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Chemotherapy-related agranulocytosis as a predictive factor for germline BRCA1 pathogenic variants in breast cancer patients: a retrospective cohort study

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Summary

BACKGROUND: Carriers of germline pathogenic variants of the *BRCA1* gene (*gBRCA1*) tend to have a higher incidence of haematological toxicity upon exposure to chemotherapy. We hypothesised that the occurrence of agranulocytosis during the first cycle of (neo-)adjuvant chemotherapy (C1) in breast cancer (BC) patients could predict *gBRCA1* pathogenic variants.

PATIENTS AND METHODS: The study population included non-metastatic BC patients selected for genetic counselling at Hôpitaux Universitaires de Genève (Jan. 1998 to Dec. 2017) with available mid-cycle blood counts performed during C1. The BOADICEA and Manchester scoring system risk-prediction models were applied. The primary outcome was the predicted likelihood of harbouring *gBRCA1* pathogenic variants among patients presenting agranulocytosis during C1.

RESULTS: Three hundred seven BC patients were included: 32 (10.4%) *gBRCA1*, 27 (8.8%) *gBRCA2*, and 248 (81.1%) non-heterozygotes. Mean age at diagnosis was 40 years. Compared with non-heterozygotes, *gBRCA1* heterozygotes more frequently had grade 3 BC (78.1%; p = 0.014), triple-negative subtype (68.8%; p <0.001), bilateral BC (25%; p = 0.004), and agranulocytosis following the first cycle of (neo-)adjuvant chemotherapy (45.8%; p = 0.002). Agranulocytosis and febrile neutropenia that developed following the first cycle of chemotherapy were independently predictive for *gBRCA1* pathogenic variants (odds ratio: 6.1; p = 0.002). The sensitivity, specificity, positive predictive value, and negative predictive value for agranulocytosis predicting *gBRCA1* were 45.8% (25.6–67.2%), 82.8% (77.5–87.3%), 22.9% (6.1–37.3%), and 93.4% (88.9–96.4%), respectively. Agranulocytosis substantially improved the positive predictive value of the risk-prediction models used for *gBRCA1* evaluation.

CONCLUSION: Agranulocytosis following the first cycle of (neo-)adjuvant chemotherapy is an independent predictive factor for *gBRCA1* detection in non-metastatic BC patients.

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Introduction

Individuals who are heterozygous for pathogenic variants in *BRCA1* and *BRCA2* genes (*gBRCA*) face an increased lifetime risk of developing breast cancer and ovarian cancer. Early identification of *gBRCA* pathogenic variants is essential as it can lead to tailored interventions and potentially influence patients' prognosis [1–4]. Recent advances in next-generation sequencing significantly increased the accessibility and workflow of *BRCA* genetic testing; however, the identification of *gBRCA* pathogenic variants remains challenging, with financial, psychosocial, and legal implications. Current guidelines do not recommend routine *gBRCA* molecular analysis in all breast cancer patients; instead, they encourage offering genetic counselling to individuals at risk of harbouring deleterious *gBRCA* variants. In addition, this intervention facilitates a useful informed risk/benefit discussion with patients [5–7].

Several factors influence the probability of carrying *gBRCA1*/*gBRCA2* pathogenic variants, with young age at diagnosis being the most important [8]. Criteria based on tumour characteristics and individual and family history have been published by knowledgeable societies to select patients for genetic counselling referral [5–7]. A variety of risk-prediction models have been developed to assist clinicians in their informed decision-making to better identify a "high-risk" subset of breast cancer patients selected to undergo *gBRCA* genetic testing, such as the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA), the pathology-adjusted version of the Manchester score (MSS3), and BRCAPRO™ [9–17]. By accurately calculating the area under the curve on receiver operating characteristic (AUROC), complex statistical algorithms in BOADICEA and BRCAPRO assess an individual's risk of harbouring a pathogenic variant

in breast cancer susceptibility genes. Today, these risk-prediction models are largely routinely implemented in genetic counselling practice. Each model has advantages and limitations and need to be interpreted in the context of the studied populations in which they have been validated.

Breast cancer occurring in *gBRCA* heterozygotes accounts for approximatively 3–5% of all breast cancers [18–20]. breast cancers related to *gBRCA1* tend to be of higher grade and mostly present as the triple-negative molecular subtype (TNBC) [21–23]. Haplo-insufficient *BRCA* neoplastic cells have an impaired ability to repair DNA double-stranded breaks (DSBs) through homologous recombination mechanisms [24]. Neoadjuvant and adjuvant chemotherapy regimens used in breast cancer usually include alkylating agents and anthracyclines, which both induce DNA-DSBs. Because haematological cells in *gBRCA* heterozygotes harbour one defective allele, it has been hypothesised that *gBRCA* haematological cells could exhibit increased sensitivity to agents inducing DNA-DSBs (e.g., chemotherapy and radiotherapy). Several groups attempted to elucidate this question using mixed breast cancer populations and heterogeneous methodologies, leading to inconsistent results [25–36]. Analysing neutrophils counts at their nadir (mid-cycle), our group recently reported that *gBRCA1* heterozygotes but not *gBRCA2* heterozygotes with breast cancer tend to be associated with a significantly higher rate of developing grade 4 and febrile agranulocytosis during their first cycle of chemotherapy [35]. Our observation is consistent with a recent report highlighting increased haematological toxicity in *gBRCA1* but not *gBRCA2* heterozygotes [36]. Furthermore, *gBRCA1* heterozygotes require more granulocyte colony-stimulating factor (G-CSF) support than non-heterozygotes [35]. The aim of this study was to determine whether agranulocytosis occurring during the first cycle (C1) of (neo-)adjuvant chemotherapy (days 7–14) could predict the likelihood of harbouring a pathogenic *gBRCA1* variant.

