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EPIDEMIOLOGY

BRCA1/*BRCA2* germline mutations and chemotherapy-related hematological toxicity in breast cancer patients

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Abstract

Purpose BRCA1 and BRCA2 proteins are central to DNA repair process through homologous recombination. We hypothesize that *BRCA1*/*BRCA2* mutation carriers may exhibit increased hematological toxicity when receiving genotoxic chemotherapy.

Methods We included women with primary breast cancers screened for *BRCA1*/*BRCA2* germline mutations and treated with (neo)adjuvant chemotherapy in Geneva (Swiss cohort). The primary endpoint was the incidence of febrile neutropenia following the first chemotherapy cycle (C1). Secondary endpoints were the incidence of grade 3–4 neutropenia, grade 4 neutropenia and hospitalization during C1, G-CSF use and chemotherapy dose reduction during the entire chemotherapy regimen. Long-term toxicities (hematological, cardiac and neuropathy) were assessed in the Swiss cohort and a second cohort of patients from Lyon (French cohort).

Results Overall, 221 patients were assessed for acute hematological toxicity, including 23 *BRCA1* and 22 *BRCA*2 carriers. Following the C1, febrile neutropenia had an incidence of 35% ($p=0.002$), 14% ($p=0.562$) and 10% among *BRCA1, BRCA* 2 and non-carriers, respectively. Grade 4 neutropenia was found in 57% of *BRCA1* ($p<0.001$), 14% of *BRCA*2 ($p=0.861$) and 18% of non-carriers. G-CSF support was necessary in 86% of *BRCA1* ($p=0.005$), 64% of *BRCA*2 ($p=0.285$) and 51% of non-carriers. For long-term toxicity analysis, 898 patients were included (167 *BRCA1*-, 91 *BRCA2*- and 640 non-carriers). There was no difference between the 3 groups.

Conclusions *BRCA1* germline mutations is associated with greater acute hematological toxicity in breast cancer patients. These observations could have implication for primary prophylaxis with G-CSF.

Keywords Breast cancer · BRCA mutation · Toxicity · Febrile neutropenia · Haploinsufficiency · Chemotherapy

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Introduction

BRCA1/*BRCA2* germline mutations are found in up to 5% of breast cancer patients [1]. Germline mutations in *BRCA1* confer a cumulative breast and ovarian cancer risk by age 80 of 72% and 44%, respectively, while *BRCA2* mutations represent 69% and 17% risks of breast and ovarian cancer [2].

BRCA1/*BRCA2* are tumor suppressor genes that encode large, ubiquitous and multifunctional proteins. They play central roles in DNA repair, cell-cycle control and chromosomal stability [3]. Carcinogenesis in carriers of *BRCA* germline mutations implies a somatic loss of the second allele either by loss of heterozygosity [4] or somatic mutation of the second allele [5]. Without functional BRCA1/BRCA2 proteins, cells are severely impaired in their ability to perform homologous recombination (HR) of double strand breaks (DSBs) of DNA [6, 7]. The resulting genomic instability makes *BRCA* mutated tumor cells susceptible to DNA damaging agents that induce DSBs, such as interstrand cross-linking agents (platinum derivatives and alkylating agents), or anthracyclines [8].

Recently, the question has arisen as to whether non-cancerous cells would be more sensitive to DNA damaging agents due to *BRCA1*/*BRCA2* haploinsufficiency (mutation of a single allele). Preclinical data support this concept, revealing tissue and cell-type-specific genomic instability in non-tumoral tissues [9, 10]. Asymptomatic *BRCA1* carriers have increased radiosensitivity [11]. DNA repair deficiency is further impacted as BRCA1/BRCA2 stores are depleted during genotoxic and replication stress [12]. In breast cancer patients, conflicting results have been reported regarding the correlation between *BRCA1*/*BRCA2* germline mutations and chemotherapy-related acute hematological toxicity [13–15]. We hypothesized that *BRCA1*/*BRCA2* haploinsufficiency in carriers of germline mutations could increase hematological toxicity of DNA damaging agent-based chemotherapy and that the location of mutations may play a role in this effect.

