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Contralateral breast cancer in patients carrying mutations in the *BRCA1/2* gene

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Abstract

Aim: This study aim was to offer a better view of breast cancer (BC) in Romanian patients and to identify the most frequent *BRCA1/2* germline mutations in a cohort of Romanian patients with contralateral BC (CBC). This is one of the first comprehensive studies to determine the contribution of *BRCA1/2* germline mutations to CBC development in the Romanian population. *Patients, Materials and Methods*: This is a prospective study and included 281 patients with BC. We established the histological type and immunohistochemical profile for these breast tumors. We identified mutations in the *BRCA1/2* oncogenes in those patients diagnosed with CBC. We investigated correlations between the *BRCA1*/*BRCA2* genes mutation and the increased risk of collateral BC. *Results*: The most common histological type observed was ductal carcinoma. Our study group of tumors was classified into the following BC subtypes: 84.69% triple-negative BC, 9.60% Luminal A, 3.55% human epidermal growth factor receptor 2 (HER2)-positive and 2.13% Luminal B. Forty-one cases were diagnosed with collateral BC. For these 41 cases, genetic testing was performed for the *BRCA1* and *BRCA2* genes and we obtained seven cases with negative results and 34 cases with positive results for mutations in the *BRCA1* gene, describing the following types of mutations: *c.3067.C>T* (24 cases – 70.6%), *c.5266dupC* (four cases – 11.8%), *c.4035delA* (six cases – 17.6%).*Conclusions*: This study offered a better view of BC in Romanian patients and identified the most frequent *BRCA1/2* germline mutations in a cohort of Romanian patients with CBC. Also, these results demonstrate that *BRCA1* gene mutations increase the risk for CBC development.

Keywords: *BRCA1*/2 gene mutation, breast cancer, contralateral breast cancer.

Introduction

The 1990s saw the discovery of *BRCA1* and *BRCA2*, also referred to as the genes predisposed to breast cancer (BC) and ovarian cancer [1]. All humans are born with these genes as part of their regular genetic makeup, however in some people, they have a mutation that raises the risk of developing BCs and ovarian cancers. In addition to being expressed in endocrine organs, *BRCA1* has also been found in early-stage developing cells, including neuroepithelial cells. Similar to *BRCA1*, *BRCA2* is also widely expressed in many different organs. It is found to be expressed at greater frequencies in the thymus and breast and at lower levels in the ovary, lung, and spleen [2].

For *BRCA1* and *BRCA2*, more than 400 mutations have been documented. It is possible for someone to possess a mutation that is not picked up by routine testing. As a result, the majority of mutations may be regarded as unique, and a particular mutation may exist in each family. Even while family mutations vary greatly, several regions and ethnic groups have consistently reported seeing particular mutations [3]. *BRCA1* has a role in deoxyribonucleic acid (DNA) replication fork repair and restart, and defense against nucleolytic assault and degradation [4]. *BRCA2* is involved in repairing damaged DNA.

Role in tumor microenvironment

Studies have shown that heterozygous mutations in germline *BRCA1* carriers may contribute to the development of aggressive BC, indicating the critical role *BRCA1* plays in the development of BC [5]. The propensity for *BRCA1*-deficient tumor cells to metastasize is increased when stromal cells within the tumor microenvironment (TME) are impacted by *BRCA1* loss in breast epithelial cells [6]. The TME may be modified by proteins encoded by alternative

BRCA1 messenger ribonucleic acid (mRNA) transcripts. For example, overexpression of *BRCA1-IRIS* in mesenchymal stem cells (MSCs) can activate signal transducer and activator of transcription 3 (STAT3), AKT, and extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling in addition to stimulating DNA replication [7]. Most *BRCA1* associated cancers are estrogen receptor alpha (ERα)-negative, and estrogen stimulates cell proliferation and activates epithelial–mesenchymal transition to aid in the beginning and development of ER-negative *BRCA1*-deficient malignancies [8].

