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Hypermethylation of the *BRCA2* gene promoter and its co-hypermethylation with the *BRCA1* gene promoter in patients with breast cancer

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Abstract.
BACKGROUND: The *BRCA2* gene is an important tumour suppressor in breast cancer, and alterations in *BRCA2* may lead to cancer progression. The aim of the study was to investigate the association of hypermethylation of the *BRCA2* gene promoter and its co-hypermethylation with the *BRCA1* gene promoter with the development and course of breast cancer in women.
METHODS: This study included 74 women with breast cancer (tumour tissue samples and peripheral blood) and 62 women without oncological pathology (peripheral blood) – control group.
RESULTS: Hypermethylation of the *BRCA2* gene was significantly more frequently detected in the tumour tissue of women with breast cancer compared to their peripheral blood and peripheral blood of control subjects ($p = 0.0006$ and $p = 0.00001$, respectively). Hypermethylation of *BRCA2* was more frequently detected in patients with breast cancer over the age of 50 and in patients with higher Ki67 expression levels ($p = 0.045$ and $p = 0.045$, respectively). There was a high frequency of unmethylated *BRCA1* and *BRCA2* gene combination in women of the control group compared to women with breast cancer, both in blood samples and tumour tissue samples ($p = 0.014$ and $p = 0.00001$, respectively).
CONCLUSION: Our study confirms the hypothesis that *BRCA2* hypermethylation plays an important role in the pathogenesis of breast cancer and the importance of assessing its co-hypermethylation with *BRCA1* in predicting the course of the disease.

Keywords: Breast cancer, hypermethylation, *BRCA2*, *BRCA1*, gene

1. Introduction

Breast cancer is an important medical and social problem. Breast cancer in women reduces life expectancy and worsens its quality, starting from the working age. It is known that Central Asian and Eastern European countries have higher mortality rates from breast and cervical cancer and later diagnosis compared to countries in other parts of the WHO European Region [1]. In particular, in Ukraine, according to the National Cancer Registry of Ukraine, breast cancer is the leading cause of morbidity and death from malignant tumours among women [2]. Thus, in 2021, 14036 new cases of this disease were registered in women and 4732 deaths (the number of cases does not include

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the data from the Donetsk and Luhansk regions, the Autonomous Republic of Crimea and the city of Sevastopol). Only 31.9% of cases were detected during preventive check-ups and 47.3% of newly diagnosed cases had stage II disease. At the same time, the incidence of breast cancer among women in Ukraine has been steadily increasing every year over the past decade.

There are population differences in the frequencies of pathogenic variants of the *BRCA1* and *BRCA2* genes and epigenetic events, which may be associated with different incidence rates and features of breast cancer [3,4,5]. Ecology, climate, residence and lifestyle may be other factors that may also have population differences [4,6]. In addition, it is worth remembering that even hereditary cancer can be polygenic in nature, with varying degrees of contribution from modifier genes [7].

The *BRCA2* gene is an important tumour suppressor in breast cancer, and alterations in *BRCA2* may lead to cancer progression. However, the *BRCA2* gene is rarely mutated, and it is suspected that the loss of function is mediated mainly by its epigenetic regulation [8]. Since current treatment strategies aim to identify pathogenic variants in both genes, it is essential to study the combination of different "functionality" of these genes in patients with breast cancer.

The aim of the study was to investigate the association of hypermethylation of the *BRCA2* gene promoter and its co-hypermethylation with the *BRCA1* gene promoter with the development and course of breast cancer in women.

2. Materials and methods

2.1. Study population

The study involved 74 patients with newly diagnosed breast cancer who were treated at the Department of Oncology of the Bogomolets National Medical University at the Kyiv City Clinical Oncology Centre. Patients underwent a standard clinical, laboratory, instrumental and molecular pathological examination, as well as epigenetic testing for hypermethylation of the *BRCA2* gene promoter region in tumour tissue samples and peripheral blood. The study was based on the case-control principle. 62 women in the control group, who were examined for pathology of the female reproductive system, did not have malignant breast tumours. Their peripheral blood was used as a biological material for the study. Women in both groups were asked about their family history and cancer heredity.

