Male breast cancer: Specific biological characteristics and survival in a Portuguese cohort

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Abstract. Male breast cancer (BC) represents an individual subtype of BC, with therapeutic procedures based on female BC therapy results. The present study evaluated the parameters currently used for the characterization and therapy of male BC, and their association with disease-free (DFS) and overall survival (OS), aiming to obtain a comprehensive basis to improve the personalized care of male BC. A total of 196 patients from March 1970 to March 2018 (mean follow-up, 84.9 months) were profiled, using clinicopathological review, molecular assessment [BRCA1/2, DNA repair associated (BRCA1/2) status, immunohistochemistry, fluorescence *in situ* hybridization and DNA flow cytometry] and Cox regression statistical analysis. The median age of patients was 66.5 years. At presentation, 39.2% of patients with invasive carcinomas were in anatomic stage (AS) I. Patients exhibited primarily invasive carcinomas of no special type, histological grade 2, estrogen receptor α-(ERα) and progesterone receptor (PR)-positive, receptor tyrosine kinase erbB-2-negative, high Ki-67, Luminal B-like and aneuploid tumors. A total of 13 of the 44 (29.5%) BRCA-evaluated patients exhibited BRCA2 mutations, significantly associated with family history (FH), bilaterality, high Ki-67 expression, absence of PR and Luminal B-like tumors. Bilaterality was associated with the occurrence of non-breast primary neoplasms (NBPN). The 5 and 10-year DFS rates, excluding patients with distant metastasis, NBPN and *in situ* carcinomas (n=145) were 65.9 and 58.2%, respectively, and the 5 and 10-year OS rates were 77.5 and 59.2%, respectively. In the univariate analysis, Luminal B-like subtype, BRCA2 mutations, high Ki-67 expression, and AS II and III were significantly associated with shorter DFS and OS. In addition, age >70 years was associated with low OS. In the multivariate analysis, FH, AS II and III, and Luminal B-like subtypes were associated with poorer OS. In conclusion, the data from the present study emphasize the high incidence of BRCA2 mutation in male BC, and its association with FH, bilaterality, high Ki-67 expression, negative PR expression and Luminal B-like subtypes, and with shorter DFS and OS in univariate analysis.

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Introduction

Breast cancer (BC) is a heterogeneous and complex disease, with a great variation in clinical outcomes. BC is the most frequently diagnosed cancer in females and second in causes of cancer mortality in both sexes, as metastatic BC remains an almost incurable disease. Incidence of male BC is rare (~1% of numbers of female BC and 1% of all malignancies in males in Western countries); however, in previous years, a slight increase in incidence has been observed in certain countries (1-5). BC in males has become most frequently diagnosed at an early anatomic stage (AS), and an improvement in overall survival (OS) has been observed (6-9). However, the lack of information regarding male BC and the unviability of screening have contributed to a persistently high percentage of diagnoses at advanced AS. In Portugal, the annual male BC gross incidence rates in 2010 and 2011 were 1.23 and 1.77 per 100,000 people, respectively, and the gross mortality rates were 0.34 and 0.51, respectively (10).

Despite increasing interest, the biology and optimal management of male BC remain poorly understood, and contradictory data are often identified. Certain common epidemiological risk factors, which remain to be identified in either sex, may be relevant in the understanding and prevention of the disease (11). Genetic predisposition appears to be an important contributor to risk and may have clinical implications (8,12,13). Family history (FH) is also relevant, and a positive FH in a masculine family member is strong indication for genetics consultation (13). In addition, genetic mutations may be identified in patients without FH and should be routinely screened in male BC (13). In contrast to those

identified in females, mutations in the BRCA2, DNA repair associated (BRCA2) gene are predominant in male BC, while BRCA1, DNA repair associated (BRCA1) gene mutations are infrequent (8,12,14,15).

Obesity is one of the most common causes of hyperestrogenism in males, and adolescent overweight has been associated with an increased risk of male BC (2,12,16). In addition, liver diseases, alcoholism, Klinefelter's syndrome, exogenous estrogen use (namely for the treatment of prostate cancer) and androgen deficiency due to testicular disease including hypogonadism and orchitis, appear to increase the risk of disease (2,4,7). Occupational and environmental exposures to radiation, and heat and electromagnetic fields have also been implicated as potential risk factors (3,8,12).

Male BC is diagnosed by mammography or ultrasonography and confirmed by core biopsy, which are always performed following a suspicious clinical examination. Therapeutic procedures are based on the recommendations for female BC, but mastectomy rather than breast-conserving surgery is performed in the vast majority of cases. In addition, hormone therapy is less tolerated in males compared with in females, and side effects including weight gain, depression, deep venous thrombosis, decreases in libido and impotence, with high rates of discontinuation, were described (4,5,17,18).

Molecular testing in BC, through the use of sophisticated techniques including deep sequencing analysis, has led to an improved understanding of this disease. Concomitantly, the identification of targeted therapies has reinforced the requirement for improved stratification in BC subtypes (13). The present study investigated a retrospective series of male BC cases, assessed the clinicopathological and molecular characteristics that are currently the basis for therapeutic strategies, and estimated their association and significance in disease-free survival (DFS) and OS of male BC.

