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ORIGINAL RESEARCH

Anthracycline-related cardiotoxicity in patients with breast cancer harboring mutational signature of homologous recombination deficiency (HRD)

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Background: The BRCA proteins play a key role in the homologous recombination (HR) pathway. Beyond *BRCA1/2,* other genes are involved in the HR repair (HRR). Due to the prominent role in the cellular repair process, pathogenic or likely pathogenic variants (PV/LPVs) in HRR genes may cause inadequate DNA damage repair in cardiomyocytes.

Patients and methods: This was a multicenter, hospital-based, retrospective cohort study to investigate the heart toxicity from anthracycline-containing regimens (ACRs) in the adjuvant setting of breast cancer (BC) patients carrying germline *BRCA* PV/LPVs and no-*BRCA* HRR pathway genes. The left ventricular ejection fraction (LVEF) was assessed using cardiac ultrasound before starting ACR therapy and at subsequent time points according to clinical indications.

Results: Five hundred and three BC patients were included in the study. We predefined three groups: (i) *BRCA* cohort; (ii) no-*BRCA* cohort; (iii) variant of uncertain significance (VUS)/wild-type (WT) cohort. When baseline (T0) and post-ACR (T1) LVEFs between the three cohorts were compared, pre-treatment LVEF values were not different (*BRCA1/2* versus HRR-no-*BRCA* versus VUS/WT cohort). Notably, during monitoring (T1, median 3.4 months), patients carrying *BRCA* or HRR no-*BRCA* germline pathogenic or likely pathogenic variants showed a statistically significant reduction of LVEF compared to baseline (T0). To assess the relevance of HRR on the results, we included the analysis of the subgroup of 20 BC patients carrying PV/LPVs in other genes not involved in HRR, such as mismatch repair genes (*MUTYH, PMS2, MSH6*). Unlike HRR genes, no significant differences in T0-T1 were found in this subgroup of patients.

Conclusion: Our data suggest that deleterious variants in HRR genes, leading to impaired HR, could increase the sensitivity of cardiomyocytes to ACR in early BC patients. In this subgroup of patients, other measurements, such as the global longitudinal strain, and a more in-depth assessment of risk factors may be proposed in the future to optimize cardiovascular risk management and improve long-term survival.

Key words: anthracycline, *BRCA*, breast cancer, cardiotoxicity, homologous recombination deficiency

INTRODUCTION

Breast cancer susceptibility gene 1 (*BRCA1*) and breast cancer susceptibility gene 2 (*BRCA2*) play a prominent role in homologous recombination repair (HRR). HRR is a high-fidelity system involved in the DNA repair pathway that acts on DNA double-strand breaks (DSBs).[1] In the absence of functional HRR, for example, when either BRCA1 or BRCA2 is defective, the preferential use of error-prone systems to repair DSBs leads to an increased burden of

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genomic alterations. When these alterations occur in key tumor driver genes, they may result in the promotion of tumorigenesis, thus amplifying the loss of the cancer-suppressive effects of *BRCA1/2* genes.[2]

BRCA pathogenic or likely pathogenic variants (PV/LPVs) account for the most identifiable hereditary breast cancer (BC) and increase the risk of developing various other cancers, mainly ovarian, but also prostate and pancreatic cancers.[3] For women who carry a germline PV, the cumulative risk of developing BC by age 70 years is 45%-66%.[4]

Following technological progress and deeper knowledge of BRCA-related cancers, the demand for genetic testing is rapidly increasing.[5] *BRCA* mutational status provides useful information on prognostic, preventive, and therapeutic value.[6] Despite germline *BRCA1/2* being currently the main genetic biomarkers of homologous recombination deficiency (HRD), other HRR pathway genes are now recognized to contribute to hereditary BC risk, including ataxia telangiectasia mutated (*ATM*), partner and localizer of BRCA2 (*PALB2*), checkpoint kinase 2 (*CHEK2*), RAD51 recombinase (*RAD51*), BRCA1 interacting helicase 1 (*BRIP1*), and BRCA1 associated RING domain 1 (*BARD1*) genes.[2]

Although the exact magnitude of HRR-associated gene cancer risk has not yet been defined, these genes are often included in multi-gene panel testing,[7] leading to the recent dramatic shifts that occurred in the genetic testing landscape while impacting the clinical management of BC patients carrying PV/LPVs in *BRCA* and no-*BRCA* HRR pathway genes.[3,8]

Due to the important role in the cellular repair process, deleterious variants in HRR genes may cause inadequate DNA damage repair in cardiomyocytes.[9] In preclinical studies using murine models, loss of cardiomyocyte *BRCA1* or *BRCA2* has resulted in impaired DNA DSB repair, with subsequent accumulation of DNA damage, increased cardiomyocyte apoptosis, and heart dysfunction following genotoxic (doxorubicin) stress.[10,11] These observations in animal models highlighted the role of *BRCA* genes as 'caretakers' of genome stability, but also as 'gatekeepers' of cardiac function, and suggested the potential predisposition of human *BRCA* mutation (*BRCA*m) carriers to anthracycline-induced cardiac failure.[10]

Despite limited subsequent studies testing this hypothesis in women with *BRCA1/2*-associated BC treated with anthracycline-based chemotherapy,[12-14] the role of *BRCA1/2* deleterious variants as a predisposing condition to cardiac dysfunction is still debated, and the contribution by no-*BRCA* genes is unknown.