Patients and methods

Patient cohort

We analysed the files of all breast cancer patients referred for genetic counselling and *gBRCA1/gBRCA2* testing between January 1998 and December 2017 at the Unit of Oncogenetics, Hôpitaux Universitaires de Genève (HUG), Switzerland. As per edited Swiss criteria [37], patients were referred for counselling if they presented with i) newly diagnosed ovarian cancer, ii) family history of breast cancer and/or ovarian cancer, or iii) age ≤60 years with TNBC or age <40 years independent of breast cancer subtype. The Geneva Ethics Committee approved the research protocol in 2015 (CCER 15-158), and an amendment to the study protocol was obtained in 2017. Written informed consent was obtained from living patients and a waiver was applied for deceased patients. The research was conducted in accordance with the Declaration of Helsinki and Swiss regulations for human research.

Patients included in the present analysis met the following inclusion criteria: (1) non-metastatic breast cancer, (2) having received (neo-)adjuvant chemotherapy, and (3) available blood count between day 7 and 14 after their first cycle of chemotherapy.

The exclusion criteria were (1) metastatic disease at diagnosis, (2) absence of (neo-)adjuvant chemotherapy, (3) absence of genetic testing, and (4) no follow-up.

Data collection

Clinical data (age at diagnosis, chemotherapy regimen and timing [neoadjuvant or adjuvant]), tumour characteristics (TNM stage, grade, oestrogen/progesterone receptors, HER2 status), and laboratory results (blood count, including neutrophils) at baseline and day 7–14 after C1 were collected from patients' medical records in a dedicated deidentified case report form. The key list was accessible only by the principal investigator. No software libraries, frameworks, or packages were used in the current work. Haematological toxicity was graded according to the Common Terminology Criteria for Adverse Events version 5.0 [38], with agranulocytosis defined as absolute neutrophil count $<0.5 \times 10^9$ cells/L. Febrile neutropenia was defined as absolute neutrophil count $<1 \times 10^9$ cells/L and fever >38.3 °C.

Pedigree data were collected from the genetic counselling charts, provided in standard linkage format with year of birth, age at last follow-up or death, and details of any cancer diagnosis (cancer site, age at diagnosis) for each family member. When age was missing but the year of birth was provided, age was calculated as years from the date of birth to the date the pedigree was drawn for living probands and the date of death for deceased probands. Cancers were recorded only when there was no ambiguity in patients' charts and age information was available. The following family history characteristics were collected for first-, second-, and third-degree relatives: (1) ≥1 breast cancer at age 40 years, (2) ≥3 female relatives with breast cancer aged ≤50 years, (3) ≥1 ovarian cancer at any age, (4) ≥1 breast cancer associated with ovarian cancer at any age (in the same relative or not), and (5) ≥1 bilateral breast cancer at any age. First-degree relatives were considered as direct filiation (biological parents and children), and second-degree relatives included aunts/uncles, grand-daughters/grand-sons, and grand-parents. Third-degree relatives include cousins and other family members.

Prediction of comprehensive genetic risk models was generated from pedigree data of genetic counselling charts: i) BOADICEA v. 3.0, a computer-based risk assessment incorporating detailed personal risk factors, pathological molecular markers, and individual and familial information from both lineages up to the third relative generation degree [39], and ii) MSS3, a paper-based risk model including several individual and familial characteristics from only one lineage, not applicable to individuals ofAshkenazi Jewish descent [14]. BOADICEA risk was prospectively calculated for probands enrolled between 2016 and 2017 and retrospectively for all other probands. MSS3 score was retrospectively generated for all probands; each variable had a dedicated numerical weight, with the sum providing a score in points that was converted into a percentage probability of finding a *gBRCA* mutation in a breast cancer patient. There is no formal consensus on the thresholds to apply for *gBRCA1*/*gBRCA2* mutation testing [12, 15]. In the current study, we choose a 10% pre-test cut-off (15 points equivalent for MSS3) in patients diagnosed with breast cancer. Germline molecular blood results

of *BRCA1* and *BRCA2* variants were classified as pathogenic according to the ENIGMA BRCA1/2 Gene Variant Classification Criteria. Women with variants of uncertain significance were considered non-heterozygotes [40, 41].