Patients and methods

Patient selection and clinicopathological evaluation. The present retrospective study involved 196 male patients with BC diagnosed and treated according to therapeutic protocols from March 1970 to March 2018 (mean follow-up time, 84.9 months), at the Portuguese Institute of Oncology of Lisbon (Lisbon, Portugal). The institutional Ethical Committee of the Portuguese Institute of Oncology of Lisbon approved the study. Patient data, including age, obesity, FH, bilaterality, presence of non-breast primary neoplasms (NBPN), presence of distant metastasis and therapeutic modalities were obtained by review of the clinical records. All slides were reviewed. AS classification included pathological tumor size (pT), pathological axillary nodal status (pN) and distant metastasis (M), and was registered according to the TNM classification system recommended by the 8th edition of the American Joint Committee on Cancer (AJCC) staging system (19). The histological type was re-evaluated as per the World Health Organization 2012 classification (20). Histological grade of differentiation (G) was assessed using the Elston-Ellis grading system criteria (21).

BRCA status. Nucleic acids were obtained from peripheral blood nucleated cells. DNA was extracted with the EZ1 Bio Robot and the EZ1 DNA blood kit (Qiagen GmbH, Hilden, Germany) and RNA was extracted using TRIzol® (Thermo Fisher Scientific, Inc., Waltham, MA, USA). A total of 44 patients were pre-screened for the c.156_157insAlu *BRCA2* Portuguese founder mutation, analyzed for *BRCA1/2* point mutations by next generation sequencing (NGS) with a CE-IVD MASTR BRCA molecular diagnostic assay (Multiplicom; Agilent Technologies, Inc., Santa Clara, CA, USA) in a MiSeq Instrument (Illumina, Inc., San Diego, CA, USA) and for large rearrangements with a Multiplex Ligation-dependent Probe Amplification (MLPA) assay using P002 BRCA1 and P045 BRCA2/CHEK2 kits (MRC-Holland, Amsterdam, The Netherlands). Variant Studio v.2.2 (Illumina, Inc, San Diego, CA, USA) and DNAnexus, Inc, Mountain View, CA, USA were used to analyze the NGS data, and Coffalyzer (MRC-Holland, Amsterdam, The Netherlands) software was used for the MLPA data. In addition to the information provided in the Variant Studio and DNAnexus programs, the Breast Cancer Information Core database and the Universal Mutation Database were used to clarify certain variants. Prior to NGS screening, patient samples were analyzed by conformation sensitive capillary electrophoresis or conformation sensitive gel electrophoresis, and samples with different patterns (fragment pattern comparison between the 44 patient samples analyzed in the same batch and also comparison with a negative control) were sequenced by Sanger sequencing using an ABI 3130 instrument as described previously (22,23).

Estrogen receptor α (ERα), progesterone receptor (PR), receptor tyrosine kinase erbB-2 [(ERBB2), antigen Ki-67 (Ki-67) immunohistochemistry (IHC) and ERBB2 in situ hybridization (ISH)]. IHC was performed using a peroxidase-indirect-polymer technique performed on a Ventana Benchmark™ ULTRA instrument (Ventana Medical Systems, Inc.; Roche Diagnostics, Basel, Switzerland) on formalin-fixed paraffin embedded tumor tissues. All cases were re-analyzed under the same conditions for all samples within this study and all kits were used according to the protocol of the manufacturer. The levels of ERα clone SP1 (Ventana Medical Systems, Inc.; Roche Diagnostics; cat. no. 790-4324) and PR clone IE2 (Ventana Medical Systems, Inc.; Roche Diagnostics cat. no. 790-2223) were recorded as the percentage of positively-stained neoplastic cell nuclei, using a ≥1% cut-off value as the criterion for positivity. The staining intensity was not evaluated (24). The ERBB2 clone 4B5 (Ventana Medical Systems, Inc.; Roche Diagnostics cat. no. 790-2991) was used for ERBB2 evaluation. The quantification of ERα, PR and ERBB2 oncoprotein overexpression (0, 1+, 2+ and 3+) was based on the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (25). The Ki-67 index was recorded as the percentage of positively-stained cells, using the Ki-67 clone 30-9 (Ventana Medical Systems, Inc.; Roche Diagnostics; cat. no. 790-4286), and 'hot spots' were classified as those areas containing 100 malignant cells. The threshold for high proliferation was 20%, based on the adaptation of the 2013 St. Gallen consensus guidelines (26). ERBB2 gene amplification was determined by FISH using a BenchMark™ Ventana® system (Ventana Medical Systems, Inc.; Roche Diagnostics) or, in samples collected from 2009, by silver *in situ* hybridization

(SISH) with evaluation of chromosome 17 (Dual-Color SISH red ISH/Benchmark™ ULTRA Ventana®; Ventana Medical Systems, Inc.; Roche Diagnostics) in 77 cases, which included 38 IHC-negative (0/1+), 35 equivocal (2+) and 4 positive (3+) cases, according to the latest ASCO/CAP guidelines. The IHC pattern, complemented with ERBB2 ISH, allowed the identification of clinically-defined, treatment-oriented subtypes (CS), according to AJCC, 8th Edition (19), and were as follows: Luminal A-like (high hormone receptors and low proliferation level), Luminal B-like (low hormone receptors and high proliferation level), HER2-like (ERBB2-positive and negative or positive hormone receptor expression) and Triple negative (TN; ER-, PR- and ERBB2-negative).

DNA flow cytometry. DNA flow cytometric analysis was performed on representative paraffin-embedded tissue according to the method described by Hedley *et al* (27), with slight modifications (50 μm sections and propidium iodide DNA staining were used). DNA content of the neoplastic cells was determined in 79 invasive carcinomas with no neoadjuvant therapy. The cell cycle analysis of DNA histograms was assessed using the Multicycle software program (32-bit version; Phoenix Flow Systems, San Diego, CA, USA). The male BC cases were also classified as diploid vs. aneuploid according to their nuclear DNA ploidy status.