Materials and methods

Patient selection

Swiss cohort

We included all women screened for *BRCA1*/*BRCA2* germline mutations at the Hôpitaux Universitaires de Genève, Geneva, Switzerland, and who received neoadjuvant or adjuvant chemotherapy for primary (non-metastatic) invasive breast cancer at the Hopitaux Universitaires de Genève or at seven participating clinics in Geneva between 1998 and 2016 (Swiss cohort). Exclusion criteria were a history of chemotherapy for a prior cancer, metastatic disease, primary prophylaxis with G-CSF, and treatment outside Geneva. All data were collected from medical records. Tumor characteristics and laboratory results were collected from pathology and laboratory reports. The study was approved by the local IRB in Geneva (CCER-15-158) and Lyon. Informed written consent was obtained from living patients.

French cohort

In addition to the Swiss cohort, we collected clinical data from a second cohort of patients (French cohort). Women were treated for primary breast cancer from 1990 to 2015 and screened for germline mutations of *BRCA1*/*BRCA*2 genes at the Centre Léon Bérard, Lyon, France, and at the Hospices Civils de Lyon, Lyon, France. All the patients in the French cohort received adjuvant or neoadjuvant chemotherapy. The study was approved by the local IRB. Informed written consent was obtained from all patients.

Data collection

For the Swiss cohort, we recorded tumor characteristics (TNM stage, grade, estrogen and progesterone receptors status, HER-2 status), hematological values (leukocyte count, neutrophil count, hemoglobin and platelet count) at baseline and at day 7–14 after the first chemotherapy cycle (C1), acute and long-term chemotherapy toxicity. All the patients had systematic check-up and blood counts at baseline (day 1) and day 7–14 after the C1. Hematological toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) [16].

We collected chemotherapy regimen, timing (neoadjuvant or adjuvant), number of cycles and duration of the entire treatment. We recorded prophylactic G-CSF use following neutropenia in prior chemotherapy cycles, dose reductions, febrile neutropenia and hospitalizations.

The incidence of secondary acute leukemia and myelodysplastic syndromes, and long-term consequences following chemotherapy (cardiac dysfunction and neuropathy) were collected in the Swiss and French cohorts.

Genetic analysis

BRCA1 and *BRCA2* mutations were classified as pathogenic according to the ENIGMA *BRCA1*/*BRCA2* Gene Variant Classification Criteria (http://www.enigmaconsortium.org/). Women with variants of uncertain significance were considered as non-carriers. Blood samples for germline DNA

testing were obtained when the patients were referred to clinical genetics units. All participants were screened for *BRCA1* and *BRCA2* mutations. *BRCA1* comprises 24 exons and encodes an 1863 amino-acid (AA) protein (https://www.ncbi.nlm.nih.gov/protein/AAC37594.1). The most important functional domains are the RING domain (amino acids 26–68) and the BRCT domain (amino acids 1650–1842). *BRCA2* comprises 27 exons and encodes a 3418 amino acids protein (https://www.ncbi.nlm.nih.gov/protein/AAB07223.1). RAD51 binding domain (RAD51-BD) corresponds to the region covering amino acids 1002–2085 of BRCA2 (exon 11) (Fig. 1).

Endpoints

The primary endpoint was the incidence of febrile neutropenia at day 7–14 of the C1 in the Swiss cohort. If a febrile neutropenia occurred before the check-up at day 7–14, this was considered as an event. Secondary endpoints were the incidence at day 7–14 of C1 of grade 4 neutropenia, corresponding to an absolute neutrophil count inferior to 0.5×10^9/L [17]; grade 3–4 neutropenia and hospitalization after C1; dose reductions and G-CSF use during the entire chemotherapy regimen; and long-term toxicities (acute leukemia/myelodysplastic syndromes, cardiac dysfunction and neuropathy). Long-term toxicities were assessed in both the Swiss and French cohorts.