PATIENTS AND METHODS

Study design and population

This was a multicenter, hospital-based, retrospective cohort study to investigate the heart toxicity from anthracycline-containing regimens (ACRs) in the adjuvant setting of BC patients carrying germline *BRCA* PV/LPVs and no-*BRCA* HRR pathway genes.

The study population included patients diagnosed at age $\geq$18 years with invasive early BC (stage I-III), who underwent hereditary cancer genetic testing between January 2016 and December 2021. All included patients had a known genetic testing result, completing at least four cycles of neoadjuvant or adjuvant chemotherapy, and received ACR. Women with *in situ* or stage IV *de novo* BC or lacking information on genetic testing and/or cardiac ultrasound were excluded from the present analysis.

Procedures

Genetic, pathological, and cardiovascular data were assessed locally at each participating center.

The eligibility for genetic counseling and testing was in agreement with national and international guidelines, taking into account the personal and family history of cancer: age at diagnosis, multiple primary tumors, number of affected relatives, and molecular characteristics of tumors.[4,5]

The pathological information collected on primary BC included the histological subtype, estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) status, and the tumor grade (grades I, II, and III) from pathology reports for clinical use. Hormone receptor positivity was defined by the expression of ER and/or PgR in $\geq$1% of invasive tumor cells. The clinical data on disease stages (I-III), breast surgery, previous risk-reducing surgery, and type, dose, duration of ACRs and any other antineoplastic treatments were abstracted from the clinical records.

The left ventricular ejection fraction (LVEF) was assessed using cardiac ultrasound/echocardiography before starting ACR therapy and at subsequent time points according to clinical indications, including at least one evaluation after the end of ACR and within 21 days of the last cycle. According to the American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI), anthracycline-associated cardiotoxicity was defined as a decrease in the LVEF $>$10% to an absolute value of $<$53% by echocardiography.

The University Hospital AOUP 'Paolo Giaccone' (Palermo, Italy) coordinated the study. The study has been carried out in accordance with the Declaration of Helsinki. The study protocol was approved by the ethical committee of the coordinating center (Comitato Etico Palermo 1; Study Protocol 'BReast CArdioncology', approval number: 0821-15092021) and by the institutional review boards of other participating centers.

Predisposition gene mutation screening. Predisposition gene mutation screening was assessed locally at each participating center as part of routine clinical care. Germline testing was carried out using next-generation sequencing (NGS) analysis on peripheral blood samples from BC patients who met the national eligibility criteria for genetic testing for the diagnosis of HRR-related hereditary cancer predisposition.[5,7] PVs and LPVs identified by NGS were

validated using Sanger sequencing, according to the local manufacturers' protocols.

Genetic variant classification and interpretation. The detected *BRCA* and other HRR gene variants were locally categorized according to the criteria developed by the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium (https://enigmaconsortium.org/) and the International Agency for Research on Cancer (IARC) recommendations.[15] The gene variants were classified into five classes: benign (class I), likely benign (class II), variant of uncertain significance (VUS, class III), likely pathogenic (class IV), and pathogenic (class V). The databases used were BRCA Exchange, LOVD, Varsome, and ClinVar.[16] The detected variants were named based on the recommendations for the description of sequence variants supplied by the Human Genome Variation Society.[17]

The presence/absence of deleterious gene variants, and the mutated gene (*BRCA1/BRCA2* or HRR-gene no-*BRCA*), was the criterion used to distinguish three cohorts of patients: (i) individuals carrying germline PV/LPVs (classes IV and V) on *BRCA1* or *BRCA2* genes (BRCA cohort); (ii) individuals carrying germline PV/LPVs (classes IV and V) on HRR pathway genes beyond *BRCA1* and *BRCA2* (HRR-no-BRCA cohort); (iii) individuals carrying VUS (class III), or showing uninformative genetic test results and referred as wild type (VUS/WT cohort).

Statistical considerations

Descriptive analyses were used to assess patients' characteristics. The prevalence of HRR gene variants, the clinicopathological characteristics of patients, and the LVEF by cardiac ultrasound were assessed for each cohort of patients. The differences between subgroups were evaluated by Fisher's exact test, Pearson's correlation coefficient, and analysis of variance test. P values <0.05 were considered statistically significant. Statistical analysis was carried out using IBM SPSS Statistics for Windows Version 27.0 (IBM Corporation, Armonk, NY).

RESULTS

Patient population

Five hundred and three BC patients, aged 21-82 years, were included in the study. We predefined three groups within our population: (i) *BRCA* cohort; (ii) no-*BRCA* cohort; (iii) VUS/WT cohort. A summary of the patient and tumor cohort characteristics is reported in Table 1.