Statistical analysis. A survival study with an initial descriptive analysis and subsequent nonparametric, semiparametric and parametric statistical techniques was elaborated. The statistical analysis was performed using the software R Core Team 2018 (28). Pearson's χ^2 and Fisher's exact tests of independence were used to evaluate the association between categorical variables. Fisher's exact test was used when the number of observations was small (n<20). Identical conclusions were obtained following the use of each test. Survival curves were estimated using Kaplan-Meier analysis, and the differences between curves were assessed by the log-rank test. P<0.05 was considered to indicate a statistically significant difference. The DFS represented the remission time until a relapse event. The OS was defined as the interval between pathological diagnosis and the occurrence of mortality due to BC. Patients without disease recurrence during the study period and those who succumbed to other causes, or those who were lost to follow-up, were considered as censored observations. The OS was evaluated in the subgroup of patients with M1 disease, and the DFS and OS in the whole series, excluding patients with M1 disease, NBPN and *in situ* carcinomas. OS and DFS were also evaluated and compared in the following groups: Patients with vs. patients without NBPN, and patients diagnosed in the years 1970-1998 (group A; n=84) vs. those diagnosed in the years 1996-2018 (group B; n=132) (prior and subsequent to the introduction of taxane chemotherapy). The variables describing the type of treatment were excluded due of the variability of protocols used. A Cox proportional hazards regression model was employed to assess the independent prognostic value of the variables. Initially, the model was fitted for each variable to evaluate their effects on OS time and remission time of disease, as a simple regression analysis. Following the determination of significant variables, a Cox regression model was performed with all variables simultaneously, as a multiple regression analysis. A backward stepwise procedure based on Akaike's Information Criterion (29) was used to select auxiliary variables. The statistical significance was obtained by the Wald test, and complementary inference was calculated as relative risk and 95% confidence intervals for each category. The quality of the fitted models was assessed using a residual analysis, and a test of proportionality of risk functions was conducted for Cox regression models associated with OS and DFS.

Results

Descriptive analysis and associations. The clinicopathological and therapeutic characteristics of the series are summarized in Table I. BRCA mutation, hormone receptors and Ki-67 protein expression, ERBB2 overexpression and amplification, CS and DNA ploidy data are presented in Table II. Significant associations between variables are indicated in Table III.

The mean and median age of patients at diagnosis was 65.2 and 66.5 years (range, 31-89 years), respectively. The majority of the patients (n=108; 55.1%) were between 40 and 69 years, and older patients (≥70 years) comprised 41.3% of the sample. Old age (≥70 years) exhibited a significant association with pT4 (P=0.021). Body mass index was not evaluated in the majority of the cases; however, from review of the clinical records, obesity was observed in ~20% of the patients.

A confirmed FH of BC was obtained in 30 patients (15.3%). FH is significantly associated with G2/G3 carcinomas, high Ki-67, Luminal B-like subtype, high anatomical stage, presence of BRCA2 mutations and bilaterality. A total of 7 patients (3.6%) exhibited bilateral carcinomas, and 1 patient exhibited synchronous tumors. FH and bilaterality were significantly associated with BRCA2 mutations. Bilaterality was also associated with the presence of NBPN. The occurrence of NBPN was identified in 28 patients (14.3%). One patient exhibited 3 other carcinomas, in the prostate, colon and kidney, and 2 patients exhibited 2 carcinomas, in the prostate and bladder and in the prostate and kidney. From the remaining patients, 12 had prostate carcinomas, 3 presented with colon-rectal carcinomas, 3 exhibited head and neck squamous cell carcinomas, 3 had gastric carcinomas, 1 had papillary thyroid carcinoma, 1 exhibited chronic lymphocytic leukemia, 1 had Hodgkin disease and 1 exhibited soft tissue histiocytic sarcoma. In the majority of cases, BC was the first neoplasm recorded. In 5 patients, it was the second neoplasm observed; 2 of these patients had exhibited lymphoma previously. A total of 2 patients with prostate carcinoma also had bilateral BC.

A total of 79 patients (40.3%) presented with pT4 carcinomas and 110 (56.4%) with axillary lymph node metastasis. The majority of the patients (n=178; 90.8%) had no distant metastasis at presentation (M0). At diagnosis, 88 patients (44.9%) were diagnosed with AS II disease. Nodal status and AS were significantly associated with Ki-67. Distant metastases at presentation (M1) were associated with ERBB2-positive carcinomas.

Regarding the histological type, 177 (90.3%) invasive carcinoma of no special type (NST) were identified, ~25% of which exhibited a range of proportions of *in situ* components, and the other most frequent invasive subtypes were mucinous carcinoma (1 pure and 4 mixed) and papillary carcinoma (4 cases). The case of pure mucinous carcinoma belonged to a

Table I. Clinicopathological and therapeutic characteristics of the patient cohort.

Characteristics	N	N (%)
Age (years)	196	
31-39		7 (3.6)
40-69		108 (55.1)
70-89		81 (41.3)
Family history (FH)	196	
No		166 (84.7)
Yes		30 (15.3)
Bilaterality	196	
No		189 (96.4)
Yes		7 (3.6)
Non-breast primary neoplasms (NBPN)	196	
No		168 (85.7)
Yes		28 (14.3)
Tumor size (pT)	196	
pTis		7 (3.6)
pT1		52 (26.5)
pT2-3		58 (29.6)
pT4		79 (40.3)
Axillary nodal status (pN)	195	
pN0		85 (43.6)
pN1		110 (56.4)
Distant metastasis (M)	196	
M0		178 (90.8)
M1		18 (9.2)
Anatomic stage (AS)	196	
In situ (is)		7 (3.6)
I		77 (39.2)
II		88 (44.9)
III		6 (3.1)
IV		18 (9.2)
Histological type (HT)	196	
In situ		6 (3.1)
Invasive no special type (NST)		177 (90.3)
Other invasive subtypes		13 (6.6)
Histological grade (G)	190	
G1		45 (23.7)
G2		110 (57.9)
G3		35 (18.4)
Therapy	196	
Surgery		177(90.3)
Radiotherapy		124 (63.3)
Hormonotherapy		118 (60.2)
Chemotherapy		73 (37.2)

N, number of patients; FH, family history; NBPN, non-breast primary neoplasia; pT, tumor size; pN, axillary nodal status; M, distant metastasis; AS, anatomic stage; NST, no special type; G, histological grade.