In the general population, the average risk of developing cancer is 0.13% for male BC (one case in 726 men), 13% for female BC, and 1% to 2% for ovarian cancer [9]. The *BRCA1* and *BRCA2* mutations are responsible for 45% to 85% of hereditary BC, respectively [10]. Each mutation increases the risk of developing ovarian cancer by 10% to 46% [10]. The *BRCA1* mutation increases the risk of developing BC by age 70 to 44% to 78%, ovarian cancer by 18% to 54%, and male BC by 0.22 to 2.8%. The *BRCA2* mutation increases the risk of developing BC by age 70 to 31% to 56%, ovarian cancer by 2.4% to 19%, and male BC by 3.2% to 12% [11].

Clinical and pathological characteristics

Different clinicopathological features are present in BC, including spontaneous BC and *BRCA* pathogenic variants. Whereas *BRCA2* pathogenic variant BC are more common, *BRCA1* pathogenic variant BC are mostly invasive ductal carcinomas [12]. Based on the status of the human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and ER, breast tumors may be categorized into four subtypes: Luminal A, Luminal B, HER2-positive, and triple-negative BC (TNBC) [13]. Compared to *BRCA2* pathogenic variant BCs, *BRCA1* pathogenic variant BCs often have a higher histological grade. In carriers of the *BRCA1* pathogenic variation, ductal carcinoma *in situ* (DCIS) is uncommon, while in carriers of the *BRCA2* pathogenic variant, it is frequent [14].

There is disagreement about the prognosis of pathogenic mutation *BRCA1*/*BRCA2* for BC. While most people believe that prognoses are the same, several pieces of research link *BRCA* pathogenic mutations to poor prognoses [15].

To determine the chance that a person or family has a germline pathogenic variation in either *BRCA1* or *BRCA2*, probability models have been created [16, 17]. Molecular genetic testing is used to identify a heterozygous germline pathogenic (or possibly pathogenic) variation in *BRCA1* or *BRCA2* in order to establish the diagnosis of *BRCA1*- and *BRCA2*-associated hereditary breast and ovarian cancer (HBOC) in a proband [18].

There are a number of guidelines and recommendations available for BC monitoring in carriers of the hereditary *BRCA* mutation. Concern has been raised over the possibility that early diagnostic radiation exposure increases the risk of BC in *BRCA* carriers [19]. The choice to begin mammography before the age of 40 should consider both the availability of yearly screening magnetic resonance imaging (MRI) and the greater density of breast tissue at younger ages [20]. Increased surveillance is required for individuals with confirmed *BRCA* mutations according to National Comprehensive Cancer Network (NCCN) recommendations, which seek to discover high-risk premalignant lesions and cancer early.

Bilateral BC is rare. A second primary cancer in the contralateral breast can be either synchronous or metachronous. The family history of BC, ER negativity, and HER2 positivity are risk factors for the development of contralateral breast malignancy, although some studies do not suggest a major role for genetic determinants in the majority of cases of bilateral breast cancer (BBC) [21]. In metachronous bilateral breast cancer (MBBC), usually a single histological variant is observed at different times [22]. There is an increase in the incidence of metachronous cancer, as well as in the long-term survival of patients with BC in general, due to increased life expectancy and improved management and prognosis of this neoplasm [23]. The etiology of bilateral BCs is not well understood; however, it seems that a familial-genetic link is more likely in patients with MBBC than in those with bilateral or unilateral synchronous BCs [24, 25]. Also, some studies have shown that the risk of developing contralateral breast cancer (CBC) is increased in patients with mutations in the *BRCA1/2* genes.

Individualized risk profiles, with identification of *BRCA1*/*BRCA2* mutations, may help select those women who would derive the greatest benefit from contralateral mastectomy to reduce the risk of CBC, compared with surveillance strategies [26].

Aim

This study aim was to offer a better view of BC in Romanian patients and to identify the most frequent *BRCA1/2* germline mutations in a cohort of Romanian patients with CBC. This is one of the first comprehensive studies to determine the contribution of *BRCA1/2* germline mutations to CBC development in the Romanian population.

Patients, Materials and Methods

Our study is a prospective analysis of 281 Romanian patients with BC diagnosed and treated at the Regional Institute of Oncology and at the Cuza Vodă Obstetrics and Gynecology Hospital, Iași, Romania, from 2015 to 2022. This study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Ethics Board (Approval No. 27/13.12.2023, Cuza Vodă Obstetrics and Gynecology Hospital). The clinical information, such as tumor grade and stage, histological report was obtained from the patient registries, oncological reports and from operative protocols.