Statistical analyses

Proportions were calculated for categorical data, whereas median and interquartile range (IQR) were calculated for continuous data. Statistical significance for categorical data was assessed using a χ^2 test or a Fisher's exact test, as appropriate. Continuous variables were compared using the Kruskall Wallis test. Patient's characteristics and toxicity frequencies were compared pair by pair (*BRCA1* carriers vs. non-carriers; *BRCA2* carriers vs. non-carriers). Missing data or inapplicable responses were excluded when calculating *p* values. *p* values < 0.05 were considered as significant. Calculations were done with STATA software (version 14.0).

Results

Patients in the Swiss cohort

We reviewed the medical records of 666 patients and 231 met our inclusion criteria. Among eligible patients, ten refused to participate and 221 patients were included (Fig. S1): 23 were *BRCA1* mutation carriers, 22 *BRCA2* carriers and 176 non-carriers. The median age was 40 years (IQR 35–50), comparable in the non-carriers, *BRCA1* and *BRCA* 2 carriers (Table 1).

Tumor characteristics and treatment

The proportion of triple negative (estrogen and progesterone receptors and HER-2 status negative) breast cancers (TNBC) was higher among *BRCA1* carriers (77%, $p < 0.001$). Tumor staging according to TNM and chemotherapy regimens was similar in all groups, with the majority of patients receiving two DNA damaging-agents, an anthracycline and an alkylating agent (Table 1). Very few patients received platinum derivatives. The majority of patients received 3*FEC100/3*docetaxel or 6*FEC100 regimens. None received dose-dense regimen (cycle every 14 days). No differences were seen in baseline counts of leukocytes, platelets and neutrophils between *BRCA* carriers and non-carriers (Table S1).

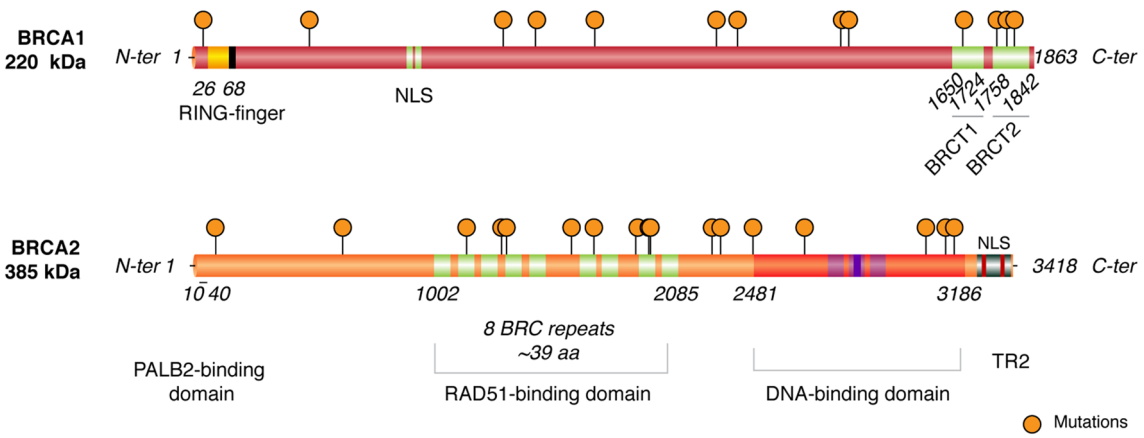

Fig. 1 Location of pathogenic mutations in *BRCA1* and *BRCA2* genes

Table 1 Clinical, pathological and treatment characteristics at diagnosis by *BRCA1*/*BRCA2* status