Table II. Molecular characteristics of the series.

Characteristics	N	N (%)
BRCA2 mutation	44	
Indeterminate		31 (70.5)
Positive		13 (29.5)
Estrogen receptors (ER)	190	
Positive		177 (93.1)
Negative		13 (6.9)
Progesterone receptors (PR)	190	
Positive		143 (75.3)
Negative		47 (24.7)
ERBB2 (IHC + ISH)	190	
Negative		179 (94.2)
Positive		11 (5.8)
Ki67	190	
Low		78 (41.1)
High		112 (58.9)
Clinical defined subtypes (CS)	190	
Luminal A-like		77 (40.5)
Luminal B-like		86 (45.3)
HER2-like		13 (6.8)
Triple negative		14 (7.4)
DNA ploidy	79	
Diploid		9 (11.4)
Aneuploid		70 (88.6)

N, number of patients; BRCA2, BRCA2, DNA repair associated; IHC, immunohistochemistry; ISH, fluorescence *in situ* hybridization; ERBB2/HER2, receptor tyrosine kinase erbB-2; Ki-67, antigen Ki-67; CS, clinical subtype; TN, triple negative.

37 years old patient with no FH, diagnosed with a pT2/pN0/M0 tumor, G2, ER/PR-positive, ERBB2-negative and BRCA indeterminate, who survived with no recurrence during a follow-up of 96 months. A total of 2 patients (1%), at 51 and 64 years old, were diagnosed with lobular invasive carcinoma, one with a FH and the other with a pathogenic BRCA2 mutation.

The majority of the male BC cases were graded as G2. A total of 45 cases (23.7%) were classified as G1, and only a minority of the cases (18.4%) was integrated in the G3 group. High grades were associated with high Ki-67 levels.

During the present study, therapeutic strategies for male BC followed the patterns of the recommendations for BC in females. The majority of patients (90.3%) underwent surgery, but only 9 patients (4.6%) performed breast-conserving surgery. A total of 33 patients (16.8%) received neoadjuvant therapy. Adjuvant radiotherapy was used in 63.3% of the patients, adjuvant hormonotherapy in 60.2% and chemotherapy in 37.2% of the patients. ERBB2-targeting agents were used in 2 patients in the cohort.

BRCA2 mutations were identified in 13 (29.5%) of the 44 patients examined and, in addition to the associations with FH and bilaterality, were also significantly associated with high Ki-67 and negative PR expression levels. A total of 10

Table III. Significant associations between clinical and molecular characteristics of the patient cohort (Pearson's chi-square test).

Characteristics	P-value
Age (<40, 40-69, ≥70 years)	
pT	0.021
Family history (FH) (no vs. yes)	
G	0.009
AS	0.011
Ki67 index	0.002
CS	0.001
BRCA2 mutation	0.002
Bilaterality (no vs. yes)	
FH	0.009
Non-breast primary neoplasms (NBPN)	<0.001
DNA ploidy	0.004
BRCA2 mutation	0.008
Tumour size (pT) (pT1 vs. pT2-3 vs. pT4)	
pN	<0.001
M	<0.001
AS	<0.001
PR	0.029
Nodal status (pN) (pN0 vs. pN1)	
M	0.002
AS	<0.001
Ki67 index	0.003
CS	0.030
Distant metastasis (M) (M0 vs. M1)	
AS	<0.001
CS	0.030
ERBB2	0.009
Anatomic stage (AS) (I vs. II/III vs. IV)	
Ki67 index	0.004
CS	0.009
ERBB2	0.015
Histological type (HT) (NST vs. others)	
pT	<0.001
pN	0.012
AS	<0.001
Grade (G) (G1 vs. G2 vs. G3)	
Ki67 index	<0.001
CS	0.002
Estrogen receptors (ER) (positive vs. negative)	
PR	<0.001
Progesterone receptors (PR) (positive vs. negative)	
BRCA2 mutation	0.010
Ki-67 (low vs. high)	
CS	<0.001
ER	0.004
PR	<0.001
ERBB2	0.011
BRCA2 mutation	0.047

Table III. Continued.

Characteristics	P-value
Age	
pT	0.021
Family history (FH)	
G	0.009
AS	0.011
Ki67 index	0.002
CS	0.001
BRCA2 mutation	0.002
Bilaterality	
FH	0.009
Non-breast primary neoplasms (NBPN)	<0.001
DNA ploidy	0.004
BRCA2 mutation	0.008
Tumour size (pT)	
pN	<0.001
M	<0.001
AS	<0.001
PR	0.029
Nodal status (pN)	
M	0.002
AS	<0.001
Ki67 index	0.003
CS	0.030
Distant metastasis (M)	
AS	<0.001
CS	0.030
ERBB2	0.009
Anatomic stage (AS)	
Ki67 index	0.004
CS	0.009
ERBB2	0.015
Histological type (HT)	
pT	<0.001
pN	0.012
AS	<0.001
Grade (G)	
Ki67 index	<0.001
CS	0.002
Estrogen receptors (ER)	
PR	<0.001
Progesterone receptors (PR)	
BRCA2 mutation	0.010
Ki-67	
CS	<0.001
ER	0.004
PR	<0.001
ERBB2	0.011
BRCA2 mutation	0.047

FH, Family history; G, Grade; AS, anatomic stage; KI-67, antigen Ki-67; CS, subtype; BRCA2, BRCA2, DNA repair associated; M, Distant metastasis; PR, Progesterone receptors; ERBB2, receptor tyrosine kinase erbB-2; ER, Estrogen receptors.