The aims of this study were: (*i*) to establish the histological type and immunohistochemical (IHC) profile of tumors from 281 BC patients; (*ii*) to identify mutations in the *BRCA1/2* oncogenes in 41 patients diagnosed with CBC; (*iii*) to highlight correlations between the *BRCA1*/*BRCA2* gene mutations and the increased risk of CBC.

For all 281 invasive BCs, classification into one of the histopathological types and subtypes was achieved after performing serial sections of the surgical resection piece fixed in formalin and processed by the usual paraffin embedding technique. Subsequently, the sections were stained by one of three methods: Hematoxylin–Eosin (HE)

staining method; light green staining method – Goldner–Szekely (GS) technique for selective highlighting of collagen fibers; Periodic Acid–Schiff (PAS)–Hematoxylin staining method for highlighting glycosaminoglycans in some tumors. Also, the histological degree of malignancy (degree of tumor differentiation) was assessed according to the combined Nottingham score.

The biological material for the optical microscopy and immunohistochemistry study was collected immediately after surgery, consisting of tumor fragments harvested from the surgical excision material, fixed immediately after harvesting in 10% neutral buffered formalin solution. The expression of the following IHC markers was studied: ER (Figure 1A), PR (Figure 1B) and HER2.

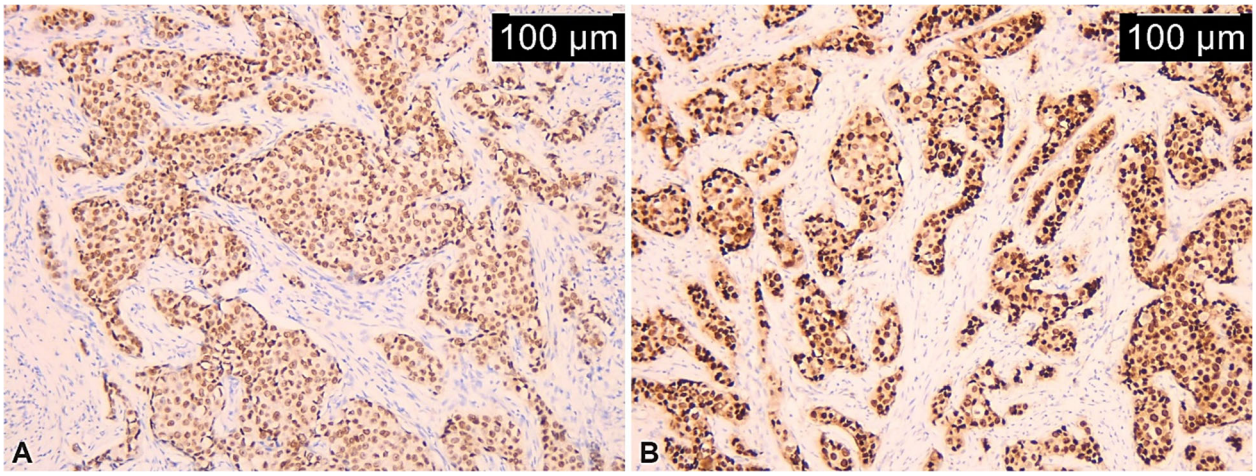

Figure 1 – ***Immunohistochemistry of estrogen (A) and progesterone (B) receptors in breast cancer. Scale bar, 100 µm.***

Peripheral blood was also collected from patients with CBC for the detection of mutations in the *BRCA1* and *BRCA2* genes by next-generation sequencing (NGS) using the Ion GeneStudio S5 Plus device.

The subjects signed the informed consent in writing after the purpose and procedures of the study were carefully explained to each woman in accordance with the Helsinki Declaration.

The study was approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania (Approval No. 379/17.01.2024).

Results

Histological study

Two hundred eighty one women diagnosed with BC were included in the study. The most common histological type observed was ductal carcinoma, found in 82.2% of cases; in the other cases, lobular carcinoma (pleomorphic in 9.6% of cases), mixed ductal and lobular (in three cases – 1.1%) and squamous metaplastic (in five cases – 1.8%) was observed (Table 1). In the vast majority of the pieces, in 273 cases, the histological grade GIII (97.2%) was observed; in eight cases (2.8%), the GII grade was observed.