The study was approved by the Commission on Bioethical Expertise and Research Ethics of Bogomolets National Medical University (0120U100871). Informed consent was obtained from each participant included in this study.

2.2. Sample collection

The materials used to determine the hypermethylation of the *BRCA2* gene promoter region were peripheral blood from women in the control group and paired samples of tumour tissue and peripheral blood from patients with breast cancer. All tumour tissue samples obtained during resection/biopsy and peripheral blood samples from the main and control groups were collected and stored using "DNA/RNA Shield" preservative (Zymo Research Irvine, CA, USA).

2.3. DNA extraction

Genomic DNA was extracted using "Quick-DNA™ Miniprep Plus Kit" (Zymo Research Irvine, CA, USA). The extracted DNA was stored at −18°C.

2.4. Methylation-specific polymerase (MSP) chain reaction

Sodium bisulfite conversion of genomic DNA was carried out using the "EZ DNA Methylation-Gold Kit" (Zymo Research Irvine, CA, USA) following the manufacturer's instructions. The modified DNA was then used as a matrix. The PCR reaction was performed using "ZymoTaq PreMix kit" (Zymo Research, US Irvine, CA, USA) and specific primer pairs (Metabion, Bayern, Germany). The methylated primers were as follows: GACGGTTGGGATGTTTGATAAGG and reverse: AATCTATCCCCTCACGCTTCTCC. The unmethylated primers were as follows, forward: AGGG TGGTTTGGGATTTTTAAGG, and, reverse: TCACAC TTCTCCCAACAACAACC [9].

The reaction products were separated by agarose gel electrophoresis and analysed according to the presence or absence of amplification of fragments of methylated DNA (M) – 250 bp and unmethylated DNA (U) – 337 bp (Fig. 1) [10]. The "Human Methylated & Unmethylated DNA Set" (Zymo Research Irvine, CA, USA) was used as methylated and unmethylated controls.

2.5. Analysis of the status of combined hypermethylation (comethylation) of the promoter regions of the BRCA1 and BRCA2 genes

In order to analyse the effect of the presence or absence of cometylation of the *BRCA1* and *BRCA2* gene

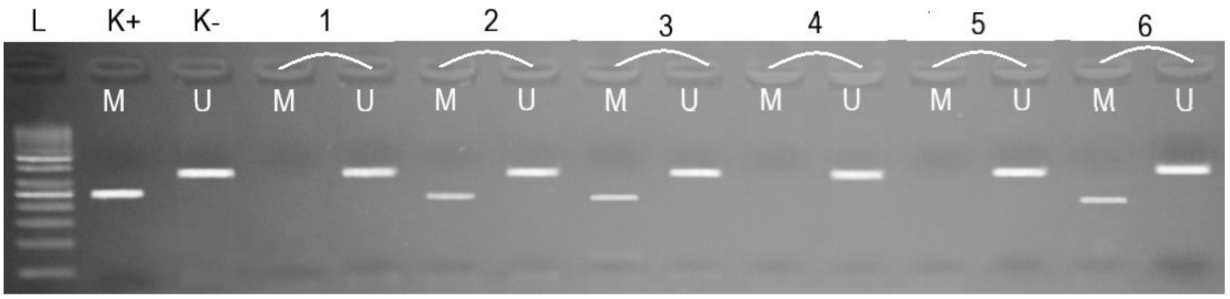

Fig. 1. Electrophoregram of methylated (M) and unmethylated (U) DNA amplification products in the promoter region of the *BRCA2* gene. L – 50-bp DNA ladder; K+ – methylated control fragment; K- – unmethylated control fragment; Samples 1, 4, 5 – UU; Samples 2, 3, 6 – MU.