	Non-carriers ($N=176$)	*BRCA1* mutation carriers ($N=23$)	*p*	*BRCA2* mutation carriers ($N=22$)	*p*
Age years; median (IQR)	41 (35–50)	38 (36–51)	0.95	40 (35–50)	0.99
Histological type			0.27		0.70
Ductal	158 (92%)	20 (87%)		21 (95%)	
Lobular	7 (4%)	1 (4%)		0 (0%)	
Medullary	4 (2%)	2 (9%)		1 (5%)	
Other	3 (2%)	0 (0%)		0 (0%)	
Unknown	4	0		0	
Grade			0.006		0.54
1	13 (7%)	0 (0%)		0 (0%)	
2	70 (40%)	3 (13%)		9 (41%)	
3	91 (52%)	20 (87%)		13 (59%)	
Unknown	2	0		0	
Estrogen receptor status			< 0.001		0.48
Negative	65 (37%)	18 (78%)		6 (27%)	
Positive	111 (63%)	5 (21%)		16 (72%)	
Progesterone receptor status			0.001		0.50
Negative	79 (45%)	19 (83%)		8 (36%)	
Positive	97 (55%)	4 (17%)		14 (64%)	
HER-2			0.010		0.41
Negative	132 (77%)	21 (91%)		19 (86%)	
Positive	39 (23%)	1 (4%)		3 (14%)	
Unknown	5	1		0	
Molecular subtype			< 0.001		0.55
Luminal	80 (47%)	4 (18%)		13 (59%)	
Triple negative	52 (30%)	17 (77%)		6 (27%)	
HER-2	39 (23%)	1 (5%)		3 (14%)	
Unknown	5	1		0	
Tumor size (TNM)			0.13		0.16
T0	1 (1%)	0 (0%)		1 (5%)	
T1	71 (40%)	15 (65%)		11 (50%)	
T2	84 (48%)	7 (30%)		9 (41%)	
T3	15 (9%)	0 (0%)		0 (0%)	
T4	5 (3%)	1 (4%)		1 (5%)	
Axillary nodal metastasis			0.05		1.00
Absent (N0)	74 (42%)	15 (65%)		9 (41%)	
Present (N+)	101 (58%)	8 (35%)		13 (59%)	
Unknown	1	0		0	
Chemotherapy regimen					
Alkylating agents	170 (97%)	23 (100%)	1.00	21 (95%)	0.57
Anthracyclines	160 (91%)	22 (96%)	0.70	18 (82%)	0.25
Taxanes	131 (74%)	18 (78%)	0.80	15 (68%)	0.61
Platinum-based	3 (2%)	0 (0%)	1.00	1 (5%)	0.37
Regimen type			0.65		0.48
Neoadjuvant	66 (37%)	7 (30%)		6 (27%)	
Adjuvant	110 (63%)	16 (70%)		16 (73%)	
Chemotherapy					
3*FEC100/3*D	115 (65.3%)	17 (73.9%)		11 (50.0%)	
4*AC	4 (2.3%)	0		0	
4*EC/12*P	7 (4.0%)	1 (4.4%)		0	

Table 1 (continued)

	Non-carriers ($N=176$)	*BRCA1* mutation carriers ($N=23$)	p	*BRCA2* mutation carriers ($N=22$)	p
4*EC/4*Carboplatin-P	0	0		1 (4.6%)	
6*AP	2 (1.1%)	0		0	
6*Carboplatin-P	3 (1.7%)	0		0	
6*CMF	9 (5.1%)	0		1 (4.6%)	
6*FEC100	30 (17.1%)	4 (17.4%)		5 (22.7%)	
6*TAC	2 (1.1%)	0		1 (4.6%)	
No taxane and no anthracycline	2 (1.1%)	1 (4.4%)		1 (4.6%)	
Taxanes and no anthracycline	2 (1.1%)			2 (9.1%)	

A adriamycin, *C* cyclophosphamide, *D* docetaxel, *E* epirubicin, *F* 5-fluorouracil, *IQR* interquartile range, *M* methotrexate, *P* paclitaxel, *T* docetaxel