Table 1 – ***Distribution of pieces according to histological type***

Histological type	*n*	%
Ductal carcinoma	231	82.2
Classic lobular carcinoma	12	4.3
Alveolar lobular carcinoma	3	1.1
Pleomorphic lobular carcinoma	27	9.6
Metaplastic carcinoma	5	1.8
Mixed ductal and lobular carcinoma	3	1.1
Total	281	100.0

Immunohistochemical study

ER expression was negative in all cases of ductal carcinoma, metaplastic carcinoma and mixed ductal and lobular carcinoma. Among the cases of lobular carcinoma, 3/4 had ER-positive expression, and 25% had strong ER-positive expression, while all cases of classic lobular carcinoma, alveolar lobular carcinoma and pleomorphic lobular carcinoma have ER-positive expression. Also, among the cases of pleomorphic lobular carcinoma, equal percentages had negative or strongly ER-positive expression (44.4% each), the remaining 11.1% having positive expression; and these differences between histological types were statistically significant (Table 2).

Table 2 – ***Comparative study of estrogen receptor (ER) expression according to histological type***

Histological type (Pearson's *chi*-squared: 295.392, *p*<0.001**)	ER							
	Negative		*Positive*		*Strong positive*		*Total*	
	n	%	*n*	%	*n*	%	*n*	%
Ductal carcinoma	231	100.0%					231	100.0%
Classic lobular carcinoma			9	75.0%	3	25.0%	12	100.0%
Alveolar lobular carcinoma			3	100.0%			3	100.0%
Pleomorphic lobular carcinoma	12	44.4%	3	11.1%	12	44.4%	27	100.0%
Metaplastic carcinoma	5	100.0%					5	100.0%
Mixed ductal and lobular carcinoma	3	100.0%					3	100.0%
Total	251	89.4%	15	5.3%	15	5.3%	281	100.0%

A similar phenomenon was observed in the case of PR expression; again, the differences between the histological types are statistically significant. All cases with ductal carcinoma, mixed classic lobular and alveolar, squamous metaplastic and mixed ductal and lobular carcinoma had PR-negative expressions. Among the cases with lobular carcinoma, three quarters (75.0%) were evaluated with PR-positive expression, and 25.0% with intensely positive expression; also, among the cases with pleomorphic lobular carcinoma, equal percentages have negative or intensely PR-positive expression (44.4% each), the remaining 11.1% have positive expression (Table 3).

In the case of HER2 expression, statistically significant differences are also observed between the histological types. The highest percentage of pieces evaluated with HER2-positive expressions was observed among those with lobular carcinoma (25.0%), followed by those with pleomorphic lobular carcinoma (11.1%) and 3.9% of those with ductal carcinoma. For the remaining histological types, HER2 expression was evaluated as negative (Table 4).

Table 3 – ***Comparative study of progesterone receptor (PR) expressions according to histological type***

Histological type (Pearson's *chi*-squared: 348.558, *p*<0.001)**	**PR**									
	Negative		***Low positive***		***Positive***		***Strong positive***		***Total***	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Ductal carcinoma	231	100.0%							231	100.0%
Classic lobular carcinoma					9	75.0%	3	25.0%	12	100.0%
Alveolar lobular carcinoma	3	100%							3	100.0%
Pleomorphic lobular carcinoma	12	44.4%	3	11.1%			12	44.4%	27	100.0%
Metaplastic carcinoma	5	100.0%							5	100.0%
Mixed ductal and lobular carcinoma	3	100.0%							3	100.0%
Total	254	90.4%	3	1.1%	9	3.2%	15	5.3%	281	100.0%

Table 4 – ***Comparative study of human epidermal growth factor receptor 2 (HER2) expression according to histological type***

Histological type (Pearson's *chi*-squared: 12.532, *p*<0.0028*)	**HER2**					
	Negative		***Positive***		***Total***	
	n	*%*	*n*	*%*	*n*	*%*
Ductal carcinoma	222	96.1%	9	3.9%	231	100.0%
Classic lobular carcinoma	9	75.0%	3	25.0%	12	100.0%
Alveolar lobular carcinoma	3	100.0%			3	100.0%
Pleomorphic lobular carcinoma	24	88.9%	3	11.1%	27	100.0%
Metaplastic carcinoma	5	100.0%			5	100.0%
Mixed ductal and lobular carcinoma	3	100.0%			3	100.0%
Total	266	94.7%	15	5.3%	281	100.0%

Based on the IHC expression of hormone receptors, ER+, PR+, HER2+, and TNBC, our study group was classified into the following BC subtypes: 84.69% TNBC, 9.60% Luminal A, 3.55% HER2+ and 2.13% Luminal B.