(76.9%) confirmed BRCA2 mutated carcinomas belonged to the surrogate Luminal B-like subtype and 2 cases were HER2-like, according to the AJCC 8th edition classification system (19). No BRCA1 mutations were identified in the series.

The majority of male breast carcinomas were ER-positive/PR-positive/ERBB2-negative, and 14 (7.4%) were TN. All PR-positive cases were ER-positive, and 34 cases (75.6%) of PR-negative carcinomas were ER-positive (P<0.0001). Using IHC, 35 ERBB2-equivocal (2+) cases and 6 positive (3+) cases were identified. From the equivocal cases, 5 (14.2%) became positive. In total, 11 ERBB2-positive cases were identified, 10 cases of which were triple-positive and 1 case was ER-positive and PR-negative. Positive ERBB2 expression was significantly associated with M1 carcinomas at presentation, high AS and high Ki-67 expression.

High Ki-67 (n=112; 58.9%) was significantly associated with positive FH, high grades, pN1, high AS, ER-negative, PR-negative and ERBB2-positive expression, and the presence of BRCA2 mutations.

The incidence rates of the 4 clinically-defined CS using IHC, according to AJCC 8th Edition (19), were as follows: Luminal A-like (40.5%), Luminal B-like (45.3%), HER2-like (6.8%) and TN (7.4%).

DNA ploidy pattern was analyzed in 79 cases, revealing a high percentage of aneuploid carcinomas (88.6%). As shown in Table III, aneuploid carcinomas were significantly associated with bilaterality.

Survival analysis. The 5 and 10-year DFS rates of patients, excluding patients with M1 carcinomas, patients with non-primary breast neoplasms and *in situ* carcinomas (n=145) were 65.9 and 58.2%, respectively, and the 5 and 10 year OS rates were 77.5 and 59.2%, respectively. Mean and median remission times were 75.6 and 50 months (range, 0-312), respectively, and mean and median survival times were 87.8 and 72 months (range, 3-396), respectively. Of the 18 patients with distant metastasis at presentation, only 1 was alive with bone metastasis after 34 months of follow-up. All other 17 patients succumbed to the disease, with the mean and median survival times for all patients with distant metastases (M1) being 18.7 and 15.5 months (range, 1-38 months). The occurrence of NBPN did not decrease OS, as patients with NBPN exhibited 5 and 10-year OS rates of 92.3 and 92.3% compared with 75.5 and 59.2% of patients without NBPN.

Kaplan-Meier estimates indicated that a longer DFS and an improved OS were significantly associated with pT1/pN0/stage I (all P<0.001), low Ki-67 carcinomas (P=0.030 and P=0.010, respectively; Fig. 1 and Tables IV and V), while a shorter DFS and poorer OS were associated with Luminal B-like subtype (P=0.002) and the presence of BRCA2 mutations (P=0.003 and P<0.001, respectively) (Fig. 2). Patients with G3 carcinomas also exhibited a shorter DFS (P=0.020). Additionally, a longer OS was associated with young age (<40 years; P=0.010 and Fig. 3). The patients diagnosed prior to the introduction of taxane chemotherapy exhibited significantly decreased 5 and 10-year DFS (P=0.030) and OS (P=0.050) compared with those diagnosed in the years following the introduction of taxane chemotherapy.

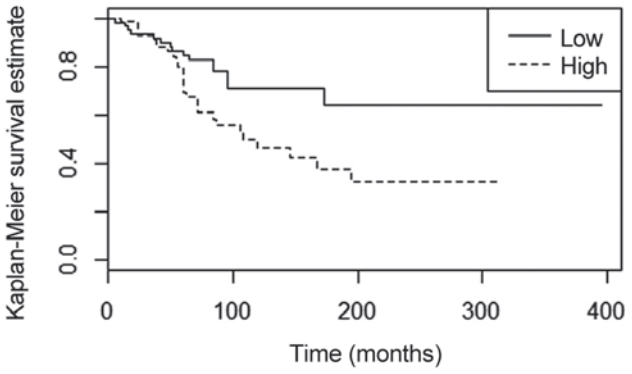

Figure 1. Kaplan-Meier overall survival curve for Ki-67 index. Log rank tests were used to analyze the curves (P=0.010). Ki-67, antigen Ki-67.

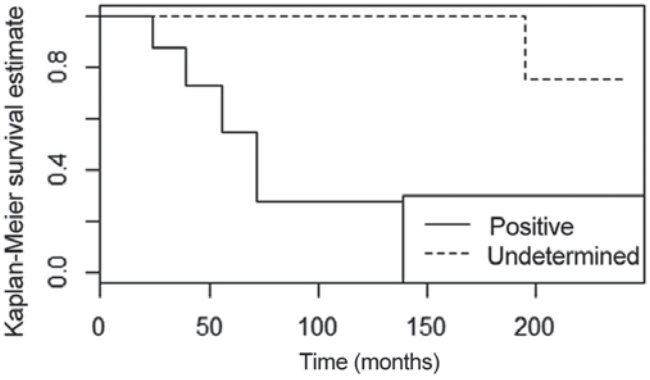

Figure 2. Kaplan-Meier overall survival curve for BRCA2 mutations. Log rank tests were used to analyze the curves (P<0.001). BRCA2, BRCA2, DNA repair associated.