Table 1
Frequency of hypermethylation of the *BRCA2* gene promoter region in the study groups

Groups of investigations	Hypermethylation of promoter region of *BRCA2* gene	
	MU	UU
Control, blood samples, ($n = 62$)	3 (4.8%)	59 (95.2%)
BC, blood samples, ($n = 74$)	12 (16.2%)	62 (83.8%)
BC, tumour samples, ($n = 74$)	31 (41.9%)	43 (58.1%)

M – methylated; U – unmethylated.

promoter regions, we used the available data on hypermethylation of the *BRCA1* gene promoter region, which were previously published for the same study groups [5].

2.6. Statistical analysis

Data were analysed using SPSS v.26 software (SPSS Inc, Chicago, IL, USA). Differences in the distribution of categorical variables between the studied groups and subgroups were assessed using χ^2 (or χ^2 with Yates correction) and odds ratio (OR) with a 95% confidence interval. Clinical and pathological characteristics of the women with BC were tested for normal distribution using the Kolmogorov-Smirnov test. Then, the probability of differences in the quantitative results was determined using ANOVA or the Kruskal-Wallis test followed by post-hoc analysis with the Bonferroni correction. P values less than 0.05 were considered statistically significant.

3. Results

In paired biological samples from patients with breast cancer (tumour tissue and peripheral blood), the frequency of detection of hypermethylation of the *BRCA2* promoter region was higher than in peripheral blood samples from women in the control group (Table 1).

Hypermethylation of the promoter region of the *BRCA2* gene in both patients of the main study group and women of the control group was detected in the heterozygous state. The frequency of *BRCA2* hypermethylation determined in tumour tissue samples of breast cancer patients was significantly higher compared to the control group ($\chi^2 = 22.77$, $p = 0.00001$, OR = 14.18 (4.07–49.41)). Although the frequency of *BRCA2* gene hypermethylation in the blood of patients with breast cancer was higher compared to the frequency in women in the control group, these differences were not significant $\chi^2 = 3.37$, $p = 0.07$, OR = 3.81 (1.02–14.17)). When comparing the frequencies of *BRCA2* hypermethylation in paired samples of breast cancer patients, a higher frequency of hypermethylation was found in tumour tissue samples ($\chi^2 = 11.83$, $p = 0.0006$, OR = 3.72 (1.72–8.06)).

When analysing the associations of *BRCA2* promoter hypermethylation with the clinical and pathological characteristics of breast cancer patients, no correlations were found between the status of *BRCA2* hypermethylation in blood samples and clinical and pathological characteristics (Table 2).

Instead, an association of *BRCA2* hypermethylation status in tumour tissue samples from breast cancer patients and such clinicopathological characteristics as age and Ki67 proliferation index was found. Thus, it was determined that *BRCA2* hypermethylation was more frequently detected (Table 2) in patients with breast cancer over 50 years of age $\chi^2 = 4.02$, $p = 0.045$, OR = 2.65 (1.01–6.96)). In patients with breast cancer with a hypermethylated *BRCA2* gene promoter, Ki67 expression levels exceeding 15% were also significantly more common (Table 2) $\chi^2 = 4.01$, $p = 0.045$, OR = 8.93 (1.07–74.77)).

The next stage of our study was to analyse the prevalence of combined hypermethylation of *BRCA1* and *BRCA2* genes in the study groups (Table 3).

Table 2
Clinical and pathological characteristics of patients with breast cancer depending on the status of hypermethylation of the *BRCA2* gene promoter region