Acute hematological toxicity

Overall, *BRCA1* carriers were more likely to develop acute hematological toxicity than *BRCA2* carriers, or non-carriers. At day 7–14 of C1, the incidence of grade 3–4 febrile neutropenia was significantly higher among *BRCA1* carriers compared to non-carriers (35% vs. 10%, $p=0.002$). Grade 3–4 neutropenia (67% vs. 36%, $p=0.01$) and grade 4 neutropenia (57% (12/21) vs. 18% (28/160), $p<0.001$) were more frequent among *BRCA1* carriers compared to non-carriers (Table 2). Unplanned hospitalizations were more frequent among *BRCA1* carriers (22% vs. 8%, $p=0.043$) than non-carriers. Incidence of anemia and thrombocytopenia was similar in all groups (Table S1). Chemotherapy dose-reductions were more frequent among *BRCA1* carriers (14% vs. 3%, $p=0.03$). G-CSF use at any time throughout the treatment was more frequent among *BRCA1* carriers (86% vs. 51% for non-carriers, $p=0.005$). For *BRCA2* carriers, they did not show increased acute hematological toxicity when compared to non-carriers (Table 2).

Among patients with TNBC, grade 3–4 febrile neutropenia (6/17, 35% vs. 6/50, 12%, $p=0.038$), grade 3–4 neutropenia (11/15, 73% vs. 13/47, 28%, $p=0.003$) and grade 4 neutropenia (9/15, 60% vs. 6/47, 13%, $p=0.001$) were significantly more frequent in *BRCA1* carriers compared to non-carriers (Table 3).

Location of mutations in BRCA1/BRCA2 genes and neutropenia

Compared to non-carriers, subgroup analyses by location of mutations revealed that among *BRCA1* carriers, the majority of patients were likely to have grade 3–4 neutropenia (14/16, 88%; $p<0.001$), but none of those having mutations located in the RING domain (0%, $p=0.165$) (Table 4).

Long-term toxicities

Data from the Swiss and French cohorts were pooled to analyze long-term chemotoxicity among 898 patients (167

Table 2 Acute chemotherapy-related hematological toxicity by *BRCA1*/*BRCA2* status

	Non-carriers ($N=176$)	*BRCA1* mutation carriers ($N=23$)	OR [95% CI]	p	*BRCA2* mutation carriers ($N=22$)	OR [95% CI]	p
Grade 3–4 febrile neutropenia after 1st cycle of chemotherapy	17/176 (10%)	8/23 (35%)	5.0 [1.8–13.5]	0.002	3/22 (14%)	1.5 [0.4–5.5]	0.56
Grade 4 febrile neutropenia after 1st cycle of chemotherapy	14/176 (8%)	5/23 (22%)	3.2 [1.0–10.0]	0.04	1/22 (4%)	0.6 [0.1–4.4]	0.57
Grade 3–4 neutropenia after 1st cycle of chemotherapy	58/160 (36%)	14/21 (67%)	3.5 [1.3–9.2]	0.01	7/21 (33%)	0.9 [0.3–2.3]	0.79
Grade 4 neutropenia after 1st cycle of chemotherapy	28/160 (18%)	12/21 (57%)	6.3 [2.4–16.3]	<0.001	4/21 (14%)	1.1 [0.3–3.5]	0.86
Dose reduction of chemotherapy	5/174 (3%)	3/22 (14%)	5.3 [1.2–24.1]	0.03	2/22 (9%)	3.4 [0.6–18.6]	0.16
G-CSF use during chemotherapy	88/171 (51%)	19/22 (86%)	6.0 [1.7–20.9]	0.005	14/22 (64%)	1.7 [0.7–4.1]	0.28

G-CSF granulocyte colony-stimulating factor

Table 3 Acute chemotherapy-related hematological toxicity in patients with triple negative breast cancer