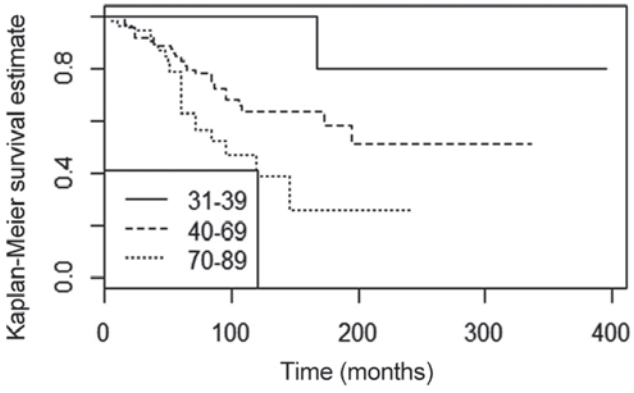

Figure 3. Kaplan-Meier overall survival curve for age (years) of the patients. Log rank tests were used to analyze the curves (P=0.010).

The results of the univariate Cox model analysis (Table IV) were consistent with the Kaplan-Meier analysis. The categories pT2-3 and 4, pN1, AS II and III, high Ki-67, Luminal B-like and BRCA2 mutations were significantly associated with shorter DFS and OS. In addition, G3 and ages >70 years were significantly associated with lower DFS and poorer OS, respectively.

In the multivariate Cox regression analysis (Table V), bilaterality, G3 and AS II and III carcinomas were the

Table IV. Univariate Cox regression analysis in relation to DFS and OS.

	Disease-free survival			Overall survival		
Variables	RR	95% CI	P value	RR	95% CI	P-value
Age (years)						
31-39	1	-	-	1	-	-
40-69	4.37	0.59-32.3	0.148	4.05	0.54-30.1	0.172
70-89	6.35	0.84-48.2	0.074	8.02	1.06-60.8	0.044
Grade (G)						
G1-2	1	-	-			
G3	2.14	1.12-4.05	0.020			
Tumor size (pT)						
pT1	1	-	-	1	-	-
pT2-3	3.19	1.23-8.27	0.017	3.55	1.14-11.0	0.029
pT4	6.10	2.50-14.9	<0.001	9.10	3.19-26.0	<0.001
Nodal status (pN)						
pN0	1	-	-	1	-	-
pN1	6.32	2.93-13.6	<0.001	6.40	2.84-14.4	<0.001
Anatomic stage (AS)						
I	1	-	-	1	-	-
II	6.71	3.11-14.5	<0.001	7.81	3.28-18.6	<0.001
III	4.80	1.01-22.7	0.048	11.1	2.75-44.9	<0.001
Ki-67						
Low	1	-	-	1	-	-
High	1.89	1.05-3.39	0.033	2.14	1.16-3.96	0.015
Clinical subtype (CS)						
Luminal A-like	1	-	-	1	-	-
Luminal B-like	2.67	1.45-4.90	0.002	2.97	1.58-5.60	<0.001
HER2-like	0.81	0.11-6.08	0.837	1.25	0.16-9.51	0.828
Triple negative	0.52	0.12-2.23	0.379	0.64	0.15-2.80	0.556
BRCA2 mutation						
Indeterminate	1	-	-	1	-	-
Positive	0.11	0.02-0.63	0.013	0.06	0.01-0.52	0.011

DFS, disease-free survival; pT, tumor size; pN, nodal status; M, distant metastasis; AS, anatomic stage; G, grade; Ki-67, antigen Ki-67; CS, clinical subtype; BRCA2, BRCA2, DNA repair associated; HER2, receptor tyrosine kinase erbB-2; TN, triple negative.

significant factors associated with a higher risk of disease recurrence. The presence of FH, AS II and III and Luminal B-like subtype were the significant characteristics associated with low OS.

Discussion

BC is a complex disease that affects females and males, and the primary known difference between sexes is incidence. According to recognized biological heterogeneity, studies comparing female and male BC have demonstrated similarities and differences (1,3,7,30,31). The understanding of the effects of the clinicopathological, molecular and genomic features associated with therapy and prognosis is progressing continuously in male BC and, as more data become available, the hypothesis that males only exhibit endocrine-associated BC identical to that in postmenopausal females becomes less plausible, and male BC emerges as a distinctive subtype of BC lacking its own guidelines (15,32-36).

Survival has been a controversial issue in male BC. The majority of studies have demonstrated a poorer outcome in male compared with female patients, but others revealed that there was no difference in the prognosis of the two sexes, when paired according to specific groups (15,30,32-38). A similar DFS and OS to pre/peri-menopausal females, but poorer compared with post-menopausal female BC, was described in male patients with BC (32), and even a lower risk of mortality compared with comparable females, despite the frequent presentation in elderly and more advanced disease in male BC (1). M1 patients have incurable disease and, in the patient cohort in the present study, all but 1 succumbed to the disease, with a median survival time of 15.5 months. The present study

Table V. Multivariate Cox regression analysis in relation to DFS and OS.