BRCA1/2 gene mutations study

Among the 281 patients included in the study, 41 cases were diagnosed with CBC. For these 41 cases, genetic testing was performed for the *BRCA1* and *BRCA2* genes and we obtained seven cases with negative results, meaning they did not present mutations in the *BRCA1/2* genes and 34 cases with positive results for mutations in the *BRCA1* gene, describing the following types of mutations: *c.3067.C>T* (24 cases – 70.6%), *c.5266dupC* (four cases – 11.8%), *c.4035delA* (six cases – 17.6%). No patients with *BRCA2* gene mutations were identified in our study group. The proportion of patients who presented a mutation in the *BRCA1* gene and developed CBC was 82.9% (34 cases out of the total CBC cases).

Discussions

BC is an oncological pathology with a continuously increasing incidence due to new diagnostic methods. The morbidity of this pathology is deeply felt worldwide, with an increased rate of incidence in most age groups. The global annual percent change for BC mortality increased by 0.23% per year since 1990 [27]. The trend is also reflected in the increasing incidence rate in Romania, with an incidence of 26.9% for women, in 2020 [28].

Histological study

BCs can be histologically classified into two major categories: *in situ* carcinoma (ductal or lobular) and invasive carcinoma, which is very important from a therapeutic standpoint. In our study, the most frequent type of BC was ductal carcinoma. This type of BC is the most commonly diagnosed cancer in women worldwide. Similar to our results, the study conducted by Cătană *et al.* on 411 Romanian patients with BC, reported ductal carcinoma as the most common histological type [29]. Also, multiple studies conducted on Iranian populations indicate that ductal carcinoma is the most prevalent subtype of BC and a major contributor to BC mortality [30]. Metaplastic carcinoma is among the rare subtypes of BC, showing an aggressive behavior and poor prognosis compared to other types of BC. Metaplastic carcinoma represented 1.8% of the total cases included in our study, which is

similar to the worldwide incidence. For example, in the US, an incidence of 0.2–2% of this type of BC has been reported [31]. In our study, the lowest incidence was represented by the three cases of mixed ductal and lobular carcinoma. Mixed BC refers to a type of BC that exhibits features of more than one histological subtype of BC. Other studies estimate mixed carcinomas incidence up to 5–10% of BCs, but this can vary depending on the population and diagnostic criteria used [32].

The histological grade of BC is a measure of how abnormal tumor cells look under a microscope compared to normal breast cells. It is an important prognostic factor and helps predict the aggressiveness of the tumor. The grading is based on the Nottingham Grading System (also called the Elston–Ellis modification of the Scarff–Bloom–Richardson grading system), with grades values from I to III. This system evaluates three features of the tumor and assigns a score: tubule/gland formation, nuclear pleomorphism, and mitotic frequency. Grade III cancers are generally more aggressive. Our results showed that 97.2% of cases presented grade III and were predominantly TNBC cases.

Immunohistochemical study

The objective of the IHC study was to histologically classify the tumors included in our study. The results showed that TNBC (84.69%) was the most common subtype of BC, followed by Luminal A (9.60%), HER2-overexpressed (3.55%), and Luminal B (2.13%). In our group of study, the presence of ER was recorded in all lobular carcinoma (42 cases) and was negative in all of ductal carcinoma, squamous metaplastic, mixed ductal and lobular (239 cases).

This finding is in discordance with data from the literature that reports that 78% of BC are ER-positive, with a projected rate increasing by 0.75% per year [33]. On the other hand, recent studies have shown that the incidence rate of ER-positive cancer has increased by 1.75% per year from 2004 to 2009 [34]. From 2009 to 2019, the incidence has slowed to a 0.87% annual increase. In addition, studies conducted in Jordan showed that the ER expression rate increased substantially from 50.8% in 2006 [35] to 76.96% in 2022 [36]. The same conclusion was reported by Khabaz, that the ER expression rate increased from 42.9% in 1995 [37] to 75.50% in 2014 [38]. These differences in the expression of the ER may be due to ethnic differences, but we encourage future studies to investigate ER expression in different populations depending on genetic inheritance and risk factors to which that population is exposed.