	Non-carriers (N = 52)	*BRCA1* mutation carriers (N = 17)	OR [95% CI]	*p*
Grade 3–4 febrile neutropenia after 1st cycle of chemotherapy	6/50 (12%)	6/17 (35%)	4.0 [1.1–14.8]	0.04
Grade 3–4 neutropenia after 1st cycle of chemotherapy	13/47 (28%)	11/15 (73%)	7.2 [1.9–26.7]	0.003
Grade 4 neutropenia after 1st cycle of chemotherapy	6/47 (13%)	9/15 (60%)	10.3 [2.7–39.2]	0.001
Hospitalization for febrile neutropenia	6/50 (12%)	4/17 (24%)	2.3 [0.6–9.2]	0.26
Dose reduction of chemotherapy	3/50 (6%)	3/16 (19%)	3.6 [0.7–20.1]	0.14
G-CSF use during chemotherapy	26/48 (54%)	13/16 (81%)	3.7 [0.9–14.5]	0.06

G-CSF granulocyte colony-stimulating factor

Table 4 Location of pathogenic mutations in *BRCA1*/*BRCA2* and incidence of grade 3–4 neutropenia

	Non-carriers (*N* = 176)	*BRCA1* mutation carriers (*N* = 23)				*BRCA2* mutation carriers (*N* = 22)			
		RING domain	*p*	Others	*p*	RAD51-binding domain	*p*	Others	*p*
Incidence of grade 3–4 neutropenia after 1st cycle of chemotherapy	58/162 (36%)	0/5 (0%)	0.16	14/16 (88%)	< 0.001	6/12 (50%)	0.36	1/9 (11%)	0.17
Incidence of grade 4 neutropenia after 1st cycle of chemotherapy	28/162 (17%)	0/5 (0%)	0.59	12/16 (75%)	< 0.001	4/12 (33%)	0.24	0/9 (0%)	0.36

BRCA1, 91 *BRCA2* mutation carriers and 640 non-carriers). The clinical characteristics of this cohort are described in Table S3. Median follow-up for the entire cohort was 5.8 years. We observed a myelodysplastic syndrome in one *BRCA2* mutation carrier and an acute leukemia in one non-carrier. Regarding cardiovascular complications, we observed heart failure in one *BRCA2* carrier and 3 non-carriers. Sequelar neuropathy was observed in 3 non-carriers.

Discussion

The data from this multicenter cohort study show a significant increase in acute hematological toxicity with chemotherapy in breast cancer patients who carry *BRCA1* germline mutations. To our knowledge, this is the first study investigating the link between *BRCA* germline mutation status and hematological toxicity with systematic check-up at day 7–14 after the first cycle of chemotherapy. This method eliminates reporting bias present when patients consult only if they have fever after chemotherapy. We mainly focused on toxicity after the first cycle because patients who receive G-CSF as secondary prophylaxis after febrile neutropenia have significant decrease in the incidence of febrile neutropenia in the subsequent cycles of chemotherapy, as it was reported in the landmark G-CSF registration trial [18]. The evaluation of hematological toxicity after the first cycle seemed to us the least biased.

Here, we reported a significant increase in febrile neutropenia in *BRCA1* mutation carriers only, but not in *BRCA2* carriers. Febrile neutropenia is a frequent and life-threatening consequence of chemotherapy. Cancer patients who experience febrile neutropenia have 15% additional mortality compared to similar patients undergoing myelosuppressive chemotherapy [19]. Risk factors for febrile neutropenia are older age, performance status, comorbidities, female gender, treatment regimens, advanced disease and genetic factors such as *TP53* genotype [20]. Our cohort was homogenous in terms of age, gender, extension of the disease and chemotherapy regimen.

