV and detailed[f]), classic and surrogate subtypes, ER status
Cubasch *et al.* (2018) [15]				2009–2011	88 (17.6%)	NR	NR		411 (82.4%)		Survival
Traore *et al.* (2015) [28]	Guinea/Conakry	Yes	Retrospective, cross-sectional	2007–2012	14 (5.0%)	NR	Count <350 cells/μl: $n=2$ (33%)	NR	264 (95.0%)	NR	Stage (III/IV)
Phakathi *et al.* (2016) [6]	South Africa/ Pretoria	Yes	Prospective cohort	2009–2014	31 (19.4%)	55%[e]	Mean count: 406 cells//μl (SD 206); Count <200 cells//μl: $n=5$ (16%)	81% black; 6% mixed; 13% white	129 (80.6%)	44% black; 8% mixed; 47% white; 1% Indian	Stage (III/IV and detailed[f])
Langenhoven *et al.* (2016) [9]	South Africa/ Cape Town	Yes	Retrospective, cross-sectional	2010–2011	31 (6.9%)	39%[e]	Mean count: 477 cells/μl (SD 160)	58% black; 42% mixed	420 (93.1%)	10% black; 65% mixed; 25% white	Stage (III/IV and detailed[f]), classic subtypes, ER status
Ngidi *et al.* (2017) [25]	South Africa/ Durban	Yes	Retrospective, cross-sectional	2012–2015	21 (32.3%)	95%	Mean count: 435 cells/μl (SD 248; range 80–945)	NR	44 (67.7%)	NR	Stage (III/IV and detailed[f]), classic subtypes (HER2+ only), ER status
McKenzie *et al.* (2018) [11]	Nigeria, Uganda, Zambia, Namibia and South Africa	Yes	Prospective cohort (ABC-DO study)	2014–2016	178 (9.9%)	NR	NR	NR	1617 (90.1%)	NR	Stage (III/IV)

Table 1 (*continued*)

Author (year) [reference no.]	Location	BC only?	Study design (study name)	Inclusion dates	WLWH (%)	WLWH: % ART[a]	WLWH: $CD4^+$ cell count[a]	WLWH: race/ethnicity	HIV-neg women (%)[b]	HIV-neg women: race/ethnicity	Variables provided
Van Zyl *et al.* (2018) [26]	South Africa/ Pretoria	Yes	Retrospective, cross-sectional	2013–2017	49 (38.0%)	NR	Count <200 cells/μl: *n* = 10 (26%)	NR	80 (62.0%)	NR	Stage (III/IV), classic and surrogate subtypes, ER status
Sadigh *et al.* (2019) [14]	Botswana	Yes	Prospective cohort (Thabatse Cancer Cohort)	2010–2018	151 (31.6%)	85%	Median count: 457 cells/μl (IQR 304–618); Count <200 cells/μl: *n* = 17 (11%)	NR	327 (68.4%)	NR	Stage (III/IV and detailed[f]), classic subtypes (TNBC only), ER status, survival
Brandao *et al.* (2019) [17]	Mozambique	Yes	Prospective cohort (Moza-BC Cohort)	2015–2017	52 (25.5%)	74%[e]	Median count: 439 cells/μl (range 43–1104); Count <200 cells/μl: *3* = 5 (10.9%)	98% black; 2% other	152 (74.5%)	98% black; 2% other	Stage (III/IV and detailed[f]), classic and surrogate subtypes, ER status, survival
Phakathi *et al.* (2019) [31]	South Africa/ Johannesburg	Yes	Retrospective, cross-sectional	2015–2017	226 (22.2%)	72%	Median count: 477 cells/μl (IQR 287–670)	97% black; 0.4% white; 2% mixed; 0% Asian	790 (77.8%)	81% black; 10% white; 7% MIXED; 2% Asian	Stage (III/IV and detailed[f]), classic and surrogate subtypes, ER status
Bhatia *et al.* (2019) [32]	Botswana/ Gaborone	Yes	Retrospective, cross-sectional	2011–2015	49 (43.0%)	NR	Median count: 396 cells/μl (IQR 209–525.6; count <250 cells/μl: *n* = 13 (30%)	NR	65 (57.0%)	NR	Classic subtypes, ER status
Ayeni *et al.* (2019) [30]	South Africa	Yes	Prospective cohort (SABCHO)	2016–2018	478 (22.0%)	NR	NR	Overall cohort[g]: 77% black; 11% Asian; 7% white; 5% mixed	1695 (78.0%)	Overall cohort[g]: 77% black; 11% Asian; 7% white; 5% mixed	Stage (III/IV)

ABC-DO, African Breast Cancer – Disparities in Outcomes study; ART, antiretroviral treatment; BC, breast cancer; ER, estrogen receptor; HACM, HIV/AIDS Cancer Match study; HIV-neg, HIV-negative; NCDB, National Cancer Database; NR, not reported; SABCHO, South African Breast Cancer and HIV Outcomes Study; TNBC, triple negative breast cancer; USA, United States of America; WLWH, women living with HIV; WNH, white non-Hispanic.

[a]$CD4^+$ cells count and antiretroviral treatment uptake was assessed at the time of diagnosis of breast cancer.

[b]Only considering breast cancer patients.

[c]Including all tumor types (not restricted to breast cancer).

[d]In this study, breast tumors were classified into 'regional stage', which we considered as stage III and into 'distant stage', which we considered as stage IV.

[e]Patients not taking antiretroviral treatment at the time of breast cancer diagnosis were referred to a HIV clinic in order to start antiretroviral treatment.

[f]Detailed stage meaning: stage 0/I vs. II vs. III vs. IV at diagnosis.

[g]Including both HIV-positive and HIV-negative patients.

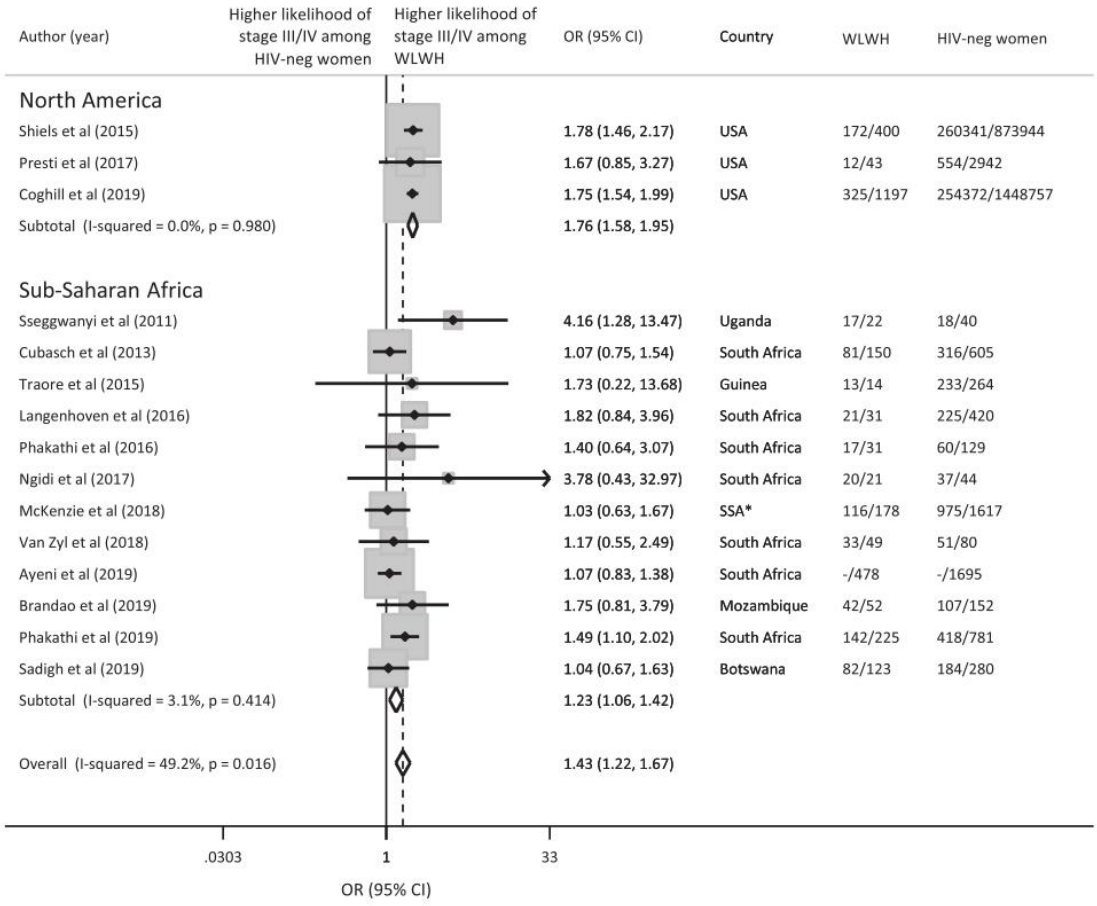


Fig. 2. Meta-analysis of the odds of presenting with locally advanced/metastatic stage (III/IV) at diagnosis. McKenzie (2018): OR adjusted for age, country, race/ethnicity, tumor characteristics (subtype, grade, histological type), marital status, education, employment, socioeconomic position, breast cancer awareness and knowledge, place of residence, number of contacts with providers, comorbidities, body mass index, symptom duration; Ayeni (2019): OR adjusted for age, race/ethnicity, highest level of education and hospital site. All the other ORs are unadjusted. Weights are from random-effects analysis; random-effects analysis *P* value: *P* less than 0.001. *Includes multiple countries from sub-Saharan Africa (SSA): Namibia, Nigeria, South Africa, Uganda, and Zambia. CI, confidence interval; HIV-neg, HIV-negative; OR, odds ratio; WLWH, women living with HIV.

Our results also suggest that when WLWH develop breast cancer, they present with a more advanced disease. One argument is that this may be because of a lower access to healthcare services/surveillance among WLWH [10,44]. As none of the North American studies provided the adjusted ORs specifically for the likelihood of presenting with stage III/IV vs. stage 0/II, our pooled OR was computed using unadjusted ORs, which is a limitation. However, in the large study by Coghill *et al.* (2019), after adjustment for age, race/ethnicity, year of cancer diagnosis, median household income, type of healthcare facility and individual health insurance, the association between HIV infection and stage IV breast cancer (vs. stage I) was significant, with a OR of 2.06 (95% CI, 1.70–2.50) [27]. Interestingly, in subgroup analysis, this association was more pronounced among women with private insurance (OR 2.22) or Medicare (OR 2.27), expected to have good access to cancer care, whereas the OR was 0.77 among women with Medicaid. Likewise, the other large study from the USA showed that WLWH had higher odds of developing stage IV (vs. stage 0–II) breast cancer, with an OR of 1.99 (95% CI, 1.40–2.83), adjusted for age, race/ethnicity and year of cancer diagnosis [10].

In studies from sub-Saharan Africa, most of which were conducted in public hospitals from low/middle-income countries with low or unavailable access to breast cancer screening, the proportion of stage III/IV disease was high among HIV-negative patients, as expected [45], but it was slightly higher among WLWH. Unfortunately, only two of those studies provided adjusted ORs for this

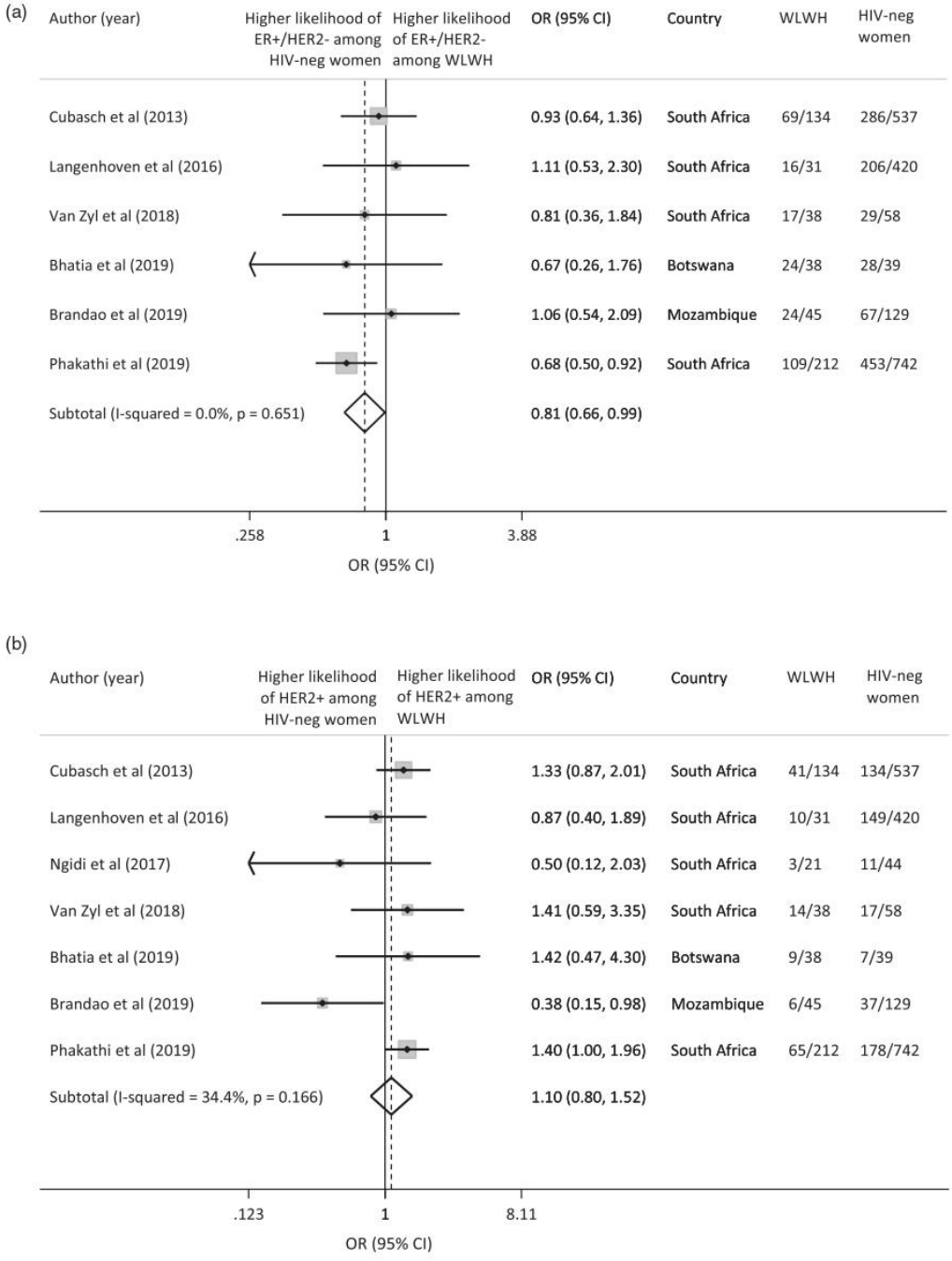


Fig. 3. Meta-analysis of the odds of presenting with each of the classic subtypes. (a) Estrogen receptor (ER)-positive/HER2-negative subtype. (b) HER2-positive subtype. (c) Triple-negative subtype. All the ORs are unadjusted. Weights are from random-effects analysis; random-effects analysis *P* value in: (a) $P = 0.043$; (b) $P = 0.553$; (c) $P = 0.269$. CI, confidence interval; ER, estrogen receptor; HIV-neg pts, HIV-negative; OR, odds ratio; TNBC, triple-negative breast cancer; WLWH, women living with HIV.

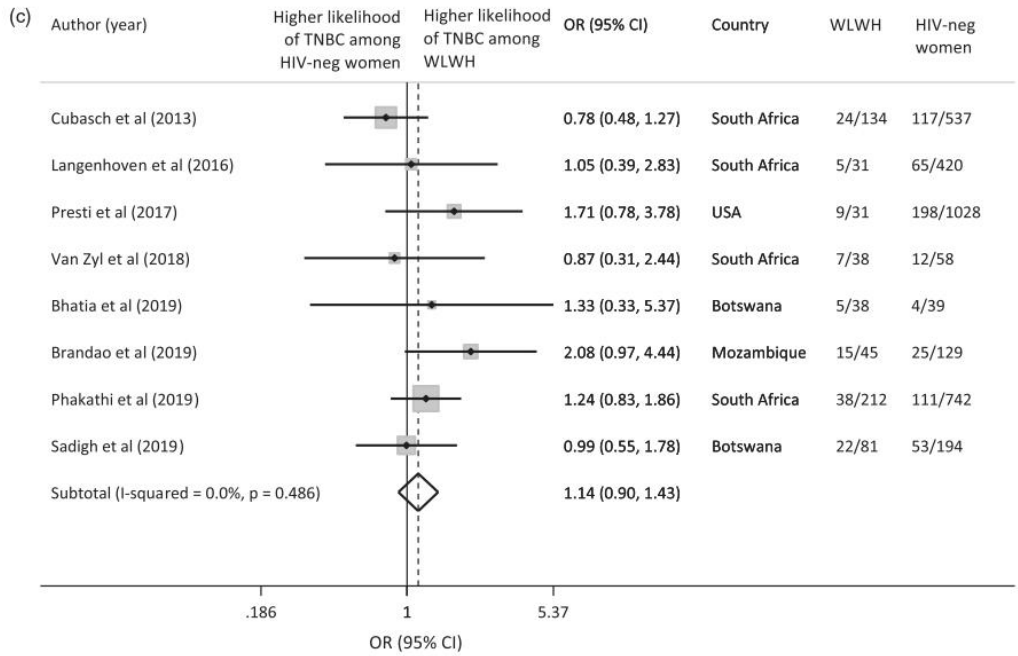


Fig. 3. (Continued).

comparison, taking into account age, race/ethnicity, education, among others, and those ORs were nonsignificant [11,30]. Thus, it is possible that among this patient population, with an already high baseline proportion of stage III/IV, that the presence of HIV infection does not significantly increase the likelihood of advanced/metastatic breast cancer if adjusted to other clinicopathological characteristics. However, more detailed data from sub-Saharan African studies would be needed to fully clarify this question.

WLWH had worse overall survival, even when adjusting for other factors, such as age, stage, ethnicity, and in some studies, breast cancer subtype, income and treatments. It may be argued that this increased mortality may be because of nonbreast cancer-related deaths (e.g. HIV-related). Nonetheless, in the Coghill *et al.* [33] study, breast cancer-specific survival was worse among WLWH compared with HIV-negative patients diagnosed from 2004 to 2010, with an adjusted hazard ratio of 3.43 (95% CI, 2.35–5.01). Another study from the USA, which was not included in this meta-analysis because of potentially overlapping population, also showed a worse breast cancer-specific survival among WLWH (adjusted hazard ratio 2.84; 95% CI, 2.29–3.52) [46]. Likewise, the Coghill *et al.* [27] study demonstrated that breast cancer patients with similar age, race, year of cancer diagnosis, median household income, stage, treatment, type of health insurance and treating cancer facility still had a higher mortality likelihood if they had HIV infection. On the other hand, patients with cancer from sub-Saharan Africa usually have worse access to effective treatments compared with patients in developed countries, leading to worse survival rates [47]. Yet, despite this baseline poor prognosis among the general breast cancer population, this meta-analysis showed that WLWH in sub-Saharan Africa do even worse in terms of survival compared with HIV-negative sub-Saharan African women, despite adjustment for age, stage and subtype. This has been recently confirmed by the survival analysis of the ABC-DO study, showing that age-stage-adjusted hazard ratio for 3-year all-cause mortality was 1.48 (95% CI, 1.22–1.81) among WLWH compared with HIV-negative women, which is similar to our findings [48].

Therefore, in addition to the potential worse socioeconomic/treatment factors and the HIV-induced morbidity/mortality, WLWH may indeed present with breast cancer that is biologically more aggressive, translating into more advanced stage at diagnosis and worse survival. This is line with our current knowledge about the importance of immune system functioning on breast cancer prognosis, as differences in the composition and function of the immune cell infiltrate can lead to distinct survival outcomes in breast cancer [49]. In addition, recent clinical trials have shown the positive effect of stimulating the immune system with immune checkpoint inhibitors, further reinforcing the importance of an intact immune system to combat the tumor [50,51]. Moreover, it is also recognized that immunosuppressed solid organ transplant recipients have worse breast cancer-specific survival comparing to the general cancer population [52].

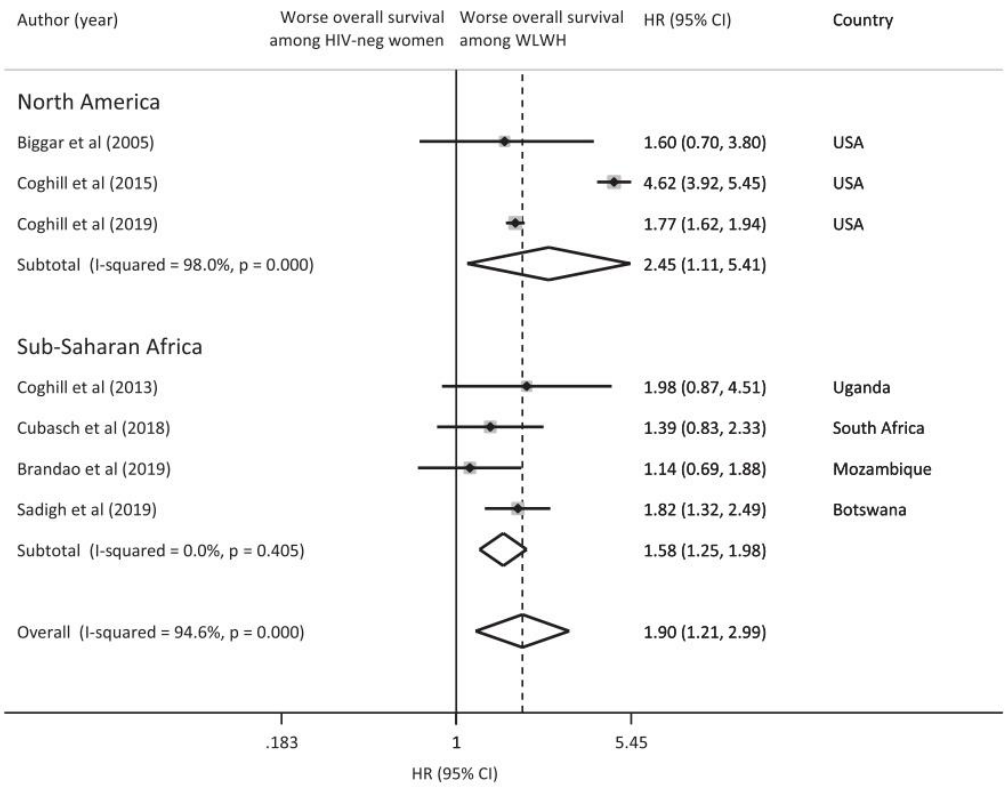


Fig. 4. Meta-analysis of adjusted overall survival. Biggar (2005): HR adjusted for sex, race, age and year of cancer diagnosis; Coghill (2013): HR adjusted for age, year of cancer diagnosis, and stage; Coghill (2015): HR adjusted for sex, age, year of cancer diagnosis, stage, and race/ethnicity; Cubasch (2018): HR adjusted for age, stage, grade, and subtype; Sadigh (2019): HR adjusted for age, cancer stage, tumor receptor expression, and income; Brandao (2019): HR adjusted for age, stage, subtype, and treatment received; Coghill (2019): HR adjusted for sex, race, age, year of cancer diagnosis, stage, median household income, treatment, type of individual health insurance, and treating cancer facility. Weights are from random-effects analysis. Random-effects analysis *P* value: $P = 0.005$. CI, confidence interval; HIV-neg, HIV-negative; HR, hazard ratio; WLWH, women living with HIV.

In addition to the limitations already mentioned, performing this meta-analysis posed other challenges. High heterogeneity was found in the meta-analysis for adjusted overall survival in North America. Nonetheless, heterogeneity was low for most of the other meta-analyses. Data regarding cancer treatment received by WLWH vs. HIV-negative patients were scarce (Supplemental Table 20, http://links.lww.com/QAD/B974). Still, unlike what has been reported in the USA [44], no differences were observed in terms of receipt of surgery, radiotherapy or chemotherapy between WLWH vs. HIV-negative women in the sub-Saharan African studies [6,14,17], but only one of them adjusted survival estimates for treatment [17]. Similarly, no study adjusted survival estimates to $CD4^+$ cell count, antiretroviral therapy or to time since HIV diagnosis. This is a limitation as a low $CD4^+$ cell count at cancer diagnosis seems to be associated with worse survival among patients with non-AIDS-defining malignancies and could be a potential factor to explain why survival outcomes are worse among some WLWH with breast cancer. On the other hand, the influence of antiretroviral therapy or time since HIV diagnosis on cancer-related survival is less clear [53,54].

All included studies were conducted in North America and sub-Saharan Africa, which may limit the generalization of these results to parts of the world with a different type/access to healthcare system. Although there are studies on WLWH with breast cancer in Latin America and Europe, they do not make a comparison to HIV-negative women [54–57]. On the other hand, sub-Saharan Africa is a very large region, in which countries' income classification vary from low-income (e.g. Mozambique) to upper middle income (e.g. South Africa), which may affect patients' outcomes. The small number of studies within each of these income categories precluded a meaningful pooled analysis of each endpoint, but reassuringly, heterogeneity was low/moderate in most of the different meta-analyses, which implies that these diverse economic backgrounds do not have a large influence in the differences between WLWH and HIV-

negative women with breast cancer in sub-Saharan Africa. Lastly, an individual patient-level data meta-analysis would allow a more precise and robust analysis of our endpoints as it would account for more confounding factors.

Our results have implications for the care of WLWH. The European AIDS Clinical Society already recommends mammographic screening for WLWH with an age of 50 years or more [58]. Nonetheless, in most regions of the world, 70% of WLWH are diagnosed with breast cancer under the age of 50 years [4] and the implementation of population-mammographic screening in low-resource settings, such as sub-Saharan Africa is very low [59]. In this region, women often present with late-stage disease, thus the use of clinical breast examinations by a health professional could be considered. These examinations detect breast cancer in symptomatic women at earlier stages (i.e. 'clinical downstaging') and could raise awareness regarding breast cancer among WLWH and health professionals; however, their survival benefit has not yet been demonstrated [60]. For WLWH who already developed breast cancer, an effort should be made to provide a similar care to the one given to HIV-negative patients, namely in terms of access to effective treatment and adequate follow-up. These patients should also be included in clinical trials testing new anticancer therapies, including immune checkpoint inhibitors, as a way to assess the safety and efficacy of innovative treatments in this population [61–63].

Conducting a systematic review and meta-analysis using data from observational studies is often more difficult to perform compared with the use of data from randomized clinical trials, given the potentially higher heterogeneity in terms of study design and methodology. Nonetheless, there are several research questions that can only be answered through the pooling of observational studies and the comparison of WLWH vs. HIV-negative women with breast cancer is one of them. Therefore, this study provides the best available evidence on this topic for the time being, and the results suggest that WLWH are diagnosed with breast cancer at a more advanced stage and have a lower likelihood of presenting with estrogen receptor-positive/HER2-negative subtype. In addition, even after adjusting for other prognostic factors, WLWH have worse overall survival compared with HIV-negative patients, both in sub-Saharan Africa and in North America. These results should raise the awareness of clinicians and policy makers regarding the detection and survival gap among WLWH who develop breast cancer. This underserved population should be the focus of more research efforts, in order to better understand the reasons behind these disparities, which can be related to viral factors, distinct breast cancer biology and anticancer immune response, and/or to a lower access to timely diagnosis of breast cancer and effective treatments.

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Conflicts of interest

M.P., L.B., Ed.A.: research grants for their Institute from Radius, AstraZeneca, Lilly, MSD, GSK/Novartis, Roche/GNE, Synthon, Servier and Pfizer. M.B., M.A.F., C.D.A., D.E., R.C.: research grants for their Institute, where they work as medical research fellows: from Radius, AstraZeneca, Lilly, MSD, GSK/Novartis, Roche/GNE, Synthon, Servier and Pfizer (none in their name). M.B.: travel grant and speaker honoraria from Roche. D.E.: funding for his ESMO fellowship (2018–2019) from Novartis. R.C.: speaker honoraria from Boehringer-Ingelheim, AstraZeneca and Janssen; travel grants from Pfizer and AstraZeneca. M.P.: consultancy honoraria for AstraZeneca, Camel-IDS, Crescendo Biologics, Debiopharm, G1 Therapeutics, Genentech, Huya, Immunomedics, Lilly, Menarini, MSD, Novartis, Odonate, Periphagen, Pfizer, Roche, Seattle Genetics; scientific board member: Oncolytics. L.B.: travel grant from Roche; speaker honoraria from BMS. N.D.: travel grants from Pfizer, MSD and Janssen's. Ed.A.: speaker honoraria and/or advisory board for Roche/GNE, Novartis, SeaGen and Zodiac; travel grants from Roche/GNE and GSK/Novartis. M.L.: consultancy for Roche and Novartis; speaker honoraria from Theramex, Roche, Novartis, Pfizer, Lilly and Takeda. All other authors declare that they have no conflict of interest (M.Br., M.C., C.C. and N.L.).

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63. Uldrick TS, Gonçalves PH, Abdul-Hay M, Claeys AJ, Emu B, Ernstoff MS, *et al.*, Cancer Immunotherapy Trials Network (CITN)-12 Study Team. **Assessment of the safety of pembrolizumab in patients with HIV and advanced cancer—a phase 1 study.** *JAMA Oncol* 2019; **5**:1332–1339.

Supplementary Material

Title: **Impact of HIV infection on baseline characteristics and survival of women with breast cancer: a systematic review and meta-analysis**

Supplementary Methods

Search strategy

Searches covering the periods from inception of each database to 1 January 2020

1. **MEDLINE**

("breast cancer" OR "breast neoplasm" OR “breast tumor” OR “breast tumors” OR “breast tumour” OR “breast tumours” OR "Breast Neoplasms"[MeSH] OR “breast”) AND (“HIV” OR “Human Immunodeficiency Virus” OR “Immunodeficiency Virus, Human” OR “AIDS” OR “Acquired Immune Deficiency Syndrome Virus” OR “Acquired Immune Deficiency Syndrome”) NOT (animals [mh] NOT humans [mh])

2. Scopus

(TITLE-ABS-KEY ("breast cancer" OR "breast neoplasm" OR "breast tumor" OR "breast tumors" OR "breast tumour" OR "breast tumours" OR "breast") AND TITLE-ABS-KEY ("HIV" OR "Human Immunodeficiency Virus" OR "Immunodeficiency Virus, Human" OR "AIDS" OR "Acquired Immune Deficiency Syndrome Virus" OR "Acquired Immune Deficiency Syndrome")) AND PUBYEAR < 2020

3. ISI Web of Knowledge

ALL=("breast cancer" OR "breast neoplasm" OR "breast tumor" OR "breast tumors" OR "breast tumour" OR "breast tumours" OR “breast”) AND ALL=("HIV" OR "Human Immunodeficiency Virus" OR "Immunodeficiency Virus, Human" OR "AIDS" OR "Acquired Immune Deficiency Syndrome Virus" OR "Acquired Immune Deficiency Syndrome")

4. **LILACS**

Breast AND HIV

5. **SciELO**

Breast AND HIV

Manual search of abstracts from major conferences, taking place from 2016 to January 2020:

- American Society of Clinical Oncology Annual Meeting
- AORTIC's International Conference on Cancer in Africa
- Conference on Retroviruses and Opportunistic Infections
- ESMO Breast Cancer Congress
- European Society for Medical Oncology (ESMO) Congress

San Antonio Breast Cancer Symposium

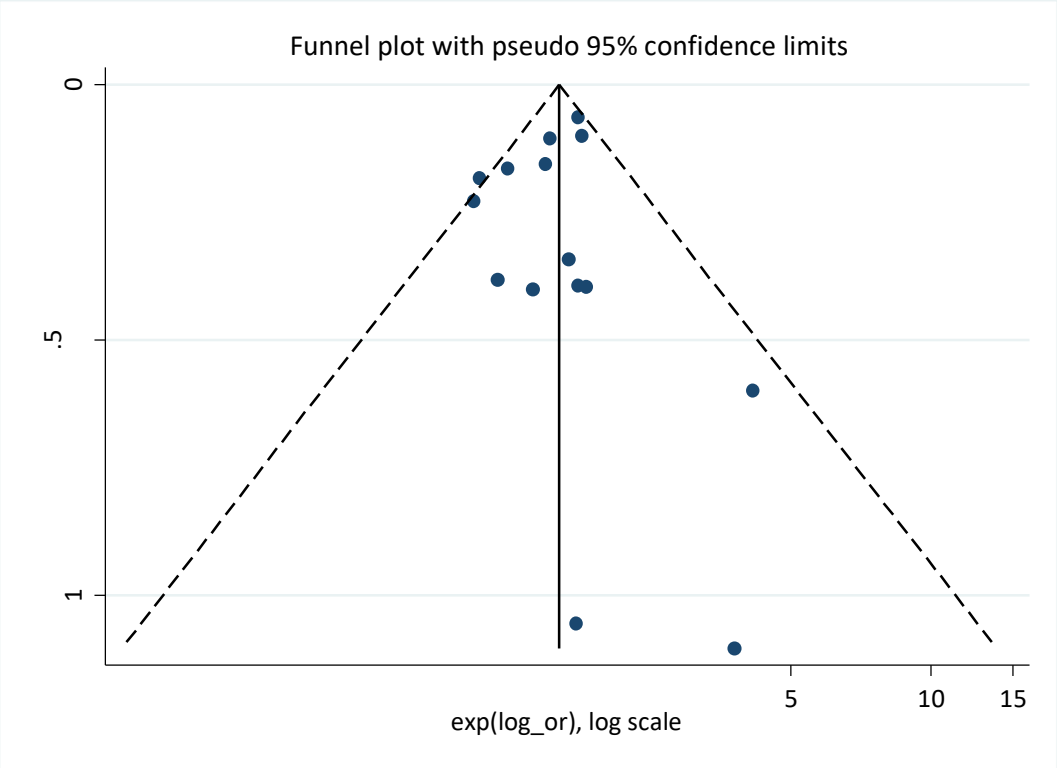
Supplementary Table 1 – Quality assessment of studies included.

First author, year	Study design	Pros-pective?	BC only?	Patient selection	Stage definition according to TNM classification	Definition of ER and HER2 status	Survival analysis: median FU and loss to FU	Survival analysis: Adjustment for confounding
Biggar et al (2005) [1]	Linkage study	No	No	Population-based study: linked data about persons from the statewide New York State Cancer Registry to persons registered in the New York City HIV/AIDS Registry to obtain information about the AIDS status of the cancer cases. **Low risk**	NA	NA	FU was limited to 24 months after cancer diagnoses. Death information was considered to be more than 95% complete by the New York State Cancer Registry. **Low-medium risk.**	Yes: for sex, race, age, year of cancer diagnosis. **Low-medium risk**
Sseggwanyi et al (2011) [2]	Case series (single-center)	Yes	Yes	One teaching hospital from Uganda. Only patients with known HIV status included. **Low risk**	Authors collected staging information from medical records, and classified patients according to the TNM staging system. **Low risk**	NA	NA	NA
Coghill et al (2013) [3]	Cancer registry	No	No	Data retrieved from a cancer registry in Uganda. HIV status was retrospectively collected and both patients with known and unknown status were included – patients with unknown HIV status were considered as "HIV negative" (and the country in which the study was conducted has a prevalence of HIV infection >5.0%). **Medium risk**	Authors collected staging information from medical records, and classified patients into 2 groups, whenever they had this data available: lower stage (I – II) and advanced stage (III – IV) according to the TNM staging system. **Low risk**	NA	Median FU was not provided; 40% of patients overall were lost follow-up. **High risk**	Yes: for age, year of cancer diagnosis, stage. **Low risk**
Cubasch et al (2013) [4]	Case series (single-center)	No	Yes	Data retrieved from an electronic database of an academic hospital in South Africa. Known HIV status, with a 2-exam algorithm. **Low risk**	Authors reported data using the TNM staging system. **Low risk**	Authors collected ER and HER2 status information from medical records, and classified patients according to the following rules: ER <1% was considered negative and HER2 0, 1+, and 2+ were considered negative. **Medium risk**	NA	NA
Cubasch et al (2018) [5]				Data retrieved from an electronic database of an academic hospital in South Africa. Among included patients, 17% had unknown HIV status, but survival analysis was reported separately for "HIV-positive", "HIV-negative" and "HIV-unknown". **Low risk**	Authors reported data using the TNM staging system. **Low risk**	Authors collected ER and HER2 status information from medical records, and classified patients according to the following rules: ER <1% was considered negative and HER2 0, 1+, and 2+ were considered negative. **Medium risk**	Median follow-up reported; 48% of patients were lost follow-up. **High risk**	Yes: for age, stage, grade and BC subtype. **Low risk**
Shiels et al (2015) [6]	Linkage study (HACM study)	No	No	Two national databases (US HIV and cancer registries) in the US, automatically matched by social security number. **Low risk**	SEER classification as local, regional, distant and unknown. **Low risk**	NA	NA	NA
Coghill et al (2015) [7]				Two national databases (US HIV and cancer registries) in the US automatically matched by social security number. **Low risk**	SEER classification as local, regional, distant and unknown. **Low risk**	NA	NA	Yes: for age, sex, stage, year of cancer diagnosis, race/ethnicity. **Low risk**
Traore et al (2015) [8]	Case series (single-center)	No	Yes	Single-center study, selection based on medical records. Authors do not describe how HIV status was evaluated among "HIV-uninfected" patients. **Medium risk**	Authors collected staging information from medical records, and classified patients into 2 groups: lower stage (I – II) and advanced stage (III – IV) according to the TNM staging system. **Low risk**	NA	NA	NA
Phakathi et al (2016) [9]	Case series (single-center)	Yes	Yes	Prospective cohort of a state hospital in South Africa. Only patients with known HIV status were included in the analysis (representing 96% of patients included in the cohort). **Low risk**	Patients were classified according to the TNM staging system. **Low risk**	NA	NA	NA
Langenhoven et al (2016) [10]	Case series (single-center)	No	Yes	Single-center study, selection based on medical records; 23% of patients had an unknown HIV status. **Medium risk**	Authors collected staging information from medical records, and classified patients according to the TNM staging system. **Low risk**	Authors collected ER and HER2 status information from medical records; they fail to report how this was classified for the study. **Medium risk**	NA	NA
Ngidi et al (2017) [11]	Case series (multicentric)	No	Yes	Retrospective analysis of medical charts from 2 South African hospitals. Analysis restricted to patients treated with chemotherapy for breast cancer. Only patients with known HIV status included; do not state the amount of patients excluded due to unknown HIV status. **Low-medium risk**	Authors collected staging information from medical records, and classified patients according to the TNM staging. **Low risk**	Authors collected ER and HER2 status information from medical records; they fail to report how this was classified for the study. **Medium risk**	NA	NA
Presti et al (2017) [12]	Case series (multi-centric)	No	Yes	Retrospective chart review from 4 hospitals in the USA. HIV status was retrospectively collected and both patients with known and unknown status were included – patients with unknown HIV status were considered as "HIV negative", but the proportion of "HIV unknown" is not provided (but the prevalence of HIV in the country where the study was conducted is <0.5%). **Low-medium risk**	Authors collected staging information from medical records, and classified patients according to TNM staging. **Low risk**	Authors collected ER and HER2 status information from medical records; they fail to report how this was classified for the study. **Medium risk**	NA	NA
McKenzie et al (2018) [13]	Cohort (multi-country – ABC-DO study)	Yes	Yes	Observational cohort study of women newly diagnosed with breast cancer in tertiary cancer centers from 5 African countries. HIV infection status was based on testing in South Africa, but it was self-reported in the other participating countries. **Medium risk**	Authors collected staging information from medical records, and classified patients into 2 groups: lower stage (I – II) and advanced stage (III – IV) according to TNM staging. **Low risk**	Authors collected ER and HER2 status information from medical records, and classified patients according to ASCO/CAP guidelines.[14–16] **Low risk**	NA	NA

First author, year	Study design	Pros-pective?	BC only?	Patient selection	Stage definition according to TNM classification	Definition of ER and HER2 status	Survival analysis: median FU and loss to FU	Survival analysis: Adjustment for confounding
Van Zyl et al (2018) [17]	Case series (single-center)	No	Yes	Single-center study, selection based on medical records. Only patients with known HIV status were included in this analysis (representing 49% of patients initially assessed). **Medium risk**	Authors collected staging information from medical records, and classified patients according to TNM staging. **Low risk**	ER and HER2 status information were retrieved from a national laboratory database; they fail to report how this was classified for the study. **Medium risk**	NA	NA
Sadigh et al (2019) [18]	Cohort (multi-centric; Thabatse Cancer Cohort)	Yes	Yes	4 sites from Botswana. Only patients with known HIV status were included in this analysis (representing 96% of patients initially assessed). **Low risk**	Authors reported data using the TNM definition. **Low risk**	Authors report ER status, but no PR status. HER2 status was assessed only by immunohistochemistry. **Medium-high risk**	Follow-up period is reported, and authors have described the number of patients who lost follow-up (1%). **Low risk**	Yes: for age, stage, BC subtype, and income. **Low risk**
Brandao et al (2019) [19]	Cohort (multi-centric; Moza-BC Cohort)	Yes	Yes	3 sites from Mozambique, but patients followed in 1 of the sites. Almost all patients had known HIV status (96% of patients in the cohort). **Low risk**	Authors reported data using the TNM definition. **Low risk**	According to ASCO/CAP guidelines.[14–16] **Low risk**	Median follow-up reported, and authors have described the number of patients who were lost follow-up (11%). **Low-medium risk**	Yes: for age, stage, BC subtype, and any treatment received (yes vs. no). **Low risk**
Phakathi et al (2019) [20]	Consecutive series (2 centers)	No	Yes	5 hospitals from South Africa. Only patients with known HIV status included in the analyses (representing 96.8% of patients initially assessed). **Low risk**	Authors reported data using the TNM definition. **Low risk**	HER2 status was defined according to ASCO/CAP guidelines, whereas ER and PR status were defined according to Allred score (3-8 considered positive). **Low-medium risk**	NA	NA
Bhatia et al (2019) [21]	Case series	No	Yes	Retrieved data from 2 national datasets. No information regarding the presence of distant metastases was available. Complete tumor and nodal staging was available for 13% of the specimens. **Medium-high risk**	For the patients who had staging data available, it was reported using the TNM definition. **Low risk**	ER and PR status reported according to ASCO/CAP guidelines. HER2 status assessed only with IHC, no ISH performed (+3 were considered HER2-positive) **Medium risk**	NA	NA
Ayeni et al (2019) [22]	Cohort (SABCHO)	Yes	Yes	Prospective cohort, 5 hospitals from South Africa. Almost all patients had known HIV status (95%). **Low risk**	Authors reported data using the TNM definition. **Low risk**	ER, PR and HER2 status retrieved from medical records; they fail to report how this was classified for the study. **Medium-high risk**	NA	NA
Coghill et al (2019) [23]	Cancer registry (NCDB)	No	No	Data was retrieved from a National database. HIV testing was not performed routinely in all of these patients (but the prevalence of HIV in the country where the study was conducted is <0.5%). **Low-medium risk**	Authors reported data using the TNM definition. **Low risk**	NA	NA	Yes: for age, sex, race, stage, year of cancer diagnosis, median household income (by zip code), treatment, type of individual health insurance and treating cancer facility. **Low risk**

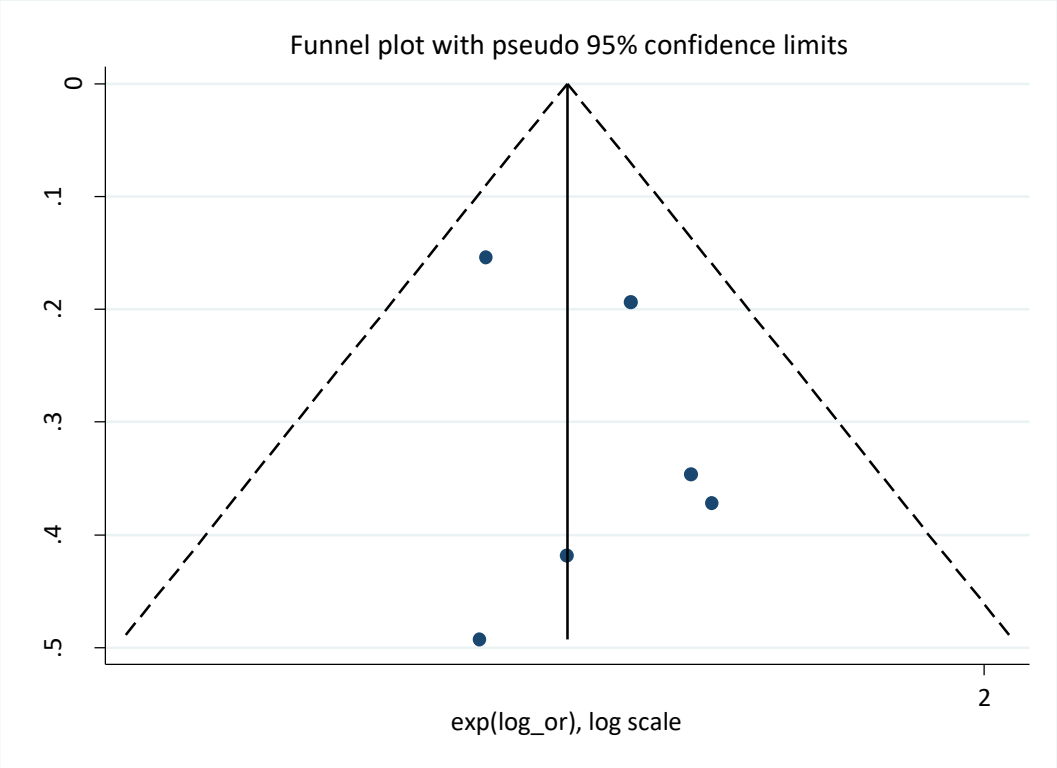
ABC-DO=African Breast Cancer - Disparities in Outcomes study. ASCO/CAP=American Society of Clinical Oncology / College of American Pathologists. BC=breast cancer. ER=estrogen receptor. HACM=HIV/AIDS Cancer Match study. NCDB=National Cancer Database. NA=not applicable (variable not evaluated in the study). SABCHO=South African Breast Cancer and HIV Outcomes Study. SEER= Surveillance, Epidemiology, and End Results Program.

Supplementary Figure 1 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with locally advanced/metastatic stage (III/IV) at diagnosis among WLWH compared with HIV-negative women with breast cancer.



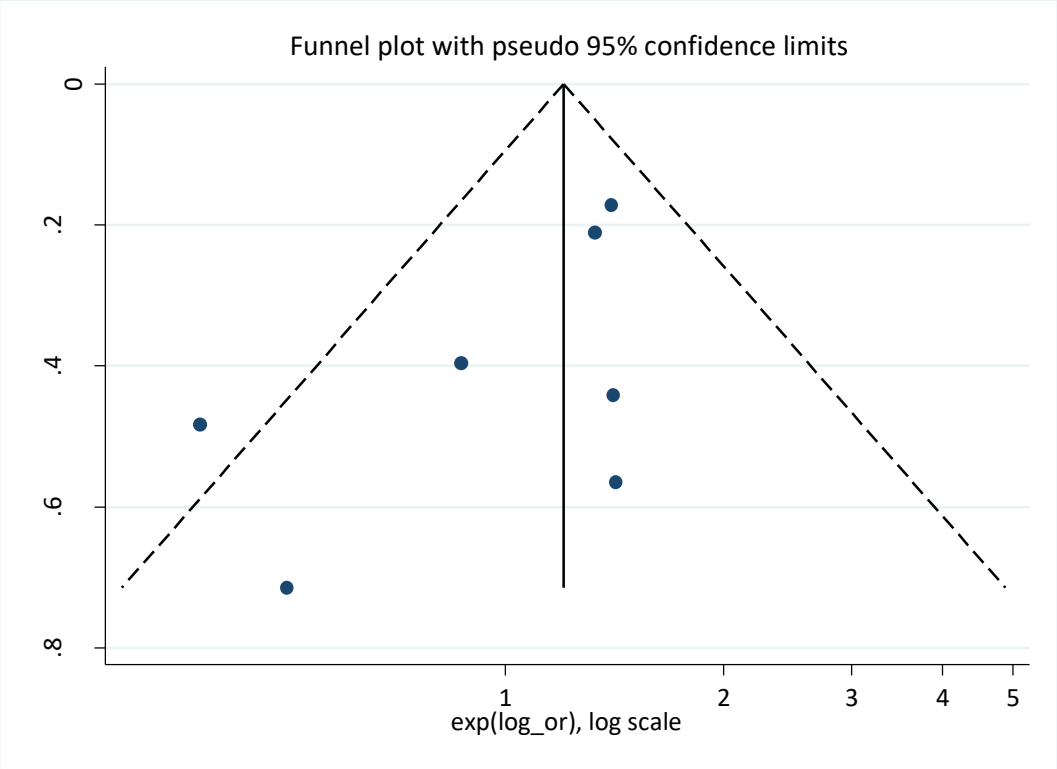
Egger's test: p=0.543

Supplementary Figure 2 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with ER-positive/HER2-negative subtype among WLWH compared with HIV-negative women with breast cancer.



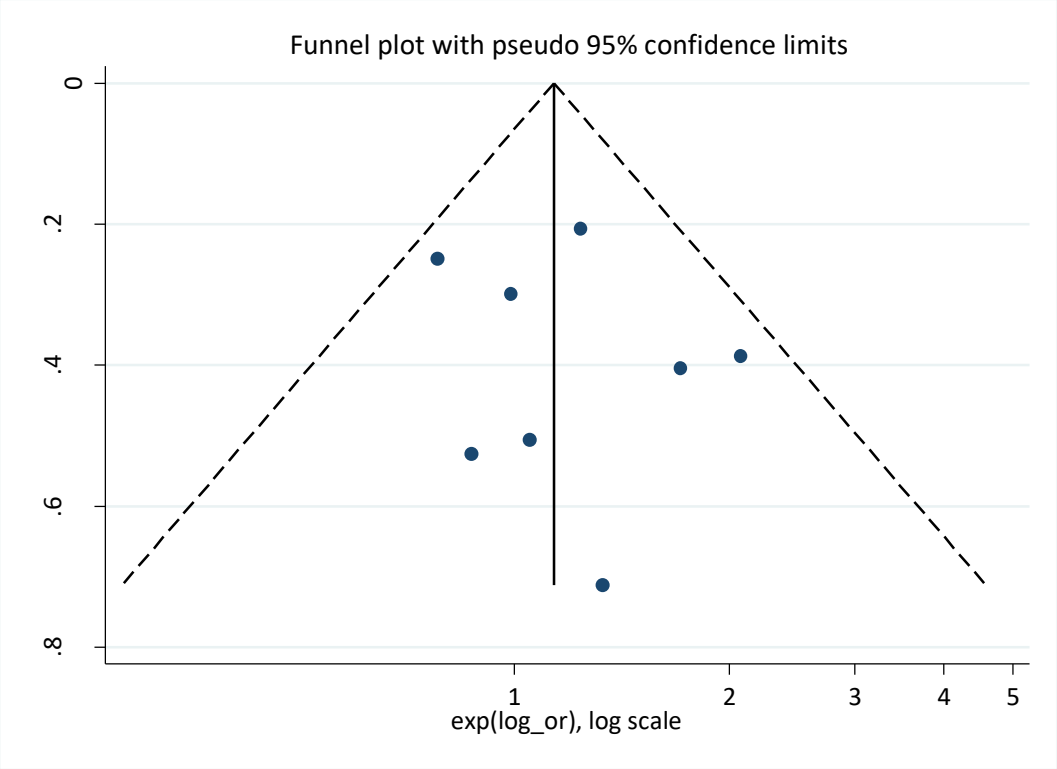
Egger's test: p=0.383

Supplementary Figure 3 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with HER2-positive subtype among WLWH compared with HIV-negative women with breast cancer.



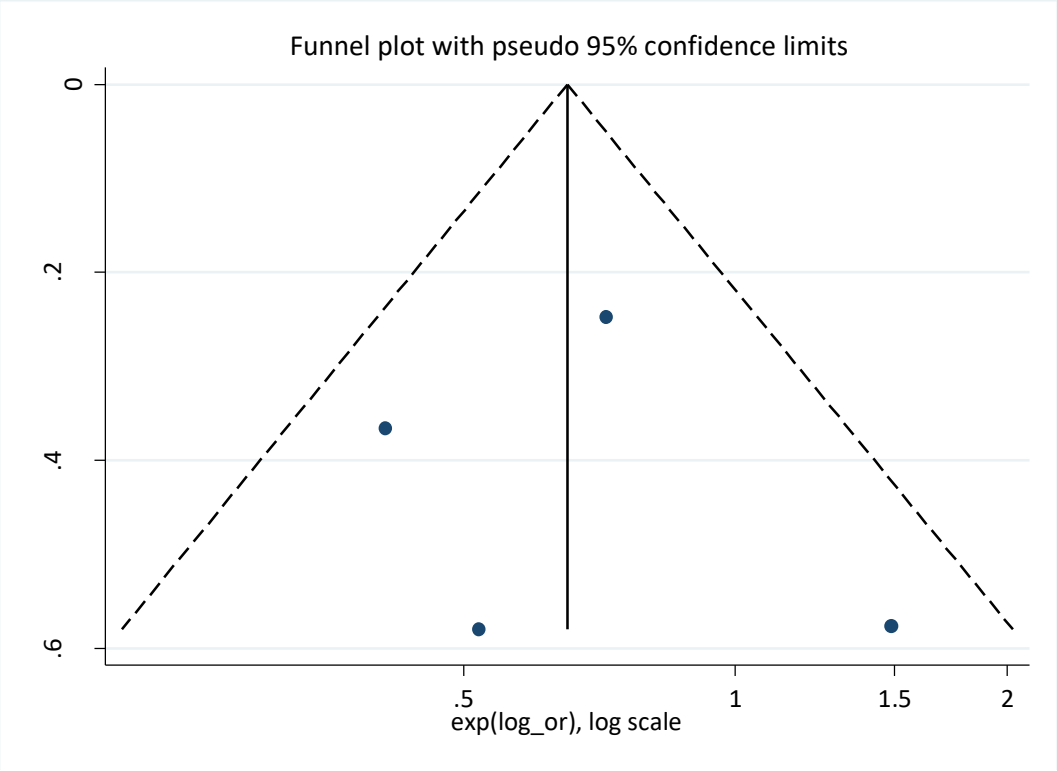
Egger's test: p=0.111

Supplementary Figure 4 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with triple-negative subtype / basal-like subtype among WLWH compared with HIV-negative women with breast cancer.