Endpoints

The primary endpoint was to assess the proportion of *gBRCA1* pathogenic variants in a non-metastatic breast cancer population presenting with agranulocytosis during C1 of chemotherapy. Secondary endpoints included comparison of the predictive value of individualised parameters (TNBC, agranulocytosis after C1) and both risk models (BOADICEA v. 3.0 and MSS3).

Statistics

This was an exploratory analysis; no sample size calculation was performed. Proportions were calculated for binary data (e.g., family history, agranulocytosis, febrile neutropenia) and categorical data (e.g., TNM stage, grade, chemotherapy regimen), whereas median and interquartile range were calculated for continuous data (age). Comparison of categorical data was performed using the χ^2 test or Fisher's exact test, when appropriate. Continuous variables were compared using the Kruskal–Wallis test. Patient characteristic frequencies were summarised according to *gBRCA* status and were compared pair by pair (*gBRCA1* heterozygotes vs. non-*gBRCA1* heterozygotes; *gBRCA2* heterozygotes vs. non-*gBRCA2* heterozygotes). Missing data or inapplicable responses were excluded when calculating p values. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression. Variables were added to the model if the two-sided significances level was less than 0.05 in the univariate analysis. A stepwise backward selection procedure removing terms with $P > 0.05$ was performed to assess the independent contribution of each studied factor to the outcome variables. All probability values were two-tailed, and the level of significance was set at 0.05. Calculations of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were performed for mid-cycle agranulocytosis (cut-off 0.5 G/L neutrophils), BOADICEA (cut-off 10%), and MSS3 (cut-off 15 points) individually in univariate analysis. Variables yielding p values less than 0.1 by univariate analysis were retained for the multivariate analysis. ORs were generated for variables that retained significance in the multivariate analysis. All analyses were conducted using STATA software (version 14.0 SE, Stata Corporation, College Station, Texas, USA).

Results

Patient characteristics

Among 892 women diagnosed with breast cancer who were referred for genetic counselling between 1998 and 2017 to the Unit of Oncogenetics at HUG, 322 met the inclusion criteria (figure S1). A total of 307 patients were included in the present analysis: 248 (81.1%) were *BRCA1/2* non-heterozygotes, 32 (10.4%) were *gBRCA1* heterozygotes, and 27 (8.8%) were *gBRCA2* heterozygotes. Our cohort include roughly one-third of breast cancer patients referred for genetic counselling from private practice and two-third from the university hospital. Patient demographics, tumour characteristics, chemotherapy regimens, and family history are summarised in table 1. Approximatively one-third of breast cancer patients were treated with neoadjuvant chemotherapy, and two-third received adjuvant chemotherapy. Nearly all patients (>95%) received alkylating agents, and 91% of non-heterozygotes, 94% of *gBRCA1* heterozygotes, and 75% of *gBRCA2* heterozygotes received anthracyclines. The median age at diagnosis was comparable between *gBRCA* heterozygotes and non-heterozygotes (40 years). TNBC phenotype was significantly more frequently observed in *gBRCA1* heterozygotes (68.8%) compared with *gBRCA2* heterozygotes (30.8%) and non-heterozygotes (25.8%) ($p < 0.001$). Carriers of *BRCA1* pathogenic variants were more likely to have a positive personal and/or familial history of breast cancer and/or ovarian cancer. No familial history was reported by 34% of *gBRCA1* and 38% of *gBRCA2* heterozygotes.

Occurrence of mid-cycle agranulocytosis

Compared with non-heterozygotes, the incidence of agranulocytosis occurring during C1 was higher among *gBRCA1* heterozygotes (45.8% versus 16.8%; $p = 0.002$) than among *gBRCA2* heterozygotes (20.8% versus 16.8%; $p = 0.576$) (Table 2). Febrile neutropenia occurred more frequently in *gBRCA1* heterozygotes (28%; $p = 0.001$) than *gBRCA2* heterozygotes (8%; $p = 0.641$) compared with non-heterozygotes (5.5%). Only 10 patients (3.3%) received primary prophylaxis with G-CSF during C1 (one *gBRCA1*heterozygote, one *gBRCA2* heterozygote, and eight non-heterozygotes). Clinical factors and therapeutic agents that may predict agranulocytosis were evaluated via multivariate analysis; however, none of these were statistically significant in our cohort (supplemental table S1 and supplemental table S2).

Prediction of gBRCA1 pathogenic variants

The median BOADICEA BRCA1 score was 9.7 (2.5–56.6) among *gBRCA1* heterozygotes compared with 0.7 (0.3–2.8) for non-heterozygotes ($p < 0.001$). This difference remained statistically significant when applying a pre-test probability threshold of 10. Median BOADICEA BRCA2 score was not statistically different between *gBRCA2* heterozygotes (2.6 [1.0–7.0]) and non-heterozygotes (1.8 [0.8–4.0]; $p = 0.113$). MSS3 scores were significantly higher in both *gBRCA1* (27.5 [20–38]; $p < 0.001$) and *gBRCA2* heterozygotes (20 [14–28]; $p < 0.001$) compared with non-heterozygotes (13 [9–17]). This difference remained significant when a 15-point cut-off was applied (equivalent to a 10% pre-test probability threshold). Scores per mutational status are summarised in table 2. AUROCs were generated per mutational status for BOADICEA and MSS3 (supplemental table S3).