Among BC subgroups, triple-negative breast cancer was more frequent in the *BRCA* cohort [*BRCA* cohort versus no-*BRCA* cohort versus VUS/WT cohort: $n = 60$ patients (40.0%) versus $n = 12$ (23.5%) versus $n = 81$ (26.8%)] ($P = 0.009$). Indeed, in the VUS/WT cohort, an increased rate of ER-positive (ER and/or PgR positive) and HER2-positive BC was observed than in the mutated cohorts. As expected, more patients in the VUS/WT cohort were treated with adjuvant trastuzumab ($P < 0.001$) and/or endocrine therapy ($P = 0.02$). At the same time, when analyzing the type of chemotherapy, patients in the *BRCA* and no-*BRCA* cohorts showed a higher number of taxane treatment, along with ACR, rather than ACR alone ($P = 0.008$). No difference between three cohorts was observed in radiation therapy (no versus yes) ($P = 0.05$) and laterality of BC (left versus right) ($P = 0.4$); notably, the number of bilateral BC was statistically higher in the *BRCA* and no-*BRCA* cohorts than in the VUS/WT cohort ($P < 0.001$).

Significant difference was observed in rates of risk-reducing salpingo-oophorectomy (RRSO) between patients carrying *BRCA* or no-*BRCA* PVs and VUS/WT patients [$n = 51$ (34.0%), $n = 3$ (5.9%), $n = 12$ (3.9%), respectively; $P < 0.001$]. In terms of comorbidities and risk factors, as shown in Table 2, patients with germline *BRCA* or no-*BRCA* PVs had a history of diabetes, dyslipidemia, hypertension, and smoking more frequently than those without PVs. However, only the differences in diabetes and history of cigarette smoking were statistically significant ($P < 0.001$ and $P = 0.02$, respectively). Conversely, the use of oral contraceptives was more frequent in the VUS/WT cohort ($P = 0.006$), as well as the number of pregnancies ($P < 0.001$).

Genetic landscape

Four hundred and eighty-three BC patients were included in the analysis. Two hundred and one patients were carriers of germline PV/LPVs: 150 patients were in the *BRCA* cohort (29.8%), and 51 patients were in the no-*BRCA* cohort (10.2%). Twenty BC patients in the no-*BRCA* cohort (39.2%) were excluded from the analysis as carriers of germline PV/LPVs in no-HRR genes, including *MUTYH*, *PMS2*, *MSH6*, *CDH1*, *STK11*, and *EpCAM*. In the cohort of 302 BC patients without PV/LPVs, 57 showed a VUS (11.3%), and 245 had genetic testing not informative (48.7%).

In the *BRCA* cohort, 77 patients were carriers of *BRCA1* PV/LPVs (51.3%), and 73 were carriers of *BRCA2* PV/LPVs (48.7%). The most frequent PVs identified in *BRCA1*-positive carriers were c.514del; p.Gln172fs, observed in five BC patients, and c.4964_4982del; p.Ser1655fs, observed in four patients, and known as potential Sicilian founder mutation.[18] The most frequent PV in *BRCA2*-mutated BC patients is named c.1238del; p. Leu413fs, detected in five probands. Other PVs were observed with lower recurrence, as a result of heterogeneous geographic areas of the BC included in the study.

In the group of no-*BRCA* patients, 31 (60.8%) were carriers of PV/LPVs in the HRR genes. The highest prevalence of HRR-no-*BRCA* gene alterations was in *CHEK2, PALB2*, and *ATM*. The most represented variants in the HRR-no-BRCA cohort were c.1229delC PV in the *CHEK2* gene (NM 001005735), and c.(3113+1_3114-1)_(3201+1_3202-1)del in the *PALB2* gene. Regarding the type of PV/LPVs identified in this setting of patients, more than a third[12] were nonsense mutations. The second type more presents were frameshift mutations (n.11). The remaining variants