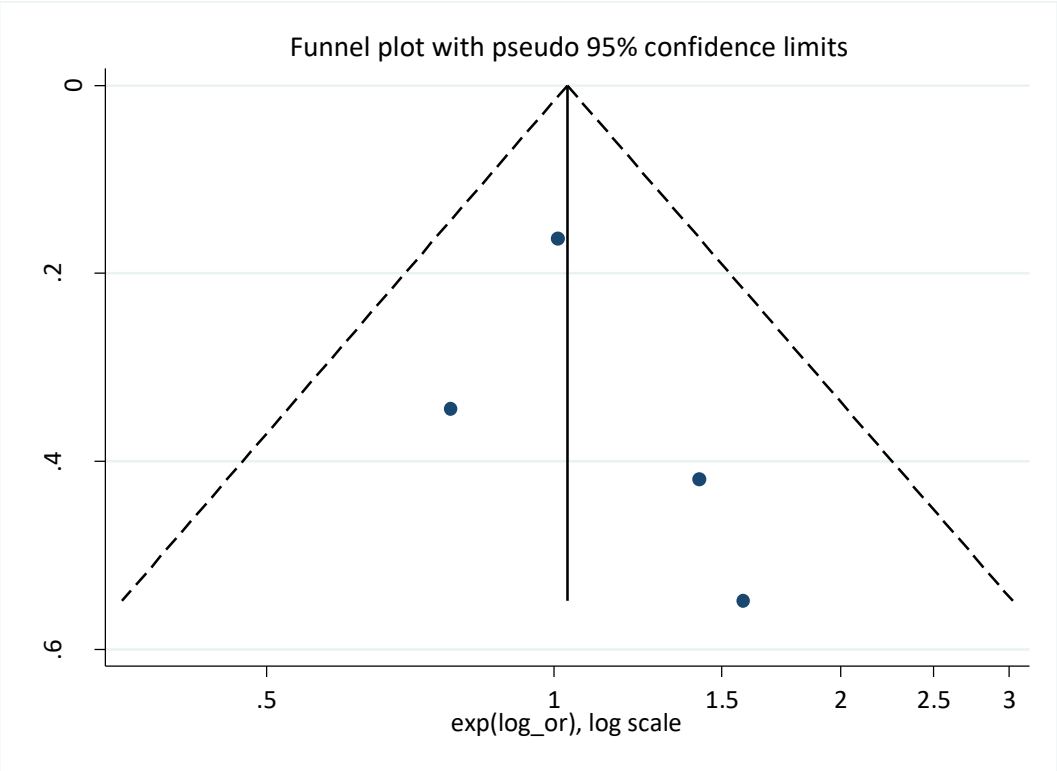
Egger's test: p=0.594

Supplementary Figure 5 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with Luminal A-like subtype among WLWH compared with HIV-negative women with breast cancer.



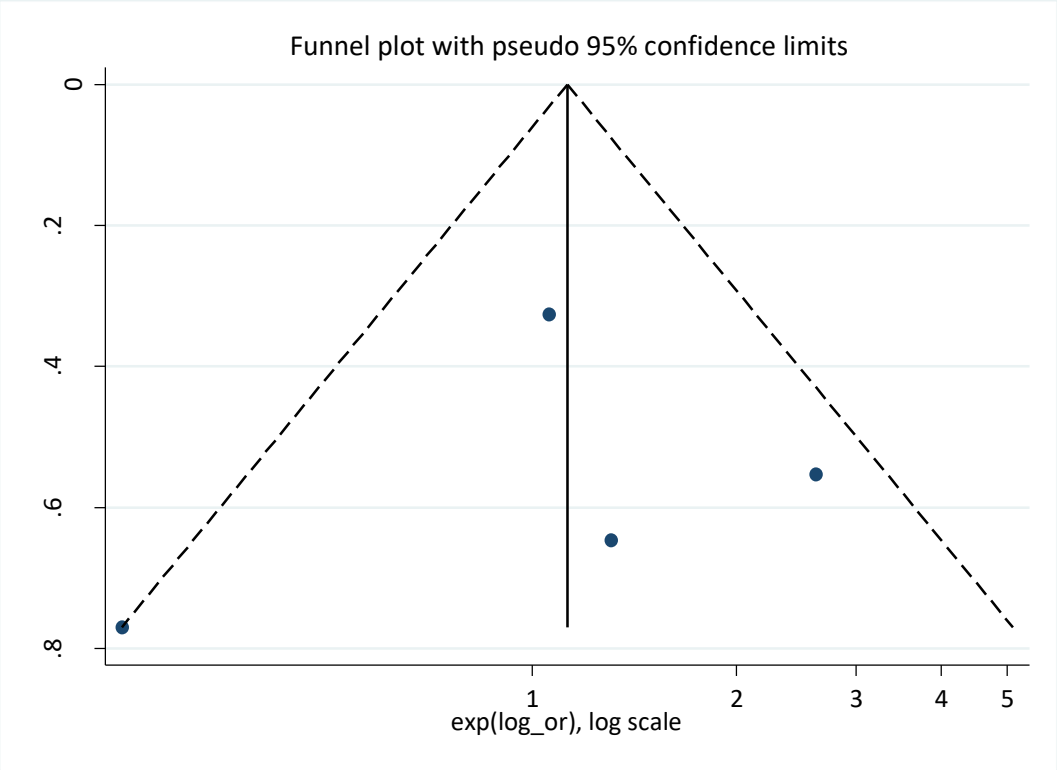
Egger's test: p=0.865

Supplementary Figure 6 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with Luminal B-like subtype among WLWH compared with HIV-negative women with breast cancer.



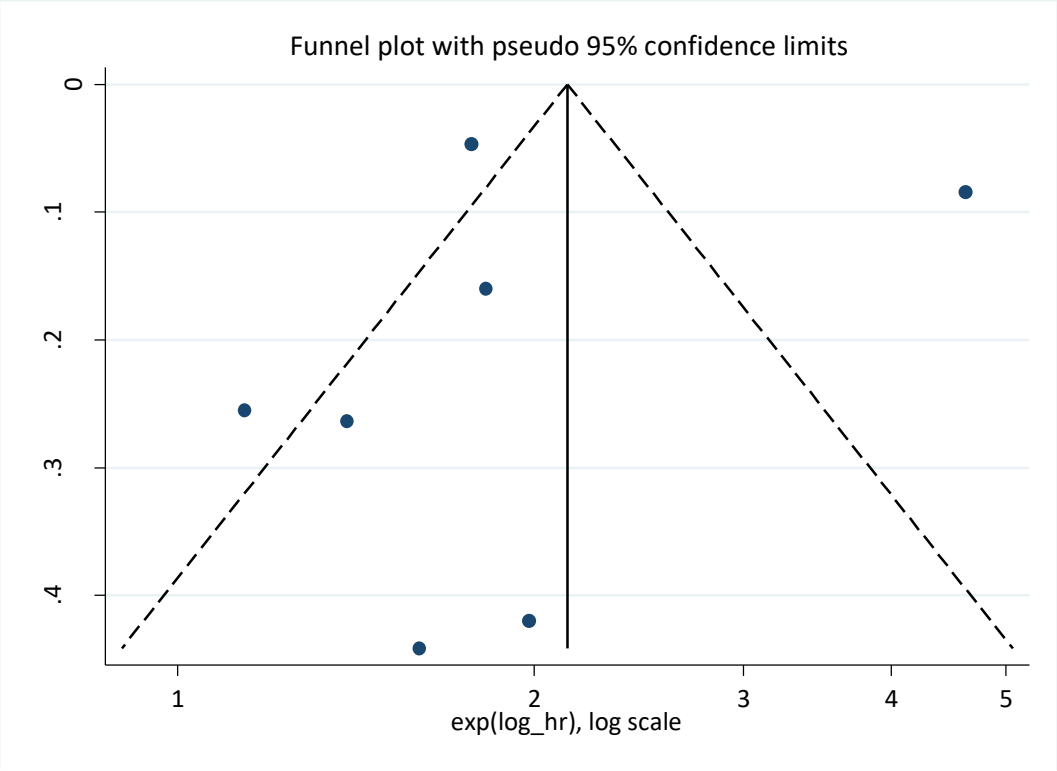
Egger's test: p=0.530

Supplementary Figure 7 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with HER2-enriched subtype among WLWH compared with HIV-negative women with breast cancer.



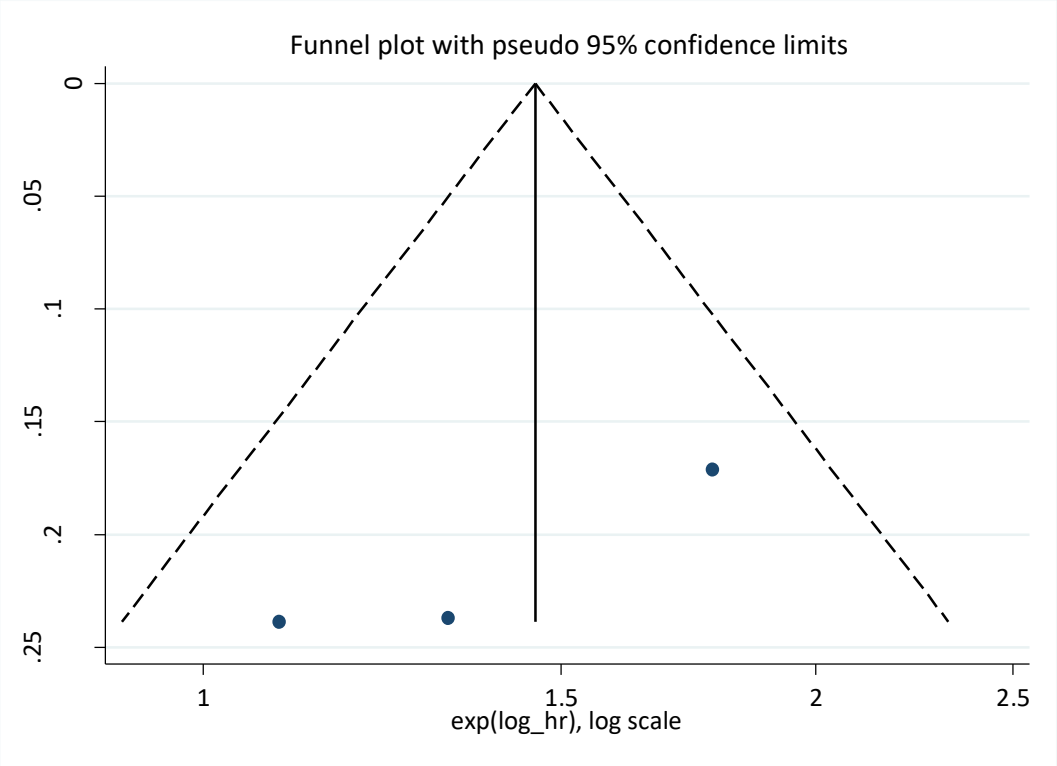
Egger's test: p=0.767

Supplementary Figure 8 – Funnel plot of publication bias among studies evaluating adjusted Overall Survival among WLWH compared with HIV-negative women with breast cancer.



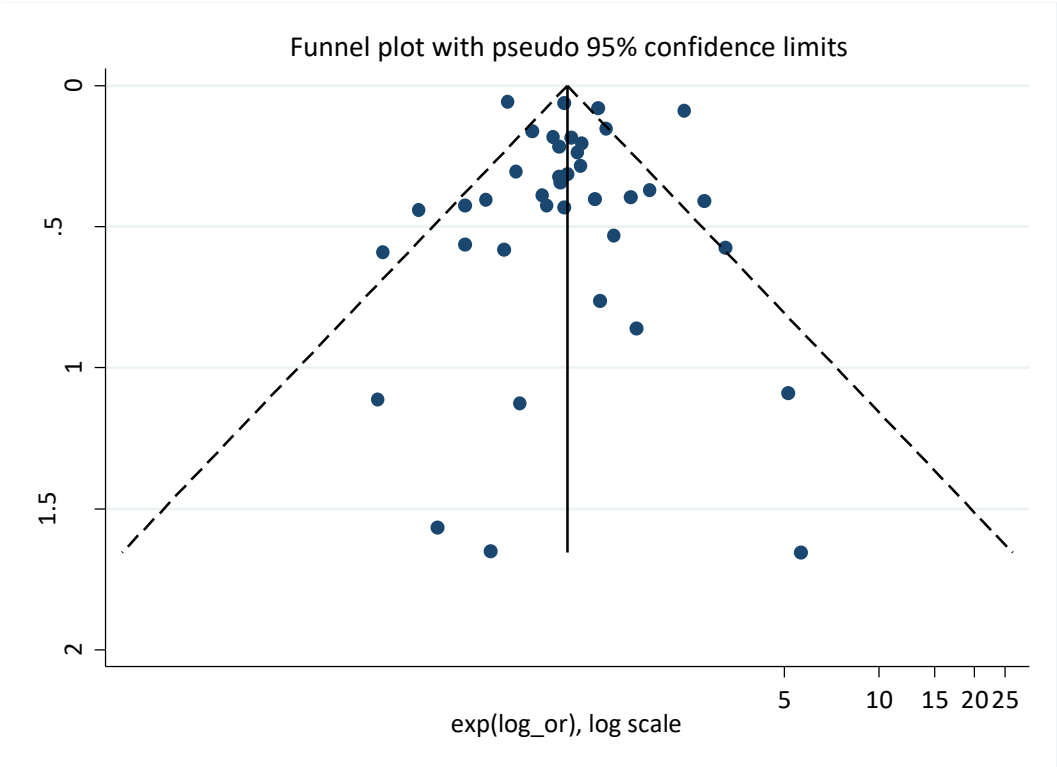
Egger's test: p=0.936

Supplementary Figure 9 – Funnel plot of publication bias among studies evaluating unadjusted Overall Survival among WLWH compared with HIV-negative women with breast cancer.



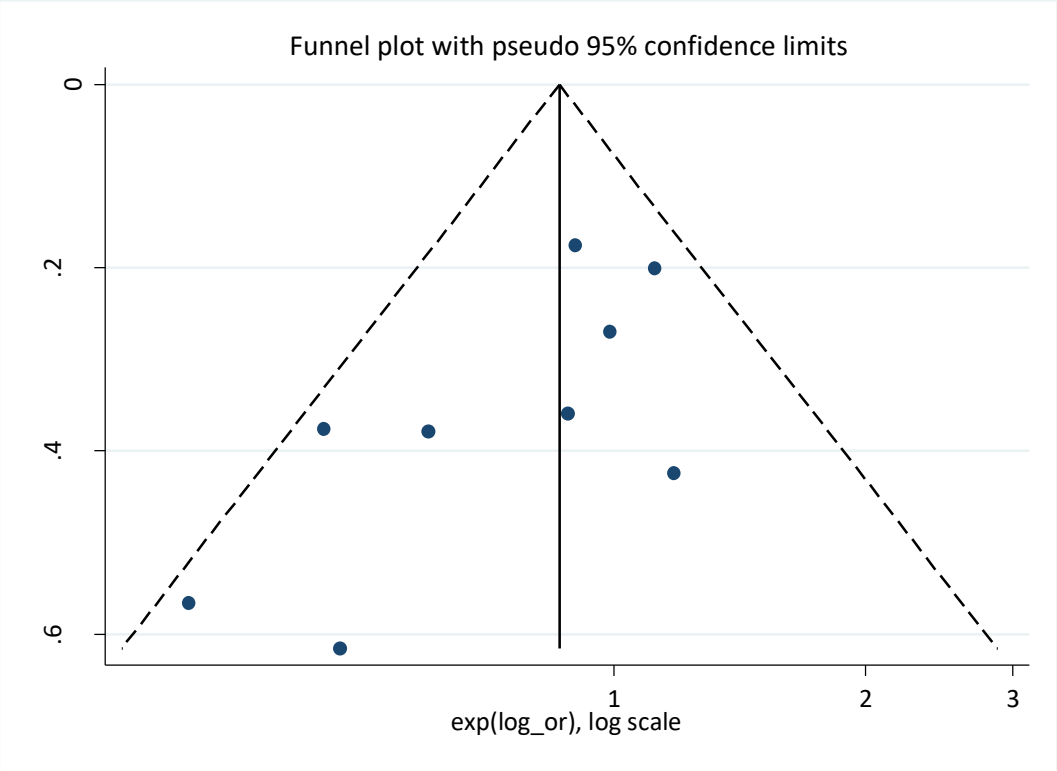
Egger's test: p=0.201

Supplementary Figure 10 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with each stage (0/I, II, III and IV) among WLWH compared with HIV-negative women with breast cancer.



Egger's test: p=0.843

Supplementary Figure 11 – Funnel plot of publication bias among studies evaluating the likelihood of presenting with estrogen receptor-positive status among WLWH compared with HIV-negative women with breast cancer.



Egger's test: p=0.047

Supplementary Table 2 – Sensitivity analysis of the odds of presenting with locally advanced/metastatic stage (III/IV) at diagnosis among WLWH compared with HIV-negative women with breast cancer (all studies).

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	Pooled OR	95% CI	P-value		
Sseggwanyi 2011	1.40	1.21-1.64	<0.001	47.5	0.025
Cubasch 2013	1.47	1.26-1.72	<0.001	44.5	0.037
Shiels 2015	1.37	1.15-1.64	<0.001	48.1	0.023
Traore 2015	1.42	1.21-1.67	<0.001	52.8	0.011
Langenhoven 2016	1.41	1.20-1.66	<0.001	52.5	0.011
Phakathi 2016	1.43	1.21-1.68	<0.001	52.7	0.011
Ngidi 2017	1.42	1.21-1.66	<0.001	51.6	0.013
Presti 2017	1.42	1.20-1.67	<0.001	52.7	0.011
McKenzie 2018	1.46	1.25-1.71	<0.001	47.6	0.024
Van Zyl 2018	1.44	1.22-1.69	<0.001	51.9	0.012
Ayeni 2019	1.51	1.31-1.74	<0.001	30.3	0.134
Brandao 2019	1.41	1.20-1.67	<0.001	52.6	0.011
Coghill 2019	1.37	1.15-1.62	<0.001	37.2	0.079
Phakathi 2019	1.42	1.19-1.69	<0.001	52.7	0.011
Sadigh 2019	1.46	1.25-1.72	<0.001	47.0	0.027

CI: confidence interval; OR: odds ratio.

Supplementary Table 3 – Sensitivity analysis of the odds of presenting with locally advanced/metastatic stage (III/IV) at diagnosis among WLWH compared with HIV-negative women with breast cancer (North American studies).

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Shiels 2015	1.75	1.54-1.98	<0.001	0.0	0.891
Presti 2017	1.76	1.58-1.96	<0.001	0.0	0.895
Coghill 2019	1.77	1.46-2.14	<0.001	0.0	0.859

CI: confidence interval; OR: odds ratio.

Supplementary Table 4 – Sensitivity analysis of the odds of presenting with locally advanced/metastatic stage (III/IV) at diagnosis among WLWH compared with HIV-negative women with breast cancer (Sub-Saharan African studies).

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	Pooled OR	95% CI	P-value		
Sseggwanyi 2011	1.20	1.05-1.39	0.010	0.0	0.711
Cubasch 2013	1.27	1.07-1.50	0.005	6.8	0.379
Traore 2015	1.24	1.06-1.45	0.008	11.1	0.339
Langenhoven 2016	1.21	1.05-1.40	0.010	3.1	0.413
Phakathi 2016	1.23	1.05-1.45	0.010	11.1	0.339
Ngidi 2017	1.22	1.06-1.41	0.006	3.0	0.414
McKenzie 2018	1.26	1.07-1.48	0.005	7.6	0.372
Van Zyl 2018	1.24	1.06-1.46	0.008	11.8	0.332
Ayeni 2019	1.30	1.10-1.54	0.002	0.0	0.460
Brandao 2019	1.22	1.05-1.41	0.011	4.8	0.397
Phakathi 2019	1.16	0.99-1.36	0.059	0.0	0.493
Sadigh 2019	1.26	1.07-1.48	0.005	7.5	0.373

CI: confidence interval; OR: odds ratio.

Supplementary Table 5 – Sensitivity analysis of the odds of presenting with ER-positive/HER2-negative subtype among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Cubasch 2013	0.77	0.60-0.97	0.030	0.0	0.630
Langenhoven 2016	0.79	0.64-0.97	0.028	0.0	0.636
Van Zyl 2018	0.81	0.66-1.00	0.050	0.0	0.506
Bhatia 2019	0.82	0.66-1.01	0.057	0.0	0.530
Brandao 2019	0.79	0.64-0.98	0.030	0.0	0.615
Phakathi 2019	0.93	0.71-1.23	0.624	0.0	0.924

CI: confidence interval; OR: odds ratio.

Supplementary Table 6 – Sensitivity analysis of the odds of presenting with HER2-positive subtype among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Cubasch 2013	0.99	0.64-1.53	0.958	43.5	0.115
Langenhoven 2016	1.12	0.78-1.61	0.523	40.5	0.135
Ngidi 2017	1.15	0.84-1.58	0.381	34.1	0.180
Van Zyl 2018	1.04	0.72-1.51	0.819	44.5	0.109
Bhatia 2019	1.06	0.74-1.51	0.759	44.8	0.107
Brandao 2019	1.29	1.02-1.62	0.032	0.0	0.687
Phakathi 2019	0.97	0.64-1.48	0.895	35.1	0.173

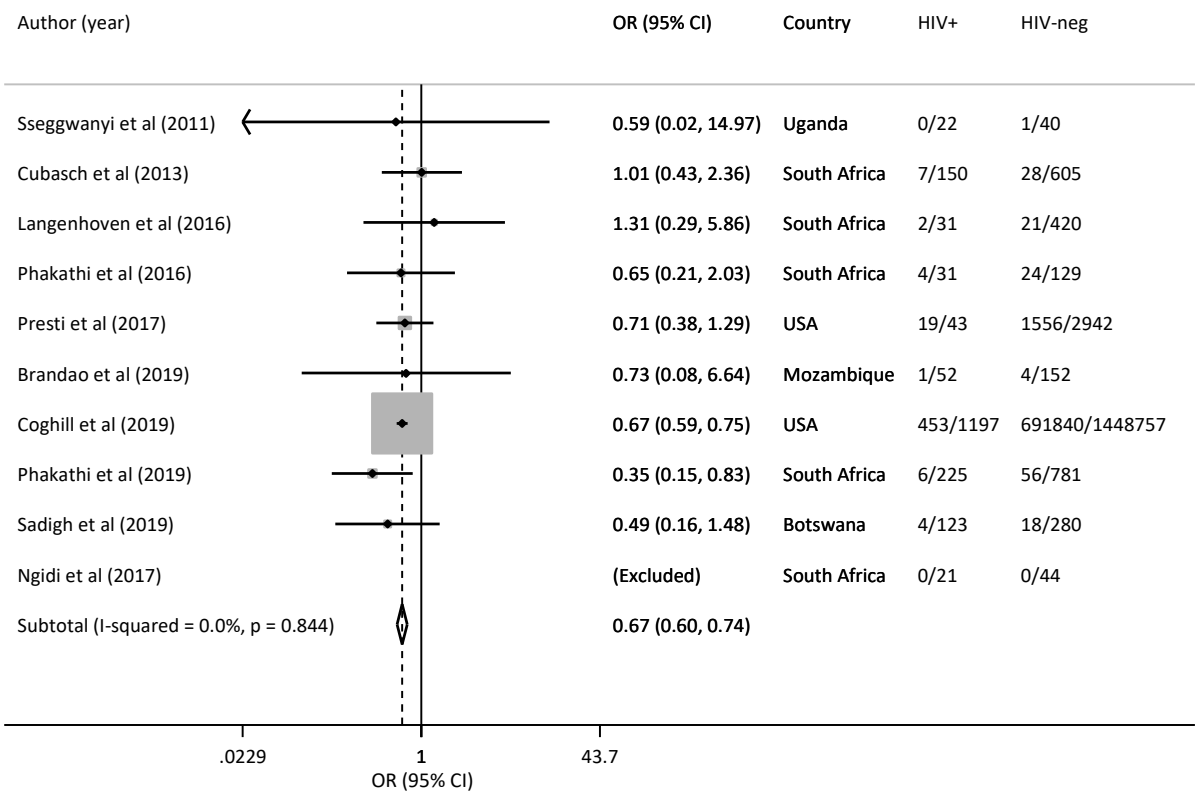
CI: confidence interval; OR: odds ratio.

Supplementary Table 7 – Sensitivity analysis of the odds of presenting with triple-negative subtype / basal-like subtype among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Cubasch 2013	1.26	0.98-1.64	0.076	0.0	0.737
Langenhoven 2016	1.15	0.89-1.47	0.279	6.9	0.376
Presti 2017	1.10	0.86-1.39	0.453	0.0	0.502
Van Zyl 2018	1.16	0.91-1.47	0.237	3.0	0.403
Bhatia 2019	1.14	0.89-1.45	0.306	6.6	0.378
Brandao 2019	1.07	0.84-1.36	0.576	0.0	0.705
Phakathi 2019	1.10	0.83-1.46	0.519	3.3	0.400
Sadigh 2019	1.17	0.90-1.51	0.233	3.5	0.399

CI: confidence interval; OR: odds ratio.

Supplementary Figure 12 – Meta-analysis of the odds of presenting with stage 0/I at diagnosis among WLWH compared with HIV-negative women with breast cancer.



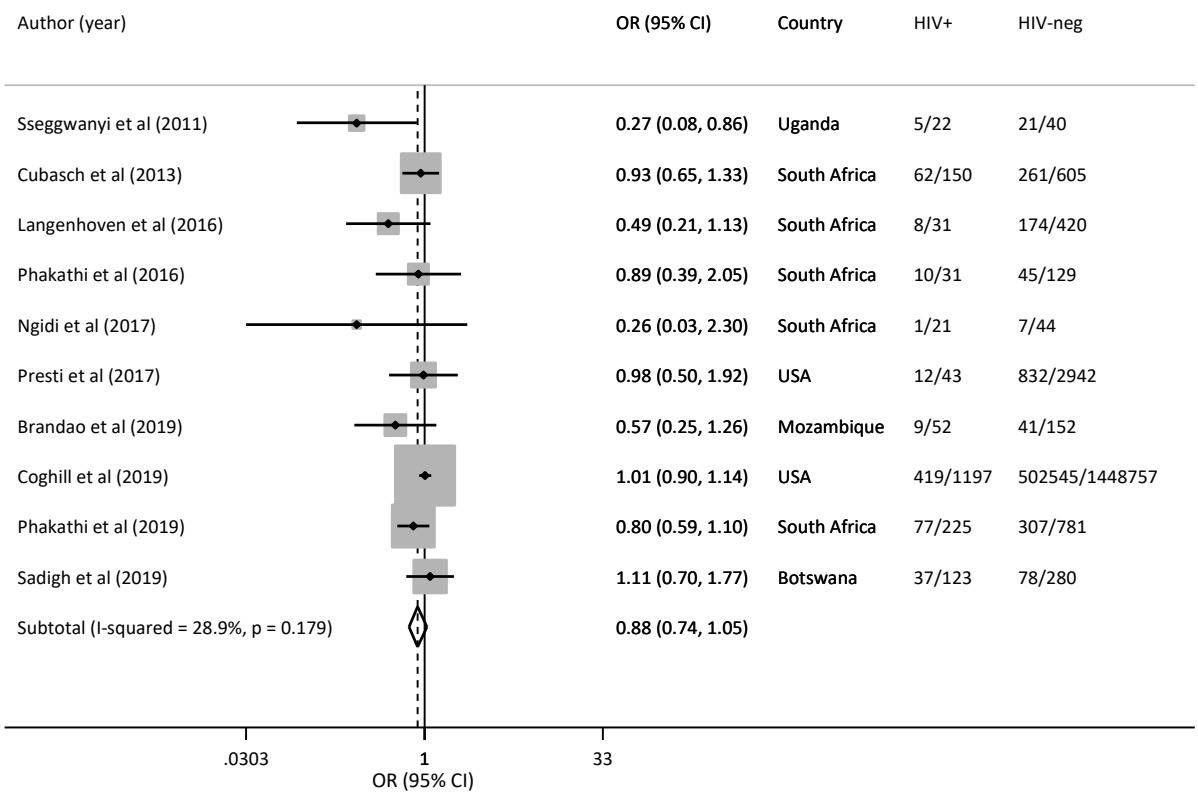
Random effect: p<0.001. CI=confidence interval; OR=odds ratio.

Supplementary Table 8 – Sensitivity analysis of the odds of presenting with stage 0/I at diagnosis among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Sseggwanyi 2011	0.66	0.59-0.74	<0.001	0.0	0.764
Cubasch 2013	0.66	0.59-0.74	<0.001	0.0	0.866
Langenhoven 2016	0.66	0.59-0.74	<0.001	0.0	0.851
Phakathi 2016	0.67	0.59-0.74	<0.001	0.0	0.763
Presti 2017	0.66	0.59-0.74	<0.001	0.0	0.768
Brandao 2019	0.66	0.59-0.74	<0.001	0.0	0.764
Coghill 2019	0.66	0.46-0.94	0.022	0.0	0.757
Phakathi 2019	0.67	0.60-0.75	<0.001	0.0	0.959
Sadigh 2019	0.67	0.60-0.75	<0.001	0.0	0.798

CI: confidence interval; OR: odds ratio.

Supplementary Figure 13 – Meta-analysis of the odds of presenting with stage II at diagnosis among WLWH compared with HIV-negative women with breast cancer.



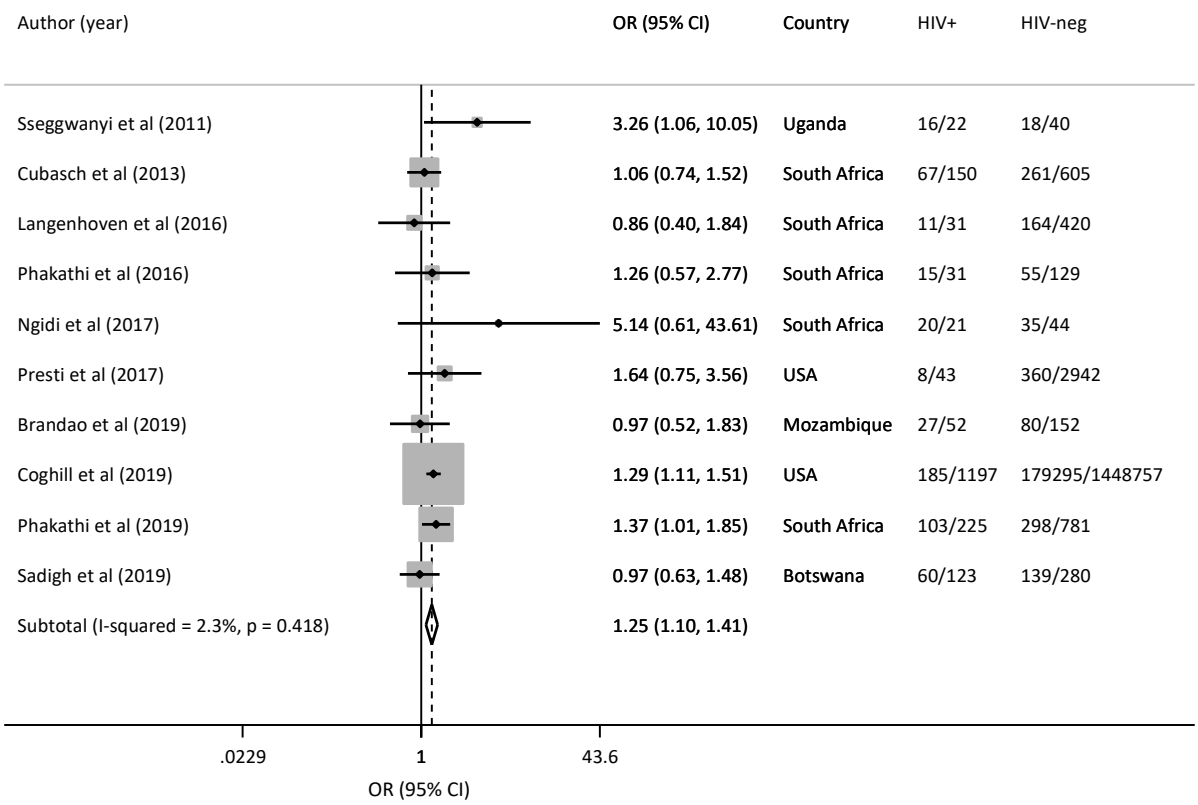
Random effect: p=0.146. CI=confidence interval; OR=odds ratio.

Supplementary Table 9 – Sensitivity analysis of the odds of presenting with stage II at diagnosis among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Sseggwanyi 2011	0.96	0.86-1.06	0.438	0.9	0.426
Cubasch 2013	0.85	0.68-1.05	0.133	36.7	0.125
Langenhoven 2016	0.91	0.78-1.07	0.266	21.2	0.255
Phakathi 2016	0.87	0.71-1.05	0.145	36.6	0.125
Ngidi 2017	0.89	0.75-1.05	0.176	29.2	0.185
Presti 2017	0.86	0.71-1.04	0.128	36.7	0.125
Brandao 2019	0.90	0.76-1.07	0.232	27.3	0.201
Coghill 2019	0.82	0.66-1.01	0.057	14.1	0.317
Phakathi 2019	0.88	0.72-1.09	0.240	29.4	0.183
Sadigh 2019	0.84	0.69-1.03	0.088	34.5	0.142

CI: confidence interval; OR: odds ratio.

Supplementary Figure 14 – Meta-analysis of the odds of presenting with stage III at diagnosis among WLWH compared with HIV-negative women with breast cancer.



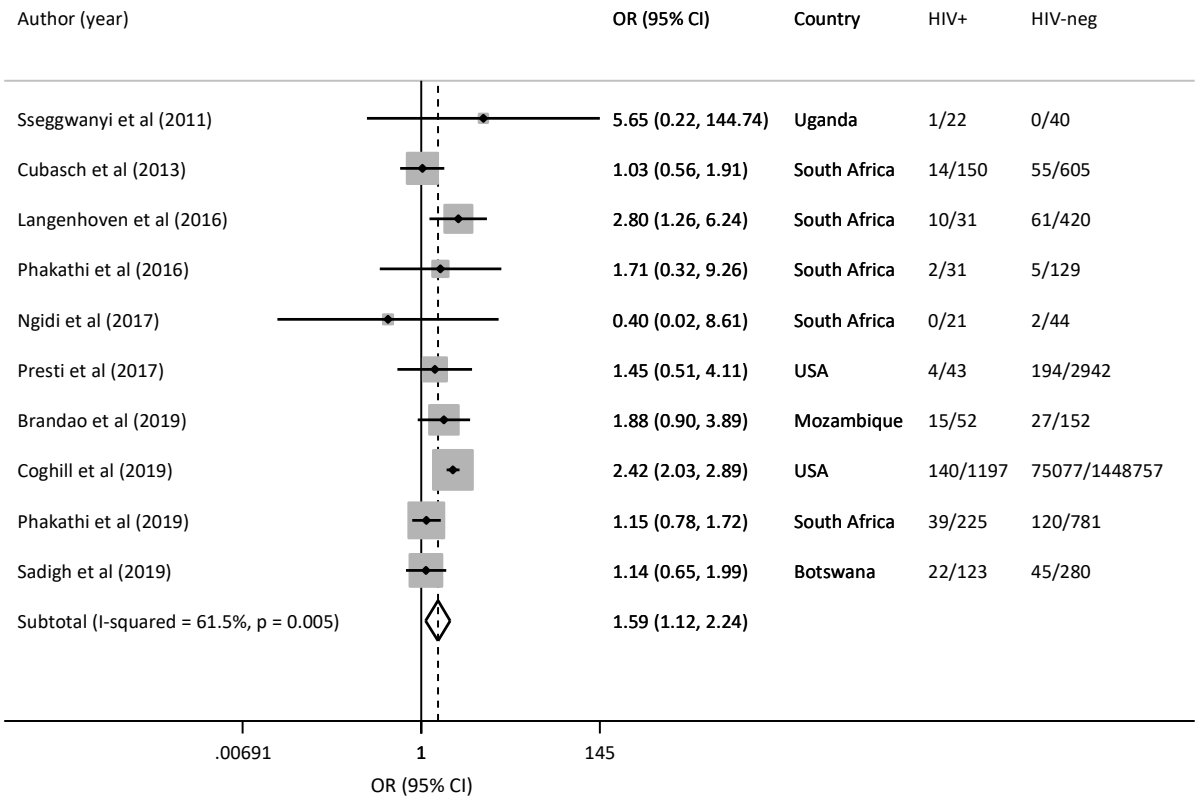
Random effect: p<0.001. CI=confidence interval; OR=odds ratio.

Supplementary Table 10 – Sensitivity analysis of the odds of presenting with stage III at diagnosis among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Sseggwanyi 2011	1.24	1.10-1.39	<0.001	0.0	0.603
Cubasch 2013	1.27	1.11-1.46	0.001	4.2	0.400
Langenhoven 2016	1.26	1.11-1.43	<0.001	3.0	0.409
Phakathi 2016	1.23	1.07-1.43	0.005	13.1	0.325
Ngidi 2017	1.24	1.11-1.40	<0.001	0.0	0.481
Presti 2017	1.23	1.07-1.41	0.003	8.4	0.366
Brandao 2019	1.25	1.10-1.44	0.001	6.7	0.379
Coghill 2019	1.20	0.99-1.45	0.065	9.0	0.361
Phakathi 2019	1.21	1.03-1.42	0.017	9.0	0.360
Sadigh 2019	1.28	1.13-1.44	<0.001	0.0	0.466

CI: confidence interval; OR: odds ratio.

Supplementary Figure 15 – Meta-analysis of the odds of presenting with stage IV at diagnosis among WLWH compared with HIV-negative women with breast cancer.



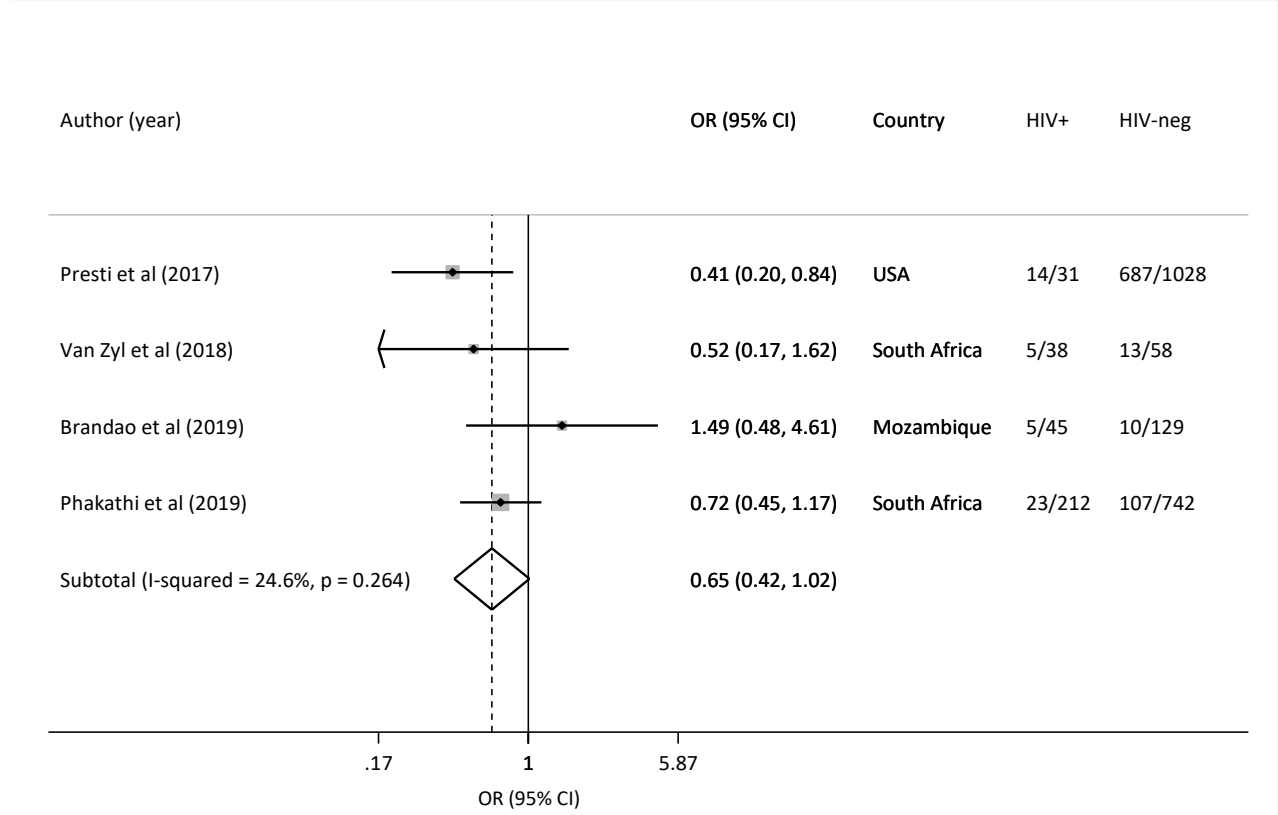
Random effect: p=0.009. CI=confidence interval; OR=odds ratio.

Supplementary Table 11 – Sensitivity analysis of the odds of presenting with stage IV at diagnosis among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Sseggwanyi 2011	1.56	1.10-2.23	0.013	65.2	0.003
Cubasch 2013	1.70	1.19-2.42	0.003	57.3	0.016
Langenhoven 2016	1.49	1.02-2.17	0.039	64.6	0.004
Phakathi 2016	1.58	1.10-2.27	0.013	65.7	0.003
Ngidi 2017	1.61	1.14-2.29	0.007	64.1	0.004
Presti 2017	1.60	1.10-2.31	0.013	65.3	0.003
Brandao 2019	1.55	1.05-2.29	0.026	65.8	0.003
Coghill 2019	1.32	1.04-1.69	0.023	0.0	0.523
Phakathi 2019	1.71	1.21-2.42	0.002	47.3	0.056
Sadigh 2019	1.68	1.16-2.42	0.006	58.6	0.013

CI: confidence interval; OR: odds ratio.

Supplementary Figure 16 – Meta-analysis of the odds of presenting with Luminal A-like subtype among WLWH compared with HIV-negative women with breast cancer.



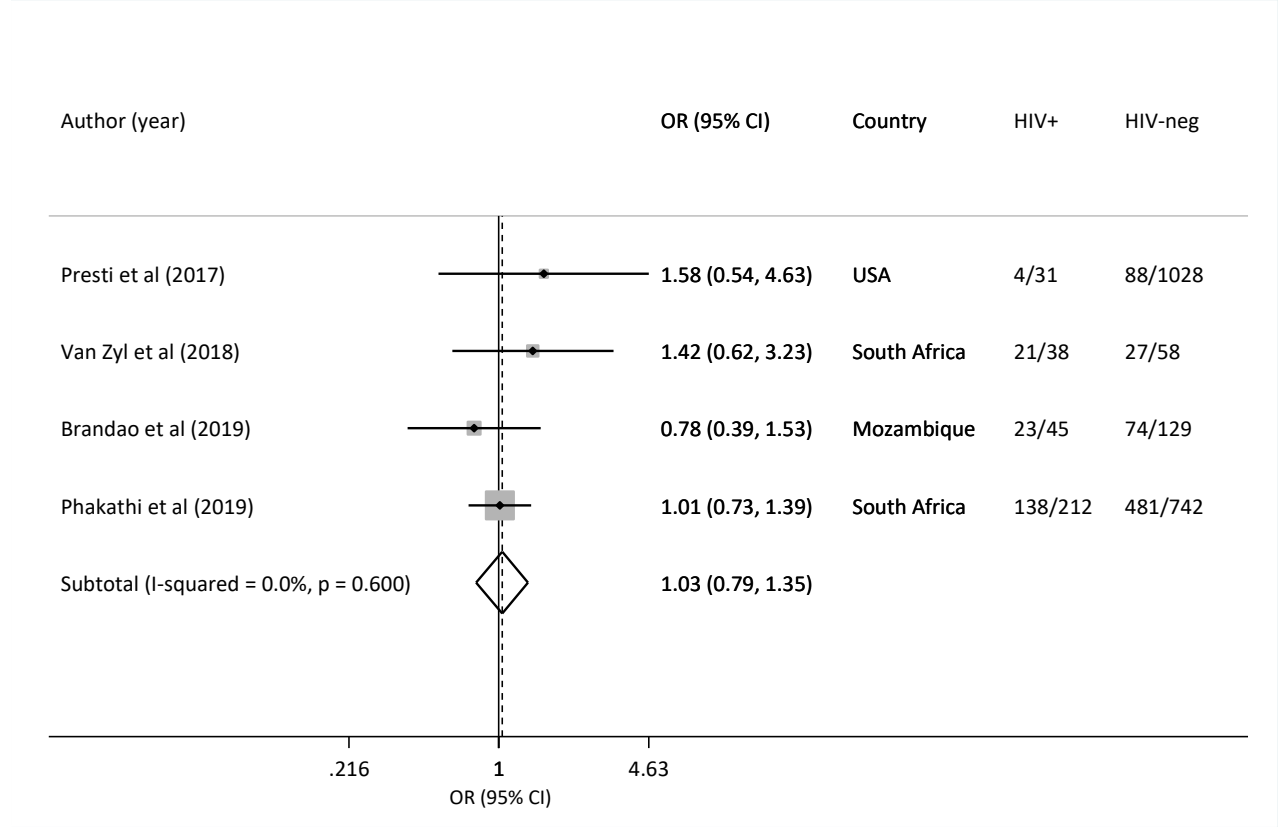
Random effect: p=0.059. CI=confidence interval; OR=odds ratio.

Supplementary Table 12 – Sensitivity analysis of the odds of presenting with Luminal A-like subtype among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Presti 2017	0.76	0.50-1.15	0.193	0.0	0.403
Van Zyl 2018	0.68	0.38-1.22	0.200	47.6	0.148
Brandao 2019	0.60	0.41-0.87	0.007	0.0	0.422
Phakathi 2019	0.63	0.29-1.33	0.226	44.4	0.165

CI: confidence interval; OR: odds ratio.

Supplementary Figure 17 – Meta-analysis of the odds of presenting with Luminal B-like subtype among WLWH compared with HIV-negative women with breast cancer.



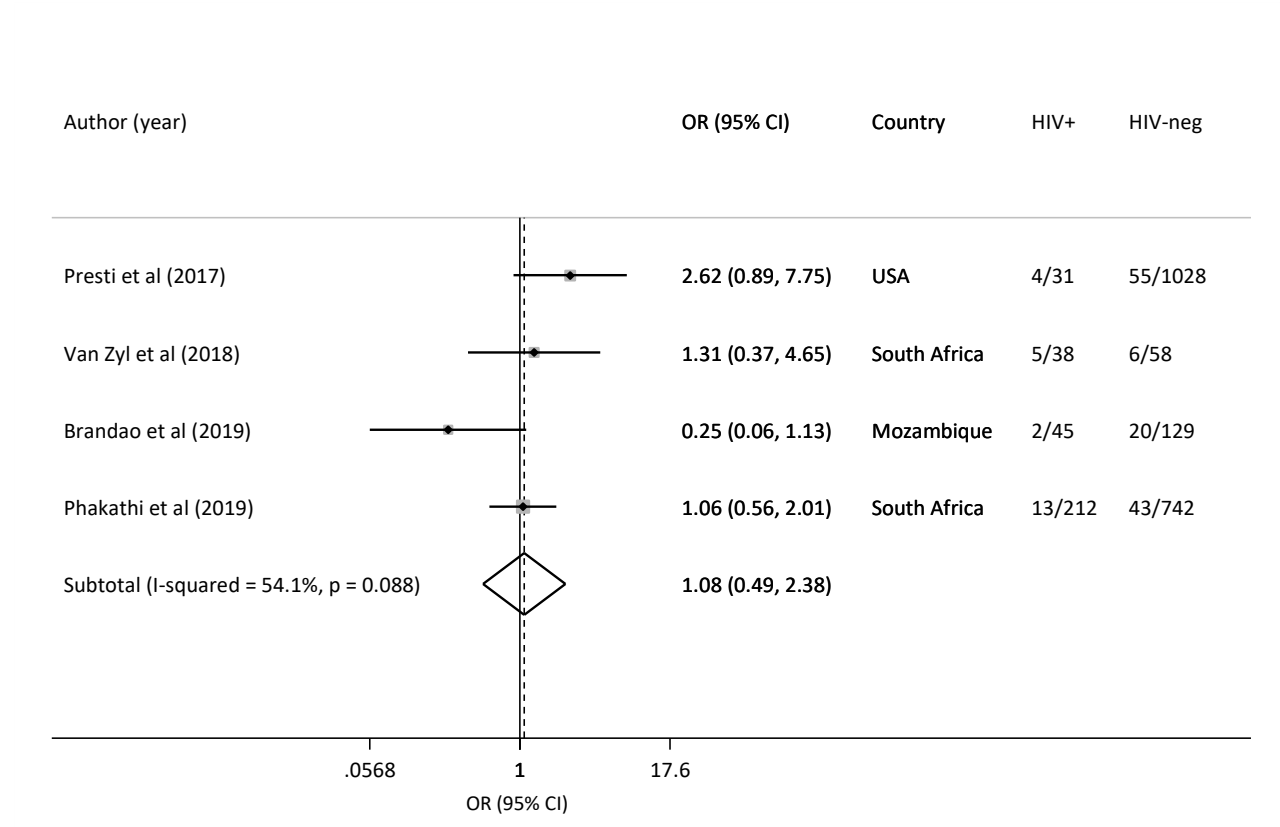
Random effect: p=0.800. CI=confidence interval; OR=odds ratio.

Supplementary Table 13 – Sensitivity analysis of the odds of presenting with Luminal B-like subtype among HIV-positive compared with HIV-negative breast cancer patients.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Presti 2017	1.01	0.77-1.32	0.962	0.0	0.542
Van Zyl 2018	1.00	0.75-1.32	0.987	0.0	0.539
Brandao 2019	1.09	0.82-1.45	0.561	0.0	0.587
Phakathi 2019	1.09	0.68-1.74	0.731	0.0	0.405

CI: confidence interval; OR: odds ratio.

Supplementary Figure 18 – Meta-analysis of the odds of presenting with HER2-enriched subtype among WLWH compared with HIV-negative women with breast cancer.



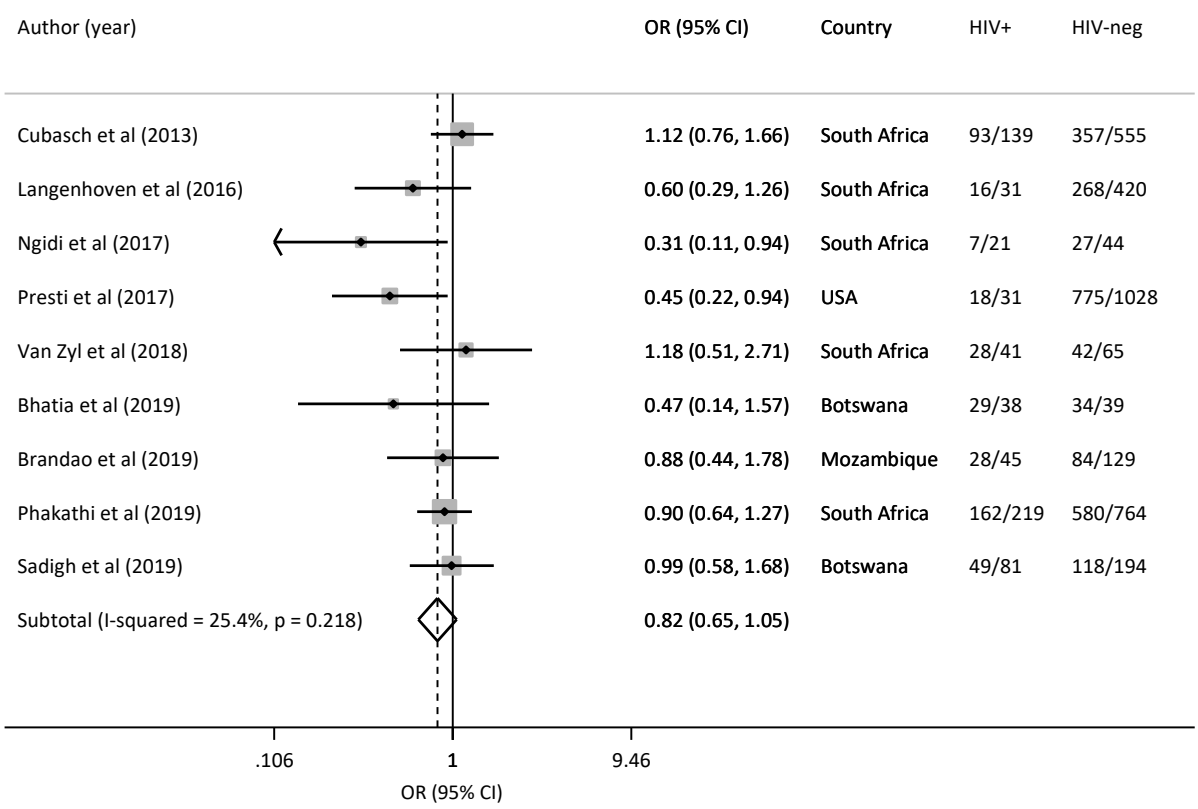
Random effect: p=0.842. CI=confidence interval; OR=odds ratio.

Supplementary Table 14 – Sensitivity analysis of the odds of presenting with HER2-enriched subtype among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Presti 2017	0.83	0.37-1.87	0.650	42.0	0.178
Van Zyl 2018	0.99	0.34-2.93	0.992	69.2	0.039
Brandao 2019	1.34	0.81-2.22	0.261	0.3	0.367
Phakathi 2019	1.03	0.27-3.99	0.966	70.5	0.034

CI: confidence interval; OR: odds ratio.

Supplementary Figure 19 – Meta-analysis of the odds of presenting with estrogen receptor-positive status among WLWH compared with HIV-negative women with breast cancer.



Random effect: p=0.112. CI=confidence interval; OR=odds ratio.

Supplementary Table 15 – Sensitivity analysis of the odds of presenting with estrogen receptor-positive status among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled OR	95% CI	P-value		
Cubasch 2013	0.77	0.59-0.99	0.043	16.9	0.297
Langenhoven 2016	0.84	0.65-1.09	0.197	28.4	0.201
Ngidi 2017	0.88	0.72-1.08	0.227	5.1	0.391
Presti 2017	0.89	0.72-1.10	0.301	6.8	0.378
Van Zyl 2018	0.79	0.61-1.03	0.081	31.0	0.181
Bhatia 2019	0.84	0.66-1.07	0.165	28.3	0.203
Brandao 2019	0.80	0.61-1.06	0.117	34.7	0.151
Phakathi 2019	0.78	0.58-1.06	0.112	34.1	0.156
Sadigh 2019	0.79	0.59-1.04	0.091	32.9	0.165

CI: confidence interval; OR: odds ratio.

Supplementary Table 16 – Sensitivity analysis of adjusted Overall Survival among WLWH compared with HIV-negative women with breast cancer (North American studies).