Univariate and multivariate analyses

Following univariate analysis, nearly all individual and familial characteristics (TNBC subtype, agranulocytosis during C1, febrile neutropenia during C1, family history of one relative with breast cancer diagnosed before 41 years of age, ≥3 relatives with breast cancer diagnosed before 51 years of age, or one relative with a positive history of

ovarian cancer with or without breast cancer) were significantly associated with *gBRCA1* mutation status (table 3). Only two variables (≥3 relatives with breast cancer diagnosed <51 years of age and bilateral breast cancer) were significant for *gBRCA2* status. Age at diagnosis was not significant in our population (based on the Swiss criteria for referral to genetic counselling). All factors listed above remained statistically independently associated with *gBRCA1* mutation statusfollowing multivariate analysis (table 4). TNBC subtype was the strongest individual predictive factor for *gBRCA1* mutation (odds ratio [OR]: 15.6 [4.3–56.6]; p <0.001). Other factors were the presence of three or more relatives with breast cancer diagnosed before 51 years of age (OR: 14.1 [2.7–74.7]; p = 0.002), one relative with breast cancer diagnosed before 41 years of age (OR: 12.8 [3.2–51.5]; p <0.001), agranulocytosis during C1 (OR: 6.1 [1.9–19.3]; p = 0.002), and a positive history of ovarian cancer (OR: 4.1 [1.0–17.1]; p = 0.05). Due to the small population size, it was not possible to estimate the OR for febrile neutropenia and the presence of breast cancer or ovarian cancer in family history.

Performance of individual and combined parameters

We evaluated the individual performance of agranulocytosis during C1, TNBC status, and BOADICEA BRCA1

Table 1:
Clinical, pathological, and family history characteristics of breast cancer patients according to *gBRCA* status.

		Non-heterozygotes (n = 248)	gBRCA1 heterozygotes (n = 32)	gBRCA2 heterozygotes (n = 27)
Median age at diagnosis, years (IQR)		42 (36–50)	38 (35–50)	43 (35–50)
	<40 (%)	102 (41.1)	18 (56.3)	12 (44.4)
	≥40 (%)	146 (58.9)	14 (43.8)	15 (55.6)
Molecular type (%)	Luminal	130 (53.3)	7 (21.9)	15 (57.7)
	TNBC	63 (25.8)	22 (68.8)	8 (30.8)
	HER2	51 (20.9)	3 (9.4)	3 (11.5)
	Missing	4	0	1
TNM stage (%)	T0	4 (1.6)	0 (0)	1 (3.7)
	T1	103 (41.5)	15 (46.9)	11 (40.7)
	T2	106 (42.7)	14 (43.8)	13 (48.2)
	T3	28 (11.3)	0 (0)	1 (3.7)
	T4	7 (2.8)	3 (9.4)	1 (3.7)
	N0	114 (46.0)	18 (56.3)	11 (40.7)
	N+	134 (54.0)	14 (43.8)	16 (59.3)
Grade (%)	1	15 (6.2)	0 (0)	0 (0)
	2	103 (42.2)	7 (21.9)	11 (40.7)
	3	126 (51.6)	25 (78.1)	16 (59.3)
Chemotherapy type (%)	Neoadjuvant	90 (36.4)	13 (40.6)	7 (25.9)
	Adjuvant	157 (63.6)	19 (59.4)	20 (74.1)
Chemotherapy regimen (%)*	Alkylating agents	242 (97.6)	31 (96.9)	26 (96.3)
	Anthracyclines	225 (90.7)	30 (93.8)	20 (74.1)
	Taxanes	188 (75.8)	24 (75.0)	19 (70.4)
	Platinum	3 (1.2)	1 (3.1)	1 (3.7)
	Primary G-CSF support	8 (3.2)	1 (3.1)	1 (3.7)
Additional oncology history (%)	Bilateral breast cancer	21 (8.5)	8 (25.0)	4 (14.8)
	Ovarian cancer	3 (1.2)	2 (6.3)	1 (3.7)
	Other *BRCA*-related cancers (pancreatic)	4 (1.6)	2 (6.5)	3 (11.1)
Family history (%)				
≥1 relative with breast cancer ≤40 years	No	230 (93.5)	23 (71.9)	23 (88.5)
	Yes	16 (6.5)	9 (28.1)	3 (11.5)
	Missing	2	0	1
≥3 relatives with breast cancer ≤50 years	No	234 (95.1)	25 (78.1)	22 (84.6)
	Yes	12 (4.9)	7 (21.9)	4 (15.4)
	Missing	2	0	1
≥1 relative with bilateral breast cancer	No	229 (93.1)	28 (87.5)	21 (80.8)
	Yes	17 (6.9)	4 (12.5)	5 (19.2)
	Missing	2	0	1
≥1 relative with ovarian cancer and breast cancer	No	243 (98.8)	29 (90.6)	26 (100)
	Yes	3 (1.2)	3 (9.4)	0 (0)
	Missing	2	0	1
≥1 relative with ovarian cancer	No	231 (93.9)	26 (81.3)	23 (88.5)
	Yes	15 (6.1)	6 (18.8)	3 (11.5)
	Missing	2	0	1