Table 1. Patient and tumor characteristics

Characteristics	*BRCA* cohort	No-*BRCA* cohort	VUS/WT cohort	*P* value
	n (%)	*n* (%)	*n* (%)	
Total patients	150 (29.8)	51 (10.2)	302 (60.0)	—
Median age, years (IQR)	45 (21-80)	52 (29-81)	49 (24-82)	0.2
Histology				
Ductal carcinoma	121 (80.7)	46 (90.3)	241 (79.8)	0.6
Lobular carcinoma	10 (6.7)	3 (5.9)	23 (7.7)	
Others	4 (2.6)	1 (1.9)	9 (2.9)	
Missing	15 (10.0)	1 (1.9)	29 (9.6)	
Tumor size				
T1 (≤2 cm)	55 (36.7)	24 (47.1)	121 (40.1)	0.4
T2-T3-T4 (>2 cm)	66 (44.0)	19 (37.2)	145 (48.0)	
Missing	29 (19.3)	8 (15.7)	36 (11.9)	
Nodal status				
Negative	57 (38.0)	21 (41.2)	137 (45.4)	0.1
Positive	68 (45.3)	23 (45.1)	110 (36.4)	
Missing	25 (16.7)	7 (13.7)	55 (18.2)	
Receptor status				
Negative (ER and PgR negative)	67 (44.7)	18 (35.3)	97 (32.1)	0.005
Positive (ER and/or PgR positive)	70 (46.7)	30 (58.8)	182 (60.3)	
Missing	13 (8.6)	3 (5.9)	23 (7.6)	
HER2 status				
HER2 negative	115 (76.7)	35 (68.6)	183 (60.6)	<0.001
HER2 positive[a]	22 (14.7)	11 (21.6)	96 (31.8)	
Missing	13 (8.7)	5 (9.8)	21 (6.9)	
TNBC				
No	85 (56.7)	37 (72.6)	209 (69.3)	0.009
Yes	60 (40.0)	12 (23.5)	81 (26.8)	
Missing	5 (3.3)	2 (3.9)	12 (3.9)	
Type of chemotherapy				
Anthracycline-containing regimen (ACR)	40 (26.7)	16 (31.4)	121 (40.2)	0.008
ACR and taxane	101 (67.3)	32 (62.7)	157 (51.9)	
Missing	9 (6.0)	3 (5.9)	24 (7.9)	
Trastuzumab				
No	109 (72.3)	39 (74.5)	180 (59.6)	<0.001
Yes	21 (14.0)	10 (19.6)	91 (30.1)	
Missing	20 (13.3)	2 (3.9)	31 (10.3)	
Adjuvant endocrine therapy				
No	67 (44.7)	13 (35.3)	101 (33.5)	0.02
Yes	69 (46.0)	32 (62.7)	181 (59.9)	
Missing	14 (9.3)	1 (2.0)	20 (6.6)	
Radiation therapy				
No	48 (32.0)	8 (15.7)	81 (26.9)	0.05
Yes	76 (50.7)	35 (68.6)	172 (56.9)	
Missing	26 (17.3)	8 (15.7)	49 (16.2)	
Laterality of breast cancer				
Left	40 (26.7)	18 (35.3)	101 (33.4)	0.002[b]
Right	53 (35.3)	14 (27.5)	112 (37.1)	
Bilateral	43 (28.7)	19 (37.2)	45 (14.9)	
Missing	14 (9.3)	0 (0.0)	44 (14.5)	

BC, breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; IQR, interquartile range; PgR, progesterone receptor; TNBC, triple-negative breast cancer; VUS, variant of uncertain significance; WT, wild type.
[a]IHC+ or amplified by FISH.
[b]Left versus right BC: $P = 0.4$; bilateral BC, yes versus no: $P < 0.001$.

identified were eight intronic variant sequences and three missense mutations (Table 3).

The overall genetic landscape of the three cohorts is shown in Figure 1.

Left ventricular ejection fraction

We examined associations between the presence of deleterious variants and LVEF decline by cardiac ultrasound. LVEFs before starting ACR therapy, and at subsequent time points, were collected.

When baseline (T0) and post-ACR (T1) LVEFs between the three cohorts were compared, pre-treatment LVEF values were not different (*BRCA1/2* versus HRR-no-*BRCA* versus VUS/WT cohort). Post-ACR LVEFs were available for $n = 321$ patients. Notably, during monitoring (T1, median 3.4 months), patients carrying *BRCA* or HRR no-*BRCA* germline pathogenic or likely pathogenic variants showed a statistically significant reduction of LVEF compared to baseline (T0). In the *BRCA1/2* cohort, median LVEF (%) T0 versus T1 was 62 (50-74) versus 58 (37-67) ($P < 0.001$) (Figure 2A); in the HRR genes no-*BRCA* cohort, median LVEF (%) T0 versus

Table 2. Risk factors between the three BC cohorts

Characteristics	*BRCA* cohort	No-*BRCA* cohort	VUS/WT cohort	*P* value
	n (%)	*n* (%)	*n* (%)	
Median body mass index (range), kg/m^2	23.1 (15.2-39.2)	23.7 (17.6-41.1)	22.0 (16.1-41.0)	ns
Comorbidities and risk factors (%)				
Diabetes	14 (9.3)	14 (27.4)	15 (4.9)	<0.001
Dyslipidemia	14 (9.3)	3 (5.9)	15 (4.9)	ns
History of smoking	49 (32.7)	12 (23.5)	64 (21.2)	0.02
Hypertension	34 (22.7)	13 (25.5)	59 (19.5)	ns
Oral contraceptives	8 (5.4)	4 (7.8)	45 (14.9)	0.006
Pregnancies	53 (35.3)	10 (19.6)	147 (48.7)	<0.001
Before BC diagnosis	53 (35.3)	9 (17.6)	146 (48.3)	—
After BC diagnosis	0 (0.0)	1 (1.9)	1 (0.3)	—
Missing	35 (23.3)	7 (13.7)	65 (21.5)	
Bilateral salpingo-oophorectomy				
Yes	51 (34.0)	3 (5.9)	12 (3.9)	<0.001
Missing	59 (39.3)	12 (23.5)	97 (32.1)	

BC, breast cancer; ns, not significant; VUS, variant of uncertain significance; WT, wild type.