Our study is unique in that we did not simply collect neutropenia and febrile neutropenia data based on medical records but rather used a methodology which mirrors that of G-CSF registration trials [21]. Indeed, our patients had systematic visits with the physician and blood tests including neutrophil counts on day 7–14 after their first chemotherapy cycle, allowing for a precise primary endpoint. While our Swiss cohort was multi-centered, the same follow-up protocol was used by all participating oncologists.

As neutrophils are abundant and have the shortest circulating half-life (6–8 h) of white blood cells [22], we suspected that neutropenia could be a surrogate marker for *BRCA1*/*BRCA2* haploinsufficiency. We identified a

significant increase in grade 3–4 and febrile neutropenia among *BRCA1* carriers. There was a significant decrease in chemotherapy dose-intensity in *BRCA1* carriers. Importantly, over the entire treatment period, *BRCA1* carriers required G-CSF support in 86% of the cases compared to only 51% in non-carriers. Thus, it is very likely that *BRCA1* carriers need G-CSF support to complete their (neo)adjuvant chemotherapy. Among patients who developed TNBC, we observed that *BRCA1* carriers had significant increased risk to develop grade 4 neutropenia compared to non-carriers. This observation, which needs to be confirmed in larger and independent cohorts of patients receiving the same regimen of chemotherapy, would have implications for screening of *BRCA1* germline mutations.

Our results are consistent with those reported by Huszno et al. who found an increased incidence in febrile neutropenia among *BRCA* carriers prior to the administration of the second cycle of anthracycline-based chemotherapy [14]. However, the authors did not detail the occurrence of febrile neutropenia in *BRCA1* and *BRCA2* separately, nor the incidence of hospitalizations or G-CSF use during chemotherapy. Additionally, they investigated Polish *BRCA1*/*BRCA2* founder mutations only, which could induce a bias regarding the location of mutations in these genes [23].

A large, single-center, retrospective study among breast cancer patients showed no difference in dose-intensity or febrile neutropenia among *BRCA1*/*BRCA2* carriers and non-carriers [13]. These diverging results with our study could have several explanations. First, the Dutch study considered "febrile neutropenia" only for patients who went to the hospital for fever. The patients did not have systematic counselling and neutrophil count at day 7–14, and this could lead to missing asymptomatic febrile neutropenia. Secondly, the authors did not consider the occurrence of febrile neutropenia in *BRCA1* and *BRCA2* separately. If we analyze *BRCA1* and *BRCA2* carriers together in our cohort, we would see less significant difference with non-carriers regarding the incidence of febrile neutropenia (24% in *BRCA* carriers *vs*. 10% in non-carriers, $p = 0.0121$).

A recent work by Kotsopoulos et al. evaluating hematological toxicity after chemotherapy in ovarian cancer, designed in a similar fashion to our study, compared *BRCA* carriers to non-carriers [24]. The authors analyzed hemoglobin, platelet and neutrophil counts before each cycle of chemotherapy. There was an increase in the incidence grade 3 or greater neutropenia among *BRCA* carriers, but no difference in dose delays, G-CSF use, anemia or thrombocytopenia rates. There was a trend toward an increase in chemotherapy dose reductions among *BRCA* carriers. The authors concluded that there were likely no clinical implications while treating ovarian cancer patients with *BRCA* mutations. There are several possible explanations for the differences observed between the results of Kotsopoulos' study and our study: (a) in terms of methodology, Kotsopoulos et al. analyzed blood cell counts before each cycle of chemotherapy, but without intermediate blood tests 7–14 days after chemotherapy which corresponds to the nadir of neutrophils [18] and thus could have underestimated acute toxicity; (b) ovarian cancer patients generally received one DNA damage agent (platinum) whereas breast cancer patients usually received two (anthracycline and cyclophosphamide); (c) *BRCA1* and *BRCA2* carriers were analyzed together, not separately. It is possible that the authors would have observed significant differences between *BRCA* carriers and non-carriers had they analyzed *BRCA1* and *BRCA2* separately or looked specifically at the incidence of grade 4 neutropenia. Overall, despite these divergences, the neutropenia results mirror our own.