IQR: interquartile range; TNBC: triple-negative breast cancer

* Chemotherapy regimen: FEC (5-fluorouracil, epirubicin, cyclophosphamide) + taxanes (docetaxel or paclitaxel); TC (docetaxel, cyclophosphamide); CMF (cyclophosphamide, methotrexate, 5-fluorouracil); EC (epirubicin, cyclophosphamide) + paclitaxel; TAC (docetaxel, doxorubicin, cyclophosphamide)

and MSS3 scores to predict *gBRCA1* pathogenic variants in our cohort by calculating sensitivity, specificity, PPV, and NPV (table 5). Furthermore, to determine whether the risk evaluation could be improved, we attempted to combine agranulocytosis during C1 with either TNBC status, BOADICEA BRCA1 score, or MSS3 score (scores being considered positive with a ≥10% threshold). Adding agranulocytosis information to TNBC status, BOADICEA BRCA1 score, or MSS3 score improved PPV from 23.4% (15.3–33.3%) to 50% (26.0–74.0%), 50% (31.3–68.7%)to 77.8% (40.0–97.2%), and 18.2% (12.4–25.4%) to 37% (19.4–57.6%), respectively (table 5).

Discussion

In the general population, the frequency of *gBRCA1* and *gBRCA2* pathogenic variants varies between 0.18% and 0.34%, respectively [42]. The presence of an increased number of relatives affected by breast cancer, ovarian cancer, prostate, or pancreatic cancers occurring at a young age usually alerts physicians to a potential hereditary breast-ovarian cancer syndrome [43]; however, most breast cancers arise in individuals with no obvious family history. The lifetime cumulative risk of developing breast cancer in *gBRCA* heterozygotes is substantially increased compared with the general population, estimated at 65% in *gBRCA1* heterozygotes and 45% in *gBRCA2* heterozygotes by the

Table 2:
Haematological toxicity during C1; MSS3 and BOADICEA scores per mutational status.

		Non-heterozygotes (n = 248)	gBRCA1 heterozygotes(n = 32)	gBRCA2 heterozygotes(n = 27)
Agranulocytosis (%)	No	183 (83.2)	13 (54.2)	19 (79.2)
	Yes	37 (16.8)	11 (45.8)	5 (20.8)
	Missing	28	8	3
Febrile neutropenia (%)	No	208 (94.6)	18 (72.0)	23 (92.0)
	Yes	12 (5.5)	7 (28.0)	2 (8.0)
	Missing	28	7	2
BOADICEA BRCA1	Median (IQR)	0.7 (0.3–2.8)	9.7 (2.5–56.6)	NA
	<10 (%)	234 (94.4)	17 (53.1)	
	≥10 (%)	14 (5.7)	15 (46.9)	
BOADICEA BRCA2	Median (IQR)	1.8 (0.8–4.0)	NA	2.6 (1.0–7.0)
	<10 (%)	225 (90.7)		23 (85.2)
	≥10 (%)	23 (9.3)		4 (14.8)
MSS3*	Median (IQR)	13.0 (9–17)	27.5 (20–38)	20 (14–28)
	<15 (%)	145 (58.9)	5 (15.6)	7 (25.9)
	≥15 (%)	101 (41.1)	27 (84.4)	20 (74.1)

IQR: interquartile range; NA: not applicable; BOADICEA: Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; MSS3: pathology-adjusted Manchester score.

* calculated based on family history in first-, second-, and third-degree relatives in one direct blood lineage (cancers on one side of the family, counsellor's choice)

Table 3:
Univariate analysis of breast cancer patients' characteristics, haematological toxicity during C1, and family history according to gBRCA1 and gBRCA2 status.

	gBRCA1			gBRCA2		
	OR	95% CI	p value	OR	95% CI	p value
TNBC	7.3	3.2–16.7	**<0.001**	1.3	0.5–3.1	0.586
Age ≤40 years	1.8	0.9–3.9	0.108	1.1	0.5–2.5	0.740
Agranulocytosis (cycle 1)	4.2	1.7–10.1	**0.001**	1.3	0.5–3.7	0.622
Febrile neutropenia (cycle 1)	6.7	2.4–19.2	**<0.001**	1.5	0.3–7.2	0.606
≥1 relative with breast cancer ≤40 years	5.6	2.2–14.1	**<0.001**	1.9	0.5–6.9	0.345
≥3 relatives with breast cancer ≤50 years	5.5	2.0–15.1	**<0.001**	3.5	1.1–11.9	**0.041**
Ovarian cancer	3.6	1.3–10.0	**0.016**	2.0	0.5–7.5	0.297
Breast cancer + ovarian cancer	8.4	1.6–43.5	**0.011**	1.3	0.1–26.1	0.858
Bilateral breast cancer	1.9	0.6–6.1	0.268	3.2	1.1–8.6	**0.037**