T1 was 62 (55-68) versus 56 (40-62) ($P < 0.001$) (Figure 2B). Conversely, in the VUS/WT cohort, median LVEF T0 versus T1 was not statistically significant [LVEF (%) 61 (45-77) versus 60 (43-76) ($P =$ not significant)] (Figure 2C).

To assess the relevance of HRR on the results, we included the analysis of the subgroup of 20 BC patients carrying PV/LPVs in other genes not involved in HRR, such as mismatch repair genes (*MUTYH*, *PMS2*, *MSH6*), and genes involved in pathways not directly associated with genome maintenance (*CDH1*, *STK11*, *EpCAM*). Unlike HRR genes, no significant differences in T0-T1 were observed in this subgroup of mutated BC patients (Figure 2D).

Finally, as expected, a marked LVEF reduction was observed in mutated patients treated with risk-reducing bilateral salpingo-oophorectomy before 40 years of age, body mass index >25 kg/m^2, and type II diabetes mellitus. The latter risk factor was probably related to the increased risk of developing insulin resistance in BRCA-mutated patients.

DISCUSSION

Germline genetic testing for women with BC will be increasingly part of clinical practice, requiring physicians to integrate genetic information into clinical decision

Table 3. PV/LPVs in HRR-no-*BRCA* genes by multi-gene panel testing

Gene	HGVS nomenclature	Protein change	Variant interpretation	Patients *n*
CHEK2	c.1229delC	p.Thr410fs	PV	3
CHEK2	c.721+3A>T	/	LPV	2
PALB2	c.661_662delGTinsTA	p.Val221Ter	PV	2
PALB2	c.(3113+1_3114-1)_(3201+1_3202-1)del	/	PV	3
PALB2	c.758dupT	p.Ser254Ilefs	PV	1
PALB2	c.1424dup	p.Arg476fs	PV	1
PALB2	c.3556del	p.Ser1186HisfsTer5	PV	1
PALB2	c.2566C>T	p. Gln856Ter	PV	1
CHEK2	c.922-1G>A		PV	1
CHEK2	c.85C>T	p.Gln29Ter	PV	1
CHEK2	c.636del	p.Phe212fs	PV	1
ATM	c.2413C>T	p.Arg805Ter	PV	1
ATM	c.8147T>C	p.Val2716Ala	PV	1
ATM	c.1065+1G>C	/	LPV	1
ATM	c.(2838+1_2839-1)_(4109+1_4110-1)del	/	PV	1
ATM	c.7792C>T	p.Arg2598Ter	PV	1
ATM	c.1463G>A	p.Trp488Ter	PV	1
ATM	c.6154G>A	p.Glu2052Lys	LPV	1
RAD51C	c.773G>A	p.Arg258His	LPV	1
RAD51C	c226_227insAT	p.Ala76Metfs26	PV	1
RAD51D	c.694C>T	p.Arg232Ter	PV	1
RAD50	c.3598C>T	p.Arg1200Ter	PV	1
NBN	c.156_157del	p.Ser53fs	PV	1
BARD1	c.1325del	p.Pro442fs	PV	1
NBN	c.2140C>T	p.Arg714Ter	PV	1
RAD51D	c.898C>T	p.Arg300Ter	LPV	1
PALB2	c.2167_2168del	p.Met723fs	PV	1
ATM	c.5932G>T	p.Glu1978Ter	PV	1

HGVS, Human Genome Variation Society; HRR, homologous recombination repair; LPV, likely pathogenic variant; PV, pathogenic variant.