Clinical and pathological characteristics	BC, blood samples ($n = 74$)		BC, tumour samples ($n = 74$)	
	MU	UU	MU	UU
Number of samples	12	62	31	43
Age, years	56.9 ± 12.8	51.7 ± 14.3	55.0 ± 14.0	50.7 ± 14.1
p value	0.15		0.11	
Age groups				
Up to 50 years	3 (25.0%)	36 (58.1%)	10 (32.3%)	24 (55.8%)
After 50 years	9 (75.0%)	26 (41.9%)	21 (67.7%)	19 (44.2%)
p value	0.07		0.045	
Diagnosis				
Bilateral	0 (0.0%)	4 (6.5%)	2 (6.5%)	2 (4.7%)
Dex	7 (58.3%)	23 (37.1%)	14 (45.2%)	16 (37.2%)
Sin	5 (41.7%)	35 (56.5%)	15 (48.4%)	25 (58.1%)
p value	0.32		0.70	
Stage				
I + II	8 (66.7%)	47 (75.8%)	21 (67.7%)	34 (79.1%)
III + IV	4 (33.3%)	15 (24.2%)	10 (32.3%)	9 (20.9%)
p value	0.51		0.27	
Histological type				
Ductal	10 (83.3%)	52 (83.9%)	27 (87.1%)	35 (81.4%)
Others	2 (16.7%)	10 (16.1%)	4 (12.9%)	8 (18.6%)
p value	0.96		0.51	
Estrogen receptor				
Positive	9 (75.0%)	48 (77.4%)	21 (67.7%)	36 (83.7%)
Negative	3 (25.0%)	14 (22.6%)	10 (32.3%)	7 (16.3%)
p value	0.86		0.11	
Progesterone receptor				
Positive	6 (50.0%)	46 (74.2%)	18 (58.1%)	34 (79.1%)
Negative	6 (50.0%)	16 (25.8%)	13 (41.9%)	9 (20.9%)
p value	0.09		0.051	
HER2/neu				
Positive	1 (8.3%)	10 (16.1%)	5 (16.1%)	6 (14.0%)
Negative	11 (91.7%)	52 (83.9%)	26 (83.9%)	37 (86.0%)
p value	0.49		0.80	
Ki67, %	31.4 ± 11.2	30.7 ± 15.2	33.7 ± 13.0	28.9 ± 15.2
p value	0.76		0.18	
Ki67 groups				
Low (0%–15%)	1 (8.3%)	10 (19.2%)	1 (3.8%)	10 (26.3%)
Intermediate (16%–29%)	7 (58.3%)	19 (36.5%)	13 (50.0%)	13 (34.2%)
High ($\geqslant$ 30%)	4 (33.3%)	23 (44.2%)	12 (46.2%)	15 (39.5%)
p value	0.35		0.045	
Molecular subtype				
TNBC	3 (25.0%)	12 (19.4%)	9 (29.0%)	6 (14.0%)
Luminal A	6 (50.0%)	25 (40.35)	11 (35.5%)	20 (46.5%)
Luminal B	3 (25.0%)	23 (37.1%)	10 (32.3%)	16 (37.2%)
HER2-positive	0 (0.0%)	2 (3.2%)	1 (3.2%)	1 (2.3%)
p value*	0.76		0.44	
Family history				
Positive	5 (41.7%)	31 (50.0%)	15 (48.4%)	21 (48.8%)
Negative	7 (58.3%)	31 (50.0%)	16 (51.6%)	22 (51.2%)
p value	0.60		0.97	

M – methylated; U – unmethylated.

There was a high frequency of unmethylated *BRCA1* and *BRCA2* gene promoters combination in women of the control group compared to women with breast cancer, both in blood samples and tumour tissue samples ($\chi^2 = 6.04$, $p = 0.014$, OR = 0.25 (0.09–0.73) and $\chi^2 = 34.84$, $p = 0.00001$, OR = 0.06 (0.02–0.18), respectively). This indicates a high protective role of this combination in reducing the risk of developing breast cancer in women. As for the main study group, in women with breast cancer, one of the most common combinations was the unmethylated *BRCA1* promoter and hypermethylated *BRCA2* promoter in tumour tis-

Table 3
Frequency of cometylation of the promoter regions of *BRCA1* and *BRCA2* genes in the study groups

Combined hypermethylation	Control, blood samples ($n = 62$)	BC, blood samples ($n = 74$)	BC, tumour samples ($n = 74$)
BRCA1 UU + *BRCA2* UU	57 (91.9%)	55 (74.3%)	31 (41.9%)
BRCA1 MU + *BRCA2* UU	2 (3.2%)	7 (9.5%)	12 (16.2%)
BRCA1 UU + *BRCA2* MU	3 (4.8%)	10 (13.5%)	17 (23.0%)
BRCA1 MU + *BRCA2* MU	0 (0.0%)	2 (2.7%)	14 (18.9%)

M – methylated; U – unmethylated.