CI: confidence interval; OR: odds ratio; TNBC: triple-negative breast cancer

Table 4:
Multivariate analysis of breast cancer patient characteristics, haematological toxicity during C1, and family history according to gBRCA1 status.

	gBRCA1		
	OR	95% CI	p value
TNBC	15.6	4.3–56.6	**<0.001**
Agranulocytosis (cycle 1)	6.1	1.9–19.3	**0.002**
Febrile neutropenia (cycle 1)	NA	NA	
≥1 relative with breast cancer ≤40 years	12.8	3.2–51.5	**<0.001**
≥3 relatives with breast cancer ≤50 years	14.1	2.7–74.7	**0.002**
Ovarian cancer	4.1	1.0–17.1	**0.05**
Breast cancer + ovarian cancer	NA	NA	

CI: confidence interval; NA: not available; OR: odds ratio; TNBC: triple-negative breast cancer

age of 70 years [2]. Identification of *gBRCA* pathogenic variants is of prime importance to implement specific medical management, such as cascade genetic screening of close relatives, specific individual surveillance [5–7], chemopreventive approaches [44–53], and personalised therapeutic strategies [4, 54, 55–60]. Even though genetic testing has been demonstrated to be cost-effective [61–65], for financial reasons, *gBRCA1*/*gBRCA2* genetic testing is restricted to newly diagnosed breast cancer patients with a high likelihood of harbouring a deleterious genetic variant [5–7].

Taking advantage of the previously demonstrated susceptibility of haematopoietic cells in *gBRCA1* breast cancer heterozygotes after DNA-damaging chemotherapies [66, 67], we hypothesised that agranulocytosis occurring during C1 could predict *gBRCA1* mutation.We chose to investigate agranulocytosis after C1 to avoid the bias introduced with the use of secondary G-CSF prophylaxis. In line with our previously reported results, the incidence of agranulocytosis during C1 was higher among *gBRCA1* heterozygotes, confirming the sensitivity of *gBRCA1* haploinsufficient haematopoietic cells to DNA-damaging agents [35]. This could be due to the requirement of BRCA1 for haematopoietic stem cell function and normal haematopoiesis [67]. Following multivariate analysis, we found that agranulocytosis during C1 was an independent predictive factor for *gBRCA1* but not *gBRCA2* pathogenic variants. This finding reinforces the weight of individual pathological and biological features in predicting *gBRCA1* pathogenic variants [68–71].

Our cohort was representative of Geneva's breast cancer population and was relatively well-balanced in terms of age, stage, and therapeutic regimens. In line with the reported literature, we noted an enrichment of high-grade tumours and the TNBC subtype among *gBRCA1* heterozygotes [68–71]. A median age of 40 years in our study underlined the fact that young age at diagnosis is the strongest predictive factor of genetic counselling and g*BRCA* testing [72]. In a study conducted by Beck et al., age ≤45 years at the time of breast cancer diagnosis and age ≤60 years at the time of TNBC diagnosis facilitated the detection of 9.3% and 9.7% of *gBRCA* pathogenic variants, respectively [73]. In a similar population to ours, Grindedal et al. found that age of onset <40 years or TNBC status facilitated the identification of 32–34% of *gBRCA* heterozygotes, highlighting the importance of these individualised parameters in *gBRCA* risk prediction [74].

In addition to well-known risk criteria [5–7, 37], several models can predict a subject's individual probability of harbouring a *gBRCA* pathogenic variant and were demonstrated to be superior to counsellors' estimated probability [7, 10–18]. The most recent versions of these risk algorithms integrate the tumour pathological characteristics of the index case, leading to increased discrimination performance [12, 15]. However, these models still rely on the fulfilment of a detailed familial history; therefore, their value is limited in the absence of any positive familial history [21]. As in previous cohorts, the BOADICEA model performed well in its prediction of *gBRCA1* pathogenic variant detection (AUC:0.71) and relatively poorly for *gBRCA2* pathogenic variants (AUC: 0.53) [10, 66–67, 75]. This could be explained by the fact that breast cancer in *gBRCA1* heterozygotes is mainly TNBC, whereas *gBRCA2* breast cancer tends to resemble sporadic breast cancer and is predominantly hormone receptor positive. Consequently, genetic predictive models considering pathological characteristics are usually better at predicting the risk of *gBRCA1* than *gBRCA2* [75]. With AUCs of 0.79 for *gBRCA1* and 0.66 for *gBRCA2,* theMSS3 model demonstrated slightly better performance than the BOADICEA model in our cohort of breast cancer patients, achieving comparable results to those reported in the literature [13, 14]. This discrepancy between the predictive risk models could be due to the presence of a high proportion of *gBRCA* heterozygotes lacking a positive family history in our cohort (34% of *gBRCA2* and 38% of *gBRCA1* heterozygotes) [74]. This finding reflects the low birth rate and tendency for smaller family sizes in Switzerland and in developed countries in general. Furthermore, young women are less likely to have close relatives who are affected due to their age [72, 76]. MSS3 tests both lineages, taking in account the highest score, and incorporates proportionally more individual cancer-based information, relying less on family history. Therefore, MSS3 is expected to perform well in breast cancer patients lacking a strong family history.