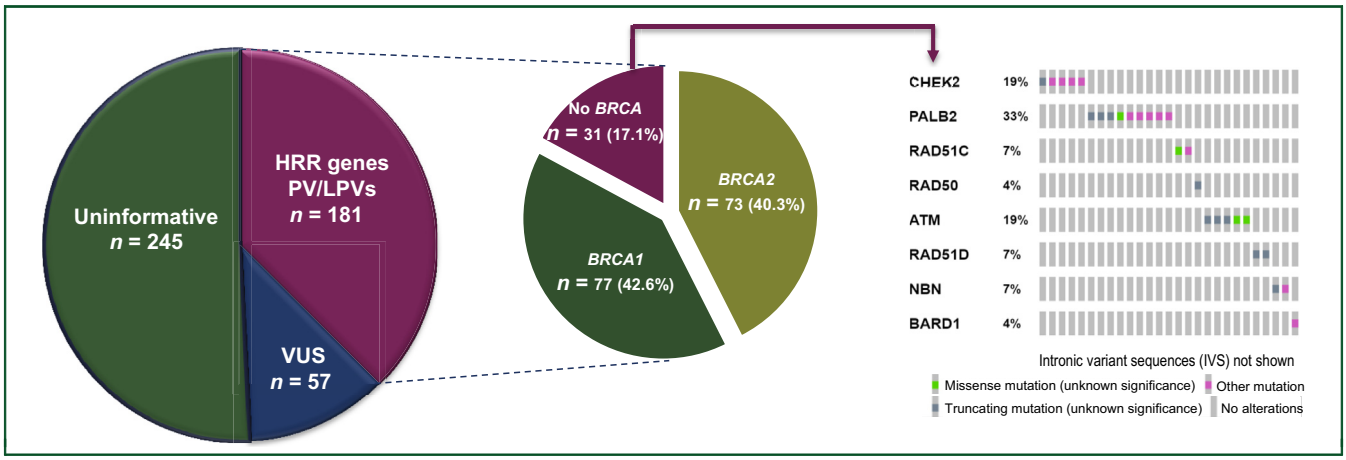

Figure 1. The genetic landscape of the 483 BC patients included in the study. A total of 181 patients were carriers of germline PV/LPVs: 77 patients were carriers of *BRCA1* PV/LPVs (42.6%), 73 were carriers of *BRCA2* PV/LPVs (40.3%), and 31 patients were carriers of germline PV/LPVs in no-*BRCA* HRR genes (17.1%). In the cohort of 302 BC patients without PV/LPVs, 57 showed a variant of uncertain significance (VUS) (18.9%), and 245 had genetic testing not informative (81.1%). Further 20 BC patients were excluded from analysis as carriers of germline PV/LPVs in no-*BRCA*, no-HRR genes, including *MUTYH*, *PMS2*, *MSH6*, *CDH1*, *STK11*, and *EpCAM*.
BC, breast cancer; HRR, homologous recombination repair; PV/LPVs, pathogenic or likely pathogenic variants.

making.[19,20] *BRCA1/2* deleterious variants confer high-penetrance susceptibility to breast and ovarian cancers.[3,21,22] However, the wider use of gene panels leads to evolving knowledge of HRR genes with sufficient clinical validity to be considered BC susceptibility genes.[23,24] Predisposition genes to BC have been studied in detail in a recent analysis of 54 555 invasive tumors, detecting germline deleterious variants in 10.1% of patients: notably, more than half of the PVs occurred in cancer predisposition genes other than *BRCA1* and *BRCA2*, e.g. *RAD51C*, *RAD51D*, *ATM*, *BARD1*, *PALB2* (*BRCA1/2* PVs versus others: 4.4% versus 5.7%).[25]

Although with a lifetime risk of cancer lower than *BRCA*, and a clinical spectrum not fully clarified, PV/LPVs in moderate-penetrance genes are equally associated with BC predisposition.[19,26-28] *PALB2*, *ATM*, *CHEK2*, *RAD51*, *BRIP1*, and *BARD1* are the genes involved in the DNA damage response pathway for DNA DSB repair, together with *BRCA1* and *BRCA2*.[2]

ACRs are frequently used in the treatment of BC, with many patients with key HRR-modulating gene alterations being exposed to anthracyclines as part of their adjuvant therapeutic regimen.

Cardiotoxicity is a dose-limiting adverse effect of ACRs that can manifest in varying severity, from an asymptomatic decline in LVEF on echocardiogram to overt congestive heart failure (HF).[29,30] Despite the pathophysiologic mechanism being multifactorial, the anthracycline effect on DNA damage is well renowned.[31] DNA topoisomerase IIβ (Top2β) inhibition and damage to mitochondrial DNA in cardiomyocytes seemed to be the cardinal element responsible for anthracycline-induced progressive HF, causing accumulation of DNA DSBs, ultimately leading to apoptosis.[29,31]

As *BRCA1/2* are key components in DNA DSB repair, it was hypothesized that *BRCA* loss of function may increase apoptosis and subsequent susceptibility to anthracycline-induced cardiotoxicity.[32,33]

This concept has driven the development of preclinical studies that corroborated the increased risk of HF and cardiac mortality in murine models with homozygous loss of *BRCA1* or *BRCA2* genes compared to wild type, after anthracycline exposure.[10,11]