	Disease-free survival			Overall survival		
Variables	RR	95% CI	P-value	RR	95% CI	P-value
Bilaterality						
No	1	-	-			
Yes	6.16	1.30-29.3	0.022			
Family history (FH)						
No						
Yes				0.33	0.13-0.90	0.030
Grade (G)						
G1-2	1	-	-	1	-	-
G3	2.20	1.10-4.42	0.026	2.06	1.0-4.24	0.051
Anatomic stage						
I	1	-	-	1	-	-
II	4.08	1.53-10.9	0.005	8.95	3.65-21.9	<0.001
III	6.79	1.15-40.2	0.035	45.7	9.92-211	<0.001
Clinical subtype						
Luminal A-like	1	-	-	1	-	-
Luminal B-like	1.72	0.92-3.23	0.091	2.05	1.07-3.93	0.030

OS, overall survival; pT, tumor size; pN, nodal status; M, distant metastasis; AS, anatomic stage; G, grade; Ki-67, antigen Ki-67; CS, clinical subtype; PR, progesterone receptor; BRCA2, BRCA2, DNA repair associated; HER2, receptor tyrosine kinase erbB-2; TN, triple negative.

encompassed a long time period, with the 10-year OS rates of cases (stages I-III; 59.2%) measuring slightly longer compared with those demonstrated by Leone *et al* (53.7%) (6), Chen *et al* (40.1%) (38) and Tural *et al* (52.5%) (39).

The risk of developing BC increases with age, similar to the majority of carcinomas at all sites. In the present study, the percentage of the patients aged ≥70 years (41.3%) confirms the high incidence of BC in older males, and also that the average age at diagnosis is ~5-10 years older compared with in females (1,12,36). The high frequency in the elderly population is important, as the therapeutic approach in older male patients is based on studies performed in females of different ages, and comorbidities in the elderly population may result in inadequate treatment. In the present study, elderly patients exhibited larger carcinomas and higher Ki-67 expression levels compared with younger patients, and old age was a prognostic factor significantly associated with low 5 and 10-year OS in Kaplan-Meier estimates, which were concordant with data from previous studies (6,7,33,38,39). Poorer prognoses in older males may be associated with tumor biology, late diagnosis, comorbidities and/or inadequate therapeutic management, and constitutes a persistent clinical problem (33,39). Similar to older patients, obese patients have unknown risk factors affecting the accurate prediction of toxicity of treatments and prognosis (35). Obesity is an important risk factor and the proportion of obese male patients with BC observed in the present study was similar to that identified by Gargiulo *et al* (8,40).

FH appears to be particularly relevant in male BC. Bouchardy *et al* (41) identified a positive FH in ~20% of male patients with BC, but no significant differences in OS in patients with FH compared with sporadic cases were observed. As the present study included patients diagnosed from 1970 onwards, the majority of patients had no information regarding FH in their clinical records. However, a confirmed family history of BC in a first-degree relative was significantly associated with BRCA mutations, and also to high AS, high grade, high Ki-67 and Luminal B-like subtype. Additionally, in the multivariate analysis, a positive FH was associated with OS. In concordance with previous data (41), positive FH was also associated with bilateral male BC. Bilaterality occurred in 3.6% of the patients in the cohort within the present study, and was significantly associated with BRCA2 mutations and with the presence of NBPN. Male patients with BC also have an increased risk of NPBN, and the long survival times currently observed should be observed cautiously (42-44). A total of ~14% of the patients in the present study exhibited NBPN, and 2 with bilaterality and prostate carcinoma. As described previously (45), prostate carcinoma is the most frequently observed non-breast primary tumor. The risk of head and neck, colon and thyroid carcinomas were demonstrated to be high in male BC (43,44), and their occurrence was also observed in the present study. A total of 2 patients had previous lymphoma, supporting the observation that males who survive lymphoma may have an increased risk of developing BC (44,46). Among the factors identified to be responsible for causing a second neoplasia, genetic factors appear to represent an important contribution. These data suggest the requirement of a genetic consultation in male BC. BRCA2 is one of the most frequently mutated genes in male BC, ranging between 4 and 40% depending on the population studied (15); 29.5% of the 44 patients included in the present study exhibited this mutation, while BRCA1 mutations are infrequent; none were observed in the present study, suggesting

a dissimilar genetic etiology between sexes (13). As described previously (8,16), the majority of BRCA2-mutated carcinomas in the present study belonged to the Luminal B-like subtype, and a significant association between BRCA2 mutations and poorer prognosis was observed.

The AS classification systems represents one of the most important established prognostic tools for male BC, as demonstrated in the present study and in previous studies (6,8,34). Male BC is increasingly diagnosed earlier (6,7), and a predominance of early stages was observed in the present study. However, high rates of advanced stages are frequently observed (1,6,8,12,33,36,37). The unviability of screening due to low incidence rates, the high occurrence of gynecomastia that may exhibit identical presentation symptoms, the fact that males are less likely to report symptoms that would lead to early diagnosis, the absence of publicly-available information about the disease, the incidence in old age with an associated suboptimal access to healthcare, and anatomic and biological differences, may explain the number of diagnoses at high stages observed (4,36,39).

The proportion of pure *in situ* carcinomas, one-half with papillary morphology, varies in previous studies, but is significantly decreased compared with the proportion described in females (30,33,47). The relative frequency of papillary morphology, either *in situ* or invasive carcinomas, may be associated with the common subareolar localization in male BC. The heterogeneous histological type of invasive carcinoma NST, with an occurrence between 85 and 95% described in previous studies (5,37), was diagnosed in 90.3% of cases in the present study. The percentage of associated *in situ* components was similar to the proportion demonstrated in females (48). Mucinous carcinoma accounts for 1-4% of male BC, has a favorable prognosis in the pure form, but the pathogenesis is not understood (30,39,49,50). In the present study, 5 cases (2.5% of all cases) were observed, one being the patient with a pure form, unusually young for the described in mucinous carcinomas. Invasive lobular carcinoma, the second most frequent histological type in females (10% of the cases), is exceptionally rare in males (1%) and its etiology remains unexplained considering the lack of development of terminal lobules in males (6,51). A total of 2 invasive lobular carcinomas (1%) were identified in the present study, both with negative epithelial-cadherin staining.