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled HR	95% CI	P-value		
Biggar 2005	2.85	1.11-7.30	0.029	99.0	<0.001
Coghill 2015	1.77	1.62-1.93	<0.001	0.0	0.816
Coghill 2019	2.96	1.06-8.25	0.038	82.8	0.016

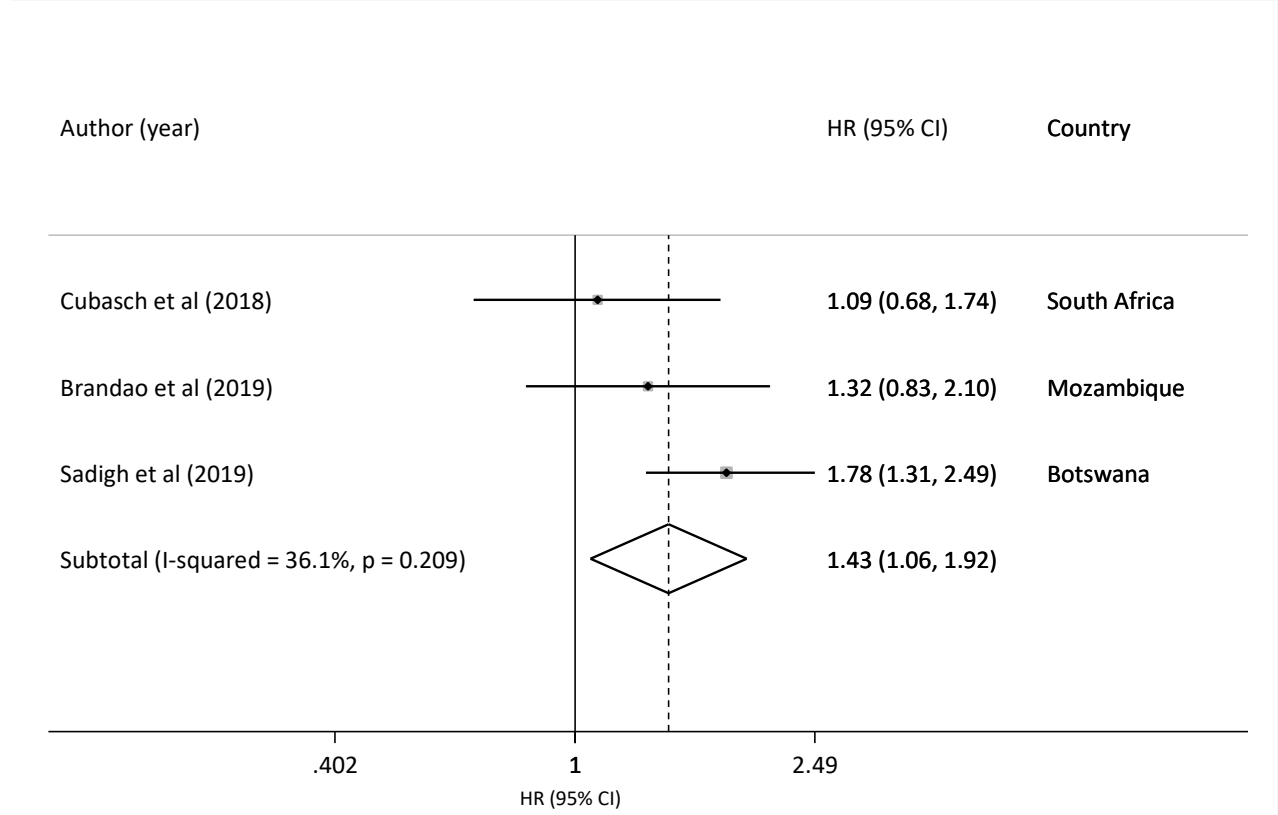
CI: confidence interval; HR: hazard ratio.

Supplementary Table 17 – Sensitivity analysis of adjusted Overall Survival among WLWH compared with HIV-negative women with breast cancer (Sub-Saharan African studies).

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled HR	95% CI	P-value		
Coghill 2013	1.51	1.14-2.00	0.004	23.0	0.273
Cubasch 2018	1.59	1.16-2.19	0.004	24.1	0.268
Brandao 2019	1.72	1.33-2.22	<0.001	0.0	0.641
Sadigh 2019	1.35	0.97-1.88	0.074	0.0	0.527

CI: confidence interval; HR: hazard ratio.

Supplementary Figure 20 – Meta-analysis of unadjusted overall survival among WLWH compared with HIV-negative women with breast cancer.



Random effect: p=0.019. CI=confidence interval; HR=hazard ratio.

Supplementary Table 18 – Sensitivity analysis of unadjusted Overall Survival among WLWH compared with HIV-negative women with breast cancer.

Study excluded	Random effect			I^2 (%)	I^2 P-value
	Pooled HR	95% CI	P-value		
Cubasch 2018	1.61	1.22-2.12	0.001	7.2	0.299
Brandao 2019	1.44	0.89-2.31	0.136	65.0	0.091
Sadigh 2019	1.20	0.86-1.67	0.277	0.0	0.570

CI: confidence interval; HR: hazard ratio.

Supplementary Table 19 – Sensitivity analysis of adjusted and unadjusted Overall Survival (OS) among WLWH compared with HIV-negative women with breast cancer, excluding studies with poor-quality follow-up.

Adjusted OS					
Studies excluded	**Random effect**			**I-squared (%)**	**I-sq. P-value**
	HR	**95% CI**	**P-value**		
Coghill 2013 & Cubasch 2018	1.50	0.96-2.36	0.077	58.1	0.122
Unadjusted OS					
Study excluded	**Random effect**			**I-squared (%)**	**I-sq. P-value**
	HR	**95% CI**	**P-value**		
Cubasch 2018	1.61	1.22-2.12	0.001	7.2	0.299

CI: confidence interval; HR: hazard ratio.

Supplementary Table 20 – Age at diagnosis and anti-cancer treatment received by WLWH and HIV-negative women with breast cancer in each of the included studies.

	Age at diagnosis		Anti-cancer treatment received by WLWH (n, %)						Anti-cancer treatment received by HIV-negative women (n, %)					
Author (Year)	WLWH	HIV-negative women	Total nb pts	Any tx received	Surgery	CT	Comple-ting CT	RT	Total nb pts	Any tx received	Surgery	CT	Comple-ting CT	RT
Biggar et al (2005)[1]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Sseggwanyi et al (2011)[2]	Mean: 32.4 (range 18-52)	Mean: 45.0 (range 21-80)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Coghill et al (2013)[3]	NR¥	NR¥	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Cubasch et al (2013)[4]	NR¥	NR¥	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Cubasch et al (2018)[5]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Shiels et al (2015)[6]	NR¥	NR¥	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Coghill et al (2015)[7]	NR¥	NR¥	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Traore et al (2015)[8]	Median: 36.5 (range 26-58)	Median: 49.0 (range 20-85)	14	7 (50%)	3 (21%)	6 (43%)	NR	NR	264	202 (77%)	NR	NR	NR	NR
Phakathi et al (2016)[9]	Median: 41.0 (SD 8.6)	Median: 55.0 (SD 13.9)	31	NR	29 (94%)	27 (87%)	25 (93%)	14 (45%)	129	NR	124 (96%)	113 (88%)	106 (94%)	41 (32%)
Langenhoven et al (2016)[10]	Median: 42.0 (range 29-60)	Median: 54.0 (range 20-91)	31	NR	NR	19 (61%)	16 (84%)	NR	39	NR	NR	39 (100%)	33 (85%)	NR
Ngidi et al (2017)[11]	Mean: 40.6 (SD 9.6)	Mean: 52.0 (SD 13.1)	21	NR	NR	21 (all)	20 (95%)	NR	44	NR	NR	44 (100%)	44 (100%)	NR
Presti et al (2017)[12]	Mean: 53.2 (SD 9.5)	Mean: 60.6 (SD: NR)	43	NR	38 (88%)	26 (61%)	NR	30 (70%)	3012	NR	2697 (90%)	1273 (43%)	NR	1869 (62%)
McKenzie et al (2018)[13]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Van Zyl et al (2018)[17]	Mean: 44.9 (SD 9)	Mean: 53.2 (SD 12.5)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Sadigh et al (2019)[18]	Median: 47.2 (IQR 41-54)	Median: 55.9 (IQR 45-66)	151	NR	111 (76%)	76 (50%)	NR	64 (43%)	327	NR	262 (82%)	147 (45%)	NR	166 (50%)
Brandao et al (2019)[19]	Mean: 45.2 (SD 10.1)	Mean: 50.8 (SD 14.3)	52	52 (100%)	41 (79%)	49 (94%)	35 (71%)	0	152	148 (97%)	125 (82%)	143 (94%)	103 (73%)	9 (6%)
Phakathi et al (2019)[20]	Median: 45.0 (IQR 40-52)	Median: 57.0 (46-67)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Bhatia et al (2019)[21]	NR¥	NR¥	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Ayeni et al (2019)[22]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Coghill et al (2019)[23]*	NR¥	NR¥	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Legend: CT=chemotherapy; IQR=interquartile range; NR=not reported; RT=radiotherapy; SD=standard deviation; tx=treatment; WLWH=women living with HIV.
*Not reported as a continuous variable, but in categories. *Overall (all tumor types): Receipt of surgery, radiotherapy or chemotherapy among HIV-positive patients: 11,598 (80.2%) vs. among HIV-negative: 5,747,187 (90.3%).

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Discussion and Conclusion

This thesis provided new insights on how the heterogeneity of breast cancer at the molecular level (*i.e.* tumor subtypes), but also in terms of socio-economic, lifestyle and demographic factors, and patients' clinico-pathological characteristics (*e.g.* age and stage at diagnosis; previous cancer history; presence of HIV infection) has repercussions in terms of the etiology, clinical management, health resource use and survival of women with breast cancer – **Figure 3**.

We evaluated these outcomes along two axes, in a high- and in a low-income setting, with different breast cancer epidemiology and healthcare organization, in order to have a more comprehensive overview of this heterogeneity. Given the marked differences between the two settings (demonstrated in **Paper IV**), most of the studies presented in this thesis consisted of independent analyses in each country, which explored their specificities and addressed the knowledge gaps in each context.

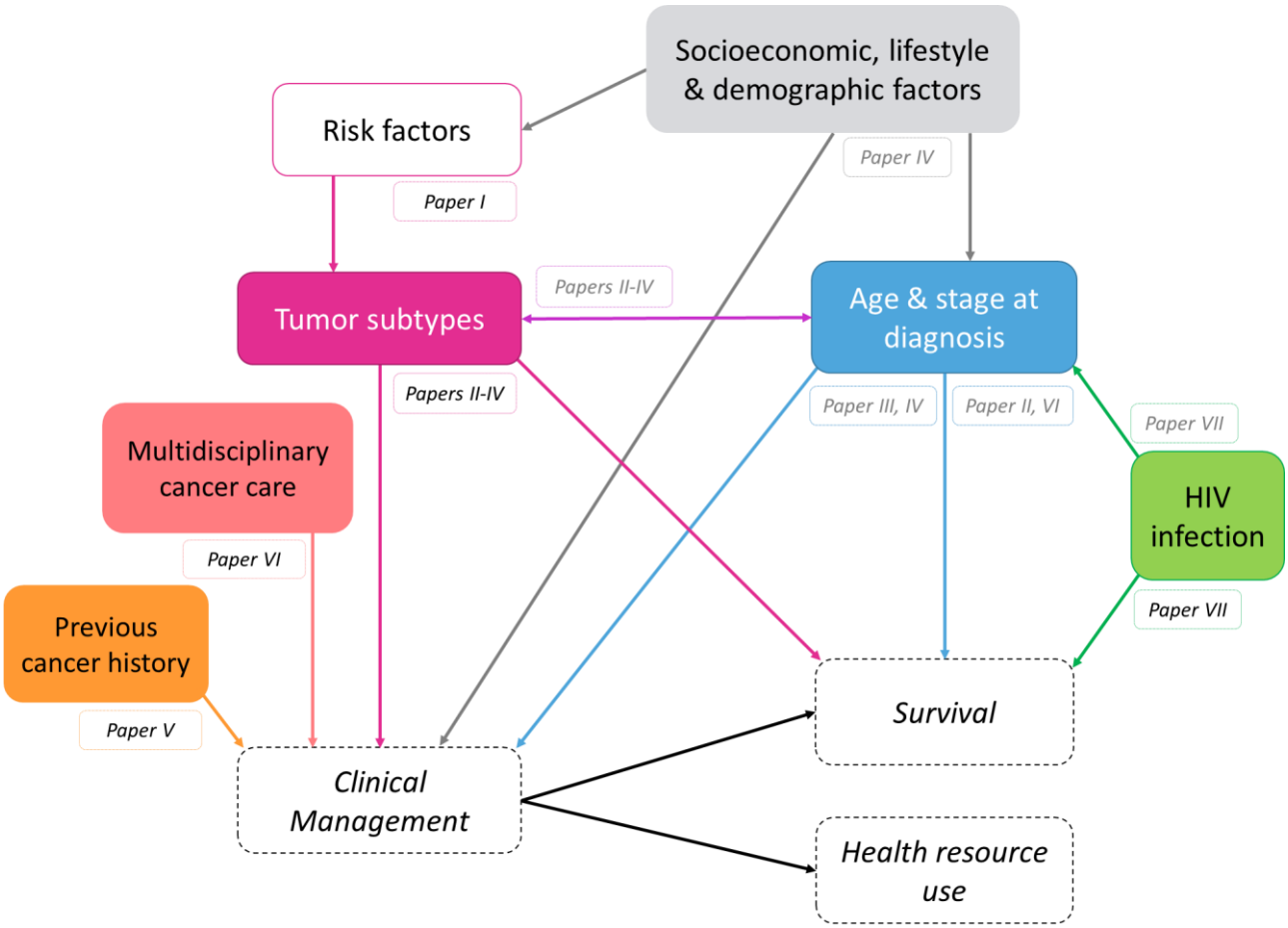


***Figure 3**: Interconnection between the heterogeneity of breast cancer in terms of the setting (socioeconomic, lifestyle and demographic factors), organization of healthcare (multidisciplinary cancer care implementation), patient characteristics (age at diagnosis, previous cancer history, presence of HIV infection) and tumor characteristics (stage at diagnosis, tumor molecular subtype), and the clinical management, health resource use and survival of patients with breast cancer; the papers addressing each of these connections are also depicted.*

With the first papers of this thesis (**Papers I-IV**), we demonstrated that the proportion of patients with tumors classified as TNBC is indeed higher among patients from Mozambique (around 25%), compared to Western populations (around 10-15% in the US[28] and 8% among patients with early breast cancer from Portugal). Moreover, the proportion of women with HER2-positive tumors in Mozambique was also higher than expected (24% in Mozambique *vs* 16% in Portugal). Subtype determination was made in a prospective manner, in a centralized lab and according to the ASCO/CAP guidelines,[24,25] thus avoiding the potential biases related to pre-analytic (*e.g.* inadequate tissue fixation, antigen degradation) and analytical conditions (*e.g.* lack of ISH for HER2, use of different cut-offs to define ER/PR positivity) verified in other African series.[30,46] Moreover, we showed for the first time in Africa that it is possible to use cell blocks created from FNAC to perform these determinations, which may help circumvent the logistical issue of the unavailability of core biopsies at diagnosis.

Then, in **Paper I,** we unraveled for the first time the etiological heterogeneity of breast cancer according to tumor subtypes in a Sub-Saharan African population. We demonstrated that multiparity is protective for HR-positive breast tumors, while no association was seen with the development of HR-negative tumors (including TNBC), which may be related to the high rates of breastfeeding in the country.[129] This finding may partly explain why the proportion of HR-positive tumors is lower among African populations, where multiparity is a common phenomenon,[130] protecting women from the development of this tumor subtype, compared to Western populations, where fertility rates are much lower.[130] This means that, if fertility rates start decreasing in Sub-Saharan Africa, it is likely that the proportion of HR-positive breast tumors will increase. On the other side, we also showed that higher BMI is associated with the development of breast cancer among post-menopausal women, regardless of the subtype. As the prevalence of overweight and obesity is rising in Africa,[131,132] this may contribute to an increase in the incidence of breast cancer among this population – both of HR-positive and HR-negative tumors. Therefore, we highlighted how it is paramount to invest in awareness campaigns to reverse this potential obesity epidemic, in order to prevent an increase in other health conditions, such as breast cancer.

Afterwards, we looked at how tumor subtype heterogeneity translates into differences in clinical management in a low-income (in Mozambique, **Paper II**) and in a high-income setting (in Portugal, **Paper III**). We saw that Portuguese women with TNBC were younger and had a more advanced stage at diagnosis compared to women with HR-positive/HER2-negative tumors, which is line with Western literature.[14] However, there were very few clinico-pathological differences at baseline across subtypes among Mozambican women and, actually, age and stage at diagnosis were similar among them. Concerning treatment, Mozambican patients with TNBC were less likely to receive endocrine therapy (as expected), but there were no differences in terms of chemotherapy or other treatments used. Surprisingly, the proportion of patients with HR-positive/HER2-negative early breast cancer receiving chemotherapy was much higher in Mozambique than in Portugal (96% *vs* 53%, respectively). This is partly due to the fact that most of the

Mozambican patients had stage III disease, but also to the absence of information regarding tumor subtype at diagnosis, which makes physicians play "on the safe side" and prescribe chemotherapy to all patients. On the other hand, in Portugal, subtype determination is made before treatment in virtually every patient and there are many available treatment options. Thus, in **Paper III**, we observed a much more tailored approach to breast cancer care, as almost all Portuguese patients with HR-positive tumors received endocrine therapy and all women with HER2-positive tumors received trastuzumab, unless there was a contra-indication. Hence, we demonstrated that the main driver of health resource use and cost of care among Portuguese women with early breast cancer was tumor subtype, and not stage at diagnosis. These findings were derived from the analysis of women treated between 2012 and 2015, but if we repeat the same analysis nowadays, this conclusion will probably be reinforced, given the approval of several agents for early HER2-positive breast cancer (pertuzumab,[133] neratinib,[134] T-DM1[81]) in the last few years, and soon for HR-positive/HER2-negative (abemaciclib[74]) and TNBC (pembrolizumab,[65] atezolizumab[66]), which will increase the relevance of tumor subtypes in terms of health resource use. This will pose important questions regarding the management of "financial toxicity" and, therefore, our findings may be used as a benchmark to which compare future health resource use and cost analyses.

As it may be difficult to directly estimate the cost of care in developed *vs* developing countries, given differences in purchasing power, we analyzed health resource use among Portuguese *vs* Mozambican women with early breast cancer and then converted the cost of their care from 2015 Euros and 2019 Mozambican Meticais to 2019 International dollars (**Paper IV**). With this, we were able to confirm our preliminary impressions: that there was an underfunding of breast cancer care in Mozambique compared to Western standards and that there was virtually no difference in health resource use according to stage or subtype.

Finally, in **Paper II**, we also assessed survival according to each subtype among Mozambican women, as there are scarce data about this in Africa. Despite the overall poor prognosis (three-year overall survival of 53%), we demonstrated that TNBC conferred an even worse prognosis, in comparison with the HR-positive/HER2-negative subtype. Sadly, we also saw that patients with HER2-positive breast cancer had a clearly worse survival compared to women with HR-positive/HER2-negative tumors, a situation similar to the 1990's and early 2000's in Western countries, before the introduction of adjuvant trastuzumab as a standard-of-care.[33] Therefore, we make the plea for a systematic determination of tumor subtype at diagnosis, as this is feasible and may help tailoring treatment even in low-resource settings, potentially leading to savings in the cost of care (*e.g.* avoidance of endocrine therapy, while supporting the use of platinum salts for TNBC) and improvements in survival outcomes.

Then, we looked to other factors that may influence breast cancer management, health resource use and survival (**Papers V-VII**). In **Paper V**, we compared women with a previous history of cancer (breast or other type) to women with breast cancer as their first primary malignancy. As expected, women with a previous

breast cancer were older, but there were no other differences in clinico-pathological characteristics, such as in stage or distribution of tumor subtypes. Yet, women with a previous breast cancer were possibly surgically overtreated, with a three-fold higher likelihood of receiving mastectomy compared to patients without a previous cancer history, while women with a previous non-breast cancer had a lower chance of receiving anthracycline-based chemotherapy, probably because they had received these agents before. Nonetheless, there were no differences regarding other kinds of therapy, namely radiotherapy, endocrine or targeted therapy and there was no significant impact on relapse-free survival, which is reassuring. Still, this Paper adds to previous research on this topic, by improving our understanding of the clinical pathway of patients with a breast cancer who had a previous primary malignancy.

Given the dismal prognosis of women with breast cancer in Sub-Saharan Africa, it is important to identify strategies that may improve it. Besides approaches to reduce the prevalence of risk factors and to detect the disease at earlier stages, there is also room for improvement in the organization of care *after* a diagnosis of breast cancer. In **Paper VI**, we demonstrated for the first time in a developing country that the implementation of an MTB for breast cancer improves the survival of women with early stage disease and that it is a cost-effective intervention. We did not find major differences in clinico-pathological characteristics or in the treatment received between patients before or after the implementation of the MTB, and we observed that its benefit was consistent among most subgroups of patients. These results can empower patients and health professionals to ask for a more effective organization of breast cancer care, influencing policymakers and scaling-up the implementation of multidisciplinary cancer care in developing countries. Additionally, these findigns could be a source of reverse learning from developing countries to high-income countries, where the coverage of MTB remains sub-optimal and where their added value is being questioned.[135]

In **Paper VII**, we focused on WLWH who develop breast cancer. We have done so because 25% of the patients included in the Moza-BC cohort had HIV infection and, when comparing them to HIV-negative women, we verified that WLWH were younger and had a higher proportion of TNBC, but similar stage at diagnosis. Their survival appeared to be worse, but given the low total number of WLWH included (n=52), that difference was not significant. Therefore, we decided to combine our results with other cross-sectional and cohort studies, both from Sub-Saharan Africa and other regions and performed the first systematic review and meta-analysis on this topic. We demonstrated that WLWH present with more advanced stage at diagnosis and have a worse overall survival, even after adjustment for other prognostic factors – both in North America and in Sub-Saharan Africa. These findings should raise the awareness of clinicians and policymakers regarding the detection and survival gap among WLWH with breast cancer, and will certainly fuel additional research efforts in order to better understand the reasons behind these disparities.

Finally, stage at diagnosis has been included in most of the analyses performed in this thesis, as it is known to determine both treatment and survival.[2] Mirroring previous findings from Western countries,[136] we

confirmed that it influences health resource use and the cost of care of early disease in Portugal (**Paper III**), but we demonstrated that its influence is much smaller in Mozambique (**Paper IV**). Nonetheless, increasing stage at diagnosis was associated with worse prognosis in patients from Mozambique, as expected (**Papers II and VI**).

In conclusion, this thesis showed how breast cancer heterogeneity, at multiple levels, influences etiology, clinical management and prognosis. Therefore, stakeholders across the world should move away from the vision of breast cancer as a single disease and understand that women with breast cancer are a very heterogeneous group, with different needs in terms of diagnosis, treatment and follow-up. This finer understanding of its complexity, both at the molecular, and at the patient and country level, may inform the design of better policies in terms of resource allocation and healthcare organization, translating into a more effective delivery of breast cancer care and, ultimately, in improved patient outcomes.

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Supplementary Papers

Supplementary Paper I: *Cancer in Sub-Saharan Africa*

Cancer in Sub-Saharan Africa

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Cancer in Sub-Saharan Africa

Sub-Saharan (SS) Africa consists of all African countries that are fully or partially located south of the Sahara Desert. It includes 48 countries and, in 2016, its population consisted of almost 1 billion people, of which 47% live on less than $1.25 a day. However, the United Nations predicts that, in 2050, it will be between 1.5 and 2 billion people.

Burden of Cancer

In SS Africa, the overall incidence of cancer (all cancers, excluding nonmelanoma skin cancer) is in general much lower than in more developed regions; among the latter, the estimated incidence rates in 2012 were nearly ninefold higher in men and fivefold higher in women. This primarily reflects differences in countries' age distributions, with SS Africa presenting a lower proportion of the population in the older age-groups, among whom most cancers tend to be more frequent. However, even the age-standardized rates, using the world standard population (ASIR-W), were more than twice higher in more developed regions. These differences are depicted in Fig. 1. The overall cancer incidence is also heterogeneous among SS African countries; for example, in South Africa, the incident rates (per 100,000) were 148.7 in men and 156.5 in women, yet they were below 60 in several other countries, such as Angola, Djibouti, or Tanzania.

Limited access to diagnosis contributes to lower incidence rates in many SS African settings due to underdiagnosis. This is illustrated by a report of a steep increase in the incidence of stomach cancer in rural Kenya soon after an endoscope became available, or by the fact that the 2006 report of the population-based registry of Beira, in Mozambique, referred no cases of prostate cancer. Further, overdiagnosis of breast and prostate cancers is substantially lower than in developed countries, since mammography screening and prostate specific antigen (PSA) testing are available to a much lesser extent in SS Africa, and are also unevenly distributed throughout the region. Risk factors such as tobacco smoking, obesity or low parity have remained infrequent in many SS

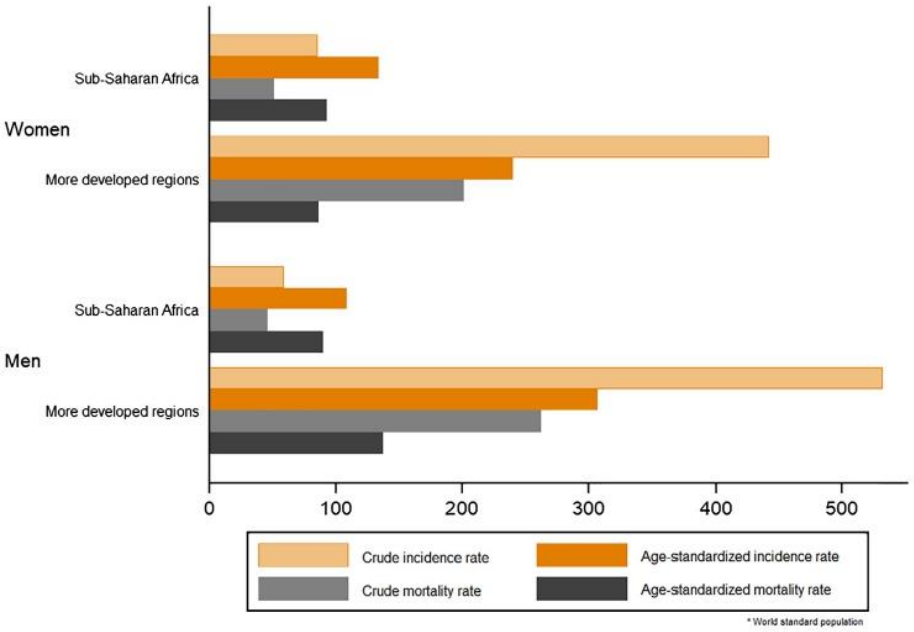


Fig. 1 Incidence and mortality rates in Sub-Saharan Africa and more developed regions. From Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D. M., Forman, D., Bray, F. (2013). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. Available from: http://globocan.iarc.fr, accessed on 30/01/2018.

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African regions, contributing to the low incidence of some of the cancers that account for a large proportion of cases in developed countries. In SS Africa and in more developed regions, the ASIR-W (per 100,000) for lung cancer among men were 4.8 and 44.7, respectively, for breast cancer among women were 33.8 and 73.4, respectively, and for colorectal cancer in both sexes were 5.8 and 29.2, respectively. Conversely, infection-related cancers are frequent in SS Africa; for example, for Kaposi sarcoma among men, the ASIR-W (per 100,000) were 7.2 in SS Africa and 0.3 in more developed regions, the corresponding figures for cervical cancer were 34.8 and 9.9, and for liver cancer, both sexes, were 7.6 and 5.4.

Taken together, these factors contribute to explain the lower cancer rates in SS Africa, but also the distinct patterns of occurrence of different cancers compared with other regions. In SS Africa, it was estimated that, in 2012, the most frequent were prostate and liver cancers and Kaposi sarcoma among men, and breast, cervical and liver cancers among women. In more developed regions, the leading cancers were prostate, lung and colorectal cancers among men, and breast, colorectal and lung cancers among women. In SS African countries, in men, prostate and liver cancers and Kaposi sarcoma were the most frequent in 23, 12, and 6 countries, respectively. Among women, cervical (28 countries) and breast (20 countries) cancers were the most frequent (Fig. 2).

In SS Africa, low priority is usually given to cancer prevention and control, because other conditions may constitute more immediate threats to public health. However, the absolute number of cases of cancer diagnosed and requiring treatment is currently high in SS Africa—over 600,000 cases were estimated in 2012—and in many settings, the health systems are unable to promote early diagnosis or to provide appropriate care to these patients, as will be discussed in more detail along this article. This leads to cancer patients' low survival, as shown in some of the few population-based estimates available from SS African regions (Table 1). Compared to the incidence gap, this results in much smaller differences in mortality estimates between SS African countries and more developed regions. Among men, the age-standardized mortality rates, world standard population (ASMR-W), were higher in more developed regions by only 50%, whereas among women the ASMR-W were actually nearly 10% higher in SS Africa. For cervical cancer, the estimated ASMR-W was nearly sevenfold higher in SS Africa, whereas for the ASIR-W the ratio was 3.5.

Disability adjusted life years (DALYs) are computed by combining the number of years of life lost (YLLs) due to premature mortality in the population and the years lost due to disability (YLDs) in people living with cancer, and may be interpreted as the years of "healthy" life lost. Therefore, the low survival of cancer patients results in a small contribution of YLDs to this more comprehensive indicator of the burden of cancer. For 2016, the Institute of Health Metrics estimated an overall number of nearly 16.5 million DALYs in SS Africa, from which just over 200,000 were from YLDs (1.2%). However, for high-income countries, the YLDs accounted for 4.4% of the DALYs; this gap is wider when considering cancers with higher potential survival, such as female breast (2.0% vs. 8.8%) or prostate (2.8% vs. 14.8%) cancers.

Mortality trends in more developed countries have been characterized by declining age-standardized rates, reflecting improvements in living conditions, specific preventive measures to decrease the exposure to risk factors, vaccination, earlier detection of cancer and improved access to effective treatments. This also shows the potential and points to strategies for controlling cancer in SS Africa. Yet, this will be challenging due to regional specificities of the epidemiology of cancer and its risk factors, the shortage of financial and human resources to tackle cancer, competing priorities in already overburdened health systems, and the need to tailor prevention and control measures to the SS African setting. Despite the progress that has been observed in more developed regions, the growth and aging of the population have contributed to an increasing absolute number of cancer deaths in most countries, showing that advances in prevention and control remain insufficient to overcome the impact of demographic changes in cancer mortality. This will bring additional difficulties to the prevention and control of cancer in SS Africa, since its population is expected to increase substantially in absolute numbers and average age over the next decades. Taking into account only the expected demographic changes, the GLOBOCAN projections for 2035 show 100% increases in the number of incident cancers and cancer deaths, in relation to 2012.

Monitoring the Burden of Cancer

The currently high frequency of cancer and its projected variation in SS Africa emphasize the importance of monitoring its burden and management in the region. Cancer registries are essential for this purpose, but in SS Africa these dedicated structures are often unavailable, or do not produce quality data. In the context of *Cancer Incidence in Five Continents*, volume X, it was estimated that 10.5% of the population was covered by population-based cancer registries with a completeness of case finding equal to or higher than 70%, and only four registries met the standards to be cited in this report, corresponding to less than 2% of the total population. This also results in scarce population-based survival estimates, as depicted in Table 1.

The GLOBOCAN project provides incidence and mortality estimates for most SS African countries, based on the best sources available for each country and sometimes on data from neighboring countries or registries in the same area. However, 19 countries had no incidence data, and only three had high quality regional data, with a coverage lower than 10%. Mortality data was only available in three countries, with complete vital registration classified as low or medium quality.

Although it is not surprising that low priority may be given to demanding cancer registration activities, when specialized human resources are lacking and financial capacity is limited, accurate population-based data are difficult to obtain when there is limited access to cancer health care services by the population and when cancer mortality statistics are not reliable. In these settings, hospital-based registries are important sources of data for population-based registries, and may support the evaluation of cancer management in each hospital, towards an efficient use of the available resources.

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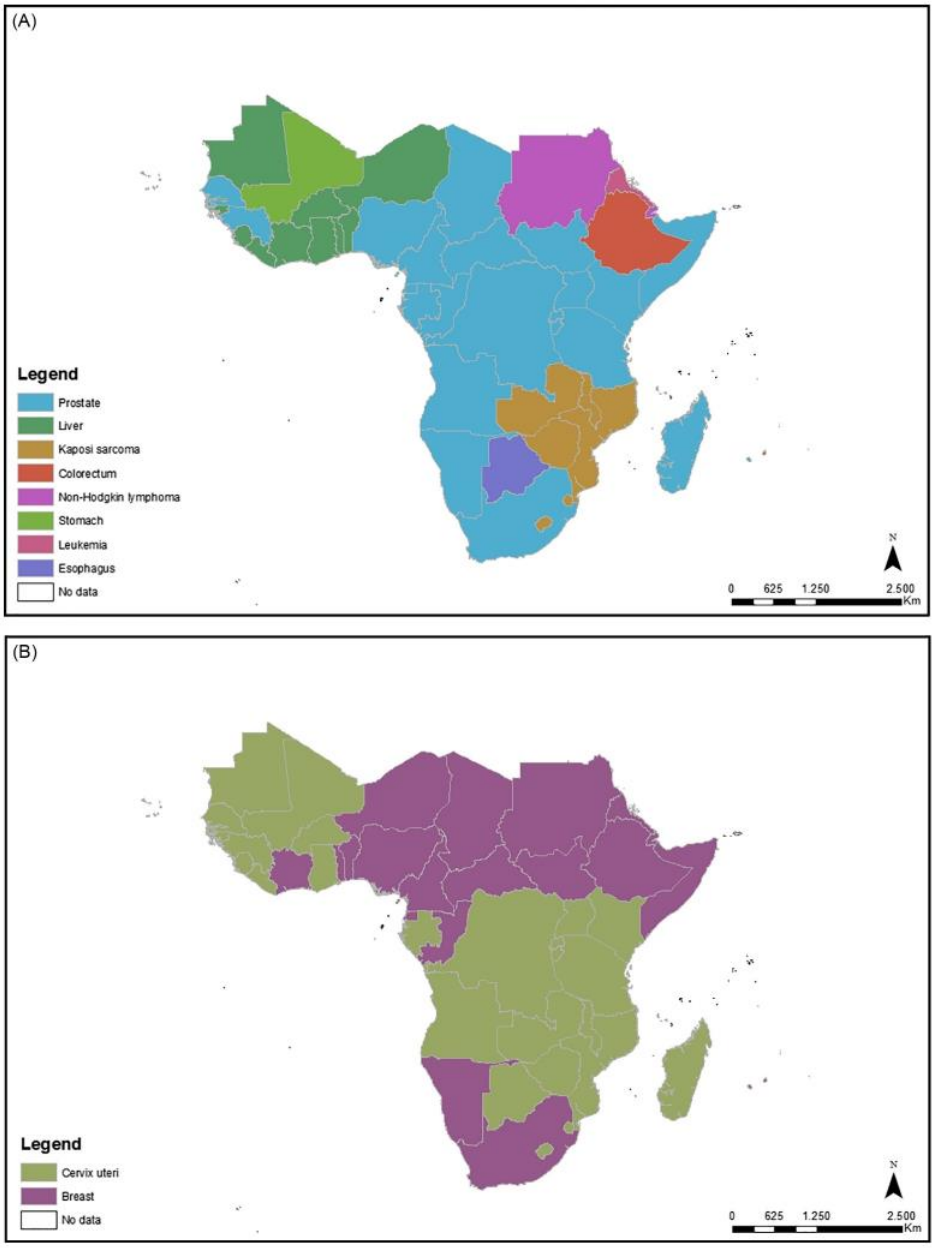


Fig. 2 Cancers with the highest absolute number of cases in Sub-Saharan African countries, among men (A) and women (B). From Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D. M., Forman, D., Bray, F. (2013). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. Available from: http://globocan.iarc.fr, accessed on 30/01/2018.

Cancer Prevention and Screening

The burden of some of the most frequent cancers in SS Africa can be reduced through decreasing the exposure to risk factors, vaccination, or early diagnosis and treatment. In fact, there is robust evidence on the causes of cancer and interventions that have proven effective for both preventing and reducing its morbidity and mortality through screening. Many SS African countries have already started to develop cancer prevention programs, often with foreign help. However, these efforts are challenged by competing problems, such as the human immunodeficiency virus (HIV) and malaria infections, and limited resources. This has a potential impact both on the perception of cancer as a major health problem and on the allocation of resources for its prevention and control. Additionally, adapting to local culture and traditions constitute a great challenge to the implementation of cancer prevention strategies.

Table 1 Age-standardized survival (95% confidence intervals), by country and type of cancer

	Country						
Cancer	*Mali (Bamako)*	*Mauritius*	*Nigeria (Ibadan)*	*South Africa (Eastern Cape)*	*The Gambia*	*Uganda (Kampala)*	*Zimbabwe (Harare)*
Breast	13.6 (0.0–30.1)[a] 0.0 (0.0–0.0)[b]	83.6 (75.9–91.3)[c]	98.8 (95.6–100.0)[c] 97.5 (89.9–100.0)[b]	71.5 (51.4–91.6)[d] 53.0 (23.4–82.7)[e] 32.0 (23.3–40.7)[c] 40.1 (30.7–49.6)[b]	9.5[f] 11.9 (0.0–24.7)[d]	36.1[f]	54.8[f]
Cervix uteri		80.8 (76.0–85.6)[c]	93.6 (83.7–100.0)[a] 58.6 (46.5–70.7)[c] 49.8 (36.5–63.1)[b]	37.0 (24.7–49.4)[d] 70.7 (56.7–84.7)[e] 40.2 (32.2–48.1)[c] 37.1 (31.4–42.9)[b]	22.9[f] 19.5 (11.0–28.0)[d]	18.7[f]	44.1[f]
Colon		65.9 (56.7–75.1)[c] 57.9 (48.5–67.2)[b]	41.2 (16.9–65.6)[c] 17.4 (0.1–34.8)[b]	31.9 (10.7–53.1)[c] 12.3 (4.3–20.2)[b]		6.2[f]	24.9[f]
Eye						35.7[f]	69.9[f]
Kaposi Sarcoma						21.9[f]	4.4[f]
Leukemia (adults)		57.2 (37.4–76.9)[c]	82.7 (59.8–100.0)[g]				
Liver		52.6 (28.9–76.3)[c] 17.0 (2.6–31.3)[b]		10.2 (0.0–22.9)[g] 27.1 (0.0–57.1)[e] 0.0 (0.0–0.0)[b]	2.6[f] 4.5 (0.2–8.8)[d]	1.1[f]	3.0[f]
Lung		31.7 (25.9–37.6)[c] 20.4 (13.2–27.7)[b]		16.7 (0.8–32.5)[c] 15.0 (6.1–24.0)[b]	49.8[f] 30.0 (3.6–56.4)[d]	0.0[f]	16.0[f]
Non-Hodgkin lymphoma					22.5[f]	26.1[f]	31.4[f]
Esophagus		28.1 (14.6–41.5)[b]		12.1 (0.0–27.0)[e] 19.2 (12.0–26.4)[c] 18.0 (12.6–23.4)[b]		4.6[f]	9.2[f]
Ovary		79.7 (69.6–89.8)[c]	59.4 (24.9–93.9)[c] 49.1 (33.8–64.4)[b]	90.9 (67.8–100.0)[g] 82.5 (42.5–100.0)[e] 81.0 (58.8–100.0)[c] 67.8 (47.4–88.2)[b]		10.2[f]	39.1[f]
Pancreas		24.5 (11.4–37.6)[b]		21.8 (0.0–49.5)[b]			
Prostate		61.8 (54.1–69.4)[c] 63.5 (54.7–72.4)[b]	97.4 (89.5–100.0)[a] 73.9 (50.2–97.6)[c] 58.7 (40.1–77.2)[b]	85.3 (60.0–100.0)[a] 77.6 (55.0–100.0)[g] 38.6 (25.1–52.0)[c] 37.8 (25.5–50.1)[b]		45.6[f]	
Rectum		83.6 (75.0–92.1)[c] 72.9 (62.7–83.0)[b]	25.9 (0.0–53.7)[c] 16.9 (0.0–37.8)[b]	19.9 (0.0–46.4)[c] 9.1 (0.0–21.5)[b]		7.2[f]	66.3[f]
Stomach		44.3 (36.8–51.7)[c] 25.7 (18.0–33.3)[b]		25.0 (6.1–44.0)[c] 25.6 (7.2–43.9)[b]	4.0[f] 0.3 (0.0–1.0)[d]	0.0[f]	18.6[f]

From Allemani, C., Weir, H. K., Carreira, H., Harewood, R., Spika, D., Wang, X. S., et al. (2015). Global surveillance of cancer survival 1995–2009: Analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet* **385**(9972), 977–1010. Allemani, C., Matsuda, T., Di Carlo, V., Harewood, R., Matz, M., Nikšić, M., et al. (2018). Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): Analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet* **391**(10125), 1023–1075. Sankaranarayanan, R., Swaminathan, R., Lucas, E. (2011). *Cancer survival in Africa, Asia, the Caribbean and Central America (SurvCan)*. IARC Scientific Publications, Vol. 162, ISBN 978-92-832-2162-3. Lyon: International Agency for Research on Cancer.

[a]Age-standardized (International Cancer Survival Standard weights) 5-year net survival for women diagnosed between 1995 and 2004.

[b]Age-standardized (International Cancer Survival Standard weights) 5-year net survival for women diagnosed between 2010 and 2014.

[c]Age-standardized (International Cancer Survival Standard weights) 5-year net survival for women diagnosed between 2005 and 2009.

[d]Age-standardized (International Cancer Survival Standard weights) 5-year net survival for women diagnosed between 1995 and 1999.

[e]Age-standardized (International Cancer Survival Standard weights) 5-year net survival for women diagnosed between 2000 and 2004.

[f]Age-standardized (Cancer standard population) 5-year relative survival for women diagnosed between 1993 and 1997.

[g]Age-standardized (International Cancer Survival Standard weights) 5-year net survival for women diagnosed between 1995 and 2009.

Reducing Exposure to Risk Factors

Tobacco consumption increases the risk of developing different types of cancer, such as lung, head and neck or esophageal cancers, among others. Many of the tobacco-related cancers are not among the most frequent in SS Africa, but there is a large potential for an increase in the prevalence of smoking and in the frequency of these cancers. Tobacco consumption is currently one of the most targeted risk factors throughout Africa. National level legislation is already in place in some countries, such as Gambia, Uganda, Nigeria or Kenya. They comprise prohibition of smoking in public or working places, banning of tobacco advertising, restriction of sales to minors, among others. The World Health Organization (WHO) also established a Centre for Tobacco Control in Africa in 2011 that is currently focusing on policies, legislation and regulations in several countries, namely Botswana, Ethiopia, Gambia, Gabon, and Niger. Moreover, private nongovernmental organizations (NGOs) developed antitobacco campaigns targeting different communities. However, tobacco control can prove difficult in Africa due to its role as an income provider for some communities, where tobacco leaf farming is still promoted as a way to reduce poverty, leading to increased consumption. Traditional tobacco products, other than manufactured cigarettes, are also frequently consumed in some settings, and may require more specific interventions for its control.

The amount of alcohol consumed in SS Africa is the second highest in the world (19.5 L of pure alcohol per year) and this is expected to increase. Alcohol is one of the leading causes of death in SS Africa, and is also related to the development of different cancers, namely esophageal, and head and neck cancers. Alcohol consumption depends on social influence and cultural changes that are closely linked to globalization, such as the growth of urban areas, industrialization, and marketing. Community norms and beliefs towards alcohol, neighborhood characteristics and peer effect are also determinants of drinking patterns, mostly in adolescents and young adults. The lack of specific governmental policies aiming to reduce alcohol consumption, the absence of community education and the increasing exposure to marketing of alcoholic beverages may contribute to increased consumption. However, countries such as Malawi, Equatorial Guinea or Zambia have already invested in political and regulatory changes with favorable outcomes in reducing consumption levels. Policy changes encompass, among others, raising taxes on alcohol, controlling advertising and enforcing drink-driving laws.

Exposure to mycotoxins through contaminated food is an established risk factor for liver (aflatoxins) or esophageal cancer (fumonisins), which are among the most frequent in SS Africa. Environmental conditions such as high levels of humidity and temperature favor the development of fungus responsible for the production of these toxins. Moreover, the disruption of the food preservation chain and constrained economic conditions increase the consumption of such products. Harvest practices, transportation and product processing are already regulated, in some countries, to help reduce contamination and exposure. However, a high portion of the SS African population is believed to still be exposed to contaminated food.

Cancers caused by infectious agents, such as cervical cancer (human papillomavirus, HPV), liver cancer (hepatitis B virus, HBV/hepatitis C virus, HCV), or Kaposi Sarcoma (herpesvirus 8, HHV8), are among the most common in Africa. HIV coinfection further increases the risk of developing these and other cancers by impairing immune response. Thus, reducing the risk of infection with these agents is an important step for cancer prevention. Strategies based on healthcare regulation and education of healthcare personnel are currently the focus of the WHO and various NGOs across multiple SS African countries. These include changing high-risk behaviors, by promoting measures such as the use of barriers to prevent exposure to body fluids, hands hygiene, prevention of injuries, screening of blood products and sterilization of medical equipment. Furthermore, community education plays a major role in preventing infections, mostly HIV and HPV, by reducing high-risk sexual behaviors. Some community educational programs encourage abstinence, monogamy and condom use. Circumcision may reduce high-risk HPV infection and transmission, and the WHO has proposed a target rate of 80% circumcision in men in low-income countries as the fallout of different randomized controlled trials. Sociocultural and healthcare barriers are being addressed in order to achieve high circumcision rates and several programs are currently in place to achieve this goal. In fact, the Joint United Nations Programme on HIV/AIDS (UNAIDS) defined 14 priority countries to scale-up circumcision. However, in some of those countries such as Lesotho, Malawi, Namibia, Rwanda, and Zimbabwe, the prevalence of circumcised men remains below 35%. Finally, in HIV infected patients, the use of highly active antiretroviral therapy has been shown to reduce the incidence of cancer. While this is true for some types of cancer such as Kaposi sarcoma, there is still insufficient evidence to establish a clear association between HIV treatment and the reduction of other cancers, like cervical tumors.

In SS Africa, *Schistosoma haematobium* is an important risk factor for bladder cancer. It ranks as the second most common parasitic infection in the region and 11.7 million people were treated for schistosomiasis in 2008. It is considered a neglected tropical disease and is estimated to cause over half a million deaths per year in SS Africa. *Schistosoma* can be transmitted by bathing in larvae infested water and the deposition of parasitic ova in the bladder induces severe inflammation leading to carcinogenesis and the development of squamous cell carcinoma. There are effective drugs for eliminating *Schistosoma* and preventing cancer, such as praziquantel. Therefore, there are several NGOs currently improving treatment coverage in order to reduce the burden of this infection in SS Africa.

Globalization is responsible for several changes in the exposure to risk factors for cancer. Obesity is also now being considered an important risk factor among countries in SS Africa, mostly in urban areas, where the intake of highly caloric food and physical inactivity is increasing. Paradoxically, increasing levels of obesity are side-by-side with severe undernutrition and stunting. Overweight and obesity incidence in adults reaches 40% in some countries, such as Burundi, alongside a 50% prevalence of stunting. Thus, healthcare interventions may also be needed in SS Africa to prevent and control weight gain and obesity, at least in some subsets of the population.

Other risk factors that are common in high-income countries are becoming more frequent in low-income countries. Younger menarche, later age at pregnancy and low parity are known risk factors for breast cancer that are clearly increasing in low-income settings.

Vaccination

There are currently two vaccines known to reduce cancer incidence: HPV vaccine and HBV vaccine. The Global Alliance for Vaccines and Immunization (GAVI), a global vaccine alliance, working closely with the United Nations Children's Fund (UNICEF), is responsible for improving access to vaccines in low-income countries.

Vaccination against HPV proved to be highly effective in preventing the development of high-grade premalignant cervical lesions that lead to cervical cancer. Currently, several HPV vaccines are available, protecting against different high-risk HPV subtypes. They also protect against some low-risk HPV subtypes. In SS Africa, HPV vaccines are usually administered to girls aged 9–14 years, using different delivery strategies, such as school based vaccination, routine immunization in conjunction with national vaccination plans, or in coordination with other healthcare programs. GAVI started several demonstration projects in different countries for HPV vaccination before its definitive inclusion in the national vaccination plan, first in Kenya, in 2013, and then in countries such as Rwanda, Cameroon, Gambia, Kenya, Malawi, Mozambique, São Tomé & Principe, among others. The price of HPV vaccine is decreasing, which enables a potential increase in vaccination, along with the possibility of including preadolescent boys in vaccination programs. Male vaccination proved to be cost-effective and is capable of further interrupting HPV transmission. Moreover, it may offer protection against other HPV-related cancers, such as head and neck or anal cancers.

A vaccine against HBV is available since the 1980s, but the world's population coverage by this vaccine is still expanding. The WHO recommends a three-dose vaccination schedule, with the first dose being administered within 24 h of birth and the other two at least 4 weeks apart. At a global level, the three-dose HBV vaccination coverage is estimated to be 84%, and in SS African increased from 5% in the year 2000 to an estimated 76% in 2015. This ranges broadly throughout different countries in the region, starting at 16% in Equatorial Guinea and reaching 98% in Rwanda and other countries. Thus, further progress must be made in many SS African countries in order to improve vaccination coverage and control of HBV infection.

Screening

Cancer screening may contribute to reduce the incidence and mortality of cancer, by diagnosing and treating premalignant lesions, or to promote timely treatment of malignant lesions with reduced morbidity. The first is best depicted by cervical cancer screening and the second by breast cancer screening. The potential benefits of cancer screening in resource constrained countries has been thoroughly discussed but even if the screening tests are available, most SS African countries still lack trained healthcare personnel and technical capability to diagnose and treat suspected cancers. Cervical and breast cancers screening are the most frequently available in SS Africa, reflecting the fact that these have been studied in resource constrained settings, mostly after adapting strategies and methods to the available human and financial resources, and cultural characteristics.

Cervical cancer screening

Despite the increasing coverage of HPV vaccination in SS Africa, scarce resources and failure to complete full schedules make screening an essential tool for prevention and control of cervical cancer. Screening with the Papanicolaou (Pap) test contributed greatly to the reduction in cervical cancer mortality in developed countries. However, cytology testing is expensive, and requires highly trained professionals and complex logistics. Furthermore, screening with the Pap test requires a follow-up visit for treatment, which is frequently impractical in resource constrained countries. Therefore, "test & treat" strategies try to obviate these difficulties by diagnosing and treating in the same visit, and they are currently proposed by the WHO for low-income settings. Test can be accomplished with visual inspection with acetic acid (VIA), digital cervicography, HPV test or with a combination of methods (e.g., HPV test and visual inspection). Treatment can be provided using cryotherapy. This approach was shown to be cost-effective, safe and culturally adapted. Other approaches are being evaluated to improve screening uptake in different cultural settings. Self-sample collection by women has proved to be reliable and reproducible, and urinary detection of HPV is being perfected. Also, recently developed rapid and portable HPV detection tests may contribute for increasing screening coverage.

Breast cancer screening

Mammography-based screening was shown to reduce breast cancer mortality in developed regions, and organized screening programs are available in many countries, with varying coverage. However, the logistics leading to a successful detection, diagnosis and treatment of breast cancer constitute a highly complex multidisciplinary undertake. Breast screening programs are expensive and require infrastructures that are often unavailable in low-income countries. This has led to a simplified screening approach based first on awareness and education, then on self-examination of the breast and finally on clinical examination of the breast. Currently, there is no screening program based exclusively on self or clinical examination, but several programs are including these methods to increase awareness for breast cancer and screening uptake. Also, randomized controlled trials showed an increase in the detection of invasive breast cancer using clinical breast examination screening. Nevertheless, none of these strategies proved to be effective in reducing breast cancer mortality and randomized trials are underway.

Cancer Diagnosis

Cancer diagnosis requires clinical evaluation of patients through various procedures, such as imaging, clinical laboratorial tests, and microscopic examination of cells and tissue samples (cytology or histopathology). The availability of resources needed for cancer diagnosis varies across SS Africa, and diagnostic capacity is often suboptimal, mainly due to the lack of adequate pathology capacity and infrastructure, as well as low quality of these services. This situation leads to delays in diagnosis and treatment, inadequate treatment strategies and follow-up, with increased morbidity and mortality.

Pathology

All patients should have a pathologic diagnosis before cancer treatment, and both anatomic and clinical pathology are crucial for its control. Cancer screening and diagnosis, including the identification of cancer type, grading and staging, as well as guidance of surgical resections and evaluation of the effectiveness of treatment depend on pathology investigation. The accuracy of cancer registration data also relies to a large extent on the quality of the pathological diagnoses.

Histopathology and cytology, such as fine needle aspiration cytology (FNAC), are needed for microscopic diagnosis. Complementary methods, namely immunohistochemistry, flow cytometry and molecular pathology, are used for phenotyping tumors (including leukemia and lymphomas), and for the demonstration of genetic alterations. These results may be used for a finer characterization of tumors, leading to a better definition of treatment and prognosis.

The availability of pathology providers and services varies widely among SS African countries. The number of pathologists per inhabitant is less than 1:500,000 (Fig. 3), which is about one tenth of the observed in high-income-countries (about 1:15–20,000) and some countries have no pathologists at all. Therefore, the number of pathologists and technicians is clearly insufficient for the provision of adequate services. Moreover, only a few countries have more than 50% of cancers with a pathologic diagnosis and, when available, the turnaround time of diagnosis can reach 1 month.

In places where pathology services are available, the quality of diagnosis is further limited by factors such as lack of maintenance of equipment, inadequate supply chain of quality reagents, inadequate specimen fixation and transportation from the operating room to pathology services, and absence of quality control programs. Immunohistochemistry and molecular testing are available in just over half and in less than 20% of SS African countries, respectively. Consequently, and as an example, the study of hormone receptors in breast cancer, which is fundamental to its management, is limited or absent in many countries. The African Strategies for Advancing Pathology (ASAP) Group, created in 2014, generated a 5-year strategic plan aiming to increase and improve pathology services in SS Africa, including advocacy for pathology, education and training of pathologists, as well as building, strengthening and maintaining operational laboratories of pathology. Local strategies for improving pathology in a specific country or site must also be encouraged.