Correlation between *BRCA* germline mutations and increased hematological toxicities has been recently reported in a phase I trial investigating the safety and efficacy of combining interstrand cross-linking agent carboplatin and PARP inhibitor talazoparib [25]. In this trial, the authors had systematic blood count analysis every week allowing detection of significant increased toxicity in *BRCA* carriers while the number of the patients was low (7 *BRCA* carriers out of 24 patients in total). This observation is consistent with our results and highlights the need of systematic neutrophil count 1 and/or 2 weeks after starting treatment with DNA damaging agents in order to detect increased toxicity in *BRCA* carriers.

Little is known regarding the correlation between genotype and response to chemotherapy in *BRCA* carriers. We observed increased hematological toxicity among *BRCA1* carriers compared to non-carriers, consistent with preclinical data suggesting *BRCA1* haploinsufficient cells are impaired in DNA repair and hypersensitive to genotoxic stress [9]. While *BRCA1* mutations generally increase response to DNA damaging agents, mutations located in the RING domain exhibit resistance to platinum drugs and PARP inhibitors in mice [26, 27]. Similarly, we observed that patients carrying *BRCA1* mutations located in the RING domain had low incidence of hematological toxicity. We did not observe an increase in acute hematological toxicity in *BRCA2* carriers.

Regarding long-term complications, there is a known correlation between *BRCA* status and secondary cancers, especially hematological malignancies [28]. Alkylating agents, G-CSF and radiotherapy further significantly increase this risk [29]. A potential mechanism for increased hematological malignancies in *BRCA* carriers could be clonal hematopoiesis, i.e., the expansion of one clone of blood population at a rate disproportionately greater than other clones [30, 31]. We did not observe an increase in secondary myelodysplastic syndromes or acute leukemia. While a correlation exists in much larger cohorts [32], our study of 898 patients may not have allowed us

to detect a difference, also because of a limited follow-up period. For long-term cardiovascular and neurological chemotoxicity, both platinum derivatives and taxanes are potentially neurotoxic, the latter being frequently used in early breast cancer therapy. Our results are consistent with the literature [33], showing an absence of increased long-term neurotoxicity among *BRCA* carriers. The only 3 cases detected in our cohort were among non-carriers. However, it must be noted that long-term peripheral neuropathy is likely under-reported in medical files [34].

Our study has several limitations. It is a retrospective study with selection bias. All patients had criteria for genetic screening, meaning our population is not representative of all breast cancer patients. For instance, our patients were young with a median age of 40. While our methodology was strict, our study remains a retrospective analysis and certain data are missing. Hematological data collected at day 7–14 may not always reflect the toxicity nadir. *BRCA1*/*BRCA2* screening by next-generation sequencing introduced in our laboratory in 2011 is more sensitive than previous techniques used (Denaturing High-Performance Liquid Chromatography or High Resolution Melt pre-screening analysis followed by Sanger sequencing method) [35], and we did not retest all negative results with more modern techniques. The chemotherapy regimen was not uniform. We did not do a multivariate analysis. The small *BRCA* carriers sample size of the Swiss cohort prevented us from confirming the relevance of our study. Thus, our observations should be considered as exploratory and need to be validated by a large-scale prospective cohort with uniform genetic testing method and uniform treatment.

Conclusion

Among breast cancer patients, there is a significant correlation between chemotherapy-related acute hematological toxicity, febrile neutropenia, and *BRCA1* germline mutation status. Our results, which need to be confirmed in an independent prospective cohort, suggest that care should be taken and primary prophylaxis with G-CSF offered when treating women with known *BRCA1* mutations with neo(adjuvant) chemotherapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study complies with the current laws in Switzerland and France.

Informed consent Informed consent was obtained from all alive participants included in the study in Geneva. For this type of study, formal consent is not required.

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