G2 carcinomas were predominant in the present study, similar to other previous studies (5,7,30,33,52). High histological grade (G3) is commonly associated with poor prognosis, but this is not always statistically significant (6,7). In the present study, G3 carcinomas occurred in 18.4% of the cases, and were demonstrated to be significantly associated with FH, high Ki-67 expression, Luminal B clinical subtype and poorer prognosis in univariate and multivariate Cox regression analyses.

The lack of randomized trials in male BC explains why therapy is based on the guidelines for BC in females. However, due to primarily anatomical and hormonal reasons, the management is not exactly the same, highlighting the requirement to improve the personalized care of male BC (17,38). Breast-conserving surgery vs. mastectomy may be performed in early stages, but is rarely used due to the paucity of breast tissue and the frequent subareolar location of carcinomas associated with the distribution of epithelial breast tissue (6-8,49). Tamoxifen is the most frequently employed systemic treatment (38,49), but low tolerance, side effects and high rates of discontinuation have been described (4,17,38,49). The relatively low rate of hormonotherapy compared with the high percentage of ER-positive carcinomas identified in the present study, and demonstrated in previous studies (7,31), may be associated with the fact that the use of tamoxifen in males was only recently standardized (7). Different chemotherapy agents and regimens have been used and the introduction of taxanes marked a significant advance in the treatment of metastatic disease in females, but there are no specific evidence-based guidelines for male BC (33,35,49). In addition to the therapeutic effects of the treatment, the improvement in DFS and OS observed in the present study when comparing the groups of patients diagnosed prior and subsequent to taxane chemotherapy may be associated with early diagnosis, standardized clinicopathological evaluation and improved follow-up observed in recent years (1).

Biomarker evaluation by IHC has resulted in differing data among male BC studies, primarily due to different methodologies, the development of scoring systems and the range of cut-off values used, but the high frequency of ER-positive/PR-positive expression and the low frequency of TN carcinomas are concordant (7,8,38,45,52). ER-positive expression is associated with improved prognoses at 5-year OS (6), but certain clinically aggressive male BC cases do not appear to have an active ER pathway (16). In the present study, negative PR expression status was associated with BRCA mutations.

Despite the different estimates described, ERBB2 positivity has a low frequency in males (7,51). Using IHC and ISH, 6.8% of the cases in the present study were identified as HER2-like clinical subtypes, according to AJCC 8th Edition (19), and significantly associated with a high Ki-67 expression level and high AS. ERBB2 positivity is generally associated with aggressive phenotypes, but survival of HER2-like carcinomas has improved in previous years, due to specific treatment with associated ERBB2-targeting agents (19,33).

Cell proliferation is also an important biological factor, usually associated with poor outcome (53). Ki-67, a nuclear protein present during all phases of the cell cycle, is the most commonly used marker to evaluate proliferation, although it lacks standardized methodology or generally accepted cut-offs. With a cut-off of ≥20%, previous studies identified a predominance of low Ki-67 values (7,8). By contrast, the present study identified a slight predominance of high Ki-67 values, significantly associated with old age, positive FH, high grade, pN1, high AS, CS and poor prognosis in the univariate Cox regression analysis.

The criteria for the definition of BC molecular subtypes used clinically for decisions regarding therapy are continuously progressing, resulting in difficulties when comparing data (5,7,15,30,38,52). Using IHC surrogates, Luminal A-like and Luminal B-like subtypes were identified in the majority of BC in males and females, usually with a poorer outcome for Luminal B-like (7,15,26). As PR, ERBB2 and Ki-67 are important prognostic and predictive factors, the inclusion of carcinomas with positive or negative PR and/or ERBB2 expression statuses and different Ki-67 cut-offs in the same subtype are key factors contributing to discordant results.

In the present study, according to the AJCC 8th edition (19), Luminal B-like subtype exhibited the poorest OS.

Bezić *et al* (54) observed aneuploidy in 78% of 31 male patients with BC. In a previous comparative study between sexes (50 cases each), our group demonstrated a significantly higher frequency of DNA aneuploid tumors in males compared with females (80 vs. 46%) (55). The high percentage of aneuploidy, which was observed to be increased in the present study, suggested a distinctive genomic instability in the carcinogenesis of male BC.

The present study has the limitations of a retrospective study from a single institution conducted over a long time period. However, the results are consistent with those of large and/or multi-institutional studies, confirming that studies involving smaller, but well-characterized clinicopathological and molecular subgroups, diagnosed and followed in multidisciplinary departments within single institutions, are important in improving the understanding of this disease.

In conclusion, the present study demonstrated that male BC was more likely to be diagnosed in older patients (with consequent associated comorbidities and suboptimal therapy), and exhibited poorer prognosis in elderly and in high anatomical stages. BRCA2 mutations were frequent, associated with FH, bilaterality, high Ki-67, PR negativity and Luminal B-like subtype, and with shorter DFS and OS in univariate analysis. In addition, male patients with BC were at high risk for NBPN. In the multivariate analysis, FH and Luminal B carcinomas were associated with poorer OS. These data underline the importance of early diagnosis and genetic screening in male BC. As sex may be a crucial feature to improve personalized care, additional studies investigating male BC are warranted and may lead to the development of relevant management approaches for BC in males and females.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

SA and AEP discussed experimental design, interpreted and discussed the data and wrote the manuscript. TP and FS performed IHC experiments. PM and FV performed BRCA analysis. MA and GLS analyzed and interpreted statistical data.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee of the Portuguese Institute of Oncology Lisbon Center.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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