In the last decades, FNAC has gained importance as an alternative pathological method for cancer diagnosis in low-income countries. For example, in Mozambique, in 2009–10, 57% of all diagnoses of pediatric cancers in the Department of Pathology of the Maputo Central Hospital were based on FNAC. In other series, when considering Burkitt lymphoma alone, FNAC was

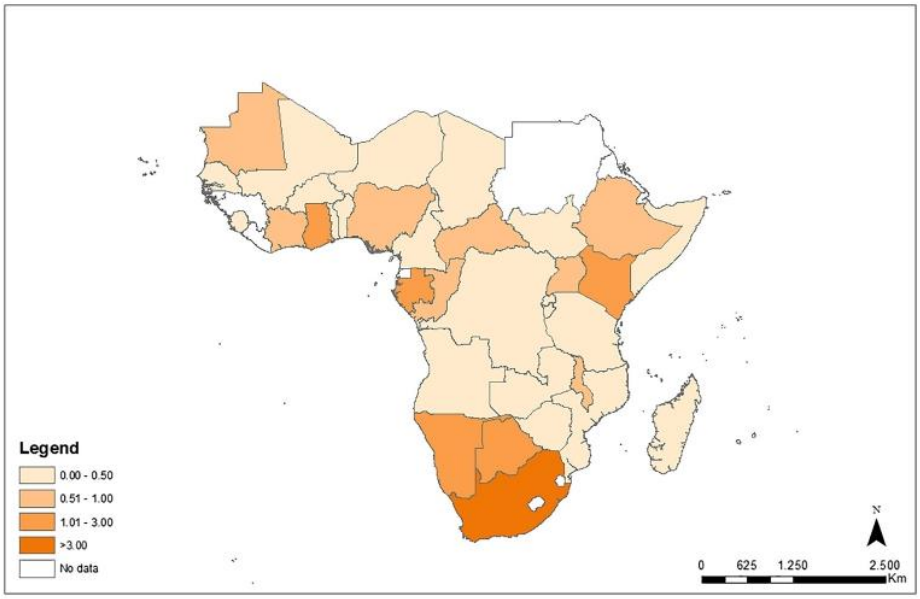


Fig. 3 Total density of pathologists per million population in 2011–13, in Sub-Saharan Africa. From African Strategies for Advancing Pathology [Internet]. Available from: http://www.pathologyinafrica.org/, accessed on 30/01/2018.

responsible for 82% of the diagnoses. Although core biopsy with histology is the preferred method for the preoperative diagnosis of breast cancer, FNAC is widely used for the initial diagnosis of palpable breast cancer in many parts of SS Africa, when core biopsy and histology are not available. Most breast cancer patients present with advanced disease and mastectomy is done many times on the basis of FNAC results, when triple assessment (clinical, radiological, and cytological findings) is conclusive for malignancy. FNAC can be conducted faster and at a lower cost than conventional histology, and with only minimal laboratory pathology infrastructures. However, diagnostic accuracy varies widely with the technique used, the specific site being sampled, and the expertise of the personnel collecting the sample and reading the slide. Samples may have poor quality when the technique is done by a person that only performs FNAC occasionally, but the proportion of inadequate samples drops from 15%30% to 12% when FNAC is performed by a trained cytopathologist. Therefore, when used appropriately and with well-trained personnel, it constitutes a quick and accurate tool for diagnosis and follow-up of cancer.

Clinical Diagnosis

Although the gold-standard for cancer diagnosis is based on tissue sample evaluation, due to the limited access to pathologic diagnosis, in some cases of specific cancers with high prevalence, treatment may start with clinical diagnosis only. Examples include bulky cutaneous Kaposi sarcoma associated with HIV, and some hepatocellular carcinomas that are diagnosed based only on ultrasonography in combination with clinical presentation and laboratorial tests. Additionally, and as already mentioned, cervical premalignant or malignant lesions can be visualized directly using acetic acid and treated with cryotherapy during the same visit. Clinical diagnosis of certain highly prevalent cancers do not require specialized personnel, are less dependent on laboratory facilities and are easier to perform.

Imaging

In the last few decades, imaging became an important tool for diagnosis, treatment planning and follow-up of patients with cancer. Currently, there are many methods available, such as conventional X-rays, ultrasound, mammography, endoscopies, computerized tomography (CT) scans, magnetic resonance imaging (MRI) and nuclear medicine, used for different purposes, namely diagnosis, staging, evaluation of response to treatment and detection of relapses.

After conventional X-rays, that are still important to detect bone tumors in many SS African countries, ultrasonography is the second most used imaging method. It is useful for the initial investigation of signs and symptoms, especially to detect and localize neoplastic lesions in the abdomen and thyroid. It is also useful to guide core needle biopsies or FNAC, and for staging and follow-up. However, its use is limited due to the lack of trained personnel.

Most of the other imaging methods, such as CT scans, MRI and nuclear medicine are scarcely available in many SS African countries. But even in countries which have this equipment, radiologists are often not adequately trained on the diagnosis and follow-up of cancer lesions, namely in the assessment of response to cancer treatment. Additionally, access to endoscopy services, which are extremely important for early detection of esophageal cancer, is also limited. Finally, the low number of mammography units in many SS African countries (Fig. 4) is a barrier for the implementation of breast cancer screening through this method. Alternatively, as mentioned, in some countries, screening is being based on clinical examination of the breast.

Treatment of Cancer

Conceptually, the management of cancer includes the treatment of local disease with surgery, radiation therapy, or both, and the treatment of systemic disease with cytotoxic chemotherapy, endocrine therapy, targeted therapy, immunotherapy or combinations of these. The choice of treatment strategy is based on the tumor extent/location and biology (pathology including biomarkers and gene expression) as well as on the age and general health status of the patient, and personal preferences. However, in SS Africa, the availability of surgery, radiation and systemic therapy varies widely between and within countries. Moreover, many patients are not managed in a multidisciplinary manner, being referred from surgeon to medical oncologist or radiation oncologist, without a proper discussion in a multidisciplinary tumor board. This impairs the achievement of the best possible treatment for the individual patient, adds delays in the patient care pathway and increases the chance of discontinuing care, all of which compromise survival outcomes. Moreover, access to different forms of treatment depends on patients' socioeconomic status.

Surgery

In the most developed countries, surgical treatment of solid tumors has evolved substantially in the last decades, which may be related to the combination with neoadjuvant and adjuvant chemotherapy, targeted therapy and radiotherapy, as well as the development of techniques such as the sentinel-node biopsy and laparoscopy. Therefore, surgery is now much less aggressive in most tumors (e.g., breast-conservation surgery, partial nephrectomy), allowing for a preservation of organ function, with decreased morbidity and mortality, and a benefit in the quality of life of cancer survivors. At the same time, the training of most surgeons on basic cancer principles (e.g., systematic lymphadenectomy in certain cancers and achievement of clear margins in most tumors), has led to an improvement in survival.

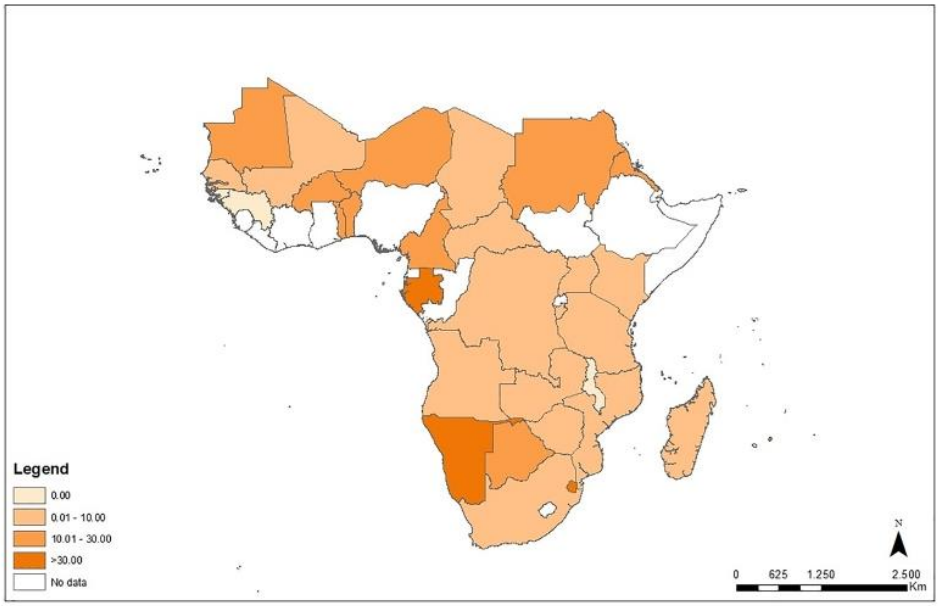


Fig. 4 Total density of mammography units per million females aged from 50 to 69 years old in 2014, in Sub-Saharan Africa. From Global Health Observatory (GHO) data [Internet]. Geneva: World Health Organization. Available from: http://www.who.int/gho/en/, accessed on 30/01/2018.

However, in SS Africa, surgery is still highly centered in cases of trauma and acute conditions. Therefore, surgical training is usually more focused on these situations and not so much on the performance of adequate oncological resections with clear margins, the achievement of a broad lymphadenectomy or the need to send the surgical specimen for proper fixation for histological assessment. Additionally, in some SS African countries, due to the absence of a proper cancer diagnosis prior to surgery and/or lack of awareness of some types of tumors, cancer surgeries may sometimes be performed by nonphysicians, who do not have the expertise to carry out these types of complex surgeries. On top of that, many of the organ-sparing approaches are also not possible due to the lack of trained cancer surgeons in organ-sparing approaches and the absence of radiotherapy. Another problem is that patients frequently present with extensive local tumors that are not suitable for surgery due to the absence of effective neoadjuvant therapies, such as radiotherapy and/or systemic treatment (e.g., trastuzumab in HER2-positive breast cancer).

As an example, currently, in Western Europe ~60%–80% of newly diagnosed breast cancers are amenable to breast conservation (wide local excision and radiation therapy). However, in low-income countries, mastectomy is still the most common intervention for breast cancer—in SS Africa, 85% of the surgeries are mastectomies. Even so, in a retrospective study from Nigeria, only 35% patients underwent mastectomy, as many of the patients presented with inoperable local or metastatic tumors at diagnosis. Also, in Rwanda, from 145 patients receiving breast cancer care, only 48% underwent mastectomy. This is in contrast with Europe, in which over 90% of women receive surgical treatment.

Radiation Therapy

Radiation therapy is an important component of cancer control programs. Given that cervical, breast, prostate, esophageal, and head and neck cancers are among the most common types of cancer in SS Africa, radiotherapy plays a very important control as a radical as well as an adjuvant therapy.

However, according to the WHO, in 2013, only 20 countries were known to have radiotherapy, and these facilities were concentrated in the southern and northern states of the region (Fig. 5). According to a survey conducted by The International Atomic Energy Agency in 2010, brachytherapy resources (high-dose rate or low-dose rate) were only available in 20 countries as well. Additionally, access can be difficult even when radiation therapy facilities are available due to the large number of people covered by each of the centers. This leads to long patient waiting lists, which results in large numbers of patients referred for radiation therapy not being treated in due time. Also, due to the out of pocket expense of traveling daily to the radiotherapy facility, some patients have a low treatment compliance, which undermines its effectiveness, especially in very radiosensitive cancers, such as head and neck tumors.

Systemic Treatment

Systemic therapy can be neoadjuvant, adjuvant or palliative, contributing to substantial improvements in survival and symptom burden, depending on its intended use. A good example is breast cancer, in which more than half of the women with localized cancer who would otherwise die of metastatic breast cancer remain disease-free when treated with the appropriate systemic regimen.

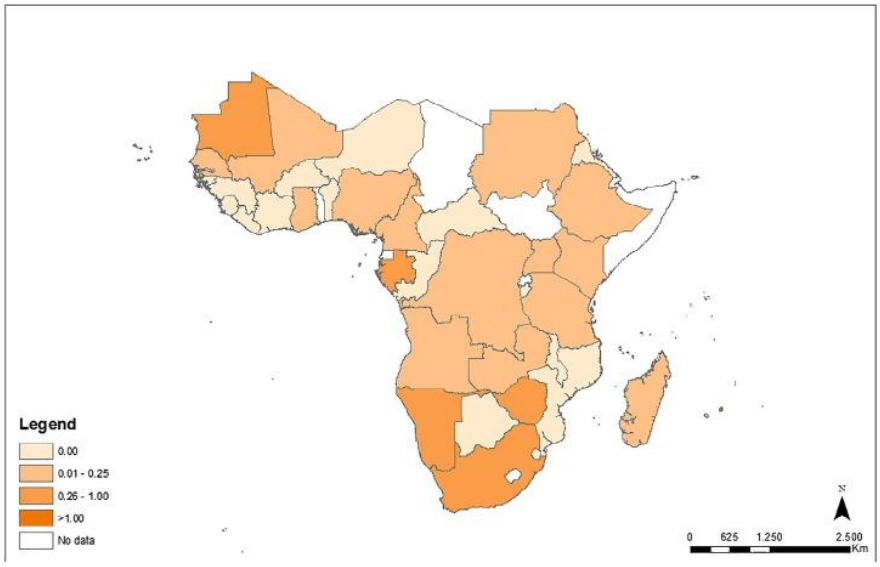


Fig. 5 Total density of radiotherapy units per million population in 2013, in Sub-Saharan Africa. From Global Health Observatory (GHO) data [Internet]. Geneva: World Health Organization. Available from: http://www.who.int/gho/en/, accessed on 30/01/2018.

However, there are multiple problems regarding the delivery of systemic treatment in SS Africa. For example, the safe administration of systemic chemotherapy, which is crucial to improve outcomes for a number of common cancers (e.g., colon, breast, cervical, and head and neck), depends on the availability of drugs, infrastructure for drug administration, and well-trained medical and nonmedical staff. Unfortunately, adequate infrastructures and trained staff are scarce in low-resource countries, and treatments are often interrupted due to the irregular supply of systemic drugs. The effectiveness of chemotherapy protocols can be further impaired by inadequate management of toxicity due to limited access to support treatments, including blood transfusions, granulocyte-colony stimulating factors, antibiotics and antiemetics.

There are examples of programs trying to overcome the absence of specialists and specialty centers in SS Africa. As an example, since 2014, the Gulbenkian Foundation has been providing support for Mozambican medical and nonmedical staff to be trained in Portugal in different cancer areas (surgery, radiation therapy, medical oncology, oncological nursing and pathology, among others) during short-term fellowships, allowing for an effective education in multidisciplinary cancer care. This has led to the implementation of new surgical cancer techniques in Mozambique and to the creation of the first multidisciplinary tumor board in the country. Also, an international partnership of Partners in Health and the Dana-Farber Cancer Institute, Harvard Medical School, and Brigham and Women's Hospital, is working in rural Malawi and Rwanda. They are operating health centers and hospitals in rural districts, supporting and training local physicians and nurse teams on the delivery of chemotherapy to patients with multiple cancers, including breast, cervical, rectal, head and neck, Hodgkin's and non-Hodgkin lymphoma, and Kaposi sarcoma.

However, the difficulty lies in scaling up these projects to a national level. One of the reasons is that the cost of present chemotherapeutic agents for all patients with cancer exceeds the healthcare budgets of low-resource countries. Therefore, many SS African countries cannot ensure that the drugs in the WHO essential drug list for cancer and newer drugs are available. This also leads to out-of-pocket payments that are unaffordable to most patients, leading to treatment nondelivery or interruption. Besides, patients that progress during first-line chemotherapy, which is usually limited to the available drugs and may not include the best possible treatment, have even more limited options for further treatment.

Regarding endocrine therapy (e.g., in breast cancer), the absence of advanced pathology services to establish hormone-receptor status continues to obstruct appropriate decision making for this treatment. However, the potential savings with the selective use of endocrine therapy are expectedly greater than the costs of hormone-receptor testing, so it would be important to develop it instead of carrying on with the blind prescription of endocrine therapy. In terms of breast cancer, tamoxifen is accessible in most countries and, in some of them, it is free of charge. Yet, newer agents, like aromatase inhibitors are mostly not available in public hospitals.

With respect to targeted therapy, in several SS African countries, agents such as trastuzumab or rituximab are not available due to their high price, even if they are part of the WHO essential drug list. However, biosimilars are already available in countries like India at a reduced cost, so they could be introduced in Africa in the near future, helping in the reduction of cancer mortality.

The development of guidelines for drug selection and administration would be very important to ensure that all patients with cancer receive the best available drugs in appropriate and therapeutic doses. For example, guidelines regarding cervical cancer treatment in limited-resource settings were issued in 2016 by the American Society of Clinical Oncology, to help with the optimization of clinical outcomes. However, it would also be important to have the technology for a better characterization of each tumor, as it could lead to a more personalized and effective treatment—for example, use of platinum drugs, that are cheap and usually available, and avoidance of endocrine therapy in triple negative breast cancer.

Clinical Trials

Clinical trials are an essential part of cancer research efforts, enable patients to have access to more treatment options and expand the possibilities of use of each drug. However, in SS Africa, the number of cancer clinical trials is still very low: in a recent review of randomized controlled trials in breast cancer registered on ClinicalTrials.gov ($n = 1099$ trials), only 6.3% occurred in one or more low- and middle-income country. Besides, among the 44 SS African countries, studies were only being conducted in Nigeria, Kenya, Ghana and South Africa; the latter had 91% of the 54 studies taking place in the region.

This is worrisome because it is paramount to undertake cancer clinical trials in SS Africa, in order to: (a) develop new drugs and treatments for cancers that are less frequent in the West, but predominant in SS Africa (e.g., esophageal cancer); (b) study diseases that are frequent in the West, but have a different presentation and course in SS Africa (e.g., higher proportion of triple negative breast cancer compared to Europe); and (c) develop more affordable ways of delivering cancer care in SS Africa (e.g., hypofractionated schemes of radiotherapy in prostate cancer).

Patients with HIV

The prevalence of HIV in SS Africa is highly variable among countries, but can be as high as 37% of the population (in urban women in Swaziland), according to the WHO. Furthermore, more than 70% of the global burden of HIV infection is in SS Africa. This is important because, since the discovery of HIV, it is known that infected patients have a higher risk of developing cancer, mainly due to the immunosuppression induced by the virus. When some of these cancers are diagnosed in an HIV-positive patient, it means that the patient has developed the stage of HIV infection called "acquired immune deficiency syndrome" (AIDS). Therefore, these cancers are called "AIDS-defining malignancies" and include Kaposi sarcoma, primary central nervous system lymphoma, peripheral intermediate- and high-grade B-cell non-Hodgkin lymphoma, and cervical cancer. HIV-positive patients also have an increased risk of developing other cancers, such as Hodgkin lymphoma or lung cancer, but these are considered non-AIDS defining malignancies. Meanwhile, due to the scale-up of highly active antiretroviral therapy (HAART) in SS Africa, HIV infection is turning into a chronic condition, with aging and growth of HIV-infected populations. Reports from countries like Brazil and United States show a decline in AIDS-defining malignancies and an increase in non-AIDS defining malignancies.

Yet, despite the high burden of HIV in SS Africa and its relation with cancer, HIV infection status is often missing for cancer patients, which is troublesome since HIV infection also influences cancer treatment and prognosis. In 2017, among a series of 1137 cancer patients from Nigeria, the HIV status was not recorded for nearly one-third of the patients, and 32% of the remaining were HIV-positive.

Regarding survival, data in HIV-positive cancer patients comes mainly from American or European series, and they all show that these patients have worse cancer-specific survival compared to those not infected. In a very large American series, even after adjustment for stage and cancer treatment, HIV-positivity was associated with higher cancer-specific mortality for colorectal cancer (hazard ratio [HR] 1.40, 95% CI 1.09–1.80), lung cancer (HR 1.28, 95% CI 1.14–1.44), melanoma (HR 1.93, 95% CI 1.14–3.27) and breast cancers (HR 2.64, 95% CI 1.86–3.73). This could be attributed to the effect of immunosuppression (as we nowadays know that an intact immune system is essential for the effectiveness of anticancer drugs) and to suboptimal therapy due to increased toxicity and higher rates of infection. On the contrary, there are known pharmacological interactions between HAART and common chemotherapy drugs, such as alkylating agents, taxanes, and anthracyclines. There are also interactions with targeted agents, like imatinib or erlotinib. Therefore, HIV-positive patients are at risk of overlapping toxicity, namely hematological, which can compromise treatment. As an example, in a case-control study in the United States (with 52 HIV-positive and 104 HIV-negative breast cancer patients), the HIV-positive women needed dose reductions and/or had chemotherapy delays due to toxicity more often (56% vs. 30%; $P = .03$). Therefore, in order to avoid immunosuppression-related infections, CD4+ cell count should be closely monitored and the use of prophylactic G-CSF/GM-CSF is strongly recommended following chemotherapy. However, these drugs may not be available in all SS African clinical centers.

Additionally, even in clinical trials regarding cancers with higher risk in HIV-positive patients (like lung or cervical cancer), these patients are usually excluded, so data regarding the efficacy and safety of new cancer therapies in HIV-positive patients is scarce. Therefore, the creation of specific guidelines to cancer treatment and management of complications in HIV-infected patients in low-resource settings, such as SS Africa, is essential in order to maintain treatment intensity and achieve the best possible survival outcomes.

Palliative Care

In SS Africa, supportive care, which broadly includes the treatment of side effects, palliative care and end-of-life care, is still an emerging specialty. Even if palliative care is now delivered in nearly 50% of SS African countries, still less than 5% of people in need receive it. This is extremely worrisome, especially because about 80% of cancer patients in SS Africa are diagnosed at advanced stages of the disease, and in many of these patients, palliative care is often the only choice of treatment.

One of the core components of palliative care is pain control. However, there are a number of barriers to the use of inexpensive opioid analgesics in SS Africa, including legal and regulatory restrictions, inadequate training of health care providers, concerns about abuse and cultural misperceptions about pain. For instance, a survey directed to medical doctors, conducted in a University Hospital in Nigeria, showed that only 40% of the respondents routinely conducted pain assessments among cancer patients, only 20% used strong opioids and over 90% of the respondents had no formal training on pain management.

According to an American Cancer Society/African Organization for Research and Training in Cancer (AORTIC) report, in 2008, the actual procurement of morphine and equivalent opioids reported by SS African governments to the International Narcotics Control Board corresponded only to about 10% of the quantity needed just for the terminal months of cancer and HIV-positive patients. This means that the vast majority of cancer patients in SS Africa do not have access to opioid treatment.

However, there are some inspiring examples, such as Kenya and Uganda that have been pointed as leaders in palliative care development in SS Africa. Through a strategy based on education and training of health care providers, improved access to opioid medications, better professional and public attitudes towards palliative care, integration of palliative care into national health care systems, and palliative care research, these countries have incorporated palliative care as part of their mainstream health service. An interesting strategy was the involvement of lay community volunteer workers, who advocate for palliative care in the community and help to clarify the myths related with the process of dying. Also, education programs were offered not only to health professionals, but also to community volunteer workers, spiritual caregivers and traditional healers.

While governments should take steps towards an easier and more comprehensive access to palliative care for their cancer patients, NGOs can lobby to put palliative care on the public agenda, and also to help on the design and implementation of palliative care programs on a local and national level.

Prospective Vision

As explained above, cancer control in SS Africa is not an easy task, due to the increasing incidence of cancer, and lack of proper healthcare networks for diagnosis and treatment. However, as this burden is growing at a fast pace, new solutions will have to be provided. First, vaccination programs, covering HBV and HPV are fortunately expanding and that could substantially decrease the burden of liver and cervical cancer, respectively, as well as head and neck cancer. Second, even if legislation control over known risk factors like tobacco and alcohol is still in its beginning, it is predictable that it will tighten in the near future, which would also be valuable for cancer control. However, "western" risk factors such as obesity are becoming more frequent and, even with educational strategies, will be hard to overcome. Third, regarding screening, diagnosis and treatment, a change of mind-set will have to occur: as it is not feasible to implement strategies used in developed countries, due to their complexity and cost, new ways of delivering cancer care will have to be pursued. For that, clinical trials in SS Africa regarding new strategies for screening (like clinical breast examination) and diagnosis (such as portable HPV detection tests) should be carried out, in order to assess their effectiveness, cost and application in SS African health systems, as well to adapt them to local habits. At the same time, trials regarding new treatment strategies with old or new drugs and combinations are also urgently needed, as a way to improve survival outcomes. Investing in strengthening pathology for early detection and diagnosis with quality is crucial. Also, a better organization of drugs' supply chain, and the use of generic drugs and biosimilars could decrease the costs and improve the delivery of systemic treatment. Finally, it is essential to train a whole new generation of physicians, nurses and technicians on cancer principles and care, in order to improve their expertise in the delivery of care and, at the same time, raise awareness for this growing problem. This would be an important step for the change of attitudes in SS Africa regarding the importance of cancer prevention and care.

See also: Cancer Disparities. Cancer in the Middle East. Cancer in Populations in Transition.

Further Reading

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Systemic treatment of patients with early breast cancer: recent updates and state of the art

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KEYWORDS

Early breast cancer
Adjuvant
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Post-neoadjuvant

ABSTRACT

This review is focused on trials generating results that potentially impacted clinical practice since the 2017 St. Gallen Consensus; the most impactful trial was KATHERINE, which revealed a 11.3% absolute iDFS improvement with T-DM1 (compared to trastuzumab) in HER2-positive breast cancer patients who presented invasive residual disease following neoadjuvant treatment. These results, if reinforced by a subsequent overall survival benefit, will consolidate neoadjuvant treatment as the standard-of-care for most HER2-positive breast cancer patients. The addition of pertuzumab to adjuvant trastuzumab (APHINITY) or extending anti-HER2 therapy with 1 year of neratinib (ExteNET) also improved outcomes, but in a more modest way. In triple-negative early breast cancer patients presenting invasive residual disease after neoadjuvant chemotherapy, the CREATE-X trial demonstrated the benefit of post-neoadjuvant capecitabine. In patients with luminal tumours, updated results of the SOFT/TEXT trials confirmed the benefit of aromatase inhibitors plus ovarian suppression in high-risk premenopausal patients, and the 12-year results of the BIG1–98 trial demonstrated a sustained trend in favour of letrozole compared to tamoxifen in the adjuvant treatment of postmenopausal patients. The TAILOR-X study showed that, overall, patients with an intermediate recurrence risk score (11–25) in the OncotypeDX 21-gene assay did not benefit from adjuvant chemotherapy. A meta-analysis performed by the EBCTCG demonstrated that extended adjuvant aromatase inhibitors modestly reduced breast cancer recurrence risks, with a more pronounced benefit in patients previously treated with tamoxifen. Finally, an EBCTCG meta-analysis including patients with all breast cancer subtypes showed that dose-dense chemotherapy improved survival when compared to conventionally-timed chemotherapy.

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Introduction

Surgery represents the standard-of-care definitive loco-regional treatment for early breast cancer patients [1]. Systemic treatments can be administered before (neoadjuvant) or after (adjuvant or post-neoadjuvant) surgery with the objective of preventing recurrences and treating micro-metastatic disease [2]. Since the last St. Gallen Consensus in 2017 [3], there were relevant updates concerning the systemic treatment of early breast cancer patients. The present review aims to depict the most relevant data published or presented during the last 2 years on this topic. The main focus of this manuscript is to discuss trials that provided results with the potential to modify clinical practice, although other recent studies of scientific interest will also be discussed.

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Adjuvant treatment

Since the pivotal study from Bonadonna et al., which demonstrated a survival improvement yielded by the administration of chemotherapy after surgery, the adjuvant setting has been the classic backbone for the development and investigation of systemic therapies for early breast cancer patients [4,5]. The most relevant studies evaluating adjuvant treatment that reported results or updates in the last 2 years will be discussed below, divided by breast cancer subtypes.

All subtypes

The EBCTCG meta-analysis including 37,298 early breast cancer patients comparing adjuvant standard chemotherapy (every 3 weeks in most cases) to dose-dense regimens has recently been published [6]. Dose-dense chemotherapy provided a significant reduction in the risk of recurrence (10-year recurrence risk rate ratio [RR] 0.86, 95% confidence interval [CI] 0.82–0.89) and mortality (10-year breast cancer mortality RR 0.87; 95% CI, 0.83–0.91). The benefit was present in all analysed subgroups. Of note, many of the studies included in the meta-analysis were conducted

before the "trastuzumab era", and most patients (85%) were node-positive. These results have consolidated the dose-dense schedule as the preferred option in the adjuvant treatment of early breast cancer patients, although in node-negative and HER2-positive patients the benefit of this strategy might be of small magnitude (Table 1).

Another EBCTCG meta-analysis comparing neoadjuvant vs. adjuvant chemotherapy ($n = 4,756$) demonstrated similar rates of distant recurrence, breast cancer mortality and all-cause mortality with both strategies. Notably, an increased risk of local recurrences was observed with neoadjuvant chemotherapy (RR 1.37; 95% CI, 1.17–1.61), however these results should be interpreted with caution, as substantial improvements have occurred in terms of staging, surgery, radiotherapy, and systemic treatment since these studies were conducted (Table 2) [2,7].

Since the pivotal study from Jones et al., which demonstrated the superiority of 4 cycles of docetaxel and cyclophosphamide (TC) in comparison to 4 cycles of doxorubicin and cyclophosphamide in the adjuvant treatment of early breast cancer patients, the possibility of de-escalating treatment by omitting anthracyclines has been debated [8]. The ABC trials ($n = 4,242$) evaluated the non-inferiority of 6 cycles of TC in comparison to an anthracycline and taxane-based regimen, with an accepted upper limit for the hazard ratio of 1.18 for non-inferiority [9]. The non-inferiority of TC in terms of invasive disease-free survival (iDFS) was not demonstrated (3.3 years median follow-up, hazard ratio [HR] 1.20; 95% CI, 0.97–1.49). Previous studies also failed to demonstrate the non-inferiority of 6 cycles of TC in comparison to anthracycline and taxane-based regimens [10,11]. In the PlanB trial (= 2,449), which tested the same hypothesis and defined an absolute 4.4% margin for non-inferiority, 6 cycles of TC were considered non-inferior to an anthracycline and taxane-based regimen: 5-year disease-free survival (DFS) of 89.9% vs. 89.6%, respectively (HR 1.004; 95% CI, 0.78–1.23) [12].

A recent meta-analysis including 12,741 patients and 7 trials demonstrated no difference between 6 cycles of TC in comparison to anthracycline and taxane-based chemotherapy in terms of DFS and overall survival (OS) [13]. Subgroup analysis suggested that patients with oestrogen receptor (ER)-negative tumours and N2-disease may benefit from anthracycline-based chemotherapy. Grade ≥3 toxicities were more frequent with anthracycline-based regimens (emesis, mucositis, thrombocytopenia and neuropathy). These results suggest that TC is a valid option as an anthracycline-free regimen in the adjuvant setting, especially in patients with ER-positive/N0–1 disease. Notably, the ideal number of TC cycles (4 or 6) is still debatable, and the subgroups of patients who benefit more from anthracyclines are yet poorly defined.

Other interesting studies also reported results in this period. The tAnGo trial confirmed no benefit from the addition of gemcitabine to anthracycline-taxane based adjuvant chemotherapy after a median follow-up of 10 years [14]. The D-CARE trial evaluated the addition of denosumab (120 mg monthly for 6 months, then every 3 months for up to 5 years) to standard (neo)adjuvant treatment in early breast cancer patients, but no benefit in terms of bone-metastasis free survival, DFS or OS was observed with denosumab [15]. The ABCSG-18 trial added denosumab (60 mg every 6 months during the endocrine treatment) to aromatase inhibitors in ER-positive postmenopausal patients, and has shown an improvement in DFS, which was a secondary endpoint of the study (HR 0.82; 95% CI, 0.69–0.98) [16]. Given the contradictory results of the studies evaluating adjuvant denosumab and the demonstrated benefit yielded by the addition of bisphosphonates in postmenopausal patients, adjuvant denosumab should not be recommended as a standard-of-care [17].

HER2-positive breast cancer

The treatment of HER2-positive patients has evolved significantly in the last 2 years. In terms of adjuvant treatment, the ExteNET trial showed that the extension of anti-HER2 treatment with neratinib after 1 year of trastuzumab improves iDFS (90.2% with neratinib vs. 87.7% with placebo; HR 0.73; 95% CI, 0.57–0.92), especially in ER-positive patients (5-year iDFS: 91.2% with neratinib *vs.* 86.8% with placebo; HR 0.60; 95% CI, 0.43–0.83) [18]. The APHINITY trial has demonstrated a modest improvement in 3-year iDFS with the addition of pertuzumab to the standard 1-year trastuzumab in the adjuvant setting (94.1% with pertuzumab vs. 93.2% with placebo, HR 0.81; 95% CI, 0.66–1.00; $p = 0.045$). A higher degree of benefit was observed in the node-positive subgroup (92.0% with pertuzumab vs. 90.2% with placebo, HR 0.77; 95% CI, 0.62–0.96) [19]. Cardiac toxicity was not increased with pertuzumab, although higher rates of diarrhoea were observed in comparison to placebo (9.8% vs. 3.7%). The management of patients who achieve a pathologic complete response (pCR) under neoadjuvant chemotherapy plus dual HER2 blockade is still an open question, as these patients were not included in the APHINITY trial. In the adjuvant treatment of HER2-positive patients, the extended treatment with neratinib or the dual HER2 blockade with trastuzumab/pertuzumab are both valid options particularly for node-positive disease, although the potential benefits and risks of both strategies need to be considered and discussed with patients.

Since 2017, four trials reported new or updated results on the reduction of trastuzumab adjuvant treatment duration [20,21]. The SOLD and Short-HER trials compared 9 weeks vs. 12 months of trastuzumab and, in both cases, non-inferiority was not demonstrated [20,21]. The final results from PHARE (12 vs. 6 months of trastuzumab), at a median follow-up of 7.5 years, also did not prove non-inferiority [22]. On the other hand, the PERSEPHONE trial tested the same hypothesis but the non-inferiority margin was more permissive: a HR of up to 1.29 for DFS was accepted as non-inferior [23]. The non-inferiority of 6-month trastuzumab was demonstrated in terms of DFS (HR 1.07; 90% CI 0.93–1.24). Nevertheless, in subgroup analysis, patients with ER-negative disease and those who received an anthracycline-free adjuvant chemotherapy regimen seemed to benefit more from 12-month trastuzumab. A recent meta-analysis combining 5 randomized trials with 11,383 patients showed that, overall, 12 months of trastuzumab led to better DFS (pooled HR 1.21; 95% CI, 1.09–1.36) and OS (pooled HR 1.23; 95% CI, 1.07–1.42) as compared to shorter treatment durations [24]. This effect was more pronounced in the subgroup of patients with node-positive disease (for DFS: HR 1.37; 95% CI, 1.17–1.60) or ER-negative tumours (for DFS: HR 1.33; 95% CI, 1.15–1.54). For these reasons, endorsement of the 6 month trastuzumab duration is not getting wide support [25].

Updated results from the APT trial (in which patients having small, node-negative tumours received 12 weeks of paclitaxel and 12 months of trastuzumab) showed a 7-year iDFS of 94.6% for ER-positive patients, suggesting that treatment de-escalation may be very safe in this subgroup. Conversely, the 90.7% 7-year iDFS rate for ER-negative patients with its lower limit of the 95% confidence interval at 84.6% suggests that these individuals may benefit from a more intensive chemotherapy regimen [26]. Finally, the NSABP B-47 trial showed no benefit of adding trastuzumab to standard adjuvant chemotherapy in HER2-low breast cancer, ending a long-standing question [27].

Triple-negative breast cancer (TNBC)

The standard treatment for most early TNBC patients is the administration of neoadjuvant chemotherapy followed by surgery [3,28,29]. Nonetheless, in TNBC patients submitted to upfront

Table 1
Clinical trials with a clear impact on clinical practice.

Adjuvant setting						
Trial	Notes	Nb. pts	Inclusion criteria	Treatment arms	Results	
					DFS	OS
All subtypes						
EBCTCG meta-analysis [6]	Meta-analysis of dose-dense chemotherapy	37,298	Early BC	A. Dose-dense chemotherapy B. Standard-schedule chemotherapy (every 3 weeks)	10-year recurrence risk: A. 28.0% B. 31.4% RR 0.86 (95% CI, 0.82–0.89)	10-year all-cause mortality: A. 22.1% B. 24.8% RR 0.87 (95% CI, 0.83–0.91)
HER2+						
APHINITY [19]	Benefit of adding Pertuzumab to adjuvant Trastuzumab	4,805	HER2+, N+ or N0 with a tumor ≥ 1.0 cm or between 0.5–1 cm if grade 3, ER- or age < 35 years	A. Chemotherapy + Trastuzumab (1 year) B. Chemotherapy + Trastuzumab + Pertuzumab (1 year)	3-year iDFS: A. 93.2% B. 94.1% HR 0.81 (95% CI, 0.66–1.00)	3.8 year OS rates: N/A; interim HR 0.89 (95% CI, 0.66–1.21)
ExteNET [18]	Benefit of extending treatment with Neratinib	2,840	HER2+, stage I-IIIC (later modified to stage II–IIIc)	A. Chemotherapy + Trastuzumab (1 year) → Placebo (1 year) B. Chemotherapy + Trastuzumab (1 year) → Neratinib (1 year)	5-year iDFS: A. 87.7% B. 90.2% HR 0.73 (95% CI, 0.57–0.92)	N/A
Luminal						
SOFT+TEXT [33]	Ovarian suppression + AI superior to ovarian suppression + tamoxifen	5,707	Premenopausal women with ER+ early BC	A. Tamoxifen alone B. Tamoxifen + ovarian suppression C. AI + ovarian suppression	8-year iDFS: A. 78.9% B. 83.2% (HR 0.76, 95% CI 0.62–0.93) C. 85.9% (HR 0.65, 95% CI 0.53–0.81)	8-year OS: A. 91.5% B. 93.3% (HR 0.67, 95% CI 0.48–0.92) C. 92.1% (HR = 0.85 95% CI 0.62–1.15)
TAILOR-X [40]	Chemotherapy not beneficial for patients with intermediate risk	6,711	Node-negative, HER2-/ER+ early BC, with recurrence score between 11 and 25.	A. Chemotherapy + endocrine treatment B. Endocrine treatment alone	9-year iDFS A. 84.3% B. 83.3% HR 1.08 (95% CI, 0.94–1.24)	A. 93.9% B. 93.8% HR 0.99 (95% CI, 0.79–1.22)
Meta-analysis EBCTCG [41]	Extended aromatase inhibitors	22,192	Postmenopausal women with ER+ early BC	A. 5 years of endocrine treatment followed by aromatase inhibitors B. 5 years of endocrine treatment	5-year DFS: A. 91.7% B. 89.6% HR 0.76 (95% CI, 0.70–0.83)	5-year breast cancer mortality A. 2.8% B. 3.1% RR 0.89, 95% CI, 0.77–1.02)
Post-neoadjuvant setting						
HER2 +						
KATHERINE [51]	T-DM1 better than 1y trastuzumab in non-pCR	1,486	HER2+ patients with invasive residual disease after neoadjuvant chemotherapy and trastuzumab ± pertuzumab	A. Trastuzumab × 14 cycles B. T-DM1 × 14 cycles	3-y iDFS: A. 77.0% B. 88.3% HR 0.50 (95% CI, 0.39–0.64)	OS data immature (HR 0.70; 95% CI 0.47–1.05)
TNBC						
CREATE-X [50]*	Capecitabine superior to observation	286	HER2-negative early BC with invasive residual disease after neoadjuvant chemotherapy	A. Capecitabine B. Observation	5-year DFS: A. 69.8% B. 56.1% HR 0.58 (95% CI, 0.39–0.87)	5-year OS: A. 78.8% B. 70.3% HR 0.52 (95% CI, 0.30–0.90)

*The CREATE-X study has enrolled 910 HER2-negative patients. Since the most significant results were observed in the TNBC subtype, the numbers and the survival rates displayed in the table refer to the subgroup analysis including only the TNBC patients.
Legend: BC: breast cancer; CI: confidence interval; DFS: disease-free survival; ER: endocrine-receptor; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; iDFS: invasive disease-free survival; MA: meta-analysis; OS: overall survival; pCR: pathologic complete response; RR: rate ratio; TNBC: triple-negative breast cancer; T-DM1: trastuzumab emtansine.

Table 2
Other clinical trials.

Adjuvant setting						
Trial	Notes	Nb. pts	Inclusion criteria	Treatment arms	Results	
					DFS	OS
All subtypes						
EBCTCG meta-analysis [2]	Meta-analysis of neoadjuvant vs. adjuvant chemotherapy	4,756	Early BC	A. Neoadjuvant chemotherapy B. Adjuvant chemotherapy (same regimen)	15-year local recurrence: A. 21.4% B. 15.9% RR 1.37 (95% CI, 1.17–1.61) 15-year distant recurrence: A. 38.2% B. 38.0% RR 1.02 (95% CI, 0.92–1.14)	15-year breast cancer mortality: A. 34.4% B. 33.7% RR 1.06 (95% CI, 0.95–1.18) 15-year all-cause mortality: A. 40.9% B. 41.2% RR 1.04 (95% CI, 0.94–1.15)
ABC trials [9]	TC non-inferiority to anthracycline-based chemotherapy was not demonstrated (upper margin in the interim analysis: 1.18)	4,242	HER2-negative early BC	A. Anthracycline + cyclophosphamide + taxane B. Docetaxel + cyclophosphamide × 6	4-year iDFS: A. 90.7% B. 88.2% HR 1.23 (95% CI, 1.01–1.50)	3.3-year OS (immature): HR 1.08 (95% CI, 0.82–1.41)
PlanB [12]	TC was non-inferior to anthracycline based chemotherapy	2,449	HER2-negative early BC (if ER-positive tumour, pN0/N1: only eligible if recurrence score by OncotypeDX was > 11)	A. Epirubicin + cyclophosphamide × 4 → Docetaxel × 4 B. Docetaxel + cyclophosphamide × 6	5-year DFS: A. 89.6% B. 89.9% HR 1.004 (95% CI, 0.776–1.229)	5-year OS: A. 94.5% B. 94.7% HR 0.937 (95% CI, 0.654–1.342)
HORG [10]	Non-inferiority of TC was not demonstrated (upper margin: 1.53)	650	HER2-negative, N+ BC (11% TNBC)	A. Docetaxel + cyclophosphamide × 6 B. ddFEC × 4 → ddDocetaxel × 4	3-year DFS: A. 91.1% B. 89.5% HR 1.147 (95% CI, 0.716–1.839)	OS rates: N/A HR 1.158 (95% CI, 0.491–2.727)
DBCG 07-READ [11]	Tried to demonstrate that TC was superior to EC-D.	2,012	Early breast cancer, TOP2A normal and ≥1 high-risk factor: age < 39 years, tumour size > 20mm, grade 2 or 3 ductal carcinoma, ER- (<10% positive), and/or HER2+	A. Docetaxel + cyclophosphamide × 6 B. Epirubicin + cyclophosphamide × 3 → Docetaxel × 3	5-year DFS : A. 88.3% B. 87.9% HR 1.00 (95% CI, 0.78–1.28)	5-year OS: A. 93.9% B. 94.8% HR 1.15 (95% CI, 0.83–1.59)
MA anthracyclines [13]	No difference between TC and anthracyclines plus taxanes.	12,741	HER2-negative early BC	A. 6 cycles of docetaxel + cyclophosphamide B. Sequential anthracyclines + cyclophosphamide → taxane	HR 1.08 (95% CI, 0.96–1.20)	HR 1.05 (95% CI, 0.90–1.22)
tAnGo [14]	No benefit of adding gemcitabine to adjuvant chemotherapy	3,152	Early BC, all subtypes	A. Epirubicin + Cyclophosphamide + Paclitaxel B. Epirubicin + Cyclophosphamide + Paclitaxel + Gemcitabine	10-year DFS: A. 65% B. 65% HR 0.97 (95% CI, 0.86–1.10)	10-year OS: A. 71% B. 70% HR 1.02 (95% CI, 0.89–1.16)
D-CARE [15]	No benefit of adding denosumab to (neo) adjuvant treatment	4,509	Early BC all subtypes	A. (neo) Adjuvant chemotherapy + placebo B. (neo) Adjuvant chemotherapy + denosumab 120 mg each 3–4 weeks × 6 doses → every 3 months for up to 5 years	Bone-metastasis free survival: HR 0.97 (95% CI, 0.82–1.14) DFS: HR 1.04 (95% CI, 0.91–1.19)	OS: HR 1.03 (95% CI, 0.85–1.25)
ABCSG-18 [16]	DFS was a secondary endpoint	3,425	Postmenopausal patients, ER+, under aromatase inhibitor therapy	A. Aromatase inhibitor + Denosumab 60 mg every 6 months B. Aromatase inhibitor + Placebo every 6 months	5 year-DFS: A. 89.2% B. 87.3%	N/A

					HR 0.82 (95% CI, 0.69–0.98)	
HER2+						
SOLD [20]	Non-inferiority was not demonstrated (pre-specified non-inferiority margin: 1.30).	2,174	Early-stage HER2+ BC	A. Trastuzumab × 12 months B. Trastuzumab × 9 weeks	5-year DFS: A. 90.5% B. 88.0%	5-year OS: A. 95.9% B. 94.7%
					HR 1.39 (90% CI, 1.12–1.72)	HR 1.36 (90% CI, 0.98–1.89)
Short-HER [21]	Non-inferiority was not demonstrated (pre-specified non-inferiority margin: 1.29).	1,254	HER2+, N+ or N0 and: T > 2 cm, G3, LVI+, Ki67 > 20%, age < 35 y or ER-	A. AC/EC × 4 → Docetaxel × 4 + Trastuzumab 12 months B. Docetaxel × 3 + T × 9 weeks → FEC × 3	5-year DFS: A. 88% B. 85%	5-year OS: A. 95.2% B. 95.0%
					HR 1.13 (90% CI, 0.89–1.42)	HR 1.07 (90% CI, 0.74–1.56)
PHARE [22]	Final results, median FU 7.5 y; non-inferiority was not demonstrated (pre-specified non-inferiority margin: 1.15)	3,380	Early-stage HER2+ BC	A. Trastuzumab × 12 months B. Trastuzumab × 6 months	7.5-year DFS: A. 79.6% B. 78.8%	OS rates: N/A, HR 1.13 (95% CI, 0.92–1.39)
					HR 1.08 (95% CI, 0.93–1.25)	
PERSEPHONE [23]	Non-inferiority was proven (overall) (pre-specified non-inferiority margin: initial: 1.17 → after modification: 1.29)	4,088	Early-stage HER2+ BC with indication for CT	A. Trastuzumab × 12 months B. Trastuzumab × 6 months	4-year DFS: A. 89.8% B. 89.4%	4-year OS: A. 94.8% B. 93.8%
					HR 1.07 (90% CI, 0.93–1.25)	HR 1.14 (90% CI, 0.95–1.37)
MA of trastuzumab duration [24]	1 year of trastuzumab was better than a shorter treatment duration in terms of DFS and OS.	11,383	Early-stage HER2+ BC	A. Trastuzumab × 12 months B. Trastuzumab × shorter duration (9 weeks – 6 months)	Pooled HR for DFS: 1.21 (95% CI, 1.08–1.37)	Pooled HR for OS: 1.31 (95% CI, 1.08–1.59)
APT [26]	Updated results, at 7 years of follow-up	410	HER2+, small tumours (≤3 cm), N0/N_{mic}	Paclitaxel 12 weeks + Trastuzumab 1 year	7-year iDFS: 93.3% (95% CI, 90.4–96.2)	N/A
NSABP B–47 [27]	No benefit of adding Trastuzumab in HER2-Low BC	3,270	HER2 IHC1+ or 2+ and negative ISH (HER2-Low IBC)	A. AC × 4→ weekly Paclitaxel × 12 or TC × 6 cycles B. (same CT) + Trastuzumab × 12 months	5-year iDFS: A. 89.2% B. 89.6%	5-year OS: A. 96.2% B. 94.8%
					HR 0.98 (95% CI, 0.77–1.26)	HR 1.33 (95% CI, 0.91–1.94)
TNBC						
BEATRICE [31]	No benefit of adjuvant bevacizumab	2,591	TNBC pT1-T3 after surgery	A. Chemotherapy × 4 cycles B. Chemotherapy × 4 cycles + bevacizumab (1 year)	5-year iDFS: A. 76.9% B. 79.6%	5-year OS: A. 87.7% B. 87.9%
					HR 0.87 (95% CI, 0.73–1.03)	HR 0.93 (95% CI, 0.74–1.17)
GEICAM / CIBOMA [32]	No benefit of extended adjuvant capecitabine	876	TNBC early-stage after standard (neo) adjuvant chemotherapy	A. Capecitabine × 8 cycles B. Observation	5-year DFS: A. 79.6% B. 76.8%	5-year OS: A. 86.2% B. 85.9%
					HR 0.82 (95% CI, 0.63–1.06)	HR 0.92 (95% CI, 0.66–1.28)
Luminal						
GEICAM/2006–10 [42]	No benefit of adding fulvestrant to AI	437	ER+/HER2- premenopausal early BC	A. Anastrozole × 5 years B. Anastrozole × 5 years + Fulvestrant 250 mg × 3 years	5-year DFS: A. 90.7% B. 91.25%	N/A
					HR 0.83 (95% CI, 0.57–1.22)	
BIG 1–98 trial [44]	Long term FU results (12 years). Trend in favour of letrozole	6,416	ER+/HER2- postmenopausal early BC	A. Tamoxifen B. Letrozole	14-year DFS: A. 61.2% B. 62.9%	14-year OS: A. 71.1% B. 72.9%
					HR 0.91 (95% CI, 0.81–1.01)	HR 0.89 (95% CI, 0.77–1.02)

(continued)

Table 2 (Continued)

Adjuvant setting						
Trial	Notes	Nb. pts	Inclusion criteria	Treatment arms	Results	
					DFS	OS
TAM-01 [45]	3 years of low-dose tamoxifen for *in situ* carcinomas	500	ER+ *in situ* BC	A. Tamoxifen B. Placebo	5-year DFS: A. 94.5% B. 88.7% HR 0.48 (95% CI, 0.25–0.89)	N/A
SOLE [85]	Intermittent versus continuous aromatase inhibitor	4,884	ER+ and node-positive postmenopausal patients with previous 4–6 years of endocrine treatment	A. Letrozole continuous × 5 years B. Letrozole 9 out of 12 months × 5 years	5-year DFS A. 87.5% B. 85.8% HR 1.08 (95% CI, 0.93–1.26)	5-year OS A. A.93.7% B. B.94.3% HR 0.85 (95% CI, 0.68–1.06)
Neoadjuvant setting						
Trial	Notes	Nb. pts	Inclusion criteria	Treatment arms	Results	
					pCR rates	EFS
All subtypes -						
GeparOcto (GBG 84) [52]	No difference between 2 chemotherapy regimens, even in each subtype	961	cT1c-cT4a-d HER2+ BC or TNBC (any N status) cT1c-cT4a-d, N+ luminal B-like tumours	A. Dose-dense epirubicin + cyclophosphamide + Paclitaxel B. Weekly Paclitaxel + non-PEGylated liposomal doxorubicin (+ carboplatin if TNBC) (Note: trastuzumab and pertuzumab were added if HER2+)	A. 48.3% B. 48.0% OR 0.99 (95% CI, 0.77–1.28)	N/A
HER2+						
KRISTINE [54]	Negative trial	444	HER2+, stage II/III, *T*>2 cm	A. Docetaxel + Carboplatin + Trastuzumab + Pertuzumab × 6 cycles B. T-DM1 + Pertuzumab × 6 cycles	A. 55.7% B. 44.4% $p = 0.016$	N/A
TRAIN-2 [53]	Superiority trial (hypothesis: anthracyclines would provide a better pCR) – negative trial	418	HER2+, stage II/III	A. FEC + Trastuzumab + Pertuzumab × 3 → Paclitaxel + Carboplatin + Trastuzumab + Pertuzumab × 6 cycles B. Paclitaxel + Carboplatin + Trastuzumab + Pertuzumab × 9 cycles	A. 67% B. 68% OR 1.07 (95% CI, 0.71–1.61)	N/A
ACOSOG Z1041 [55]	Long-term survival results: confirms no benefit of combining concurrent anthracyclines with anti-HER2 as compared to their sequential administration	280	HER2+, operable	A. FEC q3w × 12 weeks → weekly Paclitaxel + T × 12 weeks B. Weekly Paclitaxel + T × 12 weeks → FEC q3w + weekly T × 12 weeks	A. 56.5% B. 54.2% OR 0.90 (95% CI, 0.55–1.49)	5-y DFS rates: A. 82.9% B. 80% HR 1.02 (95% CI, 0.56–1.83)5-y OS rates: N/A, HR 1.17 (95% CI, 0.48–2.88)
PEONY [56]	Confirms the benefit of adding pertuzumab to trastuzumab + chemotherapy	329	HER2+, stage II/III, *T*>2 cm	A. Docetaxel + Trastuzumab × 4 cycles B. Docetaxel + Trastuzumab + Pertuzumab × 4 cycles Both arms: post-Surgery: 3 × FEC + 1 year of Trastuzumab (± Pertuzumab)	A. 20.9% B. 39.3% $p = 0.0014$	N/A

Legend: BC: breast cancer; CI: confidence interval; dd: dose dense regimen; DFS: disease-free survival; ER: endocrine-receptor; FEC: 5-fluorouracil, epirubicin and cyclophosphamide regimen; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; iDFS: invasive disease-free survival; MA: meta-analysis; N/A: not available; OS: overall survival; OR: odds ratio; pCR: pathologic complete response; TNBC: triple-negative breast cancer; T: trastuzumab; T-DM1: trastuzumab emtansine.

surgery, adjuvant chemotherapy is recommended [3,28,29]. No studies have provided practice-changing results in the adjuvant setting of TNBC, although interesting findings were reported.

The BEATRICE trial randomized 2,591 early TNBC patients to receive anthracycline and/or taxane-based chemotherapy for at least 4 cycles alone or in combination with bevacizumab. In 2017, an updated analysis after a median follow-up of 56 months confirmed the absence of an OS benefit with the addition of bevacizumab (HR 0.93; 95% CI, 0.74–1.17) [30,31].

The GEICAM/CIBOMA study enrolled 876 early TNBC patients submitted to surgery and (neo)adjuvant chemotherapy to receive additional 8 cycles of capecitabine or observation. After a median follow-up of 7.3 years, no benefit was observed with capecitabine: 5-year DFS rates were 79.6% with capecitabine vs. 76.8% with observation (HR 0.79; 95% CI, 0.61–1.03) [32].