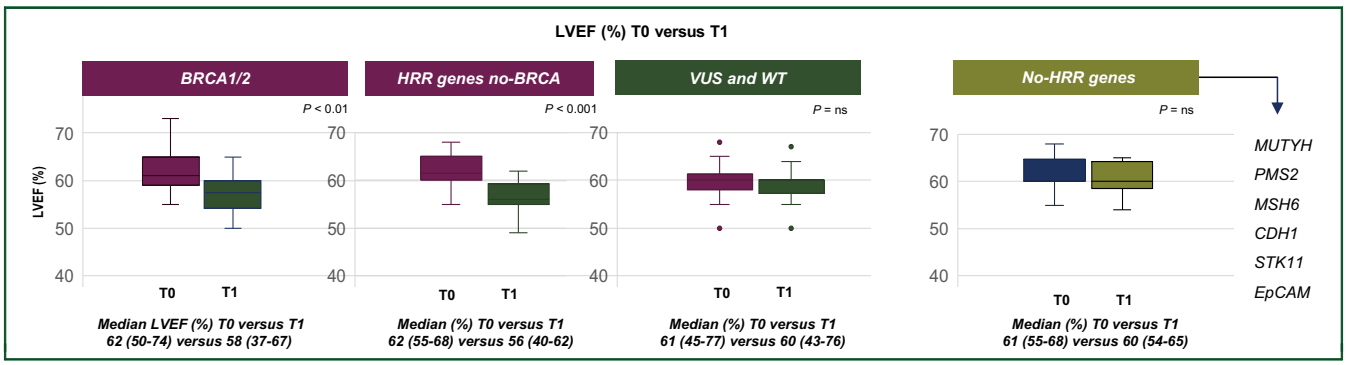

Figure 2. LVEF (%) by cardiac ultrasound and mutational landscape of patient population. (A-C) Median LVEF (%) T0 versus T1 by echocardiographic assessment between the three cohorts (*BRCA1/2* versus HRR-no-*BRCA* versus VUS/WT cohorts). (D) Explorative analysis in a subgroup of BC patients carrying PV/LPVs in other genes not involved in HRR.
BC, breast cancer; HRR, homologous recombination repair; LVEF, left ventricular ejection fraction; PV/LPVs, pathogenic or likely pathogenic variants; VUS, variant of uncertain significance; WT, wild type.

Human data addressing this issue partially contrasted with the results of murine studies.[12-14,34] However, the human studies on *BRCA*-mutated patients were partially limited by their small sample size, by wide heterogeneity in the clinical or echocardiographic diagnosis of anthracycline-related cardiotoxicity, and by surveillance protocols. Importantly, previous human studies included BC patients carrying only *BRCA*ms with lacking evidence on the impact of other HRR-associated genes on HF, particularly challenging due to their low prevalence.

Our large, unique dataset allowed an in-depth investigation of the impact of *BRCA* and no-*BRCA* genes involved in HR-mediated DNA repair on cardiac function. One main finding with potential clinical implications was found in our study. We observed a sub-clinical cardiotoxicity with an asymptomatic decline in LVEF following anthracycline treatment in BC patients carrying PV/LPVs in *BRCA* and no-*BRCA*-HRR genes.

Relative to the *BRCA* cohort, our results differ in part from previous research. The reasons could be the following. The first study by Barac et al. compared a cohort of 39 patients carrying *BRCA1/2* deleterious variants with 42 controls. The authors found no significant differences in echocardiographic parameters of cardiac function between *BRCA*-related and sporadic BC.[12] However, the study was limited by some elements. Firstly, the sample size. Despite the prospective design, the study was not adequately powered to assess differences between the two cohorts. Other limitations included the lack of prior echocardiographic data to assess individual LVEF interval variations, and the long period between the chemotherapy administration and echocardiographic examination. In fact, participants were invited for the echocardiographic analyses with an average of 69 months after adjuvant anthracycline. Furthermore, differently from our study, not all included patients in the sporadic group had known *BRCA* testing results. As a consequence, women with unknown *BRCA* PV/LPVs could have been misclassified in the sporadic cohort.

In the subsequent, retrospective study by Pearson et al., the rates of anthracycline-induced cardiomyopathy in *BRCA*m carriers compared with *BRCA* wild type were not significantly different.[13] The major limitation of this study was the small number (26/102) of *BRCA*m carriers who underwent echocardiograms after completion of adjuvant anthracyclines. Post-treatment echocardiography was carried out only in patients who either had developed clinical signs of cardiomyopathy or were treated with trastuzumab. Thus, asymptomatic declines in LVEF may have not been detected in this study population.

The results of a recent, single-center study remained consistent with the previous ones.[34] However, also in this study, statistical power was limited due to the sample size (participants treated with anthracyclines: 39 in the *BRCA*m carrier group, 14 in the *BRCA*m non-carrier group).

Conversely, results of a study on 401 *BRCA*-mutated patients were among the first to suggest that human *BRCA*m carriers may have an increased risk of HF after ACRs. The results showed that 7.7% of *BRCA*m carriers reported HF and, in addition, 9.1% of *BRCA1*m carriers and 8.2% of *BRCA2*m carriers reported arrhythmias following anthracycline exposure. These rates, compared with the overall population, were significantly higher.[14] However, this study was based on the results from an online survey in the absence of a control group of women with sporadic BC.

To our knowledge and according to the best data available today, this is the first study that investigates the impact of ACRs in patients carrying a deleterious variant within an HRR-associated gene beyond *BRCA1/2*.