The strengths of our study are its novelty and methodology. Systematic blood count check performed at mid-cycle after C1 (days 7–14) does not always reflect a precise nadir of neutrophils; however, this time window allows for the capture of most agranulocytosis events and eliminates potential reporting bias. With its inclusion of patients referred from a public university hospital and the private sector of care, our cohort is representative of the Swiss breast cancer population. As chemotherapy regimens vary in their impact on the incidence of haematological toxicity, we de-

Table 5:
Performance of TNBC status, MSS3 score, and BOADICEA score in gBRCA1 mutational status prediction alone or combined with agranulocytosis.

Variable	Sensitivity	Specificity	PPV	NPV
Agranulocytosis	45.8 (25.6–67.2%)	82.8 (77.5–87.3%)	20.8 (10.8–34.1%)	94.0 (89.9–96.7%)
TNBC	71.0 (52.0–85.8%)	72.9 (67.2–78.2%)	23.4 (15.3–33.3%)	95.6 (91.8–98.0%)
BOADICEA BRCA1	46.9 (29.1–65.3%)	94.5 (91.2–96.9%)	50.0 (31.3–68.7%)	93.9 (90.4–96.4%)
MSS3*	84.4 (67.2–94.7%)	55.7 (49.6–61.7%)	18.2 (12.4–25.4%)	96.8 (92.7–99.0%)
TNBC + agranulocytosis	39.1 (19.7–61.5%)	96.3 (93.0–98.3%)	50.0 (26.0–74.0%)	94.3 (90.6–96.8%)
BOADICEA BRCA1 + agranulocytosis	29.2 (12.6–51.1%)	99.2 (97.1–99.9%)	77.8 (40.0–97.2%)	93.4 (89.7–96.1%)
MSS3* + agranulocytosis	41.7 (22.1–63.4%)	93.0 (89.0–95.9%)	37.0 (19.4–57.6%)	94.1 (90.4–96.8%)

TNBC: triple-negative breast cancer; BOADICEA: Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; MSS3: pathology-adjusted Manchester score; PPV: positive predictive value; NPV: negative predictive value

* A threshold of 10% (≥15 points for MSS3) was used to calculate sensitivity, specificity, PPV, and NPV in this BC cohort

liberately restricted our patient population to non-metastatic breast cancer treated with neoadjuvant or adjuvant chemotherapy. This choice made our studied population quite homogenous, with nearly all breast cancer patients receiving both anthracyclines and alkylating agents in the first cycle of chemotherapy. Moreover, risk-prediction scores were uniformly retrospectively calculated with the latest version of the Manchester scoring system (MSS3) [14] and the BOADICEA model version 3 [39] based on available clinical and pathological information, with only few missing data. Finally, all patients benefited from a complete *BRCA1* and *BRCA2* sequencing, thereby avoiding potential selection bias that could be induced by analysis of founder pathogenic variants alone.

In addition to its small sample size and inherent retrospective nature, our study had several limitations. First, only breast cancer patients meeting Swiss criteria underwent genetic counselling and *gBRCA1/gBRCA2* testing. This led to recruitment bias, with substantial enrichment of *gBRCA* heterozygotes in our cohort, reflected by a mean age of 40 years. By applying Swiss guidelines, we likely missed patients whose carrier pre-test probability was too low to undergo genetic testing; therefore, our reported NPV and PPV values could not be generalised to an unselected breast cancer population. Second, the study was conducted before the implementation of 12-gene panel testing; therefore, it is important to investigate whether carriers of other genes (e.g., *FANC*), are at increased risk of acute haematological toxicity. This is a timely question given the new standard therapeutic regimen for TNBC (pembrolizumab, carboplatin, anthracyclines, and cyclophosphamide), which is associated with substantial haematological toxicity according to the recently reported KEYNOTE-552 trial results [77].

Conclusion

BRCA1 haploinsufficiency confers an increased sensitivity to DNA damaging agents in breast cancer patients, and physicians should be aware that these patients are at increased risk of developing severe acute haematological toxicity and febrile neutropenia. Although germline genetic testing is increasingly used in routine practice for young breast cancer and/or TNBC patients, our results suggest that agranulocytosis that develops during C1 in non-metastatic breast cancer patients could be an independent predictive factor of interest for g*BRCA1* pathogenic variant detection.

Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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Supplementary tables and figure

Figure S1: Patient selection flow diagram.