Luminal breast cancer

Studies with clinically relevant results in the adjuvant treatment of luminal breast cancer patients have been reported since 2017. The SOFT and TEXT trials randomized premenopausal patients whose tumours expressed ER in ≥10% of the cells to receive tamoxifen alone (SOFT), tamoxifen plus ovarian suppression (SOFT and TEXT) or exemestane plus ovarian suppression (SOFT and TEXT) for 5 years. After a median follow-up of 8 years, DFS rates were 86.8% with exemestane plus ovarian suppression vs. 82.8% with tamoxifen plus ovarian suppression (HR 0.77; 95% CI, 0.67–0.90), demonstrating the benefit of a more potent hormonal suppression in premenopausal patients [33]. Comparisons between tamoxifen plus ovarian suppression vs. tamoxifen alone (HR 0.76; 95% CI, 0.62–0.93) and exemestane plus ovarian suppression vs. tamoxifen alone (HR 0.65; 95% CI, 0.53–0.81) in the SOFT trial confirmed the benefit of adding ovarian suppression as previously reported [34]. In subgroup analysis, the benefit of exemestane plus ovarian suppression was restricted to high-risk patients who received chemotherapy, but there is as yet no OS benefit.

Notably, the administration of chemotherapy in both studies was per investigator's decision, and the risk of recurrence was mainly estimated using the clinical and pathological characteristics of the tumour. An elegant "STEPP analysis" combining these factors into a composite score clearly shows that tamoxifen alone performs very well for the patients at lowest risk [35]. Not surprisingly, an increased frequency of adverse events was observed with the addition of ovarian suppression and aromatase inhibitors, mainly musculoskeletal symptoms, osteoporosis and dyspareunia [33]. Therefore, the potential benefits and risks of this strategy need to be carefully balanced in order to decide the more appropriate adjuvant endocrine treatment for each patient.

The 21-gene breast cancer assay (OncotypeDX, Genomic Health) analyses tumour gene expression to stratify early luminal breast cancer patients into 3 different categories according to their recurrence risk (low, intermediate and high) [36–38]. As previously demonstrated, high-risk patients benefit from chemotherapy, while in the low-risk group the addition of chemotherapy does not provide any benefit [38,39]. For patients classified in the intermediate-risk category, the role of chemotherapy remained unclear. The prospective randomized phase III study TAILORx trial recruited 6,711 women with ER-positive, HER2-negative and node-negative breast cancer classified as intermediate-risk according to their gene expression profile (score between 11–25). The study's primary objective was to demonstrate the non-inferiority of endocrine treatment alone vs. the combination of chemotherapy and endocrine treatment in terms of iDFS, with an upper margin of 32.2% (HR = 1.322) accepted for non-inferiority. After a median follow-up of 90 months, non-inferiority of endocrine treatment alone was demonstrated (iDFS rate: 83.3% with endocrine treatment vs. 84.3% with chemotherapy plus endocrine treatment, HR 1.08; 95% CI, 0.94–1.24). No OS difference was observed between both groups (93.9% with endocrine treatment vs. 93.8% with chemotherapy plus endocrine treatment). Notably, a significant interaction between age, recurrence score and chemotherapy was observed for the outcome iDFS, suggesting a potential benefit from chemotherapy in women ≤50 years of age with a recurrence score between 16–25 [40]. It is not impossible that a part of this benefit is explained by chemotherapy-induced ovarian function suppression.

The TAILORx results have made a huge impact on clinical practice, by demonstrating that chemotherapy can be safely omitted in intermediate-risk patients (except those with ≤50 years and a recurrence score between 16–25), providing them an opportunity to be spared from potential harmful toxicities.

An individual-patient data meta-analysis comprising 11 studies and 22,192 patients investigated the role of extending aromatase inhibitors for more than 5 years in the adjuvant treatment of luminal breast cancer patients. In the overall population, recurrence free-survival was improved by extended aromatase inhibitor treatment (RR 0.76; 95% CI, 0.70–0.83). However, the effect was more pronounced in patients who previously received tamoxifen alone (RR 0.67; 95% CI, 0.55–0.83) or tamoxifen plus aromatase inhibitor as switch strategy (RR 0.82; 95% CI, 0.70–0.97), whereas in patients who previously received only aromatase inhibitors the benefit did not reach statistical significance (RR 0.76; 95% CI, 0.56–1.02). The absolute benefit provided by extended aromatase inhibitors in terms of recurrence rates also varied according to lymph node status: node-negative (2.5%), 1 to 3 positive lymph nodes (3.8%), and ≥3 positive lymph nodes (7.7%). The risk of bone fractures was increased with extended aromatase inhibitors (RR 1.24; 95% CI, 1.10–1.39) [41].

The GEICAM/2006-10 study randomized 437 luminal postmenopausal patients to receive aromatase inhibitor for 5 years alone or combined with fulvestrant 250 mg during the first 3 years. No benefit of the addition of fulvestrant was observed after a median follow-up of 6.3 years (recurrence-free survival rates: 90.8% with aromatase inhibitor vs. 91.3%, with fulvestrant plus aromatase inhibitor, $p = 0.357$) [42]. Of note, this study recruited less patients than anticipated, and the fulvestrant dose used (250 mg) has been demonstrated to be inferior to the 500 mg dose in the metastatic setting, leading to interruption of enrolment [43].

The BIG 1–98 was a four-arm study comparing adjuvant letrozole vs. tamoxifen (either treatment received for 5 years) and sequential treatments with both agents (2 years of one followed by 3 years of the other) in luminal postmenopausal patients. The long-term results of this study point towards a sustained superiority of the aromatase inhibitor in comparison to tamoxifen – a trend favouring letrozole was observed in terms of DFS (HR 0.91; 95% CI, 0.81–1.01) after a median follow-up of 12.6 years. Given the risk of late recurrences in luminal breast cancer patients, this study brings important long-term data on the outcomes of these patients [44].

An interesting strategy of low-dose tamoxifen has been evaluated in the TAM-01 trial, which randomized 500 patients with *in situ* breast tumours to receive either 5 mg/day of tamoxifen or placebo as adjuvant treatment for 3 years. After a median follow-up of 5.1 years, recurrence rates were 5.5% with tamoxifen vs. 11.3% with placebo (HR 0.48; 95% CI, 0.26–0.92) [45]. This study has demonstrated the feasibility of low-dose tamoxifen, although this dose has not been compared to the standard tamoxifen 20 mg/day or to aromatase inhibitors [28,46].

The HOBOE-2 trial randomized 1065 luminal premenopausal breast cancer patients to receive tamoxifen, letrozole or letrozole plus zoledronic acid (4 mg every 6 months) for 5 years (Table 3). All

Table 3
Studies with interesting findings, promising biomarkers or new treatments.

Adjuvant setting

Trial	Notes	Nb. pts	Inclusion criteria	Treatment arms	Results	
					DFS	OS
Luminal						
HOBOE-2 [47]	Adjuvant bisphosphonate in premenopausal BC	1,065	ER+ early premenopausal BC patients	A. Tamoxifen + ovarian suppression B. Letrozole + ovarian suppression C. Letrozole + ovarian suppression + zoledronic acid	5.4-year DFS: A. 85% B. 93% C. 93% HR 0.52 (95% CI 0.34–0.80) for the comparison of C. vs. A.	N/A

Neoadjuvant setting

Trial	Notes	Nb. pts	Inclusion criteria	Treatment arms	Results	
					pCR rates	EFS
HER2+						
WSG-ADAPT HER2+ /HR- [61]	Only ER negative patients. Impressive pCR with the addition of chemotherapy	134	HER2+/ER-, cT1–4c, any N	A. Trastuzumab + Pertuzumab × 12 weeks B. Paclitaxel + Trastuzumab + Pertuzumab × 12 weeks	A. 36.3% B. 90.5% Difference: 56.1% (95% CI, 42.8%-69.3%).	N/A
WSG-ADAPT HER2+ /HR+ [62]	Triple+ disease; possibility for de-escalation of CT	375	HER2+/ER+	A. T-DM1 × 12 weeks B. T-DM1 + ET × 12 weeks C. Trastuzumab + ET × 12 weeks	A. 41.0% B. 41.5% C. 15.1% $P < 0.001$	N/A
NA-PHER2 [63]	Triple+ disease; first trial to use palbociclib with anti-HER2 treatment in the early setting	36	HER2+/ER+	Trastuzumab + Pertuzumab + Palbociclib + Fulvestrant × 5 months	Ki67 geometric mean: 31.9 (SD 15·7) at baseline vs. 4.3 (15.0) at week 2 ($n = 25$; $p < 0·0001$) vs. 12.1 (20·0) at surgery ($n = 22$; $p = 0·013$); pCR: 27% (95% CI, 12–46)	N/A
PerELISA [64]	Triple+ disease; impressive pCR in non-responders	64	HER2+/ER+ (ER ≥ 10%), stage II-IIIA, post-menopausal	Letrozole × 2 weeks → biopsy (Ki67): • molecular responders (relative ↓Ki67 > 20% from baseline): Letrozole + Trastuzumab + Pertuzumab × 5 cycles • non-responders: paclitaxel + Trastuzumab + Pertuzumab × 5 cycles	Molecular responders ($n = 44$): pCR 20.5% Non-responders ($n = 17$): pCR 81.3%	N/A
NeoPHOEBE [65]	Combination of buparlisib and taxane-trastuzumab was not feasible due to liver toxicity.	50	HER2+ early BC	A. Buparlisib 6 weeks → Buparlisib + Paclitaxel + Trastuzumab B. Placebo 6 weeks → Placebo + Paclitaxel + Trastuzumab	A. 32% B. 40% ($p = 0.811$)	N/A
TNBC						
Litton et al. [68]	Neoadjuvant PARPi	20	Early BC, *BRCA* mutation (85% were TNBC)	Talazoparib single-agent for 6 months	pCR: 53% (95% CI, 32%-73%)	N/A
BrighTNess [67]	Carboplatin (not veliparib) increased pCR rates	634	TNBC stages II and III	A. Carboplatin + Paclitaxel + Veliparib B. Carboplatin + Paclitaxel C. Paclitaxel	A. 53% B. 58% C. 31% A vs. C. ($p < 0.0001$) A vs. B. ($p = 0.357$)	N/A
MA platinum [66]	Increased pCR rates with the addition of platinum compounds	2,109	TNBC patients submitted to neoadjuvant treatment and surgery	A. Platinum-based neoadjuvant chemotherapy B. Platinum-free neoadjuvant chemotherapy	A. 52.1% B. 37.0% OR 1.96 (95% CI, 1.46–2.62)	HR 0.72 (95% CI, 0.49–1.06)
Luminal						

POETIC [69]	Peri-operative aromatase inhibitors	4,480	Early-stage ER+/HER2- postmenopausal BC patients	A. Aromatase inhibitor (anastrozole or letrozole) followed by surgery B. Surgery	N/A	5-year OS: A. 89.0% B. 89.5% HR 0.98 (95% CI, 0.82–1.18)
NeoPalAna [72]	Neoadjuvant CDKi (single-arm)	50	Stage II-III ER+/HER2- BC	Anastrozole + Palbociclib	0% pCR 87% CCCA at C1D15	N/A
N007 [76]	Neoadjuvant CDKi (single-arm)	20	ER + /HER2-, $T \geq 2$ cm, post-menopausal	Letrozole + Palbociclib × 4 cycles	5% pCR; CCCA at C1D15: 71%; PEPI score 0 rate: 5%	N/A
neoMONARCH [75]	Neoadjuvant CDKi (single-arm)	223	ER+/HER2-, stage I ($T \geq 1$ cm)-IIIB, post-menopausal	A. Anastrozole B. Abemaciclib + Anastrozole C. Abemaciclib × 2 weeks → Abemaciclib + Anastrozole × 14 weeks	3.7% pCR; CCCA at C1D15: A. 14% B. 58% C. 68%	N/A
NeoPAL [73]	Neoadjuvant CDKi	106	Stage II-III ER+/HER2- postmenopausal BC patients	A. Chemotherapy (FEC + docetaxel) B. Letrozole + Palbociclib	A. 5.9% B. 3.8% (OR not provided)	N/A
PALLET [74]	Neoadjuvant CDKi	307	ER+/HER2- postmenopausal early BC patients	A. Letrozole B. Letrozole → Palbociclib + Letrozole C. Palbociclib → Palbociclib + Letrozole D. Palbociclib + Letrozole	Clinical response rate: A. 49.5% B, C and D. 54.4% $p = 0.20$	N/A
LORELEI [79]	Neoadjuvant PI3Ki	334	Stage I-III ER+/HER2- postmenopausal BC patients	A. Letrozole + Placebo B. Letrozole + Taselisib	A. 0.6% B. 1.8% $p = 0.37$	N/A
NEO-ORB [80]	Neoadjuvant PI3Ki	257	Stage I-III ER+/HER2- postmenopausal BC patients	A. Letrozole + Alpelisib B. Letrozol + Placebo	ORR: A. 43.3% B. 44.8%	N/A

Legend: BC: breast cancer; CCCA: complete cell cycle arrest, defined as Ki67 <2.7%; CI: confidence interval; DFS: disease-free survival; ER: endocrine-receptor; ET: endocrine treatment; PI3K: phosphoinositide 3-kinase; ORR: overall response rate; CCCA: complete cell-cycle arrest; pCR: pathologic complete response; CDKi: cyclin-dependent kinase inhibitor; HR: hazard ratio; HER2: human epidermal growth factor receptor 2; MA: meta-analysis; ORR: overall response rate; OS: overall survival; TNBC: triple-negative breast cancer; T-DM1: trastuzumab emtansine.

patients received ovarian suppression with triptorelin. After a median follow-up of 65 months, DFS rates were higher with zoledronic acid plus letrozole compared with tamoxifen alone (HR 0.52; 95% CI, 0.34–0.80), suggesting a benefit for the addition of bisphosphonates in the adjuvant treatment of premenopausal patients, as previously demonstrated in postmenopausal patients [17]. However, these results need to be interpreted with caution, since the superiority of zoledronic acid plus letrozole over letrozole alone has not been demonstrated (HR 0.70; 95% CI 0.44–1.12) [47]. Therefore, it is not clear if the benefit observed in the HOBOE-2 study can be attributed to a more efficient endocrine treatment with letrozole or to the addition of zoledronic acid in the experimental arm [48].

Neoadjuvant treatment

The administration of systemic treatment before surgery has many potential advantages: it allows an *in vivo* assessment of response, an early treatment of micro-metastatic disease, and it increases the rates of conservative surgical procedures [2]. The pathologic evaluation of the surgical specimen after neoadjuvant treatment also provides valuable prognostic information [49]. With the advent of effective post-neoadjuvant treatments for HER2-positive and TNBC patients with invasive residual disease after neoadjuvant treatment, this strategy is becoming more attractive and gaining importance [50,51]. Yet, one will have to power these neoadjuvant trials for event-free-survival if one is hoping to impact clinical practice.

All subtypes

The GeparOCTO was the only neoadjuvant trial published since 2017 including patients with TNBC, HER2-positive and high-risk luminal B tumours. It compared intensified dose-dense epirubicin, paclitaxel, and cyclophosphamide vs. weekly paclitaxel plus non-PEGylated liposomal doxorubicin (plus carboplatin in TNBC) [52]. HER2-positive patients also received trastuzumab and pertuzumab. The pCR rates were similar in both arms and there were no significant differences according to breast cancer subtype.

HER2-positive breast cancer

In contrast with the adjuvant and post-neoadjuvant setting, there were no practice-changing trials for the neoadjuvant treatment of HER2-positive disease. The TRAIN-2 trial tested the hypothesis that the addition of anthracyclines would improve pCR rates compared with a carboplatin–taxane regimen, in combination with the now standard dual HER2 blockade (trastuzumab and pertuzumab) [53]. The pCR rates were similar between arms (around 68%) and there were no differences according to ER status. Survival data is not yet mature, which may be needed to re-assure clinicians that a carboplatin–taxane backbone is an alternative to anthracycline-taxane based regimens for the neoadjuvant treatment of HER2-positive disease.

The KRISTINE trial compared a carboplatin–taxane backbone with dual HER2 blockade vs. T-DM1 plus pertuzumab [54]. The pCR rates were lower in the T-DM1 arm compared to the chemotherapy arm (44.4% vs. 55.7%, respectively; $p = 0.016$), both in ER-positive and ER-negative disease. Therefore, chemotherapy with dual HER2 blockade should remain the standard-of-care neoadjuvant treatment in this scenario.

Long-term survival results of the ACOSOG Z1041 trial confirmed that there was no benefit of combining concurrent anthracyclines with anti-HER2 agents [55]. The PEONY trial, conducted in Asia, showed again the benefit of adding pertuzumab to docetaxel plus trastuzumab in the neoadjuvant setting [56].

It is known that HER2-positive tumours comprise a heterogeneous population [57] and that, among them, pCR rates are higher in ER-negative tumours as compared to ER-positive tumours [58–60]. Thus, recent trials aimed to de-escalate therapy specifically in the HER2-positive/ER-negative or in the HER2-positive/ER-positive ("triple-positive disease") subgroup of patients (Table 3). The WSG-ADAPT HER2+/HR- trial tested if dual HER2 blockade only (the "chemo-free" approach) was non-inferior to paclitaxel with dual HER2 blockade in 134 HER2-positive/ER-negative patients. The pCR rates were 36.3% with the dual HER2 blockade alone vs. 90.5% in the arm with paclitaxel, demonstrating the high chemo-sensitivity of HER2-positive/ER-negative tumours [61]. The WSG-ADAPT HER2+/HR+ compared T-DM1 (with or without endocrine treatment) vs. trastuzumab with endocrine treatment in 375 patients with "triple positive" disease. Use of T-DM1 led to higher pCR rates (41.0% with TDM1 alone and 41.5% with TDM1 plus endocrine treatment) compared to the trastuzumab arm (15.1%). Long-term survival data from both WSG-ADAPT studies are needed, but its interpretation will be limited by the absence of a "standard" chemotherapy/dual HER2 blockade arm as comparator and by the fact that patients were recommended to have standard adjuvant anthracycline-taxane based chemotherapy [62].

Both WSG-ADAPT trials used Ki67 measurements after 3 weeks of treatment to classify patients as "early responders" if there was a Ki67 decrease ≥30% vs. baseline or low cellularity in the biopsy – in both trials, "early responders" had a higher likelihood of achieving a pCR. This marker could be explored to identify patients who are not benefiting from neoadjuvant treatment ("non-responders"), but it needs validation in other trials. The small NA-PHER2 trial also used a decrease in Ki67 during treatment to assess benefit from trastuzumab, pertuzumab, palbociclib, and fulvestrant in "triple positive" disease [63]. The PerELISA trial defined "molecular response" to a 2-week treatment with letrozole as a fall ≥20% in Ki67. "Molecular responders" were assigned to letrozole in combination with dual HER2 blockade and achieved a pCR rate of 20.5% [64]. More impressive, however, was the 81.3% pCR rate in the "non-responders" patients' group, who received paclitaxel plus dual HER2 blockade, suggesting that this "non-response" to endocrine therapy in HER2-positive/ER-positive patients may identify patients who benefit from chemotherapy plus dual HER2 blockade.

Mutations in the phosphoinositide 3-kinase (PI3K) gene are known mechanisms of resistance in HER2-positive breast cancer patients. The role of buparlisib (pan-class I PI3K inhibitor) in the neoadjuvant setting was evaluated in the NeoPHOEBE trial, which was prematurely interrupted due to a high incidence of severe liver toxicities with the combination of buparlisib, trastuzumab and paclitaxel (48% grade ≥3 ALT elevation and 28% grade ≥3 AST elevation) [65].

TNBC

Studies evaluating the role of platinum agents in the neoadjuvant treatment of TNBC have presented controversial results so far [66]. A 2018 meta-analysis including 9 studies and 2,109 patients has shown that the addition of platinum agents significantly increased pCR rates (52.1% with platinum-based vs. 37.0% with platinum-free chemotherapy; odds ratio [OR] 1.96; 95% CI, 1.46–2.62). No significant difference in terms of OS (HR 0.86; 95% CI, 0.46–1.63) was observed. Intriguingly, among the 96 patients harbouring a *BRCA* mutation (*BRCA*mut), no benefit in terms of pCR was observed with the addition of platinum (OR 1.17; 95% CI, 0.51–2.67), although the limited number of *BRCA*mut patients limits definitive conclusions [66].

The role of PARP inhibitors (PARPi) in the neoadjuvant setting has been investigated in the BrighTNess study, which randomized TNBC patients to paclitaxel alone, paclitaxel plus carboplatin or paclitaxel plus carboplatin and veliparib. All patients received 4 additional

cycles of doxorubicin and cyclophosphamide. The addition of veliparib did not increase pCR rates (53% with carboplatin-paclitaxel plus veliparib vs. 58% with carboplatin-paclitaxel, $p = 0.36$). Notably, only 15% of the patients included in this study were *BRCA*mut, the population in which PARPi are expected to show a higher efficacy [67]. An interesting sign of activity of PARPi was observed in a single-arm phase II study of talazoparib single-agent administered to 20 *BRCA*mut breast cancer patients (of whom 17 were TNBC). Talazoparib (1 mg) was administered for 6 months before surgery, with adjuvant chemotherapy being administered at physician's discretion afterwards. The study reported an encouraging pCR rate of 53%, highlighting the potential of PARPi for *BRCA*mut patients to be explored in future studies [68].

Luminal breast cancer

The POETIC trial randomized 4,480 postmenopausal women with luminal breast cancer to receive 4 weeks of peri-operative aromatase inhibitor or surgery. Additional treatments were allowed at investigation's discretion after surgery. In a preliminary analysis, no difference was observed in 5-year recurrence-free rates (HR 0.91; 95% CI, 0.74–1.12). The Ki67 expression (≥10% defined as high, <10% defined as low) after 2 weeks of aromatase inhibitor was evaluated as a predictive biomarker. Patients with a high Ki67 after 2 weeks of pre-operative aromatase inhibitors presented a higher recurrence risk in comparison to those with a low Ki67 (HR 2.60; 95% CI, 1.82–3.73) [69]. Although the dynamic evaluation of Ki67 identified a subgroup of patients with an excellent prognosis, it should be noted that the demonstration of its role as a way to decide whether or not aromatase inhibitor therapy is sufficient or needs to be reinforced might be compromised by the lack of protocol-defined post-surgical therapy.

It is well known that patients with luminal breast cancer present low pCR rates after neoadjuvant chemotherapy; nevertheless achieving a pCR signals a somewhat better long-term outcome, and therefore new strategies are needed to improve pCR rates in this subgroup [69,70]. Cyclin dependent kinase inhibitors (CDKi) have yielded a significant survival improvement in metastatic luminal breast cancer patients, providing a rationale for studies evaluating neoadjuvant CDKi [71–76]. These phase II studies were designed to evaluate the activity of neoadjuvant CDKi in combination with endocrine treatment, and although interesting signs of activity were observed (improved clinical response rates and decreased Ki67 expression), the addition of CDKi did not improve pCR rates and survival outcomes were not yet provided [72–76].

The occurrence of activating mutations in *PIK3CA* is a classic resistance mechanism to endocrine treatment in luminal breast cancer [77]. The combination of alpha-isoform specific PI3K inhibitors (PI3Ki) with endocrine treatment has demonstrated activity in metastatic luminal breast cancer patients [78]. Therefore, PI3Ki have also been evaluated in the neoadjuvant setting. Although a promising sign of activity was observed in the phase II study LORELEI, which demonstrated improved clinical response rates with the addition of PI3Ki to endocrine treatment, another phase II trial (NEO-ORB) did not demonstrate any benefit with the addition of PI3Ki. Moreover, treatment side effects were increased, pCR rates were not improved and survival gains are unlikely to be seen with additional follow-up. [79,80].

Post-neoadjuvant treatment

The finding of invasive residual disease after neoadjuvant treatment is associated with worse prognosis [70,81]. The concept of post-neoadjuvant treatment is built on the rationale that patients who did not achieve a pCR present a high-risk of recurrence and, therefore, may benefit from additional treatments [82].

All subtypes

The CREATE-X study evaluated post-neoadjuvant treatment in patients with HER2-negative tumours, but it will be discussed in the TNBC section below due to the significant results observed in this subtype. No other relevant studies evaluated post-neoadjuvant treatment in all-comers.

HER2-positive breast cancer

The most impactful study reported since 2017 was the KATHERINE trial, which randomized 1,486 HER2-positive early breast cancer patients with invasive residual disease after neoadjuvant treatment with chemotherapy and trastuzumab (with or without pertuzumab) to receive adjuvant T-DM1 or trastuzumab. An 11.3% absolute improvement in iDFS was observed with T-DM1 as compared to trastuzumab (3 year-iDFS: 88.3% vs. 77.0%, respectively, HR 0.50; 95% CI, 0.39–0.64) [51]. The benefit was observed in all subgroups, although 3-year iDFS rate was only 82.1% with T-DM1 in the ER-negative subgroup, showing that further improvements are needed in this subgroup. No difference in cardiac adverse events was observed, however peripheral sensory neuropathy and increased aminotransferase levels were more frequent with TDM1, suggesting that patients receiving adjuvant T-DM1 may need a closer follow-up to monitor potential adverse events.

The key to these impressive results lies in patient's selection: in trastuzumab-naïve early breast cancer patients, T-DM1 (plus pertuzumab) was not superior to the combination of chemotherapy with trastuzumab (and pertuzumab) in the KRISTINE trial [54]. Yet, in patients who had a suboptimal response to neoadjuvant chemotherapy and trastuzumab and thus have a worse prognosis, T-DM1 is an effective "salvage" therapy.

The KATHERINE study clearly shows that patients with invasive residual HER2-positive disease after neoadjuvant treatment should receive adjuvant T-DM1 instead of continuing trastuzumab. In addition, if reinforced by the subsequent demonstration of an OS benefit, these results will upgrade the neoadjuvant approach as the recommended one and restrict "pure" adjuvant systemic treatment to low-stage HER2-positive disease (cT1N0). A proposed updated algorithm for the treatment of early HER2-positive breast cancer patients is displayed in Figure 1.

TNBC

The first study to demonstrate the benefit of post-neoadjuvant treatment was the phase III CREATE-X trial, in which 910 HER2-negative early breast cancer patients who did not achieve a pCR after neoadjuvant treatment were randomized to capecitabine (1250 mg/m^2, twice per day, D1–14, every 3 weeks for 6–8 cycles) plus standard-of-care (radiotherapy per physician's discretion and endocrine therapy for ER-positive patients) vs. standard-of-care. Initially, patients received 6 cycles of capecitabine ($n = 159$), but after an interim safety analysis treatment was extended to 8 cycles. The study was positive for its primary endpoint (5-year DFS: 74.1% with capecitabine vs. 67.6% with control, HR 0.70; 95% CI, 0.53–0.92) and the 5-year OS rates were also improved by capecitabine (89.2% with capecitabine vs. 83.6% with control, HR 0.59; 95% CI, 0.39–0.90) [50].

The benefit of capecitabine was driven by the TNBC subgroup ($n = 286$) in terms of 5-year DFS (HR 0.58; 95% CI, 0.39–0.87) and 5-year OS (HR 0.52; 95% CI, 0.30–0.90). Notably, the CREATE-X enrolled exclusively Asian patients, which are known to metabolize fluoropyrimidines more efficiently than Western patients, and the

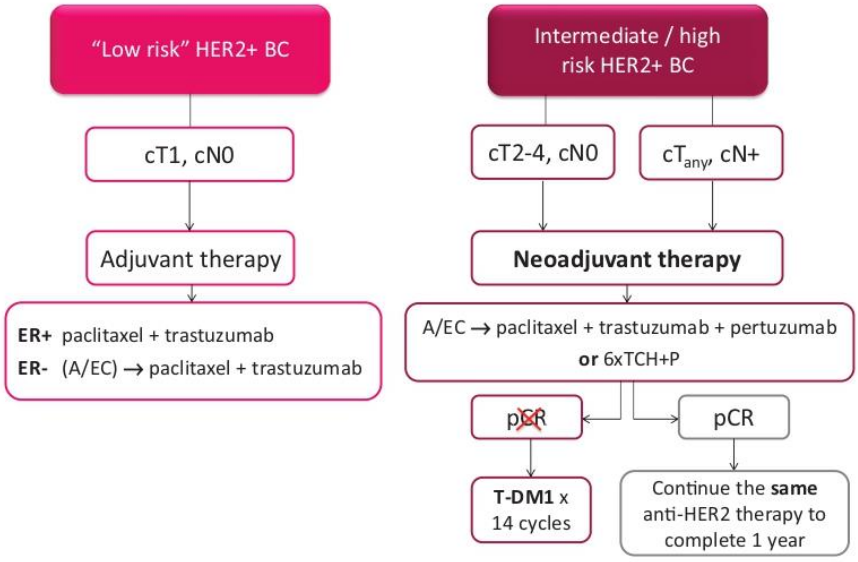


Fig. 1. Proposed algorithm for HER2-positive early breast cancer treatment as of 2019 BC: breast cancer; ER+: estrogen-receptor positive; A/EC: doxorubicin/epirubicin + cyclophosphamide; ER-: estrogen-receptor negative; N0: lymph-node negative; N+: lymph-node positive; P: pertuzumab; pCR: pathologic complete response; TCH: docetaxel + carboplatin + trastuzumab; T-DM1: trastuzumab emtansine.

capecitabine doses used in the study were higher than the usual doses used in Western individuals, raising concerns that the results observed in the CREATE-X study might not be fully transposable to a Western population.

As previously mentioned, the GEICAM/CIBOMA trial enrolled early TNBC patients independently of previous chemotherapy response, whereas the CREATE-X selected patients with incomplete response to neoadjuvant treatment. The selection of a high-risk population in the CREATE-X may account for the benefit observed with capecitabine in this study, in comparison to the negative results of the GEICAM/CIBOMA. Based on the results of the CREATE-X trial, post-neoadjuvant capecitabine is a valid option for early TNBC patients with invasive residual disease after neoadjuvant treatment [29].

Luminal breast cancer

No practice-changing studies evaluating post-neoadjuvant treatment specifically in luminal breast cancer patients have been reported. As mentioned above, in the CREATE-X trial the administration of post-neoadjuvant capecitabine improved DFS and OS in the overall study population which comprised 67.8% of ER-positive patients. However, the benefit of capecitabine in terms of DFS was not observed in ER-positive patients (HR 0.81; 95% CI, 0.55–1.17) [50]. Therefore, post-neoadjuvant capecitabine has been widely adopted only for TNBC patients [29].

Conclusions

The most important change in the treatment of early breast cancer since 2017 is that now we have the possibility to administer "salvage" treatments to HER2-positive and TNBC patients who do not achieve a pCR after neoadjuvant treatment. Thus, in intermediate and high-risk TNBC and HER2-positive disease patients (with ≥T2 and/or node-positive tumours), neoadjuvant treatment shall become the standard of care, to allow patients who do not achieve a pCR to receive post-neoadjuvant capecitabine (if TNBC) or T-DM1 (if HER2-positive).

In addition, we have seen that adding 1 year of concomitant pertuzumab to adjuvant trastuzumab or extending the duration of anti-HER2 therapy with 1 year of neratinib also improves outcomes, but in a more modest way, being alternatives for node-positive patients who did not receive neoadjuvant anti-HER2 treatment.

In patients with luminal tumours and node-negative disease, it was shown that, overall, those with an intermediate recurrence risk score (11–25) in the OncotypeDX 21-gene assay can avoid chemotherapy. The MINDACT trial, reported in 2016, also provided level I evidence of the clinical utility of a gene expression signature but in women at comparatively higher clinical risk [83]. Regarding adjuvant endocrine therapy, updated results of the SOFT/TEXT trials confirmed the benefit of a more potent endocrine treatment with aromatase inhibitors and ovarian suppression in high-risk premenopausal patients. For postmenopausal patients, long-term results of the BIG 1–98 trial demonstrated a sustained trend in favour of adjuvant letrozole compared to tamoxifen. A large individual patient meta-analysis has finally confirmed that, beyond 5 years of endocrine therapy, extended treatment with aromatase inhibitors modestly reduces the risk of breast cancer recurrence, with a more pronounced benefit observed in patients who received tamoxifen as initial treatment. A more accurate selection of patients who benefit from extended endocrine therapy is still needed, given that available multigene signatures like the PAM-50 ROR score and EndoPredict® can provide prognostic but not predictive information [84]. Finally, a large meta-analysis including patients with all breast cancer subtypes showed that adjuvant dose-dense chemotherapy improves both disease-free and overall survival compared to chemotherapy every 3 weeks, with the largest magnitude of benefit seen in patients with node-positive disease.

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None

Conflicts of interest

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Ethical approval

The present work did not involve any experiments with humans and approval from ethics committee was not necessary.

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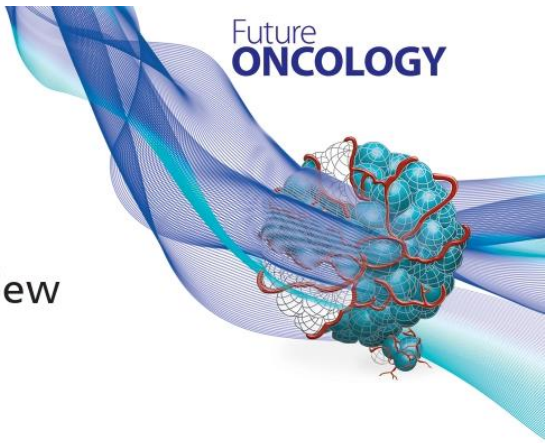
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Supplementary Paper III: *Mammaprint™: a comprehensive review*

Review

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Mammaprint™: a comprehensive review

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The number of breast cancer (BC) cases is growing worldwide, being most frequently diagnosed in the early-setting. Mammaprint™ is a 70-gene-expression signature, originally designed for selecting early BC patients with low risk of developing metastasis, so that they could be spared adjuvant chemotherapy. Its use as a prognostic biomarker has been extensively validated, both retrospectively and prospectively. However, its value as a predictive tool and as a clinically useful tool remains controversial. This review will describe how the test works, its application in the clinic and its limitations. Cost–effectiveness studies will be summarized. Finally, we will provide a perspective on the use of Mammaprint in the near future, as a valuable tool for personalizing the treatment of early BC patients.

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Keywords: adjuvant chemotherapy • breast neoplasms • gene-expression signatures

Breast cancer (BC) is diagnosed every year in approximately 1.2 million women worldwide, and is, in industrialized societies, most often localized (early BC) and curable [1]. Curative treatment of BC is based on surgical removal of the primary tumor, commonly followed by radiotherapy and, for tumors considered to be at high risk of relapse, by adjuvant chemotherapy [2]. Advances in adjuvant chemotherapy have significantly improved outcomes for women with early BC [3], but at the cost of important short- and long-term consequences for both patients and society [4,5]. Therefore, in the last 15 years, an intense debate has developed on the most appropriate means of defining which patients are at a sufficiently high risk of relapse to justify the use of chemotherapy [2].

Traditionally, a host of tumor and patient characteristics are taken into consideration to determine whether a patient is at high risk of relapse. These essentially phenotypical tumor characteristics are used to infer disease behavior. However, disease behavior is also driven by alterations occurring at the molecular level [6]. Advances in genome sequencing in the 2000s have led to multiple initiatives to go beyond disease phenotype into the genomic, transcriptomic and proteomic tumor landscapes, ultimately revealing the existence, under the overarching umbrella of BC, of multiple distinct pathologic entities [7]. This new molecular way to conceptualize BC, as well as the self-evident limitations of traditional risk assessment methods, gave birth to a number of attempts to create tools capable of providing a glimpse into disease biology to aid decision making – the gene-expression signatures (GES) [8].

Today, a number of GES measured on primary tumor tissue have been developed and are in current clinical use, holding the possibility of sparing a substantial proportion of patients not only from overtreatment but also from undertreatment. However, the expense related to performing GES and the absence, until recently, of prospective validation of their clinical utility have continued to fuel the debate surrounding their use.

Mammaprint™ was the first GES approved for use by the US FDA and was the first one to have its clinical utility validated prospectively as decision-making tool when results are discordant with classic criteria in a prospective randomized trial (MINDACT) [9,10]. This article will provide a thorough and critical review of Mammaprint, including its preclinical development, the studies which form the basis of its use in the clinic as well as how it compares to other available GES and its future prospects.

How was the signature developed?

Mammaprint was initially developed in archived fresh-frozen samples from a set of 78 young BC patients with tumor size less than 5 cm and negative lymph nodes (LN), not treated with adjuvant systemic therapy (Supplementary Table 1 & Figure 1). In this group, around half remained free of distant metastasis at five years [9]. The aim

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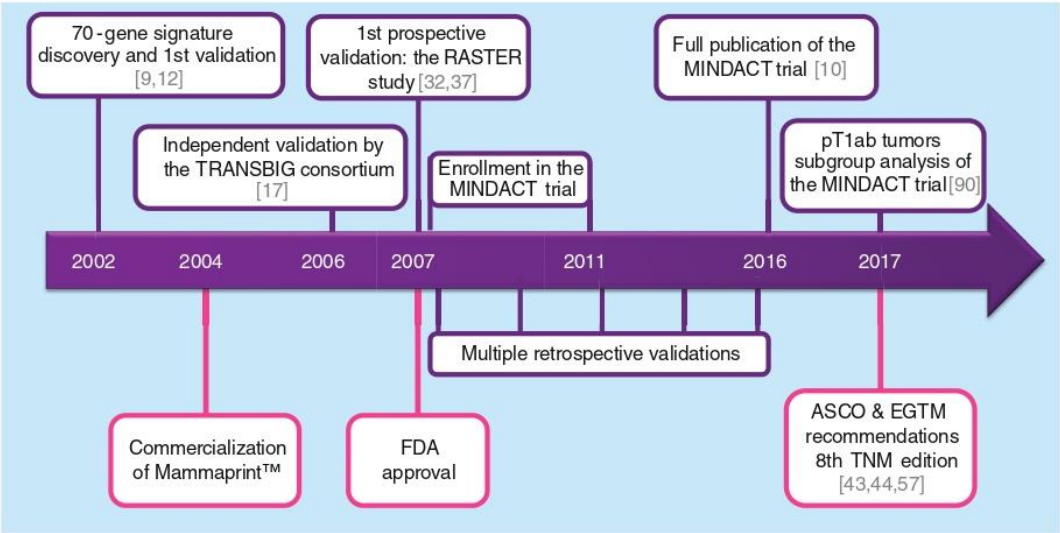


Figure 1. Clinical development of Mammaprint™, from 2002 to 2017.
ASCO: American Society of Clinical Oncology; EGTM: European Group on Tumor Marker; TNM: American Joint Committee on Cancer 8th Edition Cancer Staging Manual – Breast Cancer.

of the researchers was to develop a gene-signature that could differentiate the subgroup of patients with a low risk for distant metastasis from those experiencing metastasis in the first years after surgery. From the initial approximately 5000 genes tested by supervised analysis, a signature was created with 70 genes that best correlated with prognosis. After adjusting for classical prognostic factors, a multivariate model showed that the signature was a strong prognostic factor: patients with a 'poor-prognosis signature' had an odds ratio (OR) of 18 (95% CI: 3.3–94) of developing distant metastasis within five years compared with the ones with the 'good-prognosis' signature. The 70 genes included pertain predominantly to one of the six classical hallmarks of cancer (Figure 2) [6], largely covering proliferation, resistance to apoptosis, invasion and dissemination.

Today, the signature is marketed by Agendia under the name of Mammaprint. The test was originally developed on fresh-frozen tissue, which hampered its practical use by oncologists as well as its validation on biobanked tumor samples of clinical trials with a long follow-up, but today it can be performed in formalin-fixed paraffin-embedded tissue, with equivalent analytical validity [35].

Historical perspective on the development of Mammaprint

Early validation studies

Following initial development, most efforts consisted in retrospective validation series and pooled analysis, assessing the value of the gene-signature in different patient populations and settings [11–20,28–34] (Supplementary Table 1 & Figure 1). Patients who had neoadjuvant therapy were excluded from all these studies and as 'distant metastasis-free survival' (DMFS) was the end point used to develop the 70-gene signature, it remained the main end point of most studies. Taken together, the results of all these studies shed light not only on the potential of the test but also on important aspects of BC biology.

High prognostic value of Mammaprint for predicting distant relapse

It has long been recognized that BC has the ability to metastasize to distant sites even at an early point of tumor development. Even in small tumors (≤2 cm), Mammaprint can predict which patients have a higher risk of developing distant metastasis: 28% of 'high-genomic risk' patients by the gene-signature had a distant relapse at 10 years, compared with 13% of the 'low-genomic risk' patients (adjusted hazard ratio [HR]: 2.43; 95% CI: 1.6–3.8) [30]. This effect remained even in the very small pT1ab tumors (HR: 3.45; 95% CI: 1.0–11.5), thus further confirming the ability of Mammaprint to capture this intrinsic metastatic potential.

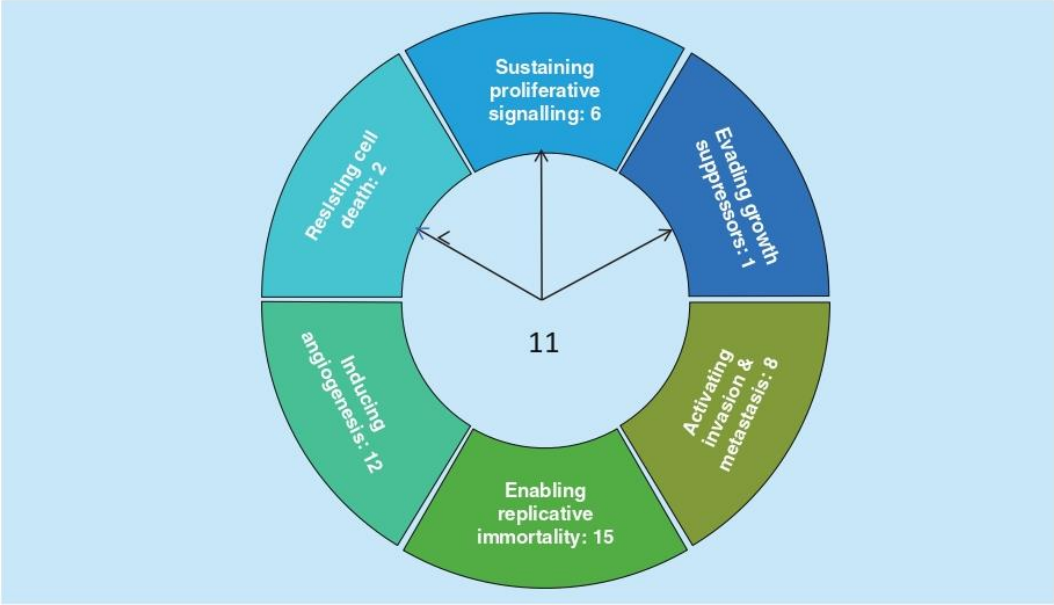


Figure 2. Hallmarks of cancer and number of genes in Mammaprint™. Mammaprint is composed by 70 genes, most of them associated with one or more hallmark. Sustaining proliferative signaling: *FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1*; Evading growth suppressors: *TGFB3, FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1; Activating invasion and metastasis: COL4A2, GPR180, MMP9, GPR126, RTN4RL1, DIAPH3, CDC42BPA, PALM2*; Enabling replicative immortality: *CCNE2, ECT2, CENPA, LIN9, KNTC2, MCM6, NUSAP1, ORC6L, TSPYL5, RUNDC1, PRC1, RFC4, RECQL5, CDCA7, DIL*; Inducing angiogenesis: *ALDH4A1, AYTL2, OXCT1, PEC1, GMPS, GSTM3, SLC2A3, FLT1, FGF18, COL4A2, GPR180, EGLN1, MMP9*; Resisting cell death: *BBC3, EGLN1, FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1*. 11 genes pertain to three hallmarks at the same time (indicated by arrows). 16 genes relate to other or unknown functions: *LOC100288906, C9orf30, ZNF533, C16orf61, SERF1A, C20orf46, LOC730018, LOC100131053, AA555029_RC, LGP2, NMU, UCHL5, JHDM1D, AP2B1, MS4A7, RAB6B*.

LN status & value of Mammaprint

LN status [11] and, in particular, the number of positive LN [20] were not correlated with a gene-signature category, indicating that dissemination to distant sites (hematogenous spread) is distinct from dissemination to LN (lymphogenic spread). LN-positive patients (between one and three positive nodes) with a low-genomic risk had a lower risk of distant metastasis (DMFS of 98% at five years and 91% at ten years), compared with high-genomic risk patients (DMFS of 80% at 5 years and 76% at 10 years) [14]. These survival data are derived from patients treated between 1984 and 1996, of whom only 18% received endocrine treatment (ET) and none received chemotherapy; therefore, the DMFS rate of low-genomic risk patients was considered favorable, indicating that this patient subgroup could possibly also be spared chemotherapy [11,12,14]. However, in patients with four to nine positive LN [20], the low-genomic risk group had a DMFS at five years of 87% and at ten years of 67%, which do not justify withholding chemotherapy, demonstrating that tumor burden is still an important prognostic factor [14].

Combination with clinical risk indexes

Clinical risk indexes like the St. Gallen [36], Adjuvant! Online (AOL) [37,38] and others, are commonly used in clinical practice to predict DMFS or overall survival (OS) and, consequently, to select patients for adjuvant therapy. However, when adjusting these clinical risk indexes for the gene-signature, most of them did not retain an independent association with DMFS or OS [13], hinting that classic clinical risk factors are mainly a reflection of the intrinsic genetic properties of the tumor. Still, when combining clinical risk factors with the gene-signature in a multivariate model for the detection of distant metastasis, the area under the curve (AUC) value increased from 0.70 (95% CI: 0.61–0.79) to 0.76 (95% CI: 0.68–0.85) and the AUC for OS increased from 0.74 (95% CI: 0.66–0.82) to 0.82 (95% CI: 0.75–0.89) [13]. Furthermore, multiple studies have demonstrated that patients

classified as having a high-clinical risk but a low-genomic risk had a similar survival compared with patients with low-clinical/low-genomic risk [15,17,22,31]. Moreover, Mammaprint presented a very high negative-predictive value for the development of distant metastasis at 5 years (over 85%) [16,18], thus confirming that a patient presenting with a low-genomic risk has a very low probability of developing distant metastasis as an early event. In Supplementary Table 1, there is a description of the rate of discordance between the gene-signature and the clinical-risk assessment in most studies, specifying in brackets the percentage of patients classified as high-clinical risk/low-genomic risk that could eventually avoid chemotherapy.

Postmenopausal patients

Is Mammaprint, initially developed in young BC patients, also useful in older, exclusively postmenopausal populations? In one series [16], only 27% of patients had a low-genomic risk, and 10-year DMFS was good both in low- and high-genomic risk groups: 100 versus 90%, respectively. However, in another series [17], 61% of patients had a low-genomic risk and it was demonstrated that Mammaprint was strongly prognostic; low-genomic risk patients had a 5-year DMFS of 93% compared with 72% in high-genomic risk patients (HR: 4.6; 95% CI: 1.8–12.0). This was further confirmed in pooled analyses in which more than half of the patients were postmenopausal [28,33,34].

Prognostic ability over time

In the updated analysis of the first validation series, after a median follow-up of 18.5 years, the HR for DMFS was 9.6 (95% CI: 4.2–22.1) in the first 5 years, but it was only 1.1 (95% CI: 0.5–2.5) in years 5–10 and 1.2 (95% CI: 0.2–6.0) in years 10–15 [12]. A similar trend was observed for OS. Therefore, the prognostic ability of Mammaprint may be reduced over time, being higher in the first five years after diagnosis and then declining. Nonetheless, the low number of long-term events could have contributed to these numbers. As the EBCTCG meta-analysis [3] has shown that the beneficial effect of chemotherapy mostly happens in the first five years after diagnosis, this does not diminish its value as an adjuvant treatment decision making tool. However, at least one study suggests that Mammaprint can identify an ultralow-risk population (score $\geq$0.355) with a breast cancer-specific survival (BCSS) rate between 94 and 97% at 20 years [21]. A meta-analysis showed that proliferation is the main component of the prognostic performance of Mammaprint and other GES [39] – in an analysis of the TransATAC study, GES based on cell proliferation showed a good performance for the prediction of late relapses, thus one can speculate that Mammaprint may have the same ability [40].

The RASTER trial

All of the aforementioned studies were retrospective and based on data from patients with different follow-up times, receiving a variable amount of adjuvant therapy, who are not representative of the modern BC patient populations, in which almost all women with estrogen receptor (ER)-positive tumors receive ET [41]. Therefore, a prospective study was still required to validate Mammaprint, but some logistical problems needed to be addressed first. The RASTER study [23] evaluated the feasibility of performing the gene-signature using fresh-frozen tissue in community hospitals in The Netherlands as well as how it changed physicians' prescription of systemic therapy. Among evaluable patients, almost half had high-genomic risk, but only 43% had high-clinical risk according to the Dutch guidelines [42]. Interestingly, due to the fact that physicians' prescription of systemic treatment was based on the Dutch guidelines, the use of Mammaprint actually resulted in an increase in the number of patients receiving chemotherapy and ET. Nevertheless, the RASTER study proved that Mammaprint is a feasible test to use in community hospitals [23]. The updated follow-up shows that among low-genomic risk patients, 5-year DMFS is identical whether or not AOL indicates a low- (94.3%) or a high-clinical risk (97.6%) [22].

The MINDACT trial

The MINDACT trial enrolled 6693 women with up to three positive LN, in nine European countries, with the goal of proving the clinical utility of adding Mammaprint to standard clinical–pathological criteria, for an improved selection of patients for adjuvant chemotherapy [10]. More specifically, the trial aimed to reduce the prescription of adjuvant chemotherapy by using the Mammaprint test, without impairing outcome. Modified AOL was used to determine high- and low-clinical risk (Figure 3) and patients were classified into four groups according to their clinical and genomic-risk: low-clinical/low-genomic, high-clinical/high-genomic, high-clinical/low-genomic and low-clinical/high-genomic. Randomization between chemotherapy versus no-chemotherapy only occurred in the discordant groups (high-clinical/low-genomic and low-clinical/high-genomic). The main end point was to prove



Figure 3. Definition of 'low-clinical risk' in the MINDACT trial for estrogen-receptor positive tumors.
LN: Lymph node; T: Breast tumor size.

that among the cohort of patients with high-clinical/low-genomic risk who did not receive chemotherapy, the lower boundary of the 95% CI for the rate of 5-year DMFS would be 92% or higher. As such, the MINDACT trial should be viewed as a single-arm 'non-inferiority' study, as it was clearly not powered to perform direct statistical comparisons between high-clinical/low-genomic risk patients who received versus who did not receive chemotherapy (these comparisons were secondary end points).

The results have shown that the primary end point was met: the 5-year DMFS in the primary-test population (high-clinical/low-genomic risk patients assigned not to receive chemotherapy and excluding the ones with a change in risk) was 94.7% with a 95% CI ranging from 92.5 to 96.2%, thus not crossing the noninferiority lower boundary (Table 1). Additionally, in the high-clinical/low-genomic intention-to-treat (ITT) population, patients who received chemotherapy had a 5-year DMFS rate of 95.9%, whereas those without chemotherapy had a 5-year DMFS rate of 94.4% – this absolute difference of 1.5% corresponded to a nonsignificant HR of 0.78 (95% CI: 0.50–1.21). As the study was not powered to make a direct comparison of outcomes between these groups, it is not strictly possible to state that their 5-year DMFS are not different. However, the magnitude of difference (1.5%) may not be viewed by many patients as valuable enough to justify the chemotherapy-associated side effects and long-term risks. On the other hand, patients with low-clinical/high-genomic risk had very similar 5-year DMFS rates whether they received chemotherapy or not (95.8 vs 95.0%, respectively), with a HR of 1.17 (95% CI: 0.59–2.28). Therefore, in patients with low-clinical risk, the clinical utility of using Mammaprint could not be demonstrated.

The authors calculated that among the high-clinical risk group, the use of Mammaprint would lead to a reduction in the use of chemotherapy in 46% of these patients (the ones with low-genomic risk), corresponding to 23% of the total population. Finally, the authors assessed the rate of 5-year DMFS if only clinical-risk or genomic-risk scores would be applied to define which patients should receive chemotherapy and the results were strikingly similar: 95.0% with clinical-risk evaluation and 94.7% with genomic-risk assessment. Thus, the added value of Mammaprint is that with its use as an aid to treatment selection, the 5-year DMFS is maintained, but an additional 23% of the overall population will not receive chemotherapy, which may decrease toxicities and costs.

Regarding 5-year disease-free survival (DFS) and OS, there were no differences between the chemotherapy versus no-chemotherapy groups within risk-discordant patients in the ITT population. However, in the per-protocol population, 5-year DFS was slightly worse in the high-clinical/low-genomic risk group without chemotherapy

Table 1. Results of the MINDACT prospective trial.