Regarding PR expression, our results showed that in all cases with ductal carcinoma, classic mixed lobular and alveolar, squamous metaplastic and mixed ductal and lobular carcinoma, PR expression was negative (242 cases). The specialized literature reported that approximately 1% to 4% of BC have PRs but not ERs. It is also reported that patients with ER-/PR+ phenotype exhibit more aggressive biological characteristics [39].

HER2 is a transmembrane glycoprotein epidermal growth factor receptor expressed in approximately 20 BCs [40], whose overexpression has been associated with an increased risk of disease recurrence and an overall worse prognosis [41]. In our study, HER2 was expressed in 5.33% of total cases. Our result showed that the most common type of BC was TNBC (84.69%). Conflicting results have been reported by Kohler *et al.* [42]. It has been reported that TNBC represents up to 12% of BC. Also, Dawood and Bauer *et al.* reported that TNBC is more common among women who are African-American [43], perimenopausal [44] and low socioeconomic status [44]. This subtype of BC is more aggressive, and according to Yau *et al.*, time to recurrence is <5 years [45]. In this study, 9.60% of the analyzed group' tumors were classified as Luminal A tumors. Typically, this subtype of tumor has a good prognosis [46].

BRCA1/2 mutations study

The prevalence of *BRCA1/2* mutation varies between ethnic groups and regions [47]. Recent studies reported that *BRCA1* germline pathogenic mutations are associated with an increased risk of 60% for BC and 83% for CBC [48]. Also, they are associated with an aggressive BC subtype, especially the TNBC subtype [49]. For *BRCA2* pathogenic mutations, the risk was reported to 55% for BC development, and to 62% for CBC development [24]. Another study conducted on 491 women reported the 10-year risk of CBC was 43.3% for patients with *BRCA1* gene mutations and 34.6% for patients with *BRCA2* gene mutations [50]. To our knowledge, this is the first study that investigates the role of *BRCA1/2* gene mutations on CBC development in Romanian women. In our CBC patient group, three distinct *BRCA1* mutational variations were identified and there were no *BRCA2* gene mutations identified. The most frequently encountered *BRCA1* mutation was *c.3067.C>T* (24 cases – 70.6%). All cases identified with this mutation were TNBC. This mutation has been identified as the second most frequent mutation in a study conducted from 2014 to 2019, on 250 women of Romanian ethnicity with BC and 240 women of Romanian ethnicity with ovarian cancer [51]. The second most prevalent *BRCA1* gene mutation encountered in our study group was *c.5266dupC* (six cases) (17.6%). Another *BRCA1* gene mutation was *c.4035delA*, found in four (11.8%) cases from our study group. We evaluated these mutations of *BRCA1* gene in ClinVar database in order to identify the pathogenicity class for each mutation, and all of three were found to be pathogenic. Also, our results showed that 82.9% of patients with *BRCA1* gene mutations developed CBC distant from the first tumor. This underscores the significance of genetic testing as a fundamental step in assessing risk, enabling informed decisions regarding whether to proceed with prophylactic bilateral mastectomy or prophylactic contralateral mastectomy. Typically, CBC is estimated to occur at a rate of 0.5% per year [52]. Another study conducted in The Netherlands on patients diagnosed between 2003 and 2008 estimated the risk for CBC development at 0.6% per year [53].

Conclusions

This study offered a better view of BC in Romanian patients and identified the most frequent *BRCA1/2* germline mutations in a cohort of Romanian patients with CBC.

The results of our study underline the importance of long-term surveillance of women diagnosed with BC, in order to detect early a tumor lesion in the contralateral breast. At the level of both breasts, the breast tissue is exposed to the same risk factors, and in the absence of prophylactic mastectomy of the contralateral breast at the first diagnosis of breast neoplasm, or in the absence of Tamoxifen treatment, according to which the risk of CBC decreases drastically, the risk of developing CBC in a woman with *BRCA1* gene mutations, is increased. In this sense, we propose a new concept of unit of the mammary gland, for good case management at the first diagnosis of BC.

Conflict of interests

The authors declare that they have no conflict of interests.

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