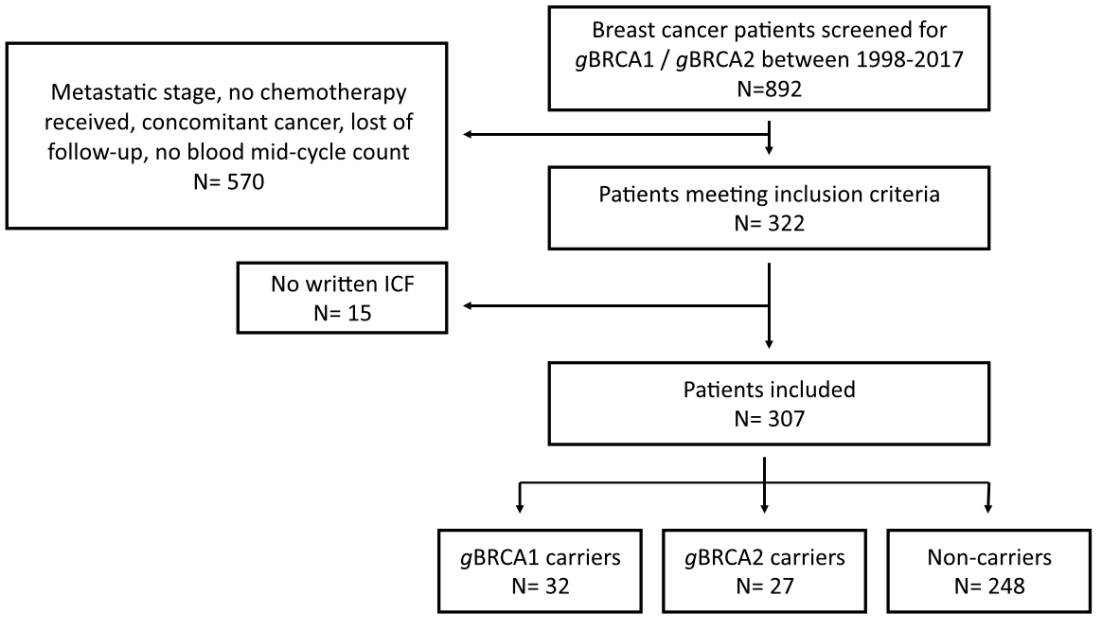

Table S1:
Multivariate analysis of breast cancer patients' characteristics, haematological toxicity during C1, and family history for gBRCA1 according to age.

Clinical factors	OR	SE	p value	95% CI
Molecular subtypes (TNBC vs. others)	1.01	0.36	0.98	0.5–2.0
T class	1.11	0.39	0.77	0.56–2.2
Grade	0.35	0.27	0.17	0.08–1.56
Age category*	0.72	0.24	0.32	0.38–1.37
Type of chemotherapy regimen (neoadjuvant vs. adjuvant)	0.72	0.25	0.34	0.36–1.41
Family history (present vs. absent)	1.09	0.36	0.79	0.12–5.76

* Age category as follows: <30 years, 30–39 years, 40–49 years, 50–59 years, 50–59 years, and >60 years.

TNBC: triple-negative breast cancer; OR: odds ratio; SE; standard error; CI: confidence interval.

Table S2:
Association between chemotherapy agent and mid-cycle neutrophil count.

Chemotherapy regimen		Agranulocytosis (%)	p value
Anthracyclines	No	3 (15.0)	
	Yes	50 (20.1)	0.42
Alkylating agents	No	2 (50)	
	Yes	51 (19.3)	0.18
Platinum	No	53 (20)	
	Yes	0	0.51
Taxanes	No	7 (14.9)	
	Yes	46 (20.8)	0.24

Table S3:
Performance of BOADICEA and MSS3 scores (AUC) according to mutational status.

	gBRCA1 + gBRCA2 versus non-heterozygotes				**gBRCA1 versus non-BRCA1 heterozygotes**				**gBRCA2 versus non-BRCA2 heterozygotes**			
	OR	**[95%CI]**	**p value**	**AUC**	**OR**	**[95%CI]**	**p value**	**AUC**	**OR**	**[95%CI]**	**p value**	AUC
BOADICEA BRCA1*	6.2	[2.8–13.7]	**<0.001**	0.61	15.3	[6.4–36.4]	**<0.001**	0.71	–			–
BOADICEA BRCA2*	1.3	[0.5–3.2]	0.548	0.51	–		–		1.7	[0.5–5.3]	0.360	0.53
MSS3*	5.6	[2.8–11.1]	**<0.001**	0.69	6.8	[2.5–18.1]	**<0.001**	0.70	3.3	[1.4–8.2]	**0.008**	0.64

* A threshold of 10% (≥15 points for MSS3) was used to calculate sensitivity, specificity, PPV, and NPV in this BC cohort.

BOADICEA: Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm; MSS3: pathology-adjusted Manchester score; AUC: area under the curve; OR: odds ratio; CI: confidence interval.