Number of patients and FU duration	Characteristics	5-year DMFS in each subgroup	5-year DMFS HR (95% CI)	5-year OS HR (95% CI)	5-year DFS (95% CI)	5-year DMFI (95% CI)
N = 6693 total High-C[†]/low-G[‡]: 23% Low-C[†]/high-G[‡]: 9% Low-C[†]/low-G[‡]: 41% High-C[†]/high-G[‡]: 27% Primary-test population: n = 644 ITT population[¶]: n = 2207 PPS population[¶]: n = 1706 Median FU: 5.0 years	≤70 years (67% were ≥50 years; median 55 years); pT1–3 N0–1 (21% N1); 88% ER^{+}[§] and/or PgR^{+}[§]; 10% HER2^{+} Hormone-R^{+}/HER2^{-}: 81% HER2^{+}/Hormone-R^{+}: 8% HER2^{+}/Hormone-R^{-}: 2% TNBC: 10%	Low-C/low-G: 97.6% High-C/low-G: 95.1% Low-C/high-G: 94.8% High-C/high-G: 90.6%	Primary-test population (high-C/low-G, no CT): 94.7% (92.5–96.2). High-C/low-G ITT population: with CT: 95.9% (94.0–97.2) vs no CT: 94.4% (92.3–95.9) – Δ 1.5%. HR: 0.78 (0.5–1.2) High-C/low-G PPS population: with CT: 96.7% (94.7–98.0) vs no CT: 94.8% (92.6–96.3) – Δ 1.9%. HR: 0.65 (0.38–1.10)	High-C/low-G ITT population: with CT: 98.4% (97.0–99.1) vs no CT: 97.0% (95.4–98.1) – Δ 1.4%. HR: 0.69 (0.4–1.4) High-C/low-G PPS population: with CT: 98.8% (97.4–99.5) vs no CT: 97.3% (95.6–98.4) – Δ 1.5%. HR: 0.63 (0.29–1.37)	High-C/low-G ITT population: with CT: 92.9% (90.5–94.7) vs no CT: 90.1% (87.5–92.1) – Δ 2.8%. HR: 0.71 (0.5–1.01) High-C/low-G PPS population: with CT: 93.3% (90.7–95.2) vs no CT: 90.3% (87.6–92.4) – Δ 3%. HR: 0.64 (0.43–0.95)	High-C/low-G ITT population: with CT: 96.6% (94.8–97.8) vs no CT: 95.3% (93.4–96.6) – Δ 1.3%. HR: 0.76 (0.5–1.2)
High-C[†]/low-G[‡] risk, only N0 patients (n = 812)			High-C/low-G ITT population: with CT: 95.7% (93.0–97.4) vs no CT: 93.2% (90.1–95.4) – Δ 2.5% ER^{+}/HER2^{-} patients in the ITT population (n = 676): with CT: 95.5% (92.5–97.3) vs no CT: 93.9% (90.6–96.1) – Δ 1.6%. HR: 0.80 (0.4–1.5)	NR	NR	NR
Only pT1abN0 patients (n = 826) [43] High-C[†]/low-G[‡]: 0.6% Low-C[†]/high-G[‡]: 24% Low-C[†]/low-G[‡]: 76% High-C[†]/high-G[‡]: 0%	≤70 years (38% were ≥60 years), 64% postmenopausal; 91% ER^{+}; 6% HER2^{+}; Hormone-R^{+}/HER2^{-}: 85% (59% Luminal A; 26.5% Luminal B) HER2^{+}/Hormone-R^{+}: 5% HER2^{+}/Hormone-R^{-}: 1% TNBC: 5%	NR	Low-C/high-G with CT: 97.3% (89.4–99.3) vs no CT: 91.4% (82.6–95.9) – Δ 5.9%	Low-C/high-G with CT: 98.5% (89.6–99.8) vs no CT: 95.8% (89.1–98.4) – Δ 2.7%	Low-C/high-G with CT: 92.3% (83.5–96.5) vs no CT: 84.5% (74.5–90.8) – Δ 7.8%	Low-C/high-G with CT: 98.8% (91.9–99.8) vs no CT: 91.4% (82.6–95.9) – Δ 7.4%

[†]Clinical risk group: as defined by the Adjuvant! Online (version 8.0 with HER2 status) – patients were considered as having high-clinical risk when the 10-year breast cancer-specific survival was predicted to be less than 88% for ER-positive tumors, and less than 92% for ER-negative tumors, respectively.
[‡]Genomic risk group: as defined by the 70-gene-expression signature (Mammaprint™)
[§]Hormone receptor positivity was defined as the presence of at least 1% of immunoreactive cells, an Allred score of more than 2 (on a scale from 0 to 8, with higher scores indicating a greater number of receptors), or a level of cytosolic protein of at least 10 fmol per milligram.
[¶]Includes both high-clinical/low-genomic risk patients and low-clinical/high-genomic risk patients.
Prospective trial: MINDACT [10] (2016). Hazard ratios compare the chemotherapy group versus the no-chemotherapy group (which is the reference).
CT: Chemotherapy; DFS: Disease-free survival (time from surgery to any recurrence [local or regional], distant metastasis, ductal carcinoma *in situ*, second breast primary, invasive second primary cancer or death from any cause); DMFI: Distant metastasis-free interval (time from surgery until the diagnosis of a distant recurrence); DMFS: Distant metastasis-free interval (time from surgery until the diagnosis of a distant metastasis or death by any cause); ER: Estrogen receptor; FU: Follow-up; Hormone-R: Hormone receptor; HR: Hazard ratio; ITT: Intention-to-treat; N0: Patients with lymph-node negative tumor; NR: Not reported; OS: Overall survival (time from surgery until death from any cause); PgR: Progesterone receptor; PPS: Per-protocol population; TNBC: Triple-negative breast cancer.

compared with patients who received chemotherapy: 90.3 versus 93.3%, respectively (HR: 0.64; 95% CI: 0.43–0.95; p = 0.03). Additionally, among patients with ER-positive/HER2-negative/LN-negative disease, the 5-year DMFS was similar between the chemotherapy and no-chemotherapy groups: 95.5 versus 93.9%, respectively (HR: 0.80; 95% CI: 0.44–1.45).

Problems & controversies regarding the test & the MINDACT trial

Technical challenges

There are several technical challenges regarding the use of Mammaprint. As proliferation rate is a continuous variable, there must be a trade-off in each cut-point used between the amount of patients spared of adjuvant systemic treatment and the proportion who may relapse in the future. It must be emphasized that the cut-point used for risk-classification applies to western populations and usually splits the population in half in terms of

risk (Supplementary Table 1); this cut-point is probably inadequate for Asian populations, as more than 80% are classified as high-genomic risk [18,19]. Furthermore, other cut-points can be used to classify patients as ultralow-risk [21] or as ultrahigh-risk [44,45], but these have been less extensively studied.

In the MINDACT trial, 162 patients were falsely identified as being high-genomic risk due to a change in RNA-extraction solution, showing that technical issues can interfere with the reliability of the test [10].

MINDACT design

Definition of the primary end point

A major criticism to the MINDACT trial is that the primary end point is based in a nonrandomized cohort of patients: high-clinical risk/low-genomic risk patients not receiving chemotherapy. Besides, a formal noninferiority design was not pursued, as it would lead to a much higher sample size, which was not feasible in terms of cost and time. However, treatment de-escalation in oncology would be impossible if we would always require a 'non-inferiority' design, due to its associated high costs and low interest from commercial companies to fund it. Therefore, other de-escalation studies have used this nonrandomized approach and their results were welcomed by both physicians and patients. An example is the single-arm APT trial, which tested the use of chemotherapy without anthracyclines plus trastuzumab for one year in 410 patients with small (≤2 cm) HER2-positive tumors [46]. Invasive DFS rate at only three years was 98.7% (95% CI: 97.6–99.8) and this leads to a practice-changing de-escalation strategy in these patients, sparing them from the long-term effects of anthracyclines. Updated 7-year results confirmed the initial favorable prognosis [47]. Therefore, this regimen was endorsed by scientific guidelines, without the need for evaluating the potential harm associated with anthracyclines removal in a randomized fashion. Likewise, Mammaprint was also endorsed by scientific societies [48,49], even if the secondary end point shows that there might be a potential small gain in DMFS/DFS with the prescription of chemotherapy, as it was recognized that this small gain would be offset by the chemotherapy-associated side effects and risks.

Definition of clinical risk

The TRANSBIG consortium chose AOL to define clinical risk, as it was a validated, standardized assessment, freely available to any clinician and based on large clinical databases [50]. The TRANSBIG consortium, with the involvement of EUROPA DONNA, agreed that a 92% chance of survival at ten years without chemotherapy was a desirable goal, as any benefit from chemotherapy beyond this threshold would be of small magnitude and counter-balanced by its risks (namely premature menopause, cognitive dysfunction, cardiac damage and leukemia [4,51]). As adjuvant ET for women with ER-positive disease provides an estimated 4% survival gain at ten years, the threshold for this group was set at 88%.

However, AOL did not include the levels of ER, PgR and Ki67 positivity to stratify Luminal A versus Luminal B tumors. Additionally, it might no longer be accessible [38]: however, the statisticians of MINDACT have provided a table that defines clinical risk as obtained with the use of AOL during the conduct of MINDACT [10].

Definition of the DMFS lower boundary

In noninferiority trials, the definition of a threshold for what is considered 'not clinically significant' is always a matter of subjectivity and debate. Therefore, when the MINDACT trial was designed, a large discussion was set within the TRBANSBIG consortium to define the 5-year DMFS lower boundary for MINDACT [52]. As explained above, it was decided that a 5-year DMFS ≥92% would be considered acceptable. However, this definition should be regarded as a trade-off in terms of the proportion of patients who have a distant relapse versus those who are safely spared from chemotherapy. Therefore, this compromise cannot be considered as an 'applies-to-all' decision and should be explained to the patient before ordering Mammaprint.

Potential chemotherapy benefit in 5-year survival rates

The MINDACT trial was not powered to show differences in survival according to the prescription of chemotherapy. However, it is true that in the high-clinical risk/low-genomic risk ITT and per-protocol populations, the HR for 5-year DMFS, OS and DFS was always below 1.0, favoring chemotherapy, including in ER-positive/HER2-negative LN-negative patients (Table 1). A reason may be that 8% of these patients had HER2-positive tumors and, probably, they were undertreated with ET alone. Another explanation for this possible positive effect of chemotherapy in low-proliferative tumors may reside in its indirect endocrine effects: 35% of the high-clinical/low-genomic risk patients were ≤50 years, likely being premenopausal, and 98% were hormone receptor-positive. Thus, chemotherapy

might have induced at least a temporary ovarian function suppression in two-thirds of these young patients and, as the SOFT trial has shown, ovarian suppression can lead to small improvements in 5-year DFS and DMFS when added to tamoxifen, but OS gain was only seen in the high-risk women who also got chemotherapy [53]. The TAILORx trial randomized patients with ER-positive/HER2-negative LN-negative tumors and an Oncotype DX® intermediate recurrence score (11–25) to ET versus chemotherapy + ET: in the ITT population, there were no survival differences between the two groups; however, in the subgroup of women ≤50 years and with a recurrence score of 16–25, there was an invasive DFS benefit with chemotherapy + ET versus ET alone, which may be attributed to a premature menopause induced by chemotherapy. Yet, it is important to keep in mind that these findings come from a nonpreplanned subgroup analysis and should not be considered as definitive [54].

Long-term survival

The recently published European Network for Health Technology Assessment report expresses the concern that the possible decrease in DMFS at five years could widen in the next five years and result in a substantial harm in terms of 10-year OS [55]. However, this increase in distant recurrences in the no-chemotherapy arm versus the chemotherapy arm seems highly unlikely: the EBCTCG meta-analysis involving 100,000 patients showed that the effect of chemotherapy is mainly observed during the first five years after treatment and that after this initial period the survival curves do not further separate [3]. Thus, it seems fair to assume that 10-year results from MINDACT will show similar rates of recurrence events in both trial arms. Moreover, it is not possible to statistically 'model' a prediction for the 10-year OS, as the population included in this trial is a new highly selected population defined by a diagnostic test not available in the past.

Additionally, in the overall population, DFS events corresponded mostly to second primary cancers (42%), followed by distant metastases (36%), locoregional recurrences (16%) or deaths due to any cause (6%). As most second primary cancers and locoregional recurrences can still be treated with curative intent, it is unlikely that substantial harm in OS will emerge in the no-chemotherapy arm.

Quality of life

Earlier studies stated that the reduction on the rate of prescription of chemotherapy among the high-clinical/low-genomic risk group could translate into reduced toxicity and thus better quality of life (QoL), but no formal evaluation of QoL was performed in the MINDACT trial [10]. The reason is that it was perceived by both authors and patient advocates that it would be too burdensome for the patients and additionally, at the time, there was no validated QoL instrument [56]. While the utility of using Mammaprint in terms of QoL cannot be stated, common clinical sense would indicate that for the 23% of patients who are spared the side effects of chemotherapy, there is a QoL benefit extending over a few months or even a few years in case of severe cognitive dysfunction, sleep disturbances and fatigue [51,57,58].

Predictive ability

Kok *et al.* [27] evaluated the ability of Mammaprint to predict which patients benefit the most from adjuvant treatment with tamoxifen. However, in these ER-positive patients receiving adjuvant tamoxifen, being 'incomplete-endocrine responsive' (either ER or progesterone receptor [PgR] between 0 and 49%, with at least ER >10%) was more predictive of a worse BCSS (HR: 7.22; 95% CI: 2.17–24.0) than having a high-genomic risk (HR; 2.78; 95% CI: 1.3–5.9). After adjustment for ER/PgR positivity and other clinical factors, Mammaprint was no longer significantly associated with BCSS (HR: 1.8; 95% CI: 0.77–4.61).

The predictive ability of Mammaprint for the benefit of adjuvant chemotherapy has not been prospectively demonstrated, as the MINDACT trial was not powered to directly compare outcomes between chemotherapy versus no-chemotherapy in the clinical/genomic-risk discordant groups [10]. This is a limitation, as the greatest utility of a genomic testing is to guide decisions regarding the use of chemotherapy and, for that, it should be able to predict which patients will benefit from chemotherapy or not. This means that, for now, Mammaprint's ability to select patients for chemotherapy is based solely on its prognostic capabilities.

Use in HER2-positive or TNBC patients

In a retrospective pooled analysis in HER2-positive disease, among 89 patients, all chemotherapy and trastuzumab-naive, 22% were classified as low-genomic risk, which is an intriguing observation as HER2-positive BC is considered an aggressive disease [29]. Low-genomic risk patients had a 10-year DMFS of 84% in the absence of chemotherapy

and trastuzumab, while high-genomic risk patients had a worse outcome (10-year DMFS of 55%). However, in the MINDACT trial, HER2-positive patients constituted only 8% of the high-clinical/low-genomic risk population (n = 124) and a separate analysis was not performed in these patients. Thus, the value of Mammaprint in this population is uncertain [10].

Likewise, the usefulness of the gene-signature is probably very low or absent in triple-negative patients, as 90–96% of them were classified as high-genomic risk in the MINDACT trial [10] and in earlier validation series [20,31,33]. This is in agreement with data from a large meta-analysis of GES, in which most of the HER2-positive and triple-negative tumors were classified as high-risk, therefore limiting its clinical utility in these patients. Another comprehensive meta-analysis using both clinicopathologic and gene-expression data and focusing on the main molecular subtypes showed that among ER-positive/HER2-negative tumors, proliferation-related genes were indeed the strongest prognostic parameter; however, in the triple-negative subset, the most important prognostic genes were the ones related with immune response and in the HER2-positive group, tumor invasion-related genes were the more relevant ones [59].

Use of Mammaprint in clinical trials

Mammaprint is being used in clinical trials, most notably in the I-SPY2 neoadjuvant trial (NCT01042379). Here, trial population is categorized into eight subtypes defined by three biomarkers: hormone receptors (ER/PgR), HER2 and Mammaprint. The gene-signature was chosen as a biomarker, as it was previously shown that a high-genomic risk was associated with higher neoadjuvant chemosensitivity [24]. For this trial, patients traditionally classified as high-genomic risk by Mammaprint were further divided in two categories: high-risk1 (MP1) and (ultra)high-risk2 (MP2) based in a higher cut-point [44]. The MINT I study also demonstrated that patients classified as MP2 had a greater sensitivity to neoadjuvant chemotherapy, as their pCR rate was 44% compared with a pCR rate of 24% in MP1 patients [45].

Practical application in the clinic

Mammaprint was the first GES approved by the FDA, in 2007. Following the publication of MINDACT, both the American Society of Clinical Oncology and the European Group on Tumour Markers published updated guidelines recommending the use of Mammaprint [48,49]. The National Comprehensive Cancer Network still favors the use of Oncotype DX, though it recognizes Mammaprint™ as a valid alternative [41]. Consensus opinion is, today, that a low-genomic risk Mammaprint result can be used in patients with ER-positive/HER2-negative early BC, with up to three positives nodes, considered as having a high-clinical risk of relapse according to traditional criteria, to safely forego the use of adjuvant chemotherapy. Additionally, Mammaprint was incorporated in the 8th TNM edition for the classification of BC, along other GES, as a way of downstaging selected ER-positive/HER2-negative LN-negative tumors with low-genomic risk into the same prognostic category as T1a-T1bN0M0 [60]. It should, nonetheless, be discussed with the patient that there might be a small loss in DMFS and DFS with the avoidance of chemotherapy in this setting. A practical approach to the application of Mammaprint in the clinic for patients with ER-positive/HER2-negative tumors is provided in Figure 4.

As Mammaprint's prognostic performance is driven by genes related to proliferation [39], an open question is if Mammaprint can be replaced by a simpler and cheaper method of evaluating cell proliferation, like nuclear grade or the Ki67 score. A study tested the Genomic Grade (a GES) in 883 tumor samples of patients enrolled in the BIG 1–98 trial and showed that among the 60% of patients who were centrally classified as nuclear grade 2, the ones classified as GG1 (low proliferative) by the Genomic Grade had a 10-year DMFS of 99%, compared with 85% for patients with GG3 (high proliferative) [61]. Thus, this molecular assessment showed to accurately define prognosis in this large proportion of patients with an intermediate nuclear grade. Regarding Ki67 positivity, it is a proven prognostic factor both in LN-negative and LN-positive early BC patients, being associated with worse DFS and OS [62]. However, its assessment is still highly variable among pathologists and no standard cut-point has been defined, making it application in clinic troublesome [63]. This was recognized by the American Society of Clinical Oncology that does not endorse the use of Ki67 for adjuvant treatment decision [49]. Even so, some guidelines state that Ki67 may be used to distinguish between Luminal A-like and Luminal B-like subtypes and recommend the use of adjuvant chemotherapy in patients with ER-positive tumors and a high Ki67 [64,65]. However, a MINDACT substudy comparing the classification of tumors by a central pathology lab using modern immunohistochemistry according to the 2013 St Gallen guidelines [66] to a molecular type classification based on Mammaprint and BluePrint™ showed provocative results [67]. In this analysis of 5806 tumor samples, 34% of tumors were classified

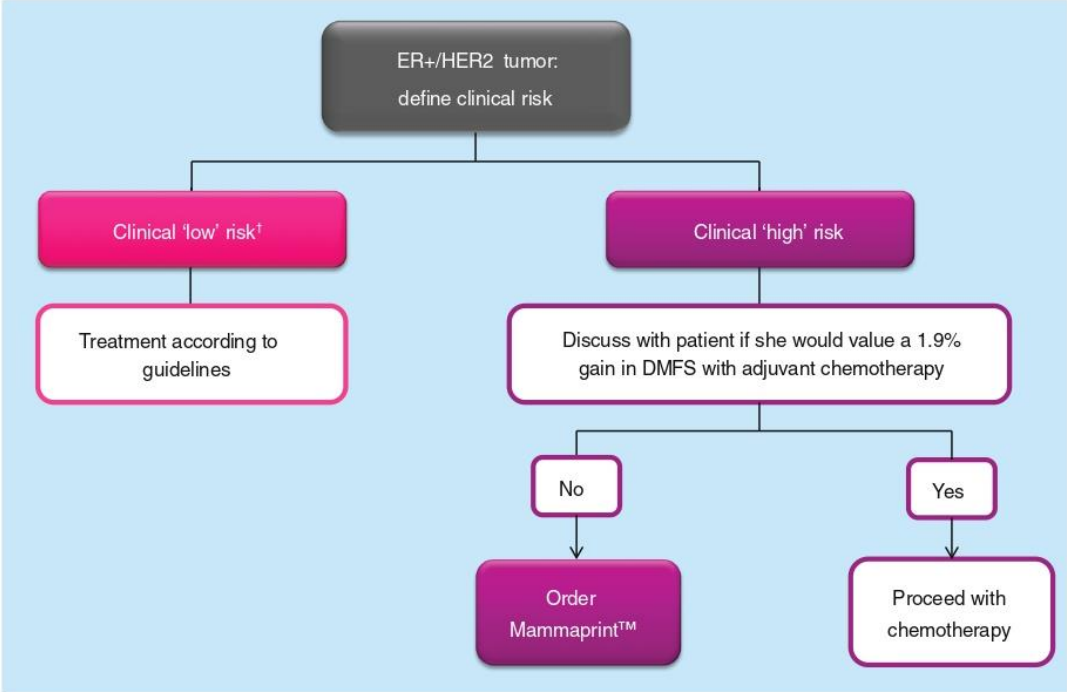


Figure 4. Practical use of Mammaprint™ in the clinic for patients with estrogen receptor-positive/HER2-negative tumors, by immunohistochemistry. With this approach, around half of the patients for which Mammaprint is ordered will be classified as low-genomic risk and could be spared chemotherapy.
†The definition of low-clinical risk used in the MINDACT trial is depicted in Figure 3.
DMFS: Distant metastasis-free survival.

Table 2. How to take Ki67 into account for the decision to order a Mammaprint™ test?

1. Assess histological grade and Ki67 score in ER-positive tumors	
• Grade & Ki67 seem to be 'concordant' – Grade 1 & Ki67 ≤10% – Grade 3 & Ki67 ≥30%	• Grade and Ki67 seem to be 'discordant' – Grade 1 & Ki67 >10% – Grade 3 & Ki67 <30%
	• Grade 2, any Ki67
2. Application in the clinic	
Consider as 'reliable information' on tumor biology and proceed as outlined in Figure 4.	Consider as 'unreliable information' → order Mammaprin™, unless the change in Grade would not impact risk as outlined in Figure 3.

The used cut-points for the Ki67 score are merely indicative; different cut-points can be used according to local pathology guidelines and practice.
ER: Estrogen receptor.

as Luminal B-like, but when applying the molecular subtyping, half of these tumors were reclassified as Luminal A (for which chemotherapy is usually not recommended). However, both classifications performed very well in predicting a DMFS rate ≥96% for the Luminal A tumors: the only difference is that the molecular classification leads to a 16% increase in tumors classified as 'Luminal A.' In the future, automated scoring of Ki67 may overcome the reproducibility issue and lead to the integration of Ki67 score in models that predict risk of BC relapse [68]. Our proposal would be to combine information regarding nuclear grade and Ki67 score, ordering a Mammaprint test for grade 2 tumors or in cases in which grade and Ki67 seem to be discordant (Table 2).

Cost–effectiveness of Mammaprint

Despite its utility, Mammaprint carries logistic and cost issues. Even if it can now be performed in formalin-fixed paraffin-embedded tissue, it is still a centralized test to be performed by Agendia, meaning that hospitals have to ship their samples to The Netherlands or to the USA, increasing the turnaround time for obtaining results. The

company is currently working to decentralize the test, creating a limited number of accredited labs across Europe. There is also an added cost to the regular pathological assessment, as the performance of the test costs, by itself, 2675€ in France [69], 3000€ in Germany [70], £2207 in the UK [71] and US$4200 in the USA [72].

Still, its utility in clinical practice was demonstrated in a pooled analysis in which the rate of prescription of chemotherapy among 790 patients decreased from 52 to 43% after performing Mammaprint [73]. Likewise, in an analysis involving more than 2000 patients, the use of Mammaprint was associated with a 10% decrease in the use of chemotherapy [74]. In the prospective WSG PRIME study [75], however, among 452 patients, there was a net increase in the use of chemotherapy of 1.9%, due to the fact that patients with low-clinical risk who were initially advised not to receive chemotherapy, were then advised to receive chemotherapy based on a high-genomic risk result, which is a strategy not proven to be beneficial in the MINDACT trial. Therefore, if this study was conducted today, probably these patients would not receive chemotherapy and the use of Mammaprint would then be associated with a reduction of 13% in the chemotherapy prescription rate. Currently, the IMPACt prospective registry (NCT02670577) is assessing the influence of Mammaprint on chemotherapy plus ET versus ET alone treatment decisions in 481 ER-positive/HER2-negative patients.

Analyses have usually shown that Mammaprint is a cost-effective test [73,76]. In the UK, the use of Mammaprint compared with standard care leads to a gain of 0.18 quality-adjusted life years (QALYs), at a cost of £1097/QALY gained [71]. In an US analysis, Oncotype DX and Mammaprint were directly compared and the results showed that Mammaprint is the most cost-effective test [77]. Another US analysis compared Mammaprint to AOL: its use spared 10% of patients from chemotherapy, with an increase of 0.15 QALYs, at a cost of US$700 to US$10,000/QALY, depending on the model used, but being cost-effective in every scenario [78]. In Spain, the use of Mammaprint compared with AOL or Oncotype DX leads to a gain in QALYs between 0.23 and 0.75, with a cost of €1457/QALY gained [79]. In contrast, a French analysis compared Mammaprint with AOL and with a chemotherapy-for-all strategy and concluded that the use of AOL would be cost-effective, but the use of Mammaprint or of chemotherapy-for-all would not [69]. However, it is important to mention that certain specificities of the French healthcare system and practice reduce the relevance of these results for other countries [72,80].

These analyses were mostly based on retrospective series and applied Mammaprint to a nonselected population. This 'blind' approach spares chemotherapy in about only a fifth of patients, corresponding to the high-clinical risk/low-genomic risk population. However, if these analyses applied the stepwise procedure recommended in the MINDACT trial of first assessing clinical risk and then ordering Mammaprint only to the high-clinical risk patients, the test would be requested to just half of the potential patients and would spare chemotherapy in half of these tested patients (the ones classified as high-clinical/low-genomic risk). Therefore, this stepwise approach would probably even further improve Mammaprint's cost–effectiveness. By contrast, in TAILORx, Oncotype DX was applied to every patient who had an indication to chemotherapy according to the National Comprehensive Cancer Network guidelines. Even after this preselection, 70% of the patients presented a low-clinical risk according to the MINDACT definition and, thus, could have been spared chemotherapy even before being tested with Oncotype DX; this suggests that for a majority of patients' traditional criteria can still determine management in the adjuvant setting [54].

Comparison with other GES

A large number of additional GES are currently available for use in clinical practice. These GES used different methods for assessing gene expression, as well as different sets of genes, generating the possibility of different performances. Though currently no data points toward better clinical outcomes for patients with one particular GES over another, several studies have compared the classification of patients according to risk by different GES with Mammaprint, when applied to the same set of samples. Table 3 summarizes the principal characteristics of the most widely used GES.

An early attempt to compare GES was made by Fan *et al.*, who tested five different GES (PAM50, Mammaprint, Oncotype DX, the 2-gene ratio component of the Breast Cancer Index and wound response) on 295 samples. Results show all tests except the 2-gene ratio predicted outcomes similarly, with a high correlation of results between patients, with concordance between Oncotype DX and Mammaprint being as high as 81% [81]. However, this was an *in silico* comparison and it was not based on the commercial GES assays, which could in part explain why these results contrast with more recent studies such as the larger OPTIMA prelim trial.

In OPTIMA prelim, 313 patients with ER-positive/HER2-negative early BC with tumors ≥3 cm and/or 1–9 positive LN were randomized between standard (chemotherapy + ET) or test-directed treatment (chemotherapy

Table 3. Available gene-expression signatures and characteristics.

Characteristics	Gene-expression signatures					
	Mammaprint™	Oncotype DX®	Prosigna® (PAM50)	Endopredict®	Breast Cancer Index	Genomic Grade
Number of genes	70	21	50	11	7	97
Method	DNA-microarray	RT-PCR	Nanostring	RT-PCR	RT-PCR	RT-PCR
Sample type	Frozen/FFPE	FFPE	FFPE	FFPE	FFPE	FFPE
Location	Central†	Central	Local	Local	Central	Central
Test results	High or low risk + subtype	High/intermediate/low risk	High/intermediate/low risk + subtype	High or low risk	High or low risk/high or low benefit	High or low risk
Clinical indication by EGTM	Predicting prognosis and guiding decision-making regarding chemotherapy for women with $ER^+/HER2^-$ EBC with LN^- or LN^+ (1–3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with $ER^+/HER2^-$ EBC with LN^- or LN^+ (1–3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with $ER^+/HER2^-$ EBC with LN^- or LN^+ (1–3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with $ER^+/HER2^-$ EBC with LN^- or LN^+ (1–3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with $ER^+/HER2^-$ EBC with LN^-	No recommendation
Prospective validation	MINDACT (positive)	TAILORx (for N0; positive)/RxPONDER (for N1; ongoing)	OPTIMA trial (ongoing)	None	None	ASTER70 (ongoing)
Agency approval	EMA, FDA	EMA, FDA	EMA, FDA	EMA, FDA	Not approved	Not approved
Original validation set	Developed in young patients (<55 years), who had not received systemic therapy following surgery	Developed in patients who had received tamoxifen only in the NSABP B-20 and B-14 studies	Postmenopausal patients in the training and develoment sets received heterogeneous treatment	Developed in postmenopausal patients, who had received ET only in the ABCSG-6 and 8 trials	Initially developed in patients treated with tamoxifen only and then further refined in heterogenously treated patients	Developed in a heterogeneous patient population, 62% aged ≤50 years, in order to permit the measurement of histologic grade via gene-expression profile
Guidelines/scientific societies that recommend the test	ASCO, EGTM, ESMO, St Gallen Panel	ASCO, EGTM, ESMO, NCCN, St Gallen Panel	ASCO, EGTM, ESMO, St Gallen Panel	ASCO, EGTM, ESMO, St Gallen Panel	ASCO, EGTM, St Gallen Panel	No recommendation

†Descentralization is ongoing to allow for local testing.
ASCO: American Society of Clinical Oncology; EBC: Early breast cancer; EGTM: European Group on Tumour Markers; ER: Estrogen receptor; ESMO: European Society for Medical Oncology; ET: Endocrine therapy; FFPE: Formalin-fixed paraffin-embedded tissue; LN: lymph node; N0: Lymph node-negative; N1: 1–3 positive lymph nodes; NCCN: National Comprehensive Cancer Network; RT-PCR: Reverse transcription polymerase chain reaction.

only if Oncotype DX recurrence score >25), with stratification by a number of other GES, including Mammaprint and Prosigna® (PAM50). Results show a significant degree of discordance between different GES, with 61% of samples having no consensus across all performed tests. Mammaprint categorized 40% patients as high-risk, while Oncotype DX only classified 18% as high-risk [71,82]. In another study, comparing Mammaprint to the 76-gene signature and Genomic Grade in 198 patients, 68% of patients were classified similarly and all three signatures added information to classic criteria and performed similarly in predicting clinical outcomes [83]. Yet, there were around 30% of patients who were assigned to different risk groups according to the test used, potentially leading to different treatment strategies, which raise important questions regarding the use of tests that have not been validated in prospective clinical trials. It is also important to note that three other large retrospective studies comparing GES, but not including Mammaprint, have concluded that Prosigna better predicted patient prognosis, including, most significantly, in terms of long-term follow-up [84–86]. The most recent of these was conducted as a preplanned comparative study in the TransATAC trial [40]. Tissue samples from women with ER-positive/HER2-negative either LN-negative (n = 591) or LN-positive with a maximum of three positives nodes (n = 183) were submitted to testing with six different prognostic prediction tools – four GES (Breast Cancer Index, Endopredict®, Prosigna, Oncotype DX) and two scores based on clinical, pathologic and imunohistochemical data (the clinical treatment score and the 4-marker immunohistochemical score). The long median follow-up available for the sample (over ten years) and mature survival data allowed for a comparative analysis of the performance of all six tools in both LN-negative and LN-positive cohorts in the 0–5 years and, critically, the 5–10-year follow-up (in which approximately 50% of the relapses occurred). The results of this study confirm that all six tools provided accurate prognostic information for LN-negative patients, especially for early relapses (<5 years). For late relapses, Breast cancer Index, Prosigna and Endopredict performed better. For LN-positive patients, however, tools integrating tumor burden

(Breast Cancer Index® and Endopredict) performed better, but the overall performance was less striking than in the node-negative subgroup. These data underscore the added value of combining both traditional criteria and GES, showing that, ideally, when determining the type and duration of adjuvant treatment, both clinical and genetic tumor factors should be taken into account to perform a comprehensive assessment of the patient's risk of relapse, leading to a truly tailored treatment.

Future perspective

Over the last 15 years, Mammaprint has proved to be a powerful prognostic tool for early BC patients, especially for ER-positive/HER2-negative with up to three positive LN. However, the first validation series showed that its ability for predicting relapse may drop after the first five years from diagnosis [12], leading to the question if this is an useful tool for the selection of patients to adjuvant treatments other than chemotherapy. Indeed, as we are still waiting for the long-term data from the MINDACT trial, an important open research question is if Mammaprint can be used for selecting the best duration of adjuvant ET. A positive hint came from the retrospective analysis of a Swedish study, in which patients with ultralow-risk tumors as defined by Mammaprint had an extremely good survival even without adjuvant ET [21]. If these findings are validated in MINDACT, this could lead to the use of Mammaprint to select patients for extended adjuvant ET.

Earlier validation studies included patients with all types of BC. However, our understanding of BC biology has evolved significantly over these past years and it is now known that the different subtypes of BC have very distinct biological and clinical behaviors [7]. Indeed, the above-mentioned meta-analysis [39,59] have shown that GES mostly based on proliferation markers are probably not useful in the triple-negative or HER2-positive group of patients as they usually have high rates of proliferation. Therefore, Mammaprint as a predictive tool for adjuvant systematic treatment should be probably used only for the ER-positive/HER2-negative subgroup of patients with high-clinical risk, as it was demonstrated in the MINDACT trial that patients with tumors with low-proliferation markers (nuclear grade 1) are usually low-clinical risk and therefore do not benefit from the use of Mammaprint [10].

On the other hand, for the neoadjuvant setting, Mammaprint is the only GES that has some data suggesting a role as predictor of benefit with neoadjuvant treatment, including in high-proliferating tumors, both with chemotherapy [24–26,45] and with the poly(ADP-ribose) polymerase (PARP) inhibitor veliparib [44], especially when a higher cut-point is used to separate high-risk from ultrahigh-risk patients. So, in the future, it is expected that Mammaprint will be increasingly used as a selection/stratifying factor in neoadjuvant trials with ER-positive/HER2-negative and/or triple-negative patients; it may also be combined with other biomarkers, like DNA-repair deficiency biomarkers, to increase its predictive ability [87].

The IMPAKT Working Group 2012 recommended the creation of registries for patients who are subjected to genomic testing in the daily clinical practice [88]. An example is the observational 'NBRST' registry (NCT01479101), including patients receiving neoadjuvant treatment after having been tested by BluePrint™/Mammaprint. It confirmed that tumors with a high-genomic risk were more chemosensitive than low-genomic risk tumors in a real-world setting [26]. However, similar registries would also be useful in other regions of the world, like Asia, in which the prognostic and clinical utility of Mammaprint has not been proven yet.

Meanwhile, new genomic profiles using DNA microarray platforms or whole genome arrays will probably be developed to explore other markers that could lead to different types of prediction (of response to treatment or of early or late relapse) in a single assay, which could be added to the Mammaprint genomic-risk [89]. Another option would be to combine a baseline tissue-based Mammaprint genomic-risk with the analysis of baseline and postneoadjuvant treatment circulating tumor-DNA to identify patients at higher risk of early relapse [90].

In conclusion, the clinical utility of Mammaprint in early BC patients has been prospectively validated by the MINDACT trial, which showed that patients with a high-clinical risk but with a low-genomic risk could be safely spared chemotherapy. Long-term survival data from MINDACT are awaited. Nevertheless, in the era of precision medicine, Mammaprint has already proven to be a useful tool for the tailoring of adjuvant chemotherapy, potentially sparing up to one in five patients from chemotherapy, with an expected decrease in toxicity and improvement of QoL.

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Executive summary

- How was the gene-expression signature (GES) developed?
  - GES were developed to define which early breast cancer patients benefit from the use of adjuvant chemotherapy, as it leads to important short- and long-term consequences for both patient and society.
  - Mammaprint™ was developed in 2002 as a 70-gene signature with the ability of distinguishing patients with high risk of distant metastasis at 5 years ('high-genomic risk') from those with a low risk ('low-genomic risk').
- Problems and controversies regarding the test and the MINDACT trial.
  - The MINDACT trial was not powered to directly compare outcomes between patients receiving chemotherapy versus no-chemotherapy, therefore the predictive ability of Mammaprint for selecting patients who would benefit the most from chemotherapy could not be proven.
  - Its prognostic ability in the adjuvant setting for HER-positive and triple-negative patients has been questioned, as most of these patients are classified as 'high-genomic risk'.
- Practical application in the clinic.
  - Mammaprint is the first GES to have its clinical utility validated in a prospective randomized trial (the MINDACT trial) as a decision-making tool when genomic-risk results are discordant with clinical criteria.
  - Mammaprint is approved by many health authorities throughout the world, based on the results of extensive studies on its analytical validity, clinical validity, and more recently, clinical utility in a prospective setting (the MINDACT trial).
  - Mammaprint was incorporated in the 8th TNM edition for the classification of breast cancer, along other GES, as a way of downstaging selected estrogen receptor-positive/HER2-negative node-negative tumors with low-genomic risk into the same prognostic category as T1a-T1bN0M0.
- Use of Mammaprint in clinical trials.
  - In the neoadjuvant setting, a higher cut-point separates patients with high-risk from ultrahigh-risk, which predicts the sensitivity to chemotherapy, even in high proliferation tumors like HER2-positive and triple-negative and lead to the use of Mammaprint as a stratification factor in the neoadjuvant I-SPY2 trial.
- Cost–effectiveness of Mammaprint.
  - Cost–effectiveness analyses have mostly shown that Mammaprint is likely to be a cost-effective test.
- Precision medicine.
  - In the era of precision medicine, Mammaprint has already proven to be an important tool for the tailoring of adjuvant chemotherapy, potentially sparing an important proportion of patients from aggressive treatments and leading to a reduction in toxicities and costs, with an expected improvement in the quality of life of early breast cancer patients.

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Supplementary Paper IV: *Patient-reported outcomes and genomic signatures: tools to tailor adjuvant endocrine treatment?*

Editorial

Patient-reported outcomes and genomic signatures: tools to tailor adjuvant endocrine treatment?

Patients with estrogen-receptor positive (ER+) breast cancer continue to be at risk of recurrence even 20 years after diagnosis, despite 5 years of adjuvant endocrine therapy (ET) [1]. The type of endocrine agent(s) prescribed and the duration are chosen according to clinicopathological factors, like menopausal status, tumor size, and lymph node status. Moreover, comorbidities and patient's preferences, based on patient perception of treatment benefit/risk ratio, are also essential in choosing optimal therapy.

The two studies [2, 3] published in this issue of *Annals of Oncology* bring new and relevant data that might further help patients and physicians in their decisions. Within the prospective, observational CANTO study, Ferreira et al. compared symptoms and quality-of-life (QoL) scores between patients who received adjuvant ET (with/without chemotherapy) with those who received adjuvant chemotherapy (with/without ET). They showed that the negative effects of chemotherapy are transient and at 2 years, chemotherapy had no detrimental effect on general QoL, although it was still associated with negative effects in some functions and symptoms. Nonetheless, what was remarkable was the persistent negative impact of ET on general QoL, as well as on many other functions and symptoms. However, it should be noted that many of the observed differences, even if they reached statistical significance, were relatively small in nature (difference <5 points). Thus, on the overall population, only the increase in insomnia for ET and the worse body image score for the CT group would be considered by most as clinically relevant [4]. Yet, the latter may be confounded by the higher incidence of mastectomy surgeries.

Data from the IBCSG VIII trial showed similar findings in premenopausal women [5] and the Mind-body Study indicated that the resolution of some chemotherapy-related symptoms may be delayed by the use of ET [6]. Table 1 provides a summary of data on the impact of ET on QoL.

Patients who experience a decrease in their QoL due to ET will be less motivated to continue adjuvant ET. Therefore, an accurate assessment of the risk of relapse beyond 5 years is critical to help physicians and patients make informed decisions. The ASCO and St. Gallen guidelines recently endorsed extended ET (up to 10 years of treatment) for all patients with node-positive BC [7, 8]. Nevertheless, the majority of women with node-positive BC who complete 5 years of ET remain free of distant recurrence, hence extended ET for all node-positive BC patients may lead to overtreatment for many [1]. Regarding node-negative patients, both guidelines recommend considering extended ET according to the patient's recurrence risk using established prognostic factors. A possible useful tool for risk assessment is the 'clinical treatment score post 5 years' (CTS5), which integrates tumor size, number of positive nodes, histologic grade, and age, which has prognostic value for late recurrence on women who are disease-free after 5 years of ET [9]. But could genomic assays help us refine the selection of women at risk of recurrence after 5 years? A comparative analysis of six prognostic signatures, including the Breast Cancer Index (BCI), showed that for women with node-positive breast cancer, genomic assays provided limited independent prognostic information beyond CTS5 [10]. The use of these genomic assays to determine which patients should receive extended ET is not endorsed by ASCO [11], while the St. Gallen consensus recommends it, but only for stage 2 node-negative patients [7].

Given the fact that extended ET leads to only a modest benefit in disease-free survival, with no demonstrated improvement on overall survival [12], coupled with increased toxicities and health costs, it is paramount to identify who are the patients for whom extended ET is not beneficial.

So far, BCI is the only assay tested in patients enrolled in extended adjuvant ET trials, first in a nested case–control study within the MA.17 trial [13] and now in node-positive patients enrolled in the aTTOm trial [3]. In the latter, about half of the patients were classified as BCI (H/I)-high and experienced a 10.2% absolute risk reduction of relapse with the extended use of tamoxifen. On the other hand, BCI (H/I)-low patients experienced no benefit from extended ET. Nonetheless, most postmenopausal women, especially those with node-positive disease, receive aromatase inhibitors (AI) for at least a part of the first 5 years of ET. For those patients, the benefit from extended adjuvant ET is considerably smaller compared with the benefit observed in patients who received 5 years of tamoxifen [8]. Thus, it would be important to test the prognostic and predictive value of BCI on trials assessing extended ET after previous AI use. Moreover, if the CDK4/6 inhibitors currently being tested in ongoing adjuvant trials are approved, the role of genomic signatures, including BCI, to predict benefit from extended adjuvant ET needs to be further reevaluated.

So, what can we conclude from these two studies? First, it was demonstrated in the largest modern observational cohort of BC patients that ET *per se* has a small yet significant adverse impact on QoL, which persists at least up to 2 years after diagnosis. This can inform discussions between oncologists and patients regarding ET's *pros* and *cons* and raise awareness regarding the need to perform a close monitoring of the impact of ET on patients' QoL and of developing strategies to minimize this impact. Second, the data from the Trans-aTTOm study and the previously reported translational MA.17 sub-study [13] provide evidence for using BCI to select those node-positive BC postmenopausal patients who need extended adjuvant ET after 5 years of tamoxifen.

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Table 1. Clinical trials and observational cohorts describing patient reported outcomes on the impact of adjuvant endocrine treatment on quality of life

Study (year)	Sample size[a]	Population	Timepoints of QoL evaluation	Regimens compared	QoL instrument(s)	PRO results
Clinical trials						
ATAC (2006) [15]	682	ER+ EBC, post-menopausal	Baseline, 3 and 6 months, and every 6 months thereafter until 5 years	Tamoxifen versus anastrozole for 5 years	FACT-Breast (FACT-B) plus endocrine symptoms subscale (ES)	At 5 years of FU: diarrhea (anastrozole 3.1% versus tamoxifen 1.3%), vaginal dryness (18.5% versus 9.1%), diminished libido (34.0% versus 26.1%), and dyspareunia (17.3% versus 8.1%) were significantly more frequent with anastrozole compared with tamoxifen. Anastrozole and tamoxifen had similar impacts on health-related QoL, which was maintained or slightly improved during the treatment period for both groups
IES (2006)[b] [16]	582	ER+ EBC, post-menopausal, after 2–3 years of tamoxifen	Baseline, 3, 6, 9, 12, 18, 24, 30, 36, 48, and 60 months	Continue tamoxifen until 5 years versus switch to exemestane	FACT-B plus ES	At 2 years of FU: prevalence of severe endocrine symptoms at trial entry was high for vasomotor complaints and sexual problems, which persisted for both groups during the study
IBCSG VIII (2007) [5]	874	ER+ EBC, node-negative, pre-menopausal	Baseline, 3, 6, 9, 12, 18, 24, and 36 months	CMF x 6 cycles versus goserelin x 24 months versus CMF x 6 cycles → goserelin x 18 months	International Breast Cancer Study Group (IBCSG) QoL core questionnaire	At 3 years of FU: overall, patients receiving goserelin alone showed a marked improvement or less deterioration in QoL measures over the first 6 months than those patients treated with CMF. There were no differences at 3 years after random assignment according to treatment except for hot flashes.
NSABP B-33 (2008)[b] [17]	454	ER+ cT1-3N1M0, post-menopausal, after 5 years of tamoxifen	Baseline and every 6 months for 5.5 years	Exemestane versus placebo for 5 years	MENQOL	At 2 years of FU: exemestane presented numerically higher symptom severity on all four scales, but no significant treatment effects in the vasomotor, psychosocial, physical, or sexual scales.
SOFT + TEXT (2015) [18]	4096	ER+ EBC, pre-menopausal; CT use was optional	Baseline, every 6 months for 24 months, and annually during years 3-6	Tamoxifen + OFS versus exemestane + OFS for 5 years	IBCSG QoL core questionnaire and a trial-specific module	At 5 years of FU: tamoxifen + OFS: more hot flushes and sweats over 5 years versus exemestane + OFS. Exemestane + OFS: more vaginal dryness, greater loss of sexual interest, and difficulties becoming aroused, more bone or joint pain versus tamoxifen + OFS. Differences between tamoxifen + OFS versus exemestane + OFS persisted over

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Table 1. *Continued*

Study (year)	Sample size[a]	Population	Timepoints of QoL evaluation	Regimens compared	QoL instrument(s)	PRO results
						time and most symptoms never got back to baseline values No differences between the CT and no CT cohorts in terms of mean scores and effect of ET.
SOFT (2016) [19]	1722	ER+ EBC, pre-menopausal; CT use was optional	Baseline, every 6 months for 24 months, and annually during years 3-6	Tamoxifen + OFS versus tamoxifen alone for 5 years	IBCSG QoL core questionnaire and a trial-specific module	At 5 years of FU: Tamoxifen + OFS: worse endocrine symptoms and sexual functioning during the first 2 years of treatment versus tamoxifen alone. Differences at 6-months in symptom-specific QoL, treatment burden, and coping effort between treatment groups were less pronounced for patients with prior CT.
MA.17R (2018)[b] [20]	1428	ER+ EBC, post-menopausal, after 5 years of AI therapy (alone or preceded by tamoxifen)	Baseline, 12, 24, 26, 48, and 60 months	(Extended) Letrozole versus placebo for 5 years	SF-36 (overall QoL), MENQOL	At 5 years of FU: no difference between the two arms on global QoL, physical, mental component, bodily pain, vasomotor domain, sexual. Worse with letrozole: role function-physical (but clinically not significant). Worse with placebo: role function-emotional.
SOLE (2018)[b] [21]	955	ER+ EBC, node-positive, post-menopausal, after 4-6 years of ET	Baseline, 6, 12, 18, and 24 months	Continuous (for 5 years) or intermittent letrozole (9 months + 3-month break in years 1–4 and then all 12 months of year 5)	BCPT Symptom Scale and 9 further symptom-specific and global QoL Indicators	At 12 months of FU: small differences, but less worsening in QoL with intermittent letrozole (versus continuous letrozole): in vaginal problems, musculoskeletal pain, sleep disturbance, physical well-being, and mood At 24 months of FU: intermittent letrozole: greater improvement in hot flushes. Changes in QoL end points did not differ significantly (24% discontinuation rate in both treatment arms).
TAILORx (2019) [22]	496	ER+/HER2- EBC, with OncoType DX RS 11-25, both pre- and post-menopausal	Baseline, 12, and 36 months	ET versus CT + ET	FACT-G and FACT-Cognitive function v3 (FACT-Cog)	FACT-Cog: worse in CT + ET versus ET at 3 and 6 months. Health-related QoL was comparable between ET and CT+ET at 12- and 36-months. Post-menopausal women under CT + ET had greater endocrine symptoms at 24 and 36 months.

Continued

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Table 1. *Continued*

Study (year)	Sample size[a]	Population	Timepoints of QoL evaluation	Regimens compared	QoL instrument(s)	PRO results
Placebo-controlled prevention trials						
MAP.3 (2014) [23]	4468	Healthy post-menopausal women	Baseline, 6 months, and yearly thereafter	Exemestane versus placebo for 5 years	SF-36, MENQOL	Exemestane had small negative effects on women's self-reported vasomotor symptoms, sexual symptoms, and pain, which occurred mainly in the first 6 months to 2 years after random assignment.
Observational cohorts						
Mind-body study (2016) [6]	186	EBC; 54% post-menopausal; 51% received CT; 66% received ET	Baseline (after primary treatment, prior to initiation of ET), at 6 and 12 months	3 groups: no ET, AI, or tamoxifen	SF-36 (physical component [PCS] and mental component scales [MCS]); BCPT symptom scale; Beck Depression Inventory II (BDI-II); Multidimensional Fatigue Symptom Inventory (MFSI); Pittsburgh Sleep Quality Index (PSQI)	No-ET group: either stable or declining symptom severity, whereas the ET groups often showed increased severity over time, and the AI group reported more severe musculoskeletal, hot flash, and cognitive problems at one or both of the FU time points compared with the no-ET group. Tamoxifen group: higher levels of hot flashes, cognitive problems, and bladder problems versus the no-ET group. Significantly reduced physical health-related QoL at the end of primary therapy compared with population norms, which improved significantly in the subsequent 12 months but which was significantly poorer in the AI group at 12 months, with a suggestion of plateau in recovery compared with the tamoxifen and no-ET groups.
CANTO (2019) [2]	4262	EBC; 62.8% post-menopausal; 52.8% received CT; 81.9% received ET	Diagnosis, end of primary treatment, (completion of surgery, CT or RT, whatever ended last) and at 2 years	4 groups: no CT nor ET; ET only; CT only; CT + ET	EORTC QLQ-C30 and BR23 questionnaires	At 2 years of FU: persistent QoL deterioration, with different trajectories by treatment received. ET, but not CT, had a major detrimental impact on C30-Summary Score, especially in postmenopausal women.

[a]Number of patients undergoing QoL assessments.
[b]Trials assessing extended endocrine treatment.
AI, aromatase inhibitor; BCPT, Breast Cancer Prevention Trial; CMF, cyclophosphamide, methotrexate, and fluorouracil chemotherapy regimen; CT, chemotherapy; DCIS, ductal carcinoma in situ; EBC, early breast cancer; ET, endocrine therapy; ER, estrogen receptor; FACT, Functional Assessment of Cancer Therapy; FU, follow-up; MENQOL, menopausal specific quality of life; OFS, ovarian function suppression; PRO, patient-reported outcomes; QoL, quality of life; RS, Recurrence Scores; RT: radiotherapy.

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However, the value of BCI or other genomic signatures to select patients for extended ET post-5 years of AI has not been convincingly documented.

Recently, studies using circulating tumor cells and circulating tumor DNA have suggested that these biomarkers might help identify patients at high risk of disease relapse who could potentially benefit from either extended adjuvant ET or newer targeted therapies [14]. The role of tumor genomic and liquid assays beyond standard clinicopathological characteristics for tailoring adjuvant ET clearly needs further evaluation. Finally, an effective monitoring of ET's side-effects will be an increasingly important component for such treatment choices.

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Supplementary Paper V: *Endocrine therapy-based treatments in hormone receptor-positive/HER2-negative advanced breast cancer: systematic review and network meta-analysis*

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Endocrine therapy-based treatments in hormone receptor-positive/HER2-negative advanced breast cancer: systematic review and network meta-analysis

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MD and ML are joint senior authors.

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ABSTRACT

Background Several endocrine therapy (ET)-based treatments are available for patients with advanced breast cancer. We assessed the efficacy of different ET-based treatments in patients with hormone receptor-positive/HER2-negative advanced breast cancer with endocrine-sensitive or endocrine-resistant disease.

Methods We searched Medline and Cochrane Central Register of Controlled Trials up to 15 October 2019 and abstracts from major conferences from 2016 to October 2019. We included phase II/III randomised trials, comparing ≥2 ET-based treatments. Progression-free survival (PFS) and overall survival (OS) were analysed by network meta-analyses using MTC Bayesian models based on both fixed-effect and random-effect models; relative treatment effects were measured as HRs and 95% credibility intervals (CrI). All statistical tests were two-sided. Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed and this systematic review is registered in the PROSPERO database.

Results 55 publications reporting on 32 trials (n=12 293 patients) were included. Regarding PFS in the endocrine sensitive setting (n=5200; 12 trials), the combination of cyclin-dependent kinases (CDK)4/6-inhibitors (CDK4/6i)+fulvestrant 500 mg (F500) was likely the most effective treatment (surface under the cumulative ranking curve (SUCRA)=97.3%), followed by CDK4/6i+aromatase inhibitor ±goserelin; there was no significant difference between them (HR 0.82; 95% CrI 0.54–1.25). Regarding OS (n=2157; five trials), the most effective treatment was probably CDK4/6i+F500 (SUCRA=97.3%); comparing CDK4/6i+F500 versus F500 held a HR of 0.77 (95% CrI 0.63–0.95). Regarding PFS in the endocrine-resistant setting (n=6635; 20 trials), CDK4/6i+F500 was likely the most effective treatment (SUCRA=95.7%), followed by capivasertib+F500, without significant difference between them (HR 0.91; 95% CrI 0.60–1.36). For OS (n=4377; 11 trials), the most effective treatments were capivasertib+F500 (SUCRA=84.7%) and CDK4/6i+F500 (SUCRA=69.9%). Comparing CDK4/6i+F500 versus F500 held a HR of 0.77 (95% CrI 0.67–0.89).

Conclusions CDK4/6i+F500 is likely the best treatment option in both endocrine-sensitive and endocrine-resistant diseases for PFS, and in endocrine-sensitive patients for OS. Concerning OS in endocrine-resistant patients, capivasertib+F500 and CDK4/6i+F500 are likely the best treatments.

PROSPERO registration number CRD42018104628.

Key questions

What is already known about this subject?

► A considerable research effort has been made in the previous years to develop more targeted and effective treatments for patients with hormone receptor-positive/HER2-negative advanced breast cancer. It is now recognised that endocrine therapy (ET) represents the cornerstone of systemic treatment for these patients. The addition of new targeted agents to ET have further improved prognosis in these patients by delaying and/or reversing resistance to endocrine treatment. Nevertheless, most of these agents have never been directly compared in clinical trials.

What does this study add?

► Our data show that, in terms of progression-free survival, the combination of a cyclin-dependent kinases 4/6 inhibitor with fulvestrant 500 mg appears to be the best treatment option for both the endocrine-sensitive and endocrine-resistant populations. In addition, we have shown that this combination significantly increases overall survival in both settings, as compared with fulvestrant 500 mg. We also analysed specific subgroup of patients, such as patients with de novo metastatic, recurrent, visceral and bone-only disease.

How might this impact on clinical practice?

► In the absence of direct head-to-head comparisons for all regimens, our results may help guiding physicians and patients in the decision regarding the choice of regimen of ET with/without targeted agents for their advanced breast cancer.

INTRODUCTION

Advanced breast cancer includes both unresectable locally advanced and metastatic breast tumours and is usually considered

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an incurable disease.[1] More than 70% of breast cancers are hormone receptor-positive (HR+)/HER2-negative (HER2−).[2] In this setting, endocrine therapy (ET) is the cornerstone of systemic treatment, being the preferred frontline therapy for these patients even in the presence of visceral metastasis, unless visceral crisis is present.[1 3] Yet, despite the significant clinical benefit of ET, almost all patients eventually acquire endocrine resistance over the course of treatment.[1]

In recent years, a considerable amount of research has allowed to better understand, define and target the mechanisms of resistance to ET.[4] Many new targeted agents have emerged, in combination with ET, and are now available in daily clinical practice or are in the late stages of drug development.[1] Some of them target the cyclin-dependent kinases (CDK)4/6, which are involved in cell-cycle regulation; or the PI3K and mTOR enzymes, which are responsible for proliferation and survival, among other functions.[4]

Nonetheless, these new combinations have never been directly compared in clinical trials. Having pair-wise comparative data for all of these regimens would not be feasible considering the large number of possible head-to-head comparisons, and the substantial expenses associated with running such clinical trials. Furthermore, thanks to the growing sequential use of ET-based regimens in order to avoid chemotherapy, and the numerous available treatment options, clinicians face the problem of deciding which are the best ET-based regimens to be sequentially prescribed to these patients. In such scenario of multiple available options, a network meta-analysis offers a unique opportunity to summarise and rank the relative efficacy of the different ET-based treatments, which could improve the treatment decision-making process in daily clinical practice. With this network meta-analysis, we assessed the efficacy of different ET-based treatments in patients with HR+/HER2− advanced breast cancer, both with endocrine-sensitive and endocrine-resistant disease.