The lack of relevant data on HRR-associated genes, beyond *BRCA1/2*, such as *ATM*, *PALB2*, *CHEK2*, *RAD51*, and *BARD1*, makes these results of high biological and clinical interest. At the therapeutic level, our findings of 5% of asymptomatic LVEF reduction are not clinically significant now. But, we do not know the potential long-term effects.[20,35] It may indicate a cardiomyocyte injury at risk to progress from silent to symptomatic left ventricular dysfunction as a result of further cardiovascular risk factors. In this context, the issue of overtreatment of early BC, and the de-escalation of ACR for selected patients, is currently being extensively debated.[36] Despite available evidence still supporting anthracycline-based chemotherapy for several patients, the issue of potential life-altering toxicities in a curative setting highlights how selected BC sub-populations may benefit from an anthracycline-free regimen, according to their risk of recurrence and death, along with the specific cardiovascular risk factor profile. Thus, the anthracycline-related cardiotoxicity can be further scaled down by improving upfront patient selection, cardiac monitoring, and preventive measures, toward the optimal and careful risk—benefit balance.[36] In addition to the previous consideration, incrementally, the treatment plan will need to take into account the biological complexity created by the poly (ADP-ribose) polymerase inhibitors (PARPis).[28,37,38] To date, it is not yet known if the synthetic lethality of PARPi might further enhance the effect of LVEF variations in patients with impaired HRR. Although in randomized trials no cardiotoxicity of olaparib was found, the cardiovascular effects of PARPis have not yet been systemically analyzed in the real world, due to the short duration of their clinical application.[39] Recently, a large variety of cardiotoxicity events, such as hypertension and increased heart rate, were reported, especially for niraparib, in a pharmacovigilance analysis using the United States Food and Drug Administration's Adverse Event Reporting System (FAERS).[40] To date, a single-center retrospective study on a population of prevalent ovarian cancers, reported by the 2021 ESMO Conference, showed cardiovascular events involving half of the patients treated with PARPi.[41]

We acknowledge several limitations of our study. Firstly, this was a retrospective study using LVEF assessed by cardiac ultrasound to detect anthracycline cardiotoxicity. According to clinical indications, echocardiography was carried out at baseline, before starting ACRs, and at subsequent time points. Relative to time points, echocardiography surveillance protocols partially varied from one center to another. Thus, although post-baseline

assessments were available for the majority of patients, in some centers follow-up echocardiography was carried out only in patients who developed clinical signs of cardiotoxicity. As a consequence, in the absence of a set period of time, the asymptomatic LVEF reduction could be underdiagnosed.

Another factor to consider is that the higher rate of RRSO and premature menopause in the *BRCA* cohort may further impact cardiac outcomes.[31] Premature onset of menopause and reduction in circulating estrogen due to bilateral RRSO, especially before the age of 45 years, may promote atherosclerosis by inducing endothelial dysfunction and metabolic changes, thus increasing the risk of cardiovascular disease.[42] In addition, even independently of the anthracycline-based treatment, literature data showed an intrinsic cardiovascular risk for *BRCA*m carriers. They may have altered levels of insulin-like growth factor-I, leading to an increased risk of developing insulin resistance and type II diabetes mellitus, and altered expression of circulating proteins associated with thromboembolic risk.[33,43] Our results confirmed these literature data, reporting an increase of diabetes mellitus in mutation carriers, and the occurrence of more frequent, although not statistically significant, hypertension and dyslipidemia, along with a more frequent history of mitral valve prolapse, aortic and tricuspid regurgitation, atrial septal aneurysm, and coronary heart disease.

Our findings highlight an increased, sub-clinical susceptibility to cardiac injury, with an asymptomatic decline in LVEF following anthracycline treatment in BC patients carrying *BRCA1/2* germline PV/LPVs or moderate-penetrance non-*BRCA1/2* germline PV/LPVs in key HRR-modulating genes, such as *RAD51*, *ATM*, *BRIP1*, *CHEK2*, *NBN*, and *PTEN*. Individually, BC patients carrying these HRR gene alterations are not common.[44] However, overall they represent a significant number of BC patients who may benefit from a more in-depth assessment of risk factors and other additional measurements such as the global longitudinal strain (GLS).[45]

Prospective validation of these findings is required to better define the impact of HRR alterations on anthracycline-related cardiotoxicity. An improved understanding will help inform decisions regarding optimal cardiac follow-up planning while enabling personalized therapeutic approaches.

Conclusion

BRCA1/2 are tumor suppressor genes extensively involved in maintaining genomic integrity as key components in DNA DSB repair mediated by the error-free HR pathway. Recently, the demand for genetic testing and the use of gene panels in clinical practice has rapidly increased, leading to an increasing population of BC patients identified with germline PV/LPVs in *BRCA1/2* and other HRR-associated genes. Our data suggest that deleterious variants in HRR genes, leading to impaired HR, could increase the sensitivity of cardiomyocytes to ACRs in early BC patients. Conversely, no significant variations in LVEF were observed in BC patients carrying PVs in genes involved in pathways not directly associated with genome maintenance. Probably, an asymptomatic LVEF reduction of 5% is not clinically significant now. However, we do not know the potential long-term effects, especially with the expanding use of PARPis. In this subgroup of patients, other measurements, such as the GLS, and a more in-depth assessment of risk factors may be proposed in the future to optimize cardiovascular risk management and improve long-term survival.

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DISCLOSURE

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