METHODS

Search strategy and selection criteria

Randomised phase II/III controlled trials including patients with HR+/HER2− advanced breast cancer, comparing at least one single-agent ET to any ET-based treatments were included. Definitions of endocrine-sensitivity and endocrine-resistance were as per the 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC4).[1] Endocrine-sensitive patients were defined as patients who never received ET in early breast cancer stage, or relapsing ≥12 months after completing adjuvant ET, or diagnosed with de novo stage IV breast cancer. Endocrine-resistant patients were defined as patients relapsing during adjuvant ET, or <12 months after its completion, or with progressive disease under ET for advanced breast cancer.

Primary endpoint was progression-free survival (PFS), defined as time from randomisation until progressive disease or death.[5] PFS was reported separately for patients with endocrine-sensitive and endocrine-resistant disease, without further molecular selection beyond HR+/HER2− status. Secondary endpoint was overall survival (OS), defined as time from randomisation until death from any cause. Pre-defined subgroup analyses for PFS benefit were: patients with de novo metastatic disease; recurrent disease; visceral disease; bone-only disease; and with specific somatic mutations (e.g., *PIK3CA*-mutated tumours).

We excluded studies without information on HER2 status; with patients with HER2+ tumours with no separate analysis for the cohort of patients with HR+/HER2− disease; with mixed endocrine-sensitive and endocrine-resistant populations for which a clear assignment was not possible; without data on PFS; or using chemotherapy as a comparator. Trial reports focusing solely on endpoints (eg, quality of life) or subgroup analyses that were not included in our protocol were also deemed not eligible.

A systematic search of the literature without language restrictions was performed up to 15 October 2019. Keywords like breast cancer and endocrine and targeted therapy drugs were combined in the search strategy (online supplementary methods), which was applied to Medline and adapted for use in the Cochrane Central Register of Controlled Trials. We also searched abstracts from major conferences (American Society of Clinical Oncology (ASCO) Annual Meeting, European Society for Medical Oncology Congress and San Antonio Breast Cancer Symposium) from 2016 to October 2019, in order to include unpublished trials.

This systematic review and network meta-analysis was based on the Cochrane Collaboration Handbook[6] and registered in the PROSPERO database. The Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for network meta-analysis guidelines was followed to report the present study.[7]

Data collection and assessment of risk of bias

Two reviewers (MB, CM) independently evaluated the screened titles and abstracts; in case of disagreement, a third author (ML) resolved it. Full papers were reviewed by five authors (MB, ML, CM, NP, MD). Multiple reports from the same trials were collated and data extraction was performed by one reviewer (MB) and verified by a second reviewer (AF). Disagreements were resolved by a third reviewer (ML).

We extracted trial name/identifier; first author; year of publication; study phase; endocrine-sensitive and/or endocrine-resistant disease population; median number of previous lines of ET and allowance of previous chemotherapy for advanced breast cancer; treatment arms; number of patients; estimates of HRs and their CIs for PFS and OS in the intention-to-treat population; and PFS parameters in the specified

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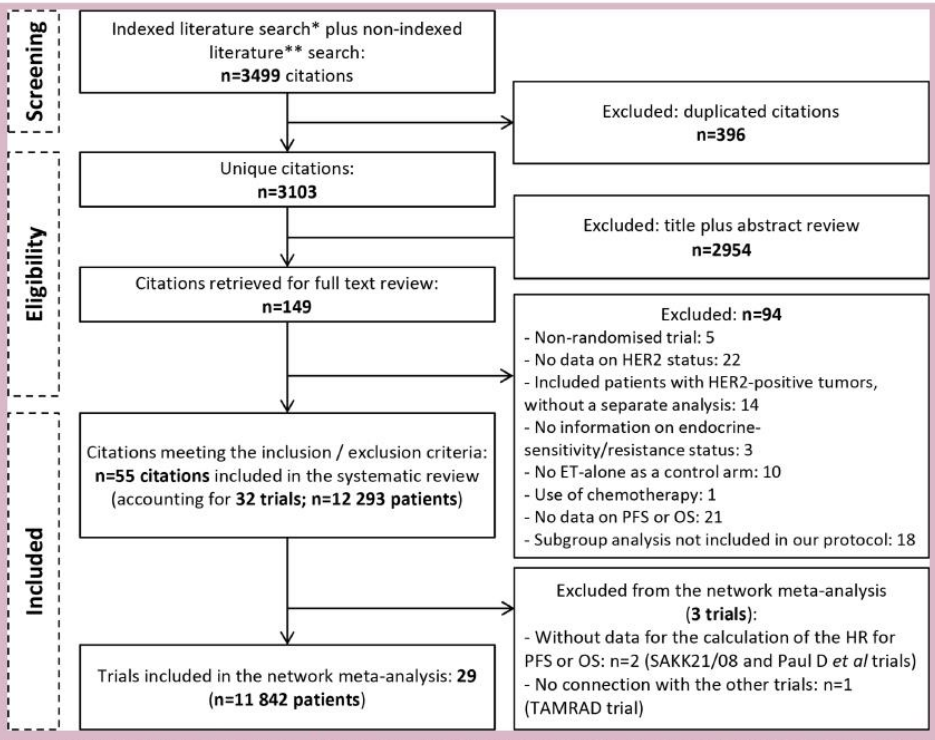


Figure 1 Adapted Preferred Reporting Items for Systematic reviews and Meta-Analyses flow diagram. *Indexed literature search (until 15 October 2019): Medline and Cochrane Central Register of Controlled Trials. **Non-indexed literature search: American Society of Clinical Oncology, San Antonio Breast Cancer Symposium, European Society for Medical Oncology annual conferences from 2016 to October 2019. ET, endocrine therapy; OS, overall survival; PFS, progression-free survival.

subgroups. For multiple reports of the same trial, we used the first publication of the primary endpoint to extract PFS data in the intention-to-treat population; for OS, we used the report with the longest follow-up data for analysis; for subgroup analysis, we used publications specifically reporting subgroup analysis (when available) or, alternatively, the first published report. Two reviewers (NP, MD) assessed the risk of bias using the Cochrane tool (version 5.1.0).[6]

Definition of treatment arms

Treatments were grouped according to their pharmacological class, with the use of clinical judgement whenever required. As examples, palbociclib, ribociclib and abemaciclib were included in the 'CDK4/6 inhibitors' (CDK4/6i) group; aromatase inhibitors (AI; anastrozole, letrozole and exemestane) were pooled together as a group, irrespective of being administered as monotherapy or combined with goserelin (the 'AI±goserelin' group), as the latter can be used to induce menopause in premenopausal patients.[1] In cases in which there was only one drug representative of a class (eg, bortezomib), the specific name of the drug was used. If different doses of the same drug were tested (eg, fulvestrant 250mg and fulvestrant 500mg) or if different compounds of the same class were compared head-to-head (eg, mTOR inhibitors vistusertib and everolimus), each drug was considered separately. All other treatment arms were then defined as single-agent ET (eg, tamoxifen), and the combinations of different ET agents between them (eg, AI+fulvestrant 250mg) and/or with targeted therapies (eg, everolimus+AI).

Data synthesis and statistical analysis

Network plots describing the geometry of all comparisons were generated. Relative treatment effects were measured as HR to compare the different treatment regimens regarding PFS and OS. Results from the included trials were pooled using both pairwise frequentist meta-analysis and network meta-analysis. Network meta-analysis is a generalisation of pairwise meta-analysis that allows all evidence to be taken into account in a single model (both direct and indirect). Direct evidence comes from head-to-head trials and indirect evidence comes from trials that has a common comparator arm. In a network meta-analysis, the final evidence for each pair of treatments will come from direct evidence only, from indirect evidence only or from a combination between direct and indirect evidence, all depending on the geometry of the network. Our network meta-analysis was performed using MTC Bayesian models based on both fixed-effect and random-effect models to yield comparative frameworks among all included arms, based on direct and indirect evidence.[8] Decision between fixed-effect

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and random-effect were made using deviance information criterion.[9] In random-effects network meta-analysis models, the same variance among studies was assumed for all pairwise comparisons. As none of our networks had pairwise comparisons with both direct and indirect evidence, consistency was not checked. Closed loops appearing in the plots come from three and four-arms individual studies.

The Meta and Gemtc packages from R (V.3.4.2) were used for the analysis. HR point estimates and their 95% CIs or 95% credibility intervals (CrI) were used to report the results. Additionally, in the network analysis, regimens were ordered based on posterior rank probabilities (which indicate the probability of each regimen being the best, the second best, and so on) as well the surface under the cumulative ranking curve (SUCRA) values. SUCRA is a value between 0% and 100% calculated based on the posterior rank probabilities (larger value indicates a more effective treatment).[10] Difference between two regimens were considered significant if their 95% CrI did not cross the value of 1.

RESULTS

Characteristics of included studies

The systematic search of the literature yielded 3499 records, of which 149 were reviewed as full text (figure 1). Among them, 55 publications reporting on 32 trials (n=12 293 patients) were included: 10 trials reported on endocrine-sensitive patients only,[10–31] 18 trials on endocrine-resistant patients only[19 29 32–56] and 3 trials on both but with distinct analyses for each group[57–60] (table 1). Publication dates ranged from 2009 to 2019, reflecting the fact that widespread standardised testing of HER2 status was only implemented in 2007,[61] rendering most trials conducted before that ineligible for this study.

Quality of the evidence

Of the 32 trials included, 11 (34%) presented a low risk in at least 6 of the 7 assessed areas of potential bias (online supplementary table 1). Regarding the 'double-blinded' and 'outcome-blind' areas, a high risk of bias was present in 14 (44%) trials. The risk of bias was frequently high/unclear in trials with results published only in the form of meeting abstracts.[13 58 62]

For both PFS and OS, most of the direct treatment comparisons had only one trial providing direct evidence, thus heterogeneity was not assessable for those comparisons. There was minimal heterogeneity (I^2=0%) in all direct comparisons with two or more trials in the PFS and OS networks, except for the comparison between fulvestrant 500 mg (F500) versus pan-PI3K inhibitors (pan-PI3Ki)+F500 in the PFS network of endocrine-resistant patients with visceral disease (I^2=42%).

Network meta-analyses results

From the 32 trials included in the systematic review, 3 were excluded from the network meta-analysis: 2 trials did not provide HR for PFS or OS (Paul *et al*[13] and SAKK21/08[41] and one trial had treatment arms that could not be connected to the rest of the network (TAMRAD[33]). In addition, the tamoxifen-containing arms of the MONALEESA-7 trial[31 32] could not be connected to the rest of the network either. Thus, 29 trials (n=11 842 patients) were included in network meta-analysis models.

Primary endpoint: PFS

The endocrine-sensitive network included 12 randomised controlled trials (n=5200 patients), testing 10 ET-based regimens (figure 2A). Using a fixed-effects model, the combination of CDK4/6i with F500 was likely the most effective treatment (SUCRA=97.3%), followed by CDK4/6i+AI±goserelin (SUCRA=89.8%)—online supplementary table 2. When comparing the efficacy of CDK4/6i+F500 versus CDK4/6i+AI±goserelin, the HR was 0.82 (95% CrI 0.54–1.25)—table 2. The endocrine-resistant network included 20 trials (n=6635 patients), comparing 20 ET-based regimens (figure 2B). Using a fixed-effects model, CDK4/6i+F500 was probably the most effective treatment (SUCRA=95.7%), followed by capivasertib+F500 (SUCRA=88.7%)—online supplementary table 3. The comparison of CDK4/6i+F500 versus capivasertib+F500 held a HR of 0.91 (95% CrI 0.60–1.36)—table 3.

Secondary endpoint: OS

The number of trials reporting on OS was smaller as compared with those reporting on PFS. In the endocrine-sensitive population, five trials were included (n=2157 patients), comparing four treatments (figure 2C). Using a fixed-effects model, CDK4/6i+F500 was likely the most effective treatment (SUCRA=97.3%), followed by CDK4/6i+AI±goserelin (SUCRA=89.8%) and F500 (SUCRA=61.8%)—online supplementary table 2. Comparing CDK4/6i+F500 to F500 held a HR of 0.77 (95% CrI 0.63–0.95) and when comparing CDK4/6i+AI±goserelin to AI±goserelin the HR was 0.70 (95% CrI 0.48–1.02)—table 2.

In the endocrine-resistant population, 11 trials (n=4377 patients), testing 12 treatments, reported data on OS (figure 2D). Using a fixed-effects model, treatments with the highest chance of improving OS were capivasertib+F500 (SUCRA=84.7%) and CDK4/6i+F500 (SUCRA=69.9%)—table 3 and online supplementary table 3. The comparison of capivasertib+F500 to F500 held a HR of 0.59 (95% CrI 0.34–1.04), and when comparing CDK4/6i+F500 to F500 the HR was 0.77 (95% CrI 0.67–0.89).

Subgroup analysis of PFS

Four trials reported subgroup analysis for PFS separately for endocrine-sensitive patients with de novo metastatic or recurrent disease.[15 23 29 63] All trials tested CDK4/6i+AI±goserelin versus AI±goserelin and the pairwise meta-analysis showed a HR of 0.50 (95% CI 0.39 to 0.63) among patients with de novo metastatic disease, favouring the combination with CDK4/6i; similarly, the HR was 0.58

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Table 1 Characteristics of the randomised controlled trials included in the systematic review

Trial name/author	Phase	Meno-pausal status	Patients (N)*	Comparisons	Median of ET (N)†	Previous CT	ITT* PFS HR (95% CI)	ITT* OS HR (95% CI)	De novo* PFS HR (95% CI)	Recurrent* PFS HR (95% CI)	Visceral* PFS HR (95% CI)	Bone-only* PFS HR (95% CI)
Endocrine-sensitive patients only												
Ibrahim *et al*[11]	II	Post-meno	110	AS1402+AI (letrozole) versus AI (letrozole)	0	No	0.95 (0.50 to 1.81)	–	–	–	–	–
SWOG S0226[12]	III	Post-meno	536	Fulvestrant 250 mg+AI (anastrozole) versus AI (anastrozole)	0	No	0.81 (0.67 to 0.98)	-‡	–	–	–	–
Paul *et al*†§[13]	II	Post-meno	120	MultiTKI (dasatinib)+AI (letrozole) versus AI (letrozole)	0	≤1 line	No HR	–	–	–	–	–
PALOMA-1[14–16]	II	Post-meno	165	CDK4/6i (palbociclib)+AI (letrozole) versus AI (letrozole)	0	No	0.49 (0.32 to 0.75)	0.90 (0.62 to 1.29)	0.34 (0.19 to 0.60)	0.54 (0.30 to 0.96)	0.55 (0.32 to 0.94)	0.29 (0.09 to 0.95)
PALOMA-2[17–20]	III	Post-meno	666	CDK4/6i (palbociclib)+AI (letrozole) versus AI (letrozole)	0	No	0.58 (0.46 to 0.72)	–	0.61 (0.44 to 0.85)	0.58 (0.41 to 0.82)	0.62 (0.47 to 0.81)	0.41 (0.26 to 0.63)
MONALEESA-2[21–24]	III	Post-meno	668	CDK4/6i (ribociclib)+AI (letrozole) versus AI (letrozole)	0	No	0.56 (0.43 to 0.72)	0.75 (0.52 1.08)	0.45 (0.27 to 0.75)	0.60 (0.45 to 0.81)	0.54 (0.39 to 0.74)	0.69 (0.38 to 1.25)
FALCON[25–27]	III	Post-meno	462	Fulvestrant 500 mg versus AI (anastrozole)	0	Allowed	0.80 (0.64 to 0.999)	0.88 (0.63 to 1.22)	–	–	0.99 (0.74 to 1.33)	–
MINT[28]	II	Post-meno	359	Sapatinib 20 mg+AI (anastrozole) versus AI Sapatinib 40 mg+AI (anastrozole) versus AI (anastrozole)	0	≤1 line	1.37 (0.91 to 2.06) 1.16 (0.77 to 1.75)	–	–	–	–	–
MONARCH 3[29 30]	III	Post-meno	493	CDK4/6i (abemaciclib)+AI (anastrozole or letrozole) versus AI (anastrozole or letrozole)	0	No	0.54 (0.41 to 0.72)	–	0.49 (0.31 to 0.76)	0.58 (0.42 to 0.81)	0.61 (0.42 to 0.87)	0.58 (0.27 to 1.25)
MONALEESA-7[31 32]	III	Pre-meno	672	CDK4/6i (ribociclib)+tamoxifen+goserelin§ versus tamoxifen +goserelin§ CDK4/6i (ribociclib)+AI (anastrozole or letrozole)+goserelin versus AI (anastrozole or letrozole)+goserelin	0	≤1 line	0.59 (0.39 to 0.88) 0.57 (0.44 to 0.74)	0.79 (0.45 to 1.38) 0.70 (0.50 to 0.98)	–	–	–	–
Endocrine-resistant patients only												
TAMRAD§[33]	II	Post-meno	111	Everolimus+tamoxifen versus tamoxifen	NR	≤1 line	0.54 (0.36 to 0.81)	0.45 (0.24 to 0.81)	–	–	–	–
BOLERO-2[34–38]	III	Post-meno	724	Everolimus+AI (exemestane) versus AI (exemestane)	NR	≤1 line	0.45 (0.38 to 0.54)	0.89 (0.73 to 1.10)	–	–	0.47 (0.37 to 0.60)	0.33 (0.21 to 0.53)
SoFEA[39]	III	Post-meno	405	Fulvestrant 250 mg+AI (anastrozole) versus fulvestrant 250 mg Fulvestrant 250 mg versus AI (exemestane)	1	≤1 line	0.95 (0.75 to 1.22) 1.06 (0.83 to 1.34)	0.85 (0.64 to 1.14) 1.26 (0.95 to 1.66)	–	–	–	–
CALGB 40302[40]	III	Post-meno	235	Lapatinib+fulvestrant 250 mg versus fulvestrant 250 mg	NR	≤1 line	1.00 (0.76 to 1.30)	–	–	–	–	–
SAKK21/08§[41]	II	Post-meno	43	Selumetinib+fulvestrant 500 mg versus fulvestrant 500 mg	1	≤1 line	No HR	–	–	–	–	–
Adelson *et al*[42]	II	Post-meno	116	Bortezomib+fulvestrant 500 mg versus fulvestrant 500 mg	1	≤1 line	0.73 (0.49 to 1.09)	–	–	–	–	–

Continued

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Table 1 Continued

Trial name/author	Phase	Meno-pausal status	Patients (N)*	Comparisons	Median of ET (N)†	Previous CT	ITT* PFS HR (95% CI)	ITT* OS HR (95% CI)	De novo* PFS HR (95% CI)	Recurrent* PFS HR (95% CI)	Visceral* PFS HR (95% CI)	Bone-only* PFS HR (95% CI)
PALOMA-3[30 45–45]	III	Both	521	CDK4/6i (palbociclib)+fulvestrant 500 mg versus fulvestrant 500 mg	1	≤1 line	0.46 (0.36 to 0.59)	0.81 (0.64 1.03)	–	–	0.47 (0.34 to 0.63)	0.63 (0.38 to 1.06)
O'Shaughnessy *et al*[46]	II	Post-meno	297	Anti-androgen (abiraterone acetate) versus AI (exemestane)	1	≤1 line	1.1 (0.82 to 1.60)	–	–	–	–	–
				Anti-androgen (abiraterone acetate)+AI (exemestane) versus AI (exemestane)			0.96 (0.70 to 1.32)				0.51 (0.32 to 0.80)	2.09 (1.04 to 4.19)
FERGI[64]	II	Post-meno	168	Pan-PI3Ki (pictilisib)+fulvestrant 500 mg versus fulvestrant 500 mg	1	≤1 line	0.74 (0.52 to 1.06)	–	–	–	0.74 (0.46 to 1.18)	–
BELLE-2[47 48]	III	Post-meno	1147	Pan-PI3Ki (buparlisib)+fulvestrant 500 mg versus fulvestrant 500 mg	1	≤1 line	0.78 (0.67 to 0.89)	0.87 (0.74 to 1.02)	–	–	0.76 (0.63 to 0.90)	0.66 (0.46 to 0.95)
MONARCH 2[30 49]	III	Both	669	CDK4/6i (abemaciclib)+fulvestrant 500 mg versus fulvestrant 500 mg	0	No	0.55 (0.45 to 0.68)	0.76 (0.61 to 0.95)	–	–	0.48 (0.37 to 0.63)	0.54 (0.36 to 0.83)
Musolino *et al*[50]	II	Post-meno	97	MultiTKI (dovitinib)+fulvestrant 500 mg versus fulvestrant 500 mg	NR	No	0.68 (0.41 to 1.14)	0.81 (0.39 to 1.65)	–	–	–	–
Zhao *et al*[51]	II	Post-meno	60	Metformin+AI (letrozole or exemestane) versus AI (letrozole or exemestane)	1	Allowed	1.20 (0.7 to 2.1)	1.10 (0.50 to 2.40)	–	–	–	–
MANTA[55]	II	Post-meno	326	Vistusertib continuous+fulvestrant 500 mg versus fulvestrant 500 mg	1	≤1 line	0.88 (0.63 to 1.24)	–	–	–	–	–
				Vistusertib intermittent+fulvestrant 500 mg versus fulvestrant 500 mg			0.79(0.55 to 1.12)					
				Everolimus+fulvestrant 500 mg versus fulvestrant 500 mg			0.63 (0.42 to 0.92)					
				Everolimus+fulvestrant 500 mg versus vistusertib continuous+fulvestrant 500 mg			0.63 (0.45 to 0.90)					
				Vistusertib continuous+fulvestrant 500 mg versus vistusertib intermittent+fulvestrant 500 mg			1.11 (0.81 to 1.52)					
PrED102[52]	II	Post-meno	130	Everolimus+fulvestrant 500 mg versus fulvestrant 500 mg	NR	≤1 line	0.61 (0.40 to 0.92)	1.31 (0.72 to 2.38)	–	–	–	–
BELLE-3[53]	III	Post-meno	432	Pan-PI3Ki (buparlisib)+fulvestrant 500 mg versus fulvestrant 500 mg	2	Allowed	0.67 (0.53 to 0.84)	–	–	–	0.56 (0.43 to 0.74)	–
KCSG BR10-04/ FLAG[54]	II	Both	138	Fulvestrant 500 mg+goserelin versus goserelin	0	Allowed	0.61 (0.37 to 0.998)	0.60 (0.28 to 1.32)	0.73 (0.26 to 2.01)	–	0.67 (0.34 to 1.34)	–
				AI (anastrozole)+goserelin versus goserelin			0.98 (0.62 to 1.55)	0.52 (0.23 to 1.19)	0.69 (0.24 to 1.96)		1.04 (0.54 to 1.97)	
ACE[56]	III	Post-meno	365	Tucidinostat+AI (exemestane) versus AI (exemestane)	NR	≤1 line	0.75 (0.58 to 0.98)	–	–	–	0.69 (0.50 to 0.96)	–
FAKTION**[62]				Capivasertib+fulvestrant 500 mg versus fulvestrant 500 mg			0.58 (0.39 to 0.84)	0.59 (0.34 to 1.05)				
Both endocrine-sensitive and endocrine-resistant patients												

Continued

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Table 1 Continued

Trial name/author	Phase	Meno-pausal status	Patients (N)*	Comparisons	Median of ET (N)†	Previous CT	ITT* PFS HR (95% CI)	ITT* OS HR (95% CI)	De novo* PFS HR (95% CI)	Recurrent* PFS HR (95% CI)	Visceral* PFS HR (95% CI)	Bone-only* PFS HR (95% CI)
EGF30008[57]	III	Post-meno	752 ES 200 ER	Lapatinib+AI (letrozole) versus AI (letrozole)	NR	No	ES: 0.94 (0.79 to 1.13) ER: 0.78 (0.57 to 1.07)	–	–	–	–	–
Krop *et al*¤‡[58]	II	Post-meno	127 ES 120 ER	Anti-androgen (enzalutamide)+AI (exemestane) versus AI (exemestane)	NR	≤1 line	ES: 0.82 (0.54 to 1.26) ER: 1.02 (0.66 to 1.59)	–	–	–	–	–
MONALEESA-3[59 60]	III	Post-meno	367 ES 345 ER	CDK4/6i (ribociclib)+fulvestrant 500 mg versus fulvestrant 500 mg	0	No	ES: 0.58 (0.42 to 0.80)	ES: 0.70 (0.48 to 1.02)	–	–	–	–
					1	No	ER: 0.57 (0.43 to 0.74)	ER: 0.73 (0.53 to 1.004)				

*Only for hormone receptor-positive/HER2-negative patients, without other molecular selection.
†Median number of ET lines for advanced breast cancer.
‡Result was not provided separately for hormone-receptor positive/HER2-negative patients, therefore it was not included in the analysis.
§Am/trial not included in the network meta-analysis.
¶Results published only in meeting abstracts (without a full publication) at the time of literature search.
AI, aromatase inhibitor; CDK, cyclin-dependent kinase; CDK4/6i, CDK4/6 inhibitor; CT, chemotherapy; ER, endocrine-resistant; ES, endocrine-sensitive; ET, endocrine therapy; ITT, intention-to-treat population; multiTKI, multi-tyrosine kinase inhibitor; NR, not reported; OS, overall survival; pan-PI3Ki, pan-PI3K inhibitor; PFS, progression-free survival; post-meno, post-menopausal patients only; pre-meno, pre-menopausal patients only.

(95% CI 0.49 to 0.70) among patients with recurrent metastatic disease (online supplementary figure 1).

Five trials reported subgroup analysis for PFS on patients with visceral disease in the endocrine-sensitive setting,[15 19 24 25 29] and nine in the endocrine-resistant setting (online supplementary figure 2).[33 42 44–46 48 52 53 55] On endocrine-sensitive patients, CDK4/6i+AI±goserelin was likely the most effective treatment (SUCRA=98.4%), with a HR of 0.59 (95% CrI 0.34–1.04) when compared with F500. On endocrine-resistant patients, CDK4/6i+F500 was probably the most effective treatment (SUCRA=94.7%), with a HR of 0.68 (95% CrI 0.53–0.88) when compared with pan-PI3Ki+F500 (online supplementary tables 4 and 5).

Patients with bone-only disease were analysed separately for PFS in nine trials, of which four were on endocrine-sensitive,[15 19 24 29] and five on endocrine-resistant patients (online supplementary figures 3 and 4).[20 35 46 47 49] In endocrine-sensitive patients, all trials compared CDK4/6i+AI±goserelin versus AI±goserelin: the pairwise meta-analysis showed a HR of 0.49 (95% CI 0.36 to 0.67) favouring the combination with CDK4/6i. In endocrine-resistant patients, treatments had to be split between two networks, due to the absence of direct or indirect connection between all tested regimens. In network 1, everolimus+AI was probably the best treatment (SUCRA=100%) and, in network 2, it was CDK4/6i+F500 which was most likely to be the best treatment (SUCRA=80.3%)—online supplementary tables 6 and 7.

No network meta-analysis was performed based on molecularly defined subgroups, such as *PIK3CA*-mutant patients, owing to the substantial degree of heterogeneity in how to define and/or identify these subgroups among different trials.[44 47 53 64]

DISCUSSION

In this network meta-analysis, all the tested ET-based treatments for patients with HR+/HER2− advanced breast cancer were compared providing a rank order for their efficacy based on clinically meaningful endpoints (PFS and OS). Results suggest that the combination of CDK4/6i+F500 is likely to be the best treatment option in terms of PFS benefit among endocrine-resistant patients and also for endocrine-sensitive patients. The second most effective treatment options were CDK4/6i+AI±goserelin in the endocrine-sensitive population, and capivasertib+F500 in the endocrine-resistant population. Notably, by directly comparing CDK4/6i+F500 to CDK4/6i+AI±goserelin or to capivasertib+AI, even if HR are below 1, the CrIs cross 1.0, meaning that these differences are non-significant. Yet, this model may still guide clinicians by indicating which treatment options are probably the most effective among all.

The robustness of OS networks was partially hampered, as half of the trials have not yet provided mature data on this endpoint and all were unpowered for it, as OS was a secondary endpoint. Even so, the analysis showed that, similarly to the PFS results, CDK4/6i+F500 is also

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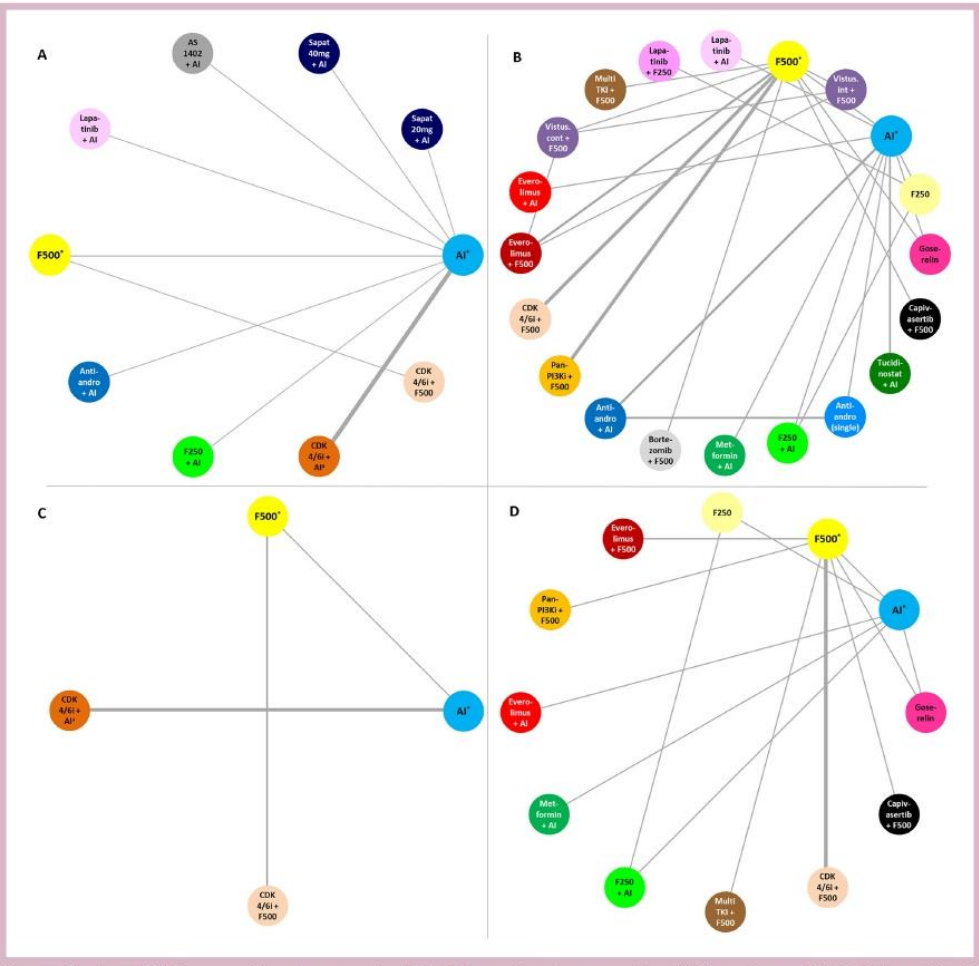


Figure 2 Network plots. (A) Progression-free survival (PFS) in endocrine-sensitive (ES) patients. (B) PFS in endocrine-resistant (ER) patients. (C) Overall survival (OS) in ES patients. (D) OS in ER patients. The width of the connecting lines is proportional to the number of trials comparing each pair of treatments, with bolder lines indicating comparisons with a higher number of trials. *±goserelin. AI, aromatase inhibitor; anti-andro, anti-androgen agent; CDK, cyclin-dependent kinases; CDK4/6i, CDK4/6 inhibitor; cont, continuous; F250, fulvestrant 250 mg; F500, fulvestrant 500 mg; int, intermittent; multiTKI, multi-tyrosine kinase inhibitor; pan-PI3Ki, pan-PI3K inhibitor; sapat, sapatinib; vistus., vistusertib.

possibly the best treatment for endocrine-sensitive patients in terms of OS, with a significantly better HR when compared with F500 (HR 0.77; 95% CrI 0.63–0.95). Interestingly, when pooling the OS data for the comparison of CDK4/6i+AI±goserelin versus AI±goserelin, the HR was non-significant (0.70; 95% CrI 0.48–1.02). In the endocrine-resistant population, the SUCRA value was higher for capivasertib+F500, but only the comparison between CDK4/6i+F500 and F500 held a significant HR (0.77; 95% CrI 0.67–0.89). These pooled results are highly relevant, as they consistently demonstrate that the addition of a CDK4/6i to F500 significantly increases the clinically important endpoint of OS, both in the endocrine-sensitive and endocrine-resistant settings. Nonetheless, we have to consider that OS gains are also influenced by post-progression therapies—in MONALEESA-3, subsequent antineoplastic therapies were received by 81.5% of patients in the ribociclib+F500 group and 84.7% of patients in the F500 group[60]; however, in MONALEESA-7, only 68.9% of patients in the ribociclib+tamoxifen/AI+goserelin group and 73.2% of patients in the tamoxifen/AI+goserelin group received subsequent antineoplastic therapy,[32] which is lower than what would be expected. Therefore, this should also be taken into account when evaluating the OS benefits of each treatment.

For the subgroup analysis of patients with endocrine-sensitive disease, data regarding the comparison between CDK4/6i+F500 versus F500 were not available and, hence, we cannot assess the combination's benefit in these subgroups. Taking that into account, results from subgroup analyses were not substantially different

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Table 2 Comparisons between treatments (HR, 95% credibility interval (CrI)) in endocrine-sensitive patients, both for progression-free survival (column vs row) and overall survival (row vs column)

Progression-free survival, HR (95% CrI)											
Treatments		**Sapat 20 mg+AI**	**Sapat 40 mg+AI**	**AI***	**Lapatinib+AI**	**AS1402+AI**	**Anti-andro+AI**	**F250+AI**	**F500***	**CDK4/6i+AI***	**CDK4/6i+F500**
Overall survival, HR (95% CrI)	Sapat 20 mg+AI		0.85 (0.56–1.28)	0.73 (0.49–1.10)	0.69 (0.44–1.07)	0.69 (0.32–1.48)	0.60 (0.33–1.08)	**0.59 (0.38–0.93)**	**0.58 (0.37–0.93)**	**0.41 (0.27–0.62)**	**0.34 (0.19–0.60)**
	Sapat 40 mg+AI	–		0.86 (0.57–1.30)	0.81 (0.52–1.27)	0.82 (0.38–1.74)	0.71 (0.39–1.28)	0.69 (0.44–1.10)	0.69 (0.43–1.10)	**0.48 (0.31–0.74)**	**0.40 (0.22–0.70)**
	AI*	–	–		0.94 (0.79–1.12)	0.95 (0.51–1.80)	0.82 (0.54–1.26)	**0.81 (0.67–0.98)**	**0.80 (0.64–1.00)**	**0.56 (0.49–0.63)**	**0.46 (0.31–0.69)**
	Lapatinib+AI	–	–	–		1.01 (0.52–1.96)	0.87 (0.55–1.39)	0.86 (0.67–1.12)	0.85 (0.64–1.13)	**0.59 (0.48–0.74)**	**0.49 (0.32–0.76)**
	AS1402+AI	–	–	–	–		0.86 (0.40–1.85)	0.85 (0.44–1.65)	0.84 (0.42–1.64)	0.59 (0.31–1.12)	0.49 (0.23–1.02)
	Anti-andro+AI	–	–	–	–	–		0.99 (0.62–1.56)	0.97 (0.60–1.56)	0.68 (0.44–1.06)	0.56 (0.31–1.00)
	F250+AI	–	–	–	–	–	–		0.98 (0.73–1.32)	**0.69 (0.55–0.86)**	**0.57 (0.36–0.88)**
	F500*	–	–	0.80 (0.47–1.38)	–	–	–	–		**0.70 (0.54–0.91)**	**0.58 (0.41–0.80)**
	CDK4/6i+AI*	–	–	0.70 (0.48–1.02)	–	–	–	–	0.87 (0.59–1.29)		0.82 (0.54–1.25)
	CDK4/6i+F500	–	–	0.62 (0.37–1.02)	–	–	–	–	**0.77 (0.63–0.95)**	0.88 (0.63–1.22)	

Treatments in the cells closer to the right-upper corner of the table are usually better than treatments in the cells closer to the upper-left corner. Cells in bold: statistically significant difference.
*±goserelin.
AI, aromatase inhibitor; anti-andro, anti-androgen agent; CDK, cyclin-dependent kinase; CDK4/6i, CDK4/6 inhibitor; F250, fulvestrant 250 mg; F500, fulvestrant 500 mg; sapat, sapatinib.

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Table 3 Comparisons between treatments (HR, 95% credibility interval) in endocrine-resistant patients, both for progression-free survival (column vs row) and overall survival (row vs column)

Progression-free survival, HR (95% CrI)																						
Treatment		**Metformin +AI**	**Anti-andro (single)**	**F250**	**Lapatinib +F250**	**Goserelin**	**AI**	**F250 +AI**	**Anti-andro +AI**	**Lapatinib +AI**	**Tucidinos-tat+AI**	**F500**	**Vistus. cont +F500**	**Vistus. int +F500**	**Bortezo-mib +F500**	**Pan-PI3Ki +F500**	**Everolim-us+AI**	**MultiTKI +F500**	**Everolim-us+F500**	**Capivaser-tib +F500**	**CDK4/6i +F500**
Overall survival, HR (95% CrI)	Metformin +AI		0.92 (0.49–1.75)	0.88 (0.48–1.61)	0.88 (0.46–1.31)	0.85 (0.42–1.71)	0.83 (0.48–1.44)	0.84 (0.46–1.53)	0.81 (0.45–1.50)	0.65 (0.35–1.22)	0.63 (0.34–1.15)	0.52 (0.25–1.07)	**0.45 (0.21–0.96)**	**0.40 (0.19–0.89)**	**0.39 (0.16–0.96)**	**0.39 (0.19–0.81)**	**0.27 (0.21–0.67)**	**0.35 (0.14–0.86)**	**0.22 (0.15–0.69)**	**0.30 (0.13–0.68)**	**0.27 (0.13–0.57)**
	Anti-andro (single)	–		0.96 (0.64–1.43)	0.96 (0.59–1.54)	0.91 (0.53–1.57)	0.90 (0.66–1.24)	0.90 (0.60–1.36)	0.88 (0.64–1.21)	0.70 (0.45–1.10)	0.67 (0.45–1.02)	**0.56 (0.31–0.98)**	**0.49 (0.25–0.92)**	**0.43 (0.22–0.83)**	**0.41 (0.20–0.82)**	**0.42 (0.23–0.75)**	**0.41 (0.23–0.58)**	**0.38 (0.18–0.81)**	**0.25 (0.18–0.66)**	**0.32 (0.16–0.64)**	**0.29 (0.16–0.53)**
	F250	1.15 (0.50–2.62)	–		1.00 (0.76–1.31)	0.96 (0.58–1.58)	0.94 (0.74–1.20)	0.95 (0.74–1.21)	0.92 (0.65–1.31)	0.74 (0.50–1.09)	0.71 (0.50–1.01)	0.59 (0.34–1.00)	**0.51 (0.28–0.94)**	**0.45 (0.24–0.84)**	**0.43 (0.22–0.83)**	**0.44 (0.25–0.75)**	**0.42 (0.22–0.57)**	**0.40 (0.19–0.82)**	**0.26 (0.20–0.66)**	**0.34 (0.18–0.65)**	**0.31 (0.18–0.53)**
	Lapatinib +F250	–	–	–		0.96 (0.54–1.69)	0.94 (0.66–1.35)	0.95 (0.66–1.35)	0.92 (0.59–1.44)	0.74 (0.46–1.19)	0.71 (0.45–1.10)	0.58 (0.32–1.06)	**0.51 (0.26–0.99)**	**0.45 (0.23–0.89)**	**0.43 (0.21–0.88)**	**0.44 (0.24–0.80)**	**0.42 (0.23–0.63)**	**0.40 (0.18–0.87)**	**0.26 (0.19–0.70)**	**0.34 (0.17–0.69)**	**0.31 (0.17–0.56)**
	Goserelin	1.76 (0.56–5.55)	–	1.53 (0.64–3.67)	–		0.99 (0.63–1.53)	0.99 (0.60–1.64)	0.96 (0.58–1.60)	0.77 (0.45–1.32)	0.74 (0.44–1.23)	0.61 (0.37–1.00)	**0.53 (0.30–0.95)**	**0.47 (0.26–0.85)**	**0.45 (0.24–0.85)**	**0.46 (0.27–0.76)**	**0.44 (0.28–0.71)**	**0.42 (0.20–0.85)**	**0.28 (0.21–0.67)**	**0.35 (0.19–0.66)**	**0.32 (0.19–0.53)**
	AI	0.91 (0.42–1.99)	–	0.79 (0.60–1.05)	–	0.52 (0.22–1.18)		1.01 (0.78–1.29)	0.98 (0.76–1.27)	0.78 (0.57–1.07)	**0.75 (0.59–0.97)**	**0.62 (0.39–0.99)**	**0.54 (0.31–0.95)**	**0.49 (0.27–0.95)**	**0.45 (0.24–0.84)**	**0.46 (0.28–0.75)**	**0.45 (0.28–0.54)**	**0.42 (0.21–0.85)**	**0.29 (0.22–0.67)**	**0.36 (0.20–0.66)**	**0.33 (0.20–0.53)**
	F250 +AI	0.97 (0.42–2.23)	–	0.85 (0.64–1.13)	–	0.56 (0.23–1.33)	1.07 (0.80–1.42)		0.97 (0.68–1.40)	0.79 (0.52–1.16)	0.75 (0.52–1.07)	0.62 (0.36–1.05)	**0.54 (0.29–0.99)**	**0.49 (0.26–0.89)**	**0.45 (0.23–0.89)**	**0.46 (0.27–0.80)**	**0.45 (0.23–0.61)**	**0.42 (0.20–0.80)**	**0.29 (0.21–0.70)**	**0.36 (0.19–0.69)**	**0.32 (0.19–0.56)**
	Anti-andro +AI	–	–	–	–	–	–	–		0.80 (0.53–1.19)	0.77 (0.53–1.11)	0.63 (0.37–1.08)	0.55 (0.30–1.02)	**0.49 (0.26–0.91)**	**0.46 (0.24–0.90)**	**0.47 (0.27–0.82)**	**0.46 (0.34–0.63)**	**0.42 (0.21–0.91)**	**0.29 (0.21–0.72)**	**0.37 (0.19–0.71)**	**0.33 (0.19–0.58)**
	Lapatinib +AI	–	–	–	–	–	–	–	–		0.96 (0.64–1.44)	0.79 (0.45–1.41)	0.69 (0.36–1.32)	0.61 (0.32–1.16)	0.59 (0.29–1.17)	0.59 (0.33–1.06)	**0.58 (0.40–0.82)**	0.54 (0.25–1.17)	**0.49 (0.26–0.92)**	**0.46 (0.23–0.91)**	**0.42 (0.23–0.75)**
	Tucidinos-tat +AI	–	–	–	–	–	–	–	–	–		0.83 (0.48–1.42)	0.72 (0.39–1.35)	0.64 (0.34–1.20)	0.60 (0.31–1.19)	0.62 (0.35–1.08)	**0.60 (0.44–0.82)**	0.56 (0.27–1.19)	**0.51 (0.28–0.95)**	**0.49 (0.25–0.93)**	**0.43 (0.25–0.76)**
	F500	1.06 (0.34–3.27)	–	0.92 (0.39–2.18)	–	0.60 (0.28–1.33)	1.16 (0.52–2.61)	1.09 (0.46–2.60)	–	–	–		0.87 (0.65–1.18)	0.77 (0.56–1.06)	0.73 (0.49–1.09)	**0.75 (0.67–0.94)**	0.73 (0.44–1.20)	0.68 (0.41–1.13)	**0.62 (0.47–0.82)**	**0.58 (0.40–0.85)**	**0.52 (0.46–0.60)**
	Vistus. cont+F500	–	–	–	–	–	–	–	–	–	–	–		0.89 (0.69–1.14)	0.84 (0.51–1.38)	0.86 (0.62–1.18)	0.83 (0.46–1.50)	0.78 (0.43–1.41)	**0.71 (0.54–0.94)**	0.66 (0.41–1.08)	**0.60 (0.40–0.84)**
	Vistus. int +F500	–	–	–	–	–	–	–	–	–	–	–	–		0.95 (0.57–1.58)	0.97 (0.69–1.36)	0.94 (0.52–1.71)	0.88 (0.48–1.61)	0.80 (0.60–1.08)	0.75 (0.46–1.24)	**0.68 (0.48–0.96)**
	Bortezomib +F500	–	–	–	–	–	–	–	–	–	–	–	–	–		1.02 (0.67–1.55)	0.99 (0.52–1.90)	0.93 (0.49–1.78)	0.85 (0.52–1.38)	0.79 (0.45–1.38)	0.72 (0.47–1.10)
	Pan-PI3Ki+F500	0.92 (0.29–2.86)	–	0.80 (0.33–1.92)	–	0.52 (0.24–1.17)	1.01 (0.44–2.32)	0.95 (0.39–2.28)	–	–	–	0.87 (0.74–1.02)	–	–	–		0.97 (0.58–1.63)	0.91 (0.54–1.53)	0.83 (0.61–1.13)	0.78 (0.52–1.16)	**0.70 (0.59–0.84)**
	Everolimus +AI	0.81 (0.36–1.83)	–	0.71 (0.50–1.00)	–	0.46 (0.20–1.04)	0.89 (0.73–1.09)	0.83 (0.58–1.18)	–	–	–	0.76 (0.53–1.77)	–	–	–	0.88 (0.39–2.08)		0.94 (0.46–1.92)	0.85 (0.48–1.52)	0.80 (0.42–1.50)	0.72 (0.43–1.22)
	MultiTKI+F500	0.86 (0.22–3.27)	–	0.75 (0.24–2.26)	–	0.49 (0.17–1.40)	0.94 (0.32–2.79)	0.88 (0.28–2.71)	–	–	–	0.81 (0.39–1.67)	–	–	–	0.93 (0.44–1.94)	1.06 (0.35–3.20)		0.91 (0.51–1.64)	0.85 (0.45–1.61)	0.77 (0.46–1.31)
	Everolimus +F500	1.38 (0.38–5.00)	–	1.21 (0.42–3.45)	–	0.79 (0.29–2.13)	1.52 (0.56–4.20)	1.43 (0.50–4.09)	–	–	–	1.31 (0.72–2.39)	–	–	–	1.51 (0.81–2.82)	1.71 (0.61–4.83)	1.61 (0.64–4.12)		0.93 (0.58–1.50)	0.85 (0.62–1.16)
	Capivaser-tib+F500	0.63 (0.18–2.20)	–	0.54 (0.20–1.53)	–	**0.25 (0.13–0.93)**	0.69 (0.26–1.85)	0.64 (0.23–1.81)	–	–	–	0.59 (0.34–1.04)	–	–	–	0.68 (0.38–1.22)	0.77 (0.28–2.13)	0.73 (0.29–1.83)	0.45 (0.20–1.02)		0.91 (0.60–1.36)
	CDK4/6i +F500	0.82 (0.26–2.54)	–	0.71 (0.30–1.71)	–	0.46 (0.21–1.03)	0.90 (0.39–2.05)	0.84 (0.35–2.02)	–	–	–	**0.77 (0.67–0.89)**	–	–	–	0.89 (0.71–1.10)	1.01 (0.43–2.35)	0.95 (0.46–1.99)	0.59 (0.32–1.09)	1.31 (0.73–2.33)	

Treatments in the cells closer to the right-upper corner of the table are usually better than treatments in the cells closer to the upper-left corner. Cells in bold: statistically significant difference.
Treatments:
AI, aromatase-inhibitor; anti-andro, anti-androgen agent; CDK, cyclin-dependent kinase; CDK4/6i, CDK4/6 inhibitor; cont, continuous; F250, fulvestrant 250 mg; F500, fulvestrant 500 mg; int, intermittent; multiTKI, multi-tyrosine kinase inhibitor; pan-PI3Ki, pan-PI3K inhibitor; vistus, visitazento.

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from the ones reported in the overall populations: CDK4/6i+AI±goserelin was likely the most effective treatment in terms of PFS both in endocrine-sensitive patients with bone-only, visceral, de novo and recurrent disease. In the endocrine-resistant setting, CDK4/6i+F500 was likely the best treatment for patients with visceral disease; for patients with bone-only disease, both CDK4/6i+F500 and everolimus+AI are suitable options.

The remaining preplanned subgroup analysis, namely in molecular-selected subgroups, was not carried out, as we did not have access to individual patient data and aggregated data from trial publications was scarce. In addition, trials testing selective PI3K-alpha inhibitors in endocrine-resistant patients, like SANDPIPER (fulvestrant with/without taselisib)[65] and SOLAR-1 (fulvestrant with/without alpelisib),[66] were not included in this systematic review, as they reported endpoints separately for patients with *PI3KCA*-mutated tumours and with *PI3KCA*-wild type tumours. Thus, our network meta-analysis only provides results regarding the use of ET-based regimens in patients with HR+/HER2− advanced breast cancer without further molecular selection. With the increasing use of multigene sequencing, clinicians will have access to the mutational landscape of the individual patient's tumour. Nevertheless, use of somatic multigene sequencing in breast cancer is still controversial as its clinical utility has not yet been proven. Hence, ASCO and ABC4 guidelines do not support the routine use of such multigene panels in routine clinical practice when deciding treatment for patients with advanced breast cancer.[1 67]

Our main conclusions are based on fixed-effect model analyses, given that all of them provided better deviance information criteria compared with the respective random-effect models. These results could have been impaired by heterogeneity among trials, but we showed that heterogeneity was minimal (I^2=0%) in all direct comparisons with ≥2 trials in the main PFS and OS networks, making the results robust, while still taking into account that these findings are derived from both direct and indirect evidence.

We have focused solely on efficacy parameters and did not include toxicity, due to the large number of regimens evaluated and the absence of one single parameter to measure it Furthermore, our group has previously published a meta-analysis on the risk of adverse events with the addition of targeted agents to ET in patients with HR+ advanced breast cancer.[68] We concluded that the use of targeted agents significantly increased the incidence of adverse events, both of grade 1–4 and grade 3–4. Therefore, the toxicity profile of each class of agents should be taken into account when deciding which treatment to prescribe to the individual patient. The systematic use of patient-reported outcomes (PROs) in clinical trials has recently become widespread practice; however, most of the trials included in our analysis did not provide information on this endpoint. Thus, we have not extracted data regarding PROs, but this should be considered in future meta-analyses. This is especially relevant nowadays, as the Magnitude of Clinical Benefit Scale of the European Society for Medical Oncology gives limited credit to a PFS gain not associated with improved quality of life or OS benefit.[69] Therefore, as this scale is used for reimbursement policies in some countries, PROs results can also influence the availability of some treatments.

We also acknowledge that there was heterogeneity in patients' populations regarding menopausal status and prior hormonal/chemotherapy treatments received, which may have influenced the benefit from each regimen. Additionally, endocrine-sensitivity should be regarded as a continuum—hence, the time cut-off to separate endocrine-sensitive from endocrine-resistant disease does not have a specific biological basis and the benefit from treatment may have changed between trials according to the proportion of patients with a 'more' endocrine-sensitive or endocrine-resistant disease. Endocrine-resistant population may also be heterogeneous, as patients might have received a variable number of previous ET lines—nonetheless, in most trials including endocrine-resistant patients, the median number of previous ET lines was just one (table 1); the only exception was the BELLE-3 trial.[53]

Publication bias was not assessed, as there are multiple limitations to its performance on network meta-analysis, especially regarding the creation of funnel plots.[70] Nonetheless, we have searched both fully published articles as well as conference abstracts, thus increasing the chances of including all potentially eligible trials.

Most of the published network meta-analyses assessing the efficacy of ET-based regimens in HR+/HER2− advanced breast cancer only included patients with endocrine-sensitive,[71–73] or with endocrine-resistant disease.[74–76] Of the network meta-analyses that have included both groups of patients, one specifically compared palbociclib+ET to different single-agent ET,[77] another compared everolimus+exemestane to several chemotherapy regimens[78] and the third evaluated palbociclib+ET versus chemotherapy,[79] making them difficult to compare with our results. Another recent and very large network meta-analysis has evaluated all chemotherapy and ET-based treatments in postmenopausal women with HR+/HER2− metastatic breast cancer.[80] Similarly to our results, it has showed that CDK4/6i plus ET are better than standard ET; in addition, it has demonstrated that no chemotherapy regimen was significantly better than CDK4/6i plus ET in terms of PFS. However, this network meta-analysis did not include premenopausal women, did not provide OS data or analysed efficacy according to endocrine-sensitivity status or different patients' subgroups (visceral disease, bone-only, etc). In addition, it comprised many trials including patients with HER2+ or triple-negative disease, partially hampering the generalisation of results.

Thanks to the high number of patients included and the geometry of the networks, we could provide ranking probabilities for each treatment, which facilitates decision-making for clinicians. Yet, these probabilities

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should be taken together with the HR and CrI of the pairwise comparisons. For instance, when analysing OS data in endocrine-resistant patients, the first rank is occupied by capivasertib+F500, followed by CDK4/6i+F500. Nevertheless, when comparing these two regimens, we see that the CrI are wide (table 3), meaning that these rankings should be interpreted with caution, as there was no significant difference between these treatments. In addition, our findings should be tailored to the individual patient, in terms of phase of disease (ie, endocrine-sensitive vs endocrine-resistant), previous therapies and tolerance to them.

Due to the rapid pace of changes in treatment options for HR+/HER2− advanced breast cancer, this network meta-analysis provides evidence-based data to patients, clinicians and policy makers to support nowadays clinical decision-making. It clearly demonstrates that CDK4/6i combined with ET is likely the best treatment option in terms of PFS and OS among patients with endocrine-sensitive and endocrine-resistant disease and across all patient subgroups, which could help facilitating the access to CDK4/6i. Yet, data of the direct comparison between CDK4/6i+F500 versus F500 among endocrine-sensitive patients comes from a subgroup analysis of a single trial (MONALEESA-3)[59 60]; therefore, these subgroup analysis results should ideally be confirmed in a dedicated randomised trial for patients with endocrine-sensitive disease. Nonetheless, while such data are not available, CDK4/6i+F500 could still be considered as an option for endocrine-sensitive patients in upcoming ASCO and ABC5 guidelines.[13] This is reinforced by the fact that, even if for many years AI have been considered the standard-of-care for these patients, the FALCON trial has demonstrated that, in terms of PFS, F500 is superior to AI.[25] However, little data are available on the effectiveness of AI or tamoxifen after F500, which leads many physicians to delay its use into the second-line—a practice which the availability of alpelisib[66] should reinforce. Ongoing trials are testing new, orally administered selective oestrogen receptor degraders, alone or in combination with CDK4/6i or AI, which should change the treatment landscape in coming years.[81]

In the future, it is expected that new genomic, proteomic, metabolomics and imaging biomarkers will be used to further tailor treatment to the individual patient. This is especially needed, not only to spare patients from the toxic effects of ineffective regimens, but also due to the potential 'financial toxicity' of these multiple ET-based treatments, given their potential impact on the individual patient and also on the sustainability of healthcare systems.[82] Therefore, new pooled analyses will probably be conducted to compare the efficacy of different ET-based treatments in biomarker-defined populations, such as patients with *PI3KCA*-mutated tumours.

In conclusion, this network meta-analysis suggests that the combination of a CDK4/6i+F500 may be the best treatment option in terms of PFS for both endocrine-sensitive and endocrine-resistant patients with HR+/HER2− advanced breast cancer. As for OS, CDK4/6i+F500 is possibly the best choice for endocrine-sensitive patients. Concerning endocrine-resistant patients, capivasertib+F500 and CDK4/6i+F500 are likely the best treatments in this setting.

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Supplementary Paper VI: *Denosumab in early-stage breast cancer*

Denosumab in early-stage breast cancer

It was with great interest that we read the survival results from the ABCSG-18 trial,[1] which randomly assigned postmenopausal patients with early-stage hormone receptor-positive breast cancer to denosumab or placebo. Results published in 2015 showed that denosumab significantly reduced clinical fractures, which was the primary endpoint of the study.[2] Now, Michael Gnant and colleagues report a modest improvement in disease-free survival with denosumab, a secondary endpoint, after a partial unmasking of the trial. Because of a variety of constraints, this analysis can only be considered descriptive, but it is implied that adjuvant denosumab or bisphosphonates should now be offered to postmenopausal women with hormone receptor-positive breast cancer. We have to disagree with this recommendation, since we believe that bisphosphonates remain the sole standard of care in this setting.

Ideally, the comparator in ABCSG-18 should have been a bisphosphonate and not placebo. Yet, in the absence of a direct head-to-head comparison, our clinical judgment must rely on the best available evidence. A meta-analysis[3] of adjuvant bisphosphonate treatment in early breast cancer showed that the use of bisphosphonates yielded an absolute reduction of 3·3% in breast cancer mortality in postmenopausal women (table). However, data on denosumab are contradictory, with the D-CARE trial[4] being negative for disease-free survival and overall survival and ABCSG-18 showing a modest benefit in disease-free survival, with no data reported on mortality. Notably, disease-free survival was a secondary endpoint of ABCSG-18, which was analysed on 3420 participants, whereas breast cancer recurrence and mortality were co-primary endpoints of the large meta-analysis,[3] analysed on 11 767 participants, thus making it a much more robust analysis.

In addition, the D-CARE trial[4] of 4509 participants had a similar follow-up time, a larger sample size, and used a more intense regimen of denosumab in higher-risk patients than did ABCSG-18. Hence, its negative results cannot be explained by suboptimal dosage or insufficient power, follow-up time, or number of disease-free survival events. Furthermore, ABCSG-18 showed that patients who started denosumab within 3 months of the start of aromatase inhibitors were the ones deriving more benefit from treatment.[1] In D-CARE,[4] it was mandatory to start treatment no later than 3 months after surgery, so patients had the ideal time window to receive denosumab.

Therefore, despite the promising role that denosumab might have in the adjuvant setting for postmenopausal patients with breast cancer, there

	ABCSG-18 trial[1,2]	D-CARE trial[4]	EBCTCG meta-analysis[3] (post-menopausal subgroup only)
Number of participants	3420	4509	11767
Recruitment period	2006–13	2010–12	Trials beginning before 2008
Key eligibility criteria	Postmenopausal patients with oestrogen receptor-positive early breast cancer receiving treatment with aromatase inhibitors	Patients with stage II or III breast cancer, any subtype	Patients with early breast cancer, any subtype
Population characteristics			
Lymph node-negative tumours	71%	6%	29%
Oestrogen receptor-positive and HER2-negative breast cancer	94%	65%	76%
HER2-positive breast cancer	6%	20%	Not reported
Triple-negative breast cancer	0	15%	Not reported
No previous chemotherapy	75%	4%	25%
Receiving aromatase inhibitors	100%	55% (within oestrogen receptor positive patients)	Not reported
Intervention	Denosumab 60 mg every 6 months, during aromatase inhibitor treatment	Denosumab 120 mg once every 3–4 weeks × 6 doses, followed by denosumab 120 mg once every 3 months for 54 months	Bisphosphonates, with mean scheduled treatment duration of 3·4 years
Comparator	Placebo	Placebo	Placebo
Timing of treatment initiation	<24 months after the beginning of aromatase inhibitor treatment	≤3 months after definitive surgery	Not specified

(Table continues on next page)

	ABCSG-18 trial[1,2]	D-CARE trial[4]	EBCTCG meta-analysis[3] (post-menopausal subgroup only)
(Continued from previous page)			
Results			
Median follow-up	73 months (IQR 58–95)	67 months (IQR 55–74)	5·6 person-years (IQR 3·7–8·0)
Primary endpoints	Time to first clinical fracture: HR 0·50 (95% CI 0·39–0·65); first clinical fracture rates at 84 months: 11·1% with denosumab *vs* 26·2% with placebo	Bone metastasis-free survival rates: 87·1% with denosumab *vs* 86·5% with placebo (HR 0·97, 95% CI 0·82–1·14)	10-year recurrence of breast cancer: 15·4% with bisphosphonates *vs* 17·6% with placebo (RR 0·86, 95% CI 0·78–0·94); 10-year distant recurrence: 12·5% *vs* 14·8% (0·82, 0·74–0·92); 10-year breast cancer mortality: 14·7% *vs* 18·0% (0·82, 0·73–0·93)
Disease-free survival (secondary endpoint)	5-year disease-free survival: 89·2% with denosumab *vs* 87·3% with placebo; 8-year disease-free survival: 80·6% *vs* 77·5%; number of disease-free survival events: 240 (14·0%) *vs* 287 (16·8%), HR 0·82 (95% CI 0·69–0·98)	5·6 year disease-free survival: 80·4% with denosumab *vs* 80·8% with placebo; number of disease-free survival events: 442 (19·6%) *vs* 433 (19·2%), HR 1·04 (95% CI 0·91–1·19)	..
Absolute gain	In disease-free survival: 1·9% at 5 years; 3·1% at 8 years	No gain in disease-free survival	At 10 years: 2·2% in breast cancer recurrence, 2·3% in distant recurrence, 3·3% in breast cancer mortality
Subgroup analysis of disease-free survival	Oestrogen-receptor positive or HER2 negative 5-year disease-free survival: 89·1% with denosumab vs 87·4% with placebo (HR 0·83, 95% CI 0·69–0·98)	Oestrogen-receptor positive or HER2 negative: HR 0·95 (95% CI 0·79–1·14); postmenopausal: 1·12 (0·92–1·36)	..
Osteonecrosis of the jaw	0	5% with denosumab *vs* <1% with placebo	Not reported

HR=hazard ratio. RR=rate ratio. EBCGTCG=Early Breast Cancer Trialists' Collaborative Group.

Table: **Comparison of phase 3 trials testing adjuvant denosumab and the meta-analysis regarding the efficacy of adjuvant bisphosphonates in early-stage breast cancer**

are still uncertainties concerning its benefit on survival. In this clinical scenario, bisphosphonates should remain the standard of care, while denosumab can be recommended to patients who have contraindications for bisphosphonates and mostly with the goal of decreasing bone fracture risk.

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1 Gnant M, Pfeiler G, Steger GG, et al. Adjuvant denosumab in postmenopausal patients with hormone receptor-positive breast cancer (ABCSG-18): disease-free survival results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019; **20:** 339–51.

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3 Early Breast Cancer Trialists' Collaborative Group. Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. *Lancet* 2015; **386:** 1353–61.

4 Coleman RE, Finkelstein D, Barrios CH, et al. Adjuvant denosumab in early breast cancer: First results from the international multicenter randomized phase III placebo controlled D-CARE study. *J Clin Oncol* 2018; **36** (suppl 15)**:** 501 (abstr).

Supplementary Paper VII: *Neoadjuvant treatment for intermediate/high-risk HER2-positive and triple-negative breast cancers: no longer an 'option' but an ethical obligation*

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Neoadjuvant treatment for intermediate/high-risk HER2-positive and triple-negative breast cancers: no longer an 'option' but an ethical obligation

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Last year, we published an editorial in this journal, advocating the use of neoadjuvant treatment (NAT) in patients with breast cancer, especially for those bearing aggressive tumours (luminal B, triple-negative and HER2-positive subtypes).[1] With the recent publication of important practice-changing data, we argue now that the use of NAT is the only ethical strategy for around one-third of women with early breast cancer.

The first reason for using NAT is that it allows surgical de-escalation, as it increases the rates of breast-conserving surgery.[2] It may also avoid a full axillary dissection in selected patients who 'convert' from cN1 to a negative sentinel lymph-node biopsy.[3] Another very important reason is that it identifies patients at a higher risk of relapse, for whom additional 'salvage' options are now available. Two large meta-analyses have demonstrated that patients who do not achieve a pathological complete response (pCR) after NAT have worse long-term survival, especially in triple-negative breast cancer (TNBC) and HER2-positive disease.[4 5] Yet, it has recently been shown that their outcome may be improved by escalating post-NAT.

The CREATE-X trial, conducted in Asia, included both patients with oestrogen receptor (ER)-positive/HER2-negative disease and TNBC, who were randomised to receive standard postsurgical treatment either with or without capecitabine.[6] Among patients with TNBC, capecitabine significantly improved 5-year disease-free survival: it was 69.8% in the capecitabine group versus 56.1% in the control group (HR 0.58; 95% CI 0.39 to 0.87); it also improved overall survival (HR 0.52; 95% CI 0.30 to 0.90). In patients with ER-positive/HER2-negative disease, the HR for disease free-survival was more modest: 0.81 (95% CI 0.55 to 1.17). Despite concerns on the extrapolation of CREATE-X results to non-Asian patients, international guidelines adopted adjuvant capecitabine as a possible treatment for patients with TNBC and invasive residual disease after NAT.[7 8]

More recently, the KATHERINE trial randomised 1486 patients with residual invasive HER2-positive disease following NAT to adjuvant T-DM1 or trastuzumab for 14 cycles.[9] Results were impressive: the 3-year invasive disease-free survival rate was 88.3% in the T-DM1 group versus 77.0% in the trastuzumab group (HR 0.50; 95% CI 0.39 to 0.64), making it clear that these patients with suboptimal responses to standard chemotherapy and anti-HER2 monoclonal antibodies (trastuzumab ± pertuzumab) should receive adjuvant T-DM1 instead of continuing trastuzumab. Nonetheless, there is space for further improvement in the ER-negative/HER2-positive subgroup, as 3-year invasive disease-free survival rate was 82.1% with T-DM1. Overall survival data are still immature.

Of note, there are several ongoing phase III trials testing the postneoadjuvant use of other drugs in patients with residual disease after NAT, like the PENELOPE-B trial in ER-positive/HER2-negative patients (standard endocrine therapy with/without 1 year of palbociclib; ClinicalTrials.gov identifier: NCT01864746) or the SWOG S1418/NRG BR006 trial in TNBC (1 year of pembrolizumab or placebo; NCT02954874).

Considering the above results—and particularly those of the very robust international KATHERINE trial—we advocate that clinicians must use tumour's response to NAT as a way to tailor adjuvant treatment of patients with intermediate to high-risk HER2-positive disease or TNBC, instead of blindly prescribing chemotherapy and/or targeted agents after surgery.

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NAT becomes the 'standard of care' for these women and not only an 'option' to discuss for the purpose of increasing the probability of less aggressive surgery, as it has an impact on disease-free survival and, possibly, on overall survival as well. A number of remaining questions will need to be addressed, like which adjuvant anti-HER2 therapy to prescribe to patients who achieve pCR after neoadjuvant chemotherapy with trastuzumab and pertuzumab and whether or not biomarkers evaluated after one or two courses of NAT might reliably identify patients who will not reach a pCR and who could benefit from an earlier introduction of a 'salvage' treatment.

The NAT strategy could also become a standard of care for high-risk luminal B disease in the near future, if it is demonstrated that those patients who do not achieve a pCR after NAT may benefit from the addition of targeted therapy to endocrine treatment. Beyond pCR 'yes or no', other prognostic markers can be used to identify high-risk patients, like the residual cancer burden[10] or the PEPI score.[11] More recently, prognostic markers like tumour-infiltrating lymphocytes in the residual tumour[12] or the persistence of circulating tumour DNA (ctDNA)[13] have also been explored. These markers may also be important for patients who achieve pCR, as we know that a part of these patients still relapse afterwards and we should find ways of identifying them.

Even though the use of NAT helps tailoring adjuvant therapy, in patients who do not achieve pCR (who are still the majority), the duration of neoadjuvant plus adjuvant treatment can be very long—for example, up to 18 months in HER2-positive disease. Thus, an earlier identification of patients who are benefiting or not from NAT is necessary in order to (de)escalate therapy accordingly. One possibility is the use of imaging during the course of NAT, like MRI[14 15] and/or ^{18}F-FDG PET/CT,[16 17] which have shown to be associated with achievement of pCR. Other possibilities are measuring the drop of Ki67 after 2–4 weeks of treatment[18–21] or assessing the fall in ctDNA levels during NAT.[22 23] Today, however, there is no proven benefit of changing the type of regimen used throughout NAT according to these markers, but there are ongoing trials testing this hypothesis (ie, ALTERNATE [NCT01953588] and ADAPT HR+/HER2- [NCT01779206]).

It should also be realised that the use of NAT demands a highly organised team of pathologists, radiologists, surgeons, medical oncologists, radiation oncologists and other professionals specialised in breast cancer care. As already recommended by the European Society for Medical Oncology,[24] we are strong believers that the model of 'breast cancer units' should now be fully implemented in Europe and abroad, as failing to do so might compromise patients' survival.[25] A courageous way of accelerating its dissemination would be to restrict breast cancer treatment reimbursement to hospitals which have an accredited breast cancer unit.

In conclusion, we claim that patients with intermediate to high-risk TNBC or HER2-positive disease (≥T2 and/or lymph-node positive tumours) must receive NAT, as this strategy not only increases the chance of less aggressive surgery, but identifies patients who will benefit from 'salvage' adjuvant therapy with an impact on long-term outcomes.

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Supplementary Paper VIII: *What is the real impact of estrogen receptor status on the prognosis and treatment of HER2-positive early breast cancer?*

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What Is the Real Impact of Estrogen Receptor Status on the Prognosis and Treatment of HER2-Positive Early Breast Cancer?

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ABSTRACT

HER2$^+$ early breast cancer is a heterogeneous disease, comprising all the intrinsic breast cancer subtypes. The only biomarker available nowadays for anti-HER2 treatment selection is HER2 status itself, but estrogen receptor (ER) status is emerging as a robust predictive marker within HER2$^+$ disease. In this Perspective, we discuss the biological and clinical differences between patients with HER2$^+$/ER-positive (ER$^+$) disease versus those with HER2$^+$/ER-negative (ER-neg) tumors, namely, short-term and long-term (>5 years after diagnosis) prognosis, response to neoadjuvant treatment and benefit from adjuvant anti-HER2–targeted therapies. We also address other possible biomarkers to be used for patient selection in future clinical trials, such as gene signatures, PAM50 subtypes, tumor-infiltrating lymphocytes, *PIK3CA* mutations, and changes in Ki67 score during treatment and discuss their limitations. Finally, we suggest new clinical trial designs that can have an impact on clinical practice, aiming to test treatment deescalation separately for patients with HER2$^+$/ER$^+$ and HER2$^+$/ER-neg tumors. We also propose an integrated classification of HER2$^+$ disease, comprising DNA, RNA, protein expression, and microenvironment characteristics, in order to identify those tumors that are truly "HER2-addicted" and may benefit the most from anti-HER2 treatment.

Perspective

It is well known that HER2$^+$ breast cancer is a heterogeneous disease (1, 2). Besides HER2 status itself, there are no validated biomarkers to identify patients likely to benefit from anti-HER2 treatments (3). It has been proposed, however, that the expression of the estrogen receptor (ER) drives variable degrees of benefit from existing anti-HER2 treatment strategies. An increasing number of trials are being developed targeting specifically HER2$^+$/ER-positive (ER$^+$) or HER2$^+$/ER-negative (ER-neg) early breast cancer, and we believe that, in the future, these may be regarded as distinct diseases or we may need additional tools to identify the varying biology within HER2$^+$ disease that is represented by ER status.

Biological differences

Distinct biological characteristics may justify the different clinical behavior observed in HER2$^+$ early breast cancer according to ER status. The ER and the HER2 pathways drive breast cancer proliferation by a complex interaction of cellular signaling processes (4). Due to the intracellular cross-talk between HER2 and ER, treatments interfering with one of the two pathways can have an effect on the other, with reactivation of ER signaling being a known mechanism of resistance to anti-HER2 agents in HER2$^+$/ER$^+$ breast cancer (5).

The distribution of intrinsic (PAM50) breast cancer subtypes within HER2$^+$ tumors also differs according to ER status (6): around 75% of HER2$^+$/ER-neg tumors and 30% of HER2$^+$/ER$^+$ are "HER2-enriched," which is the subtype associated with HER2/EGFR pathway activation (2), high proliferation rates, and an immune-activated stroma with elevated tumor-infiltrating lymphocytes (TIL) concentrations (**Fig. 1**). On the other hand, around 70% of HER2$^+$/ER$^+$ tumors are luminal A/B, which are ER-dependent tumors, with low HER2/EGFR pathway activation and a high rate of *PIK3CA* mutations (1), that are associated with lower response to anti-HER2 treatment (7).

Differences in prognosis between HER2$^+$/ER$^+$ and HER2$^+$/ER-neg early breast cancer in the "trastuzumab era"

In the first 5 years after diagnosis, HER2$^+$/ER-neg patients have worse survival compared with the HER2$^+$/ER$^+$ group. Among 42,887 patients treated between 2010 and 2015, 5-year overall survival (OS) rates were 88.2% in HER2$^+$/ER$^+$ versus 83.9% in HER2$^+$/ER-neg subgroup ($P < 0.001$; ref. 8). Yet, after 5 years, recurrence risk lowers among HER2$^+$/ER-neg patients, but is maintained among those with HER2$^+$/ER$^+$ tumors (9–11). This leads to similar long-term prognosis: in the HERA trial, 12-year OS in the trastuzumab arms was 81% in the HER2$^+$/ER$^+$ and 78%–79% in the HER2$^+$/ER-neg subgroups (9).

On the other hand, achievement of a pathologic complete response (pCR) after neoadjuvant treatment is a prognostic factor in both groups, but it has a stronger impact on event-free survival (EFS) in the HER2$^+$/ER-neg [hazard ratio (HR), 0.25] compared with the HER2$^+$/ER$^+$ subgroups (HR, 0.58; ref. 12). However, the overall median follow-up for EFS in this meta-analysis was just 5.4 years; thus, the prognostic impact of pCR on late recurrences is currently unknown.

Is ER status predictive of treatment benefit?

It is well documented that HER2$^+$/ER-neg patients treated with chemotherapy and dual anti-HER2 therapy present higher pCR rates

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Translational Relevance

HER2$^+$ early breast cancer is a heterogeneous disease, in which estrogen receptor (ER) status is emerging as a robust predictive marker. This is related to the fact that the ER and HER2 pathways drive breast cancer proliferation by a complex interaction of cellular signaling processes, including the sharing of intracellular effectors such as MAPK, PI3K, and Src3. Thus, due to the cross-talk between HER2 and ER, treatments targeting one pathway can induce the activation of the other. This leads to a different clinical behavior of HER2$^+$/ER$^+$ disease as compared with HER2$^+$/ER-neg early breast cancer. We discuss how the timing and sequencing of treatments, including anti-HER2 targeted therapy, can influence their benefit according to ER status. We also propose different deescalation trial designs, with potential impact on clinical practice, separately for HER2$^+$/ER$^+$ and HER2$^+$/ER-neg breast cancer, which should be regarded as distinctly different.

(61%–91%) compared with patients with HER2$^+$/ER$^+$ early breast cancer (26%–56%; refs. 13–20); and, compared with HER2$^+$/ER$^+$ tumors, HER2$^+$/ER-neg tumors have consistently demonstrated greater increases in pCR rates with dual anti-HER2 therapy plus chemotherapy compared with chemotherapy/trastuzumab alone (13, 18–20). Nonetheless, duration of anti-HER2 treatment seems to matter in HER2$^+$/ER$^+$ early breast cancer: in TBCRC023, increasing treatment length with trastuzumab/lapatinib and letrozole from 12 to 24 weeks led to an increase in pCR rates from 9% to 33%, whereas no significant difference was observed in HER2$^+$/ER-neg tumors (21). This suggests that in HER2$^+$/ER$^+$ tumors, in the absence of chemotherapy, prolonged inhibition of the HER2 and ER pathways may be necessary not only to decrease proliferation, but to induce apoptosis as well. Yet, adding concomitant endocrine therapy (ET) to anti-HER2 therapy and/or chemotherapy did not yield any advantage in the NSABP B-52 or ADAPT HER2$^+$/HR$^+$ studies (22, 23). This is intriguing, as one would expect that the ER/HER2 pathway blockade would suppress their cross-talk and lead to higher tumor response.

On the opposite end of the spectrum, the high chemosensitivity of HER2$^+$/ER-neg tumors was demonstrated in the ADAPT HER2$^+$/HR$^-$ study, in which the addition of paclitaxel to trastuzumab/pertuzumab increased pCR rates from 36% to 91% (17). Similarly, the KRISTINE trial showed a significantly higher pCR rate in this group with docetaxel/carboplatin plus trastuzumab/pertuzumab compared with T-DM1/pertuzumab (73% vs. 54%, respectively; ref. 16).

In the adjuvant setting, HER2$^+$/ER$^+$ and HER2$^+$/ER-neg subgroups have a similar degree of proportional risk reduction with trastuzumab (9, 24, 25). Yet, in contrast with the neoadjuvant setting, duration of adjuvant trastuzumab treatment might matter more for HER2$^+$/ER-neg early breast cancer: in a recent meta-analysis, patients receiving 9 weeks to 6 months of trastuzumab had worse EFS compared with patients treated for 12 months (26). Therefore, in HER2$^+$/ER-neg disease, longer trastuzumab treatment is probably more effective than a shorter course. However, the trials included in this meta-analysis had a median follow-up of less than 5 years, so long-term data on HER2$^+$/ER$^+$ patients are needed.

Challenging this idea, the ExteNET trial showed that sequential anti-HER2 blockade (summing 2 years of treatment) was beneficial for HER2$^+$/ER$^+$ patients, but not for HER2$^+$/ER-neg patients (27). On the other hand, updated survival results from APHINITY show that early dual HER2 blockade with trastuzumab/pertuzumab has an effect both in HER2$^+$/ER-neg and HER2$^+$/ER$^+$ diseases (28). Reasons for this difference may lie in these drugs' mechanisms of action, timing of administration, and length of follow-up. After stopping adjuvant

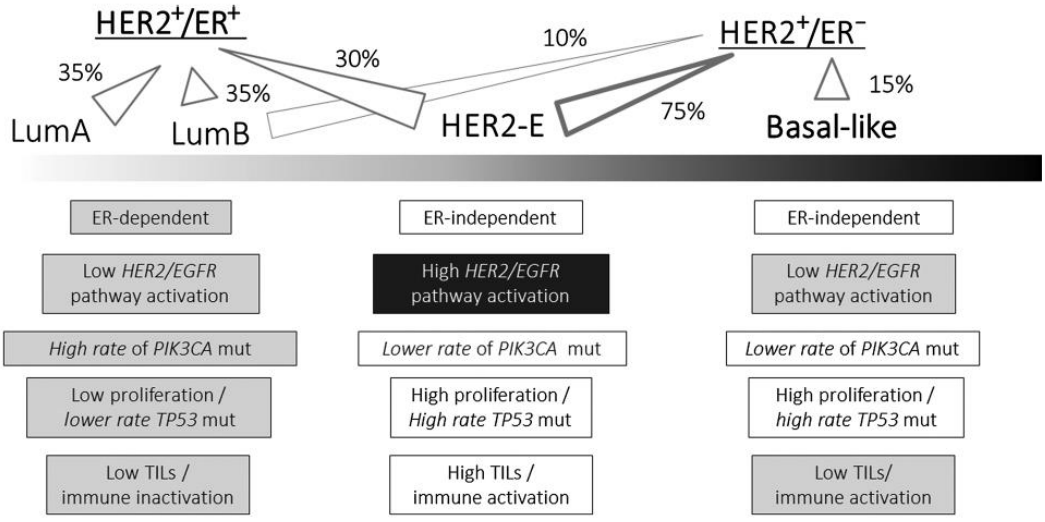


Figure 1.
Current and future classification of HER2$^+$ disease. HER2$^+$ tumors can be divided between estrogen receptor (ER)–positive and ER-negative subgroups, according to the immunohistochemical expression of ER. Yet, at the molecular level, these tumors can be divided into the four molecular subtypes (luminal A, luminal B, HER2-enriched and basal-like), showing different molecular alterations, cell pathway activation, and immune microenvironment. This leads to different responses to anti-HER2 treatment, as well as to chemotherapy and endocrine therapy. ER, estrogen receptor; HER2-E, HER2-enriched molecular subtype; LumA, luminal A molecular subtype; LumB, luminal B molecular subtype; mut, mutation; TILs, tumor-infiltrating lymphocytes; WT, wild-type.

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trastuzumab, HER2$^+$/ER$^+$ patients continue ET, but the single blocking of the ER pathway may lead to reactivation of the HER2 pathway on residual trastuzumab-resistant malignant cells (4). As neratinib was administered after trastuzumab, it may have prevented the reactivation of the HER2 pathway and, hence, benefited HER2$^+$/ER$^+$ patients, but not those with HER2$^+$/ER-neg tumors. As neratinib irreversibly inhibits several HER-family members (EGFR, HER2, HER4), it may have prevented this cross-talk in a more effective way than administration of trastuzumab for 2 years (which only inhibits HER2; ref. 9). In APHINITY, the early dual blockade has probably eliminated trastuzumab-resistant cells in part of the patients, but the benefit was seen later in HER2$^+$/ER$^+$ patients, as these tend to have later relapses, as compared with HER2$^+$/ER-neg patients.

Recently, the KATHERINE trial showed that in patients with residual disease after neoadjuvant treatment, 1 year of adjuvant T-DM1 was superior to trastuzumab, independently of ER status (29). It should be noted that this trial included selected high-risk patients, for whom suboptimal response to neoadjuvant trastuzumab could have influenced the benefit from a salvage treatment with a different anti-HER2 drug, potentially confounding the effect of baseline ER status.

How can we identify patients who benefit more or less from anti-HER2 treatment?

The biggest question nowadays is how to select patients for escalation/deescalation approaches, maintaining or increasing pCR rates and survival, with minimal toxicity. Therefore, the quest for finding prognostic and predictive biomarkers has been intense (3). One attempt was to quantify gene-expression levels of *ESR1* and *ERBB2*, showing that high levels of *ERBB2* (20, 30–32) and low levels of *ESR1* (31) are associated with higher pCR rates. Expression levels of genes involved in HER2 and ER pathways were also combined in a predictive model in an analysis of the NSABP B-31 trial. Patients were classified into three groups: (i) "high *ESR1*"/"low-intermediate *ERBB2*": no benefit from trastuzumab (HR, 1.58); (ii) "low *ESR1*"/"low-intermediate *ERBB2*": moderate benefit (HR, 0.60); (iii) "low-intermediate *ESR1*"/"high *ERBB2*": large benefit (HR, 0.28; ref. 33). This eight-gene signature, showing no linear correlation between *ERBB2* and *ESR1* mRNA levels and benefit from adjuvant trastuzumab, was later validated in the N9831 and FB-7 trials (34, 35). Nonetheless, these intriguing findings have not yet been tested in prospective studies or applied in the clinic.

Other biomarker studies focused on the correlation between PAM50 subtypes and benefit from anti-HER2 treatment. A consistent finding is that HER2-enriched tumors present higher pCR rates (between 28% and 72%) when treated with anti-HER2 therapies (with/without chemotherapy), compared with other subtypes (20, 32, 36, 37), showing that HER2-enriched subtype predicts sensitivity to anti-HER2 treatment. In fact, several studies show that ER status loses its association with pCR when subtype is taken into account, indicating that subtype captures most of the information displayed by ER status and also brings additional information (20, 32, 36, 37). One important aspect to take into account when designing deescalation trials is that HER2-enriched subtype is also chemosensitive, with overall pCR rates in HER2$^+$ disease of around 60% with versus 30% without chemotherapy, respectively (20, 32, 36, 37).

It has also been proposed that high scores of a gene-expression signature of loss of function of RB1 may identify HER2$^+$ tumors more likely to respond to chemotherapy, and this concept has been validated in HER2$^+$/ER$^+$ tumors using a refined 87-gene signature (RBsig; refs. 38, 39). Moreover, higher TIL levels are associated with improved pCR rates and survival in both HER2$^+$/ER-neg and HER2$^+$/ER$^+$ early breast cancer (40, 41), and evidence of immune activation by RNA signatures is independently associated with higher pCR (20, 31). It has also been demonstrated that high TIL levels (>75%) predicted benefit from adjuvant trastuzumab/pertuzumab versus trastuzumab alone (42). Of note, TILs levels tend to be lower in HER2$^+$/ER$^+$ compared with HER2$^+$/ER-neg tumors (40).

A decrease in Ki67 during neoadjuvant treatment is a surrogate for response and prognosis in HER2-negative/ER$^+$ early breast cancer, so its role is being evaluated in HER2$^+$/ER$^+$ disease (43). In ADAPT HER2$^+$/HR$^+$, patients with an early tumor response (Ki67 decrease ≥30% or low-cellular tumor at 3 weeks) had a higher pCR rate compared with nonresponders (36% vs. 20%, respectively; ref. 23). In the PerELISA trial, HER2$^+$/ER$^+$ patients with a Ki67 drop after 2 weeks of letrozole (molecular responders) continued on letrozole, and trastuzumab/pertuzumab were added for another 12 weeks. Nonresponders were switched to paclitaxel with trastuzumab/pertuzumab. The pCR rate was 21% among molecular responders, but even more remarkable was the 81% pCR rate among nonresponders (44). Even though the correlation between an early Ki67 drop and long-term survival is not yet validated in patients with HER2$^+$ tumors, it could become a biomarker for selecting patients with HER2$^+$/ER$^+$ who do not respond to ET and would be candidates to chemotherapy, given the impressive pCR rate achieved in this subgroup.

Thus, a dilemma for future clinical trials is how to select patients: should one be guided by HER2/ER immunohistochemistry status, by molecular subtype or by a combination of both? Based on current evidence, tumors with the highest anti-HER2 sensitivity are those HER2$^+$ by immunohistochemistry/ISH and the PAM50 HER2 enriched. Whether additional biomarkers will help identify higher anti-HER2 sensitivity is currently unknown, although TILs and *PIK3CA* mutations arise as promising candidates (**Fig. 2**). Indeed, tumors with high HER2 amplification and an intact PI3K pathway are especially sensitive to HER2-targeted therapies (30). TILs could also potentially be used to select patients, but there are no standardized cutoff points to define "high" versus "low" TIL infiltration (45). Another question is if an early biopsy to assess Ki67 levels should be performed in HER2$^+$/ER$^+$ patients receiving neoadjuvant treatment. This is limited by Ki67 low reproducibility (46) and absence of validation as a surrogate for long-term survival benefit in HER2$^+$ early breast cancer.

Deescalating strategies: a plea for separate trials according to ER status

Despite distinct biological and clinical characteristics between HER2$^+$/ER-neg and HER2$^+$/ER$^+$ early breast cancer, they are still treated similarly, except for ET. Nevertheless, different deescalation strategies for HER2$^+$ early breast cancer might be explored according to ER status: (i) to decrease anti-HER2 treatment duration, (ii) to avoid anthracyclines, and (iii) to design chemotherapy-free regimens.

As detailed before, a meta-analysis showed a worse outcome with a shorter treatment duration of adjuvant trastuzumab in patients with HER2$^+$/ER-neg disease, but this comparison was nonsignificant in patients with HER2$^+$/ER$^+$ tumors. Moreover, in the PERSEPHONE trial (the only trial so far proving its noninferiority hypothesis of 6 months vs 1 year of trastuzumab), the HR for the comparison of the two strategies was very close to 1 in the HER2$^+$/ER$^+$ subgroup (HR, 0.91; 95% confidence interval, 0.68–1.21; ref. 47). Therefore, in patients with HER2$^+$/ER$^+$ tumors, a shorter duration of trastuzumab treatment may be seen as a safe option by some—nonetheless, due to the

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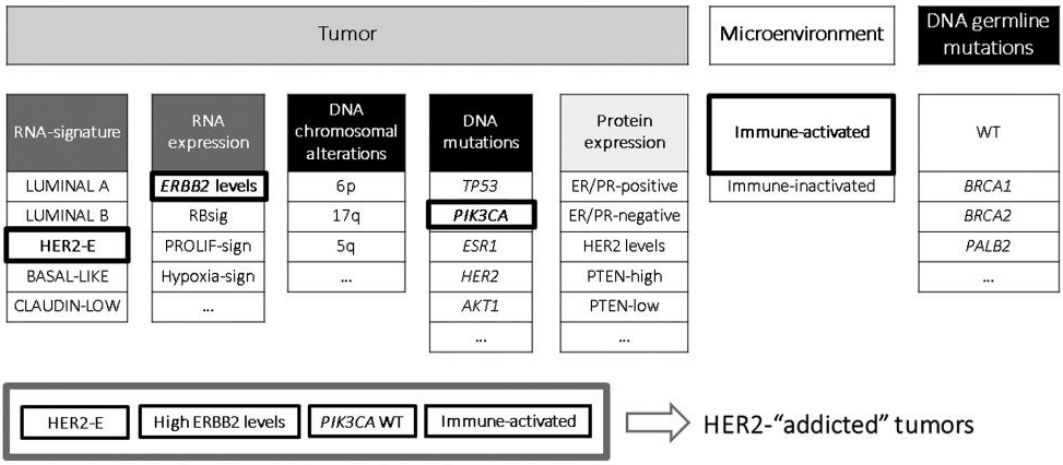


Figure 2.

Combining biomarkers in HER2$^+$ disease. Currently, protein expression assessed by immunohistochemistry is the standard method to classify breast cancer into different subtypes, according to tumor's HER2 and estrogen receptor expression. However, an integrated classification of HER2-positive breast tumors, based on their DNA, RNA, protein expression, and microenvironment, is needed to implement more precise and guided treatments and to identify those tumors "addicted" to the HER2 cellular pathway. Here, we propose a definition of "HER2-addicted" tumors that can be further refined in the future, as more data become available. ER, estrogen receptor; HER2-E, HER2-enriched molecular subtype; PR, progesterone receptor; RBsig, RB loss-of-function signature; WT, wild-type.

late relapse profile of these tumors, longer follow-up data are needed before adopting this strategy.

The APT trial (in which patients with ≤3 cm, node-negative tumors received 12 weeks of paclitaxel and 1 year of trastuzumab) showed a 7-year invasive disease-free survival of 94.6% for HER2$^+$/ER$^+$ disease and of 90.7% for HER2$^+$/ER-neg disease, with just four distant recurrences (1%; ref. 48). Thus, this treatment deescalation seems very safe for patients with low-burden tumors. Given the high chemosensitivity of HER2$^+$/ER-neg tumors, leading to a higher rate of pCR, a possible strategy for avoiding anthracyclines in patients with somewhat larger tumors would be to assess the tumor's sensitivity upfront with neoadjuvant double HER2-blockade plus a taxane. The DeCrescendo trial will test this hypothesis in patients with cT1c-T2N0 HER2$^+$/ER-neg tumors, who will receive 12 weeks of taxanes plus trastuzumab/pertuzumab. Those who achieve pCR will be spared from adjuvant anthracyclines and will only receive double HER2-blockade until completion of 1 year of treatment. The study is powered to test 3-year relapse-free survival (RFS) rate, with a narrow confidence interval, primarily in patients with PAM50 HER2-enriched tumors and then in the overall population.

The reduced chemosensitivity of HER2$^+$/ER$^+$ early breast cancer fuels the interest in alternative, targeted approaches without chemotherapy. One is the use of CDK4/6 inhibitors, which synergize with trastuzumab in HER2$^+$/ER$^+$ tumor models (49). In the small NA-PHER2 study, 20 weeks of palbociclib/ET/trastuzumab/pertuzumab provided a significant reduction in tumor proliferation, but led to a modest pCR rate of 27% (50). Long-term survival results and efficacy according to molecular subtypes are awaited. There are ongoing trials [PALTAN (NCT02907918); TOUCH (NCT03644186)] exploring this approach. In particular, TOUCH is assessing RBsig (39) as a potential biomarker to identify HER2$^+$/ER$^+$ patients who could be spared from chemotherapy and treated with ET/anti-HER2/palbociclib. On the other hand, a recent joint analysis of neoadjuvant "chemotherapy-free" trials (SOLTI-PAMELA, TBCRC023/TBCRC006, and PerE-LISA) has shown that the combination of the HER2-enriched subtype with a high mRNA expression of *ERBB2* leads to the identification of a subgroup of patients exquisitely sensitive to double HER2 blockade—with pCR rates ranging from 44.5% to 66.7% (51). Among HER2$^+$/ER$^+$ patients, only 32% were "HER2-enriched/ERBB2-high," whereas among the HER2$^+$/ER-neg subgroup, there were 68% of patients with this profile. Hence, as the HER2$^+$/ER-neg subgroup is clearly enriched with these HER2-addicted tumors, one could envision a trial restricted to HER2$^+$/ER-neg patients: they would be screened upfront and those with a HER2-enriched/*ERBB2*-high expression tumor would be proposed to receive neoadjuvant trastuzumab/pertuzumab only. Like in the DeCrescendo trial, patients achieving pCR would be spared of any adjuvant chemotherapy and the primary endpoint would be a high RFS rate with a narrow confidence interval.

Conclusion

Given the predictive value of ER status, it would be reasonable for the next wave of clinical trials to target separately HER2$^+$/ER$^+$ and HER2$^+$/ER-neg patients. This would lead to a better tailoring of deescalation treatment strategies, and hence to a greater chance of "success." In addition, this could greatly reduce the morbidity and costs associated with the long and intense systemic regimens that patients with HER2$^+$ tumors still receive, despite years of biomarker research. Thus, besides the use of ER status, we feel that an integrated classification of HER2$^+$ tumors, based on their DNA, RNA, protein expression, and immune microenvironment, would also be useful for further treatment refinement in the future.

Disclosure of Potential Conflicts of Interest

M. Brandão reports receiving other commercial research support and speakers bureau honoraria from Roche/GNE. R. Caparica reports receiving speakers bureau honoraria from AstraZeneca, Boehringer Ingelheim, and Janssen, and is an unpaid consultant/advisory board member for Pfizer and AstraZeneca. L. Malorni reports receiving commercial research grants from Pfizer and Novartis, and reports receiving speakers bureau honoraria from Novartis. A. Prat is a paid consultant for Roche,

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Authors' Contributions

Conception and design: M. Brandão, R. Caparica, M. Piccart
Development of methodology: Not applicable
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Not applicable
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Not applicable
Writing, review, and/or revision of the manuscript: M. Brandão, R. Caparica, L. Malorni, A. Prat, L.A. Carey, M. Piccart
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Supplementary Paper IX: *Cancer incidence in Mozambique in 2015– 2016: data from the Maputo Central Hospital Cancer Registry*

Cancer incidence in Mozambique in 2015–2016: data from the Maputo Central Hospital Cancer Registry

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There is scarce information on cancer incidence in Mozambique. We aimed to describe cancer incidence data from the Cancer Registry of the Maputo Central Hospital (MCH) in 2015–2016, for Maputo City dwellers, and to compare the incidence rates with those observed in 1956–1961. Cancers with incidence data in 2015–2016 were identified by the Cancer Registry of the MCH. Crude and age-standardized incident rates (ASIR, per 100 000) – direct method, world standard population – were computed for Maputo City, under the assumption that most cancer cases among this population were identified at the MCH. A total of 1707 incident cases in Maputo City dwellers were registered (76.6% confirmed histologically). Prostate cancer, Kaposi sarcoma, and liver cancer were the most frequent in men (ASIR: 24.5, 19.8, and 13.3, respectively). Cervical and breast cancers, and Kaposi sarcoma were the most common among women (ASIR: 32.4, 11.8, and 9.5, respectively). Compared with the data from 1956 to 1961, the most pronounced declines in ASIR were for bladder and liver cancers, in both sexes, and increases were greater for prostate cancer among men (172.2%) and breast cancer (237.1%) among women. Our study highlights the high frequency of infection-related cancers in Maputo, but also of those related to the ongoing demographic and socioeconomic transition. *European Journal of Cancer Prevention* **28:373–376 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.**

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Introduction

In Mozambique, efforts for cancer registration started with a 'cancer survey' of the population of Lourenço Marques (now Maputo city), conducted between 1956 and 1961 by the Department of Pathology of the Maputo Central Hospital (MCH), known at the time as the Miguel Bombarda Hospital (MBH) (Prates and Torres, 1965).

In July 2014, the MCH implemented a hospital-based registry. It aims to produce information for improving the provision of care to cancer patients in the MCH, but it is also the main source of data to a population-based cancer registry that is currently being set up in the city of Maputo.

In the present report, we describe the cancer incidence data produced by the Cancer Registry of the Maputo Central Hospital (CR-MCH) over a 2-year period (2015–2016) for Maputo City dwellers, under the assumption that most cancer cases among this population were identified at the MCH. Also, the incidence rates were compared with those observed in Maputo City in 1956–1961.

Methods

The CR-MCH is based on active identification of cases in the Departments of Pathology, Haematology, Oncology, Paediatrics, and Dermatology, in the Pain Unit and in a specific section of the hospital where private healthcare is provided. In addition, information on the death of cancer patients in the Hospital is also collected in the Hospital Morgue, as well as through the Intra-Hospital Death Registration System.

Data were collected from clinical records using a standardized form, and the date of incidence was defined according to the recommendations of the International Agency for Research on Cancer and of the International Association of Cancer Registries (Jensen *et al.*, 1991). Abstracted data were verified by a clinician and entered into CanReg5 (Coleman and Bieber, 1991) by a registry technician. Thorough routines for identification of duplicate registrations and incomplete or inaccurate records were implemented as part of the regular activities of the CR-MCH, including systematic verification by the registry staff of cases with potential errors, as identified by logical checks, or duplications, on the basis of the clinical file identification number, full name, first name or surname.

Cases receiving any type of care in the MCH, with an incidence date in 2015 and 2016, and occurring among Maputo City dwellers were considered for analysis, after excluding *in situ* and nonmelanoma skin cancers.

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We computed crude and age-standardized incident rates (ASIR) – direct method, world reference population (Doll and Smith, 1982). Comparisons with the MBH Cancer Registry data of 1956–1961 (Parkin *et al.*, 2003) are presented graphically.

The study was approved by the Joint Institutional Bioethics Committee of the Faculty of Medicine, Eduardo Mondlane University and MCH.

Results

The CR-MCH registered a total of 1707 cancer cases occurring in Maputo City dwellers, which corresponds to an incidence rate of 61.2 and an ASIR of 89.2 per 100 000 habitants.

Table 1 presents the number of cases, crude incidence rates, and ASIR for the most frequent cancers in Maputo City. The ASIR for all cancers were 95.3 and 100.1 per 100 000 in men and women, respectively. Kaposi sarcoma, and prostate and liver cancers were the most frequent in men (29.2, 16.3, and 13.8% of cases, respectively), corresponding to ASIR of 19.8, 24.5 and 13.3 per 100 000, respectively. In women, cervical cancer was the most common (31.8% of cases, ASIR 32.4 per 100 000), followed by Kaposi sarcoma (12.9% of cases, ASIR 9.5 per 100 000) and breast cancer (11.4% of cases, ASIR 11.8 per 100 000). A total of 76.6% of cases were confirmed microscopically, but for 15.2%, information could only be obtained from death certificates.

In terms of the variation in ASIR between 1956–1961 and 2015–2016 (Fig. 1), the most pronounced declines were for bladder and liver cancers, in both sexes, and increases were greater for prostate cancer among men (172.2%) and breast cancer (237.1%) among women. In the most recent years, ASIRs were at least 40% higher for colorectal and eye cancers in both sexes. Esophageal cancer varied from 4.4 to 7.0 among men and from 0.0 to 9.4 among women.

Discussion

In 2015–2016, Kaposi sarcoma was the most frequent cancer in Maputo City, in line with other recent reports of cancer incidence in eastern sub-Saharan Africa (Chokunonga *et al.*, 2000; Msyamboza *et al.*, 2012). The male/female incidence ratio was ~2 : 1, as expected for AIDS-related Kaposi sarcoma, rather than the 20 : 1 ratio found in the endemic form of the disease (Mbulaiteye *et al.*, 2011). In Mozambique, the proportion of AIDS-related cases has been estimated previously at 99% in women and at least 88% in men (Meireles *et al.*, 2015).

In agreement with other countries of sub-Saharan Africa (Parkin *et al.*, 2008), cervical cancer was the most common in women, reflecting a high prevalence of human papillomaviruses infection (Menendez *et al.*, 2010), as well as the very limited coverage of screening (Brandão *et al.*, 2018) and high prevalence of HIV infection (UNAIDS, 2008).

Our data yielded markedly lower ASIR for liver and bladder cancers than those reported by the MBH Cancer Registry in 1956–1961, but liver cancer remains the third most common cancer in Maputo. The high prevalence of chronic hepatitis B virus infection and dietary exposure to

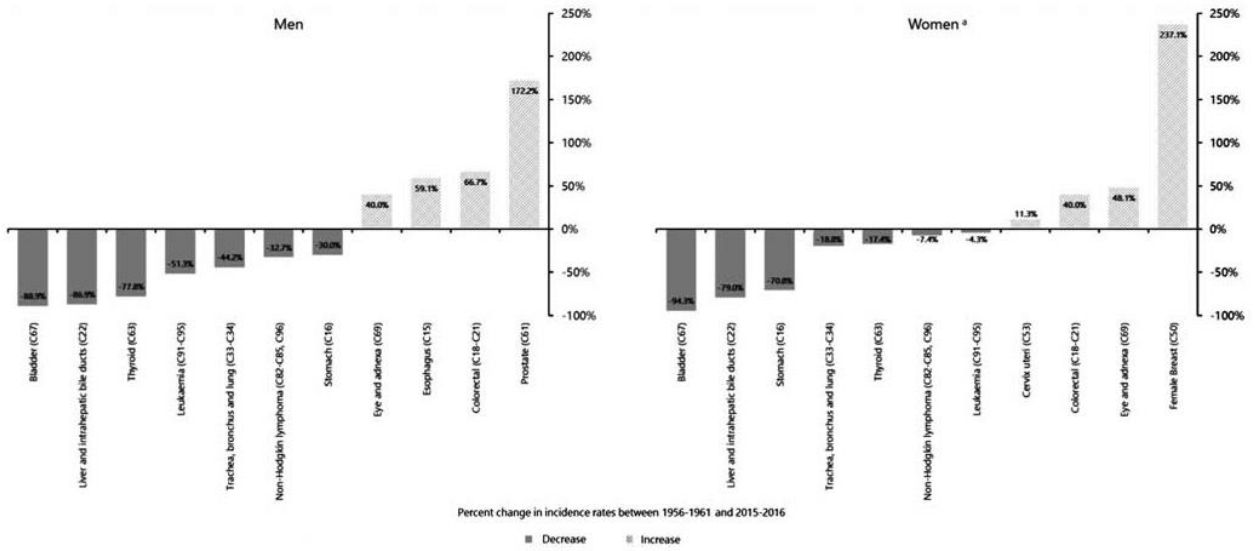
Table 1 Number of cases and crude and age-standardized incidence rates for the most frequent cancers in both sexes, excluding in situ cancers and nonmelanoma skin cancer, among those from Maputo City in 2015–2016[a]

	Men			Women				
Site (ICD-10)	Number of cases (%)	Crude rate (per 100 000)	ASIR (per 100 000)	Number of cases (%)	Crude rate (per 100 000)	ASIR (per 100 000)	MV (%)	DCO (%)
Kaposi sarcoma (C46)	212 (29.2)	17.7	19.8	126 (12.9)	9.7	9.5	67.5	13.9
Cervix uteri (C53)	–	–	–	312 (31.8)	24.0	32.4	83.3	11.9
Liver and intrahepatic bile ducts (C22)	100 (13.8)	8.3	13.3	62 (6.3)	4.8	6.6	53.7	34.6
Esophagus (C15)	44 (6.1)	3.7	7.0	76 (7.8)	5.9	9.4	82.5	10.8
Prostate (C61)	118 (16.3)	9.8	24.5	–	–	–	86.4	10.2
Female breast (C50)	–	–	–	112 (11.4)	8.6	11.8	84.8	12.5
Eye and adnexa (C69)	21 (2.9)	1.7	2.1	45 (4.6)	3.5	4.0	98.5	1.5
Non-Hodgkin lymphoma (C82–C85, C96)	34 (4.7)	2.8	3.5	31 (3.2)	2.4	2.5	72.3	24.6
Colorectal (C18–C21)	23 (3.2)	1.9	3.0	25 (2.6)	1.9	2.8	89.6	8.3
Leukemia (C91–C95)	20 (2.8)	1.7	1.9	22 (2.2)	1.7	2.2	52.4	38.1
Trachea, bronchus, and lung (C33–C34)	17 (2.3)	1.4	2.4	10 (1.0)	0.8	1.3	63.0	29.6
Connective and soft tissue (C47–C49)	14 (1.9)	1.2	1.5	13 (1.3)	1.0	1.2	92.6	0.0
Bladder (C67)	14 (1.9)	1.2	1.9	7 (0.7)	0.5	0.8	76.2	14.3
Thyroid (C73)	4 (0.6)	0.3	0.4	16 (1.6)	1.2	1.9	95.0	0.0
Stomach (C16)	11 (1.5)	0.9	2.1	5 (0.5)	0.4	0.7	62.5	37.5
Ovary (C56)	–	–	–	15 (1.5)	1.2	1.7	80.0	20.0
All cancers (except nonmelanoma skin)	725 (100.0)	60.4	95.3	980 (100.0)	75.5	100.1	76.6	15.2

ASIR, age-standardized incidence rate; DCO, death certificate only; ICD-10, International Classification of Diseases (10th ed.); MV, microscopically verified.
[a]Data are not presented for two cases of unknown age.

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Fig. 1



Variation in the age-standardized (world standard population) incidence rates between 1956 and 1961 (Miguel Bombarda Hospital) and 2015–2016 (Maputo Central Hospital Cancer Registry) in men and in women. [a]For esophageal cancer (C15), the age-standardized incidence rate was 0.0 in 1956–1961 and 9.4 in 2015–2016; therefore, the percent change could not be computed.

aflatoxins, specifically of aflatoxin B1, a carcinogenic mycotoxin, have been pointed to as the main reasons for the high frequency of hepatocellular carcinoma in African countries (Parkin *et al.*, 2003). The vaccine against hepatitis B virus was introduced in the Mozambican Extended Programme of Immunization in 2001 (Ministry of Health, 2011); however, it may have made a small contribution toward the current liver cancer incidence rate as the vaccinated population was less than 20 years of age in 2015–2016 as newborns were the target population. For bladder cancer, squamous cell carcinoma was the most frequent histological type in Maputo in 1956–1961 (Prates and Torres, 1965), as in other eastern and south-eastern African countries (Parkin *et al.*, 2008). The improvement of sanitary conditions in Maputo, leading to a decrease in chronic infection with *Schistosomiasis haematobium* (Augusto *et al.*, 2009), is likely to have contributed toward the observed decline.

Prostate and breast cancers, which are traditionally more frequent in developed countries, were the second and the third most frequent cancers in men and in women, respectively. The adoption of western lifestyles, resulting in greater exposure to factors that increase the risk of breast cancer (e.g. delayed childbearing; lower parity; obesity; physical inactivity) (Key *et al.*, 2001), and the increase in the detection of latent prostate cancers during prostatic resections because of urinary symptoms (Parkin *et al.*, 2003), may have contributed toward the increase in the ASIR. Mammography for breast cancer screening or prostate-specific antigen testing for early detection of prostate cancer is virtually nonaccessible to the general population of most sub-Saharan African countries (Pace and Shulman, 2016). However, along with the increase in detection by needle biopsies, the latter may have had some impact on the prostate cancer incidence rates among the more affluent population. The recently implemented National Program for Cervix and Breast Cancer Control in Mozambique by clinical and self-examination of the breast may have contributed toward an increased detection of breast cancer (Ministério da Saúde de Moçambique, 2009).

Although this study provides new insights into cancer incidence in sub-Saharan Africa, some limitations need to be acknowledged. We used data from a registry based on a single hospital to compute cancer incidence rates; therefore, we may have missed cancer cases among dwellers in Maputo City who were diagnosed and treated outside the MCH, leading to an underestimation of cancer incidence rates. However, the MCH is a 1500-bed hospital and national reference center for cancer, where a large proportion of cases identified in other institutions are expected to be referred. Specifically, the Department of Pathology receives most specimens from Maputo City, including tissue samples taken in public institutions and in many of the private clinics, as well as from autopsies. As the population-based registry is in its beginning and is being managed side by side with the hospital-based registry, we could estimate that ~90% of the cases occurring in this population and identified by the population-based registry were recorded by the CR-MCH.

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Therefore, the incidence rates computed in this study for the population of Maputo City may be viewed as lower bound estimates of the incidence in this population. The fact that only death certificate information was available for approximately one in every seven cases registered shows that registry procedures and the quality of the collected information may be further improved. Nevertheless, the CR-HCM is already based on a large number of sources of information, including several Departments where cancer patients may be diagnosed or treated, along with in-hospital mortality data. Furthermore, all cases of cancer are being considered in the present analysis, irrespective of the reason of their contact with the Hospital. The comparison of contemporary data with those obtained by the MBH Cancer Registry allows some insights into the variation in the burden of cancer over a period of more than half a century. However, this not only reflects temporal trends in the frequency of cancer, because of changes in the levels of exposure to risk factors, but also on the methods available for diagnosis and access to healthcare by the population.

Conclusion

Our study highlights both a high burden of cancers related to infections and the growing importance of cancers that are among the most frequent in more developed countries, reflecting the demographic and socioeconomic transition occurring in Mozambique, especially in urban areas.

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Conflicts of interest

There are no conflicts of interest.

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