Table 4
Relationship between *BRCA1* and *BRCA2* gene methylation and clinical and pathological characteristics of breast cancer patients

Clinical and pathological characteristics	BC (blood)		BC (tumour)	
	BRCA1 UU + *BRCA2* UU ($n = 55$)	*BRCA1* UU + *BRCA2* MU ($n = 10$)	*BRCA1* UU + *BRCA2* UU ($n = 31$)	*BRCA1* UU + *BRCA2* MU ($n = 17$)
Age groups				
Up to 50 years	26 (47.3%)	2 (20.0%)	18 (58.1%)	4 (23.5%)
After 50 years	29 (52.7%)	8 (80.0%)	13 (41.9%)	13 (76.5%)
p	0.20		0.046	
Ki67 groups				
Low (0%–15%)	10 (21.7%)	1 (10.0%)	9 (34,6%)	0 (0%)
Intermediate (16%–29%)	10 (21.7%)	5 (50.0%)	6 (23.1%)	5 (35.7%)
High ($\geqslant$ 30%)	26 (56.5%)	4 (40.0%)	11 (42.3%)	9 (64.3%)
p	0.33		0.035	
Progesterone receptor				
Negative	12 (21.8%)	6 (60.0%)	6 (19.4%)	7 (41.2%)
Positive	43 (78.2%)	4 (40.0%)	25 (80.6%)	10 (58.8%)
p value	0.0359		0.18	

M – methylated; U – unmethylated.

sue (in Table 3 – *BRCA1* UU + *BRCA2* MU), which was 23%. Moreover, the frequency of this combination, determined in tumour tissue samples of women with breast cancer, was significantly higher compared to the control group $\chi^2 = 7.46$, $p = 0.0063$, OR = 5.87 (1.63–21.10)).

For patients with breast cancer, we analysed differences in clinicopathological characteristics depending on the status of *BRCA1* and *BRCA2* gene methylation. The analysis was performed for all characteristics listed in Table 2. The significant differences identified in this analysis are shown in Table 4.

First of all, we noticed how the previously identified significant differences had changed for age and Ki67 proliferation index (Table 4). Thus, in women over 50 years of age, the frequency of unmethylated *BRCA1* (*BRCA1* UU) and hypermethylated *BRCA2* (*BRCA2* MU) in tumour tissue samples was significantly increased (76.5%) compared to patients with a combination of unmethylated statuses of the *BRCA1* and *BRCA2* gene promoter regions (41.9%) – $\chi^2 = 3.98$, $p = 0.046$, OR = 4.50 (1.19–16.99). In addition, patients with breast cancer who had the same combination (*BRCA1* UU + *BRCA2* MU) were significantly more likely to have Ki67 expression levels exceeding 15% compared to patients who had a combined unmethylated status of the *BRCA1* and *BRCA2* gene promoter regions – 0.0% vs. 34.6%, $\chi^2 = 4.43$, $p = 0.035$. We also determined (Table 4) a significantly lower frequency of *BRCA1* UU + *BRCA2* MU combination in blood samples from patients with breast cancer who had positive progesterone receptors – 40.0% – when compared with patients who had a compatible unmethylated status of the promoter regions of the *BRCA1* and *BRCA2* genes – 78.2% – $\chi^2 = 4.09$, $p = 0.0359$, OR = 0.19 (0.05–0.77). The results obtained indirectly indicate that the presence of a compatible unmethylated status of the *BRCA1* and *BRCA2* gene promoter regions in patients with breast cancer may be a marker of a better prognosis of the disease.

4. Discussion

Hypermethylation of the *BRCA2* promoter leads to low mRNA expression and reduced synthesis of the corresponding protein [11]. The frequency of *BRCA2* promoter hypermethylation in breast malignancies has been reported by different research groups to be 0%–69% (Table 5). Here, we report that the frequency of *BRCA2* promoter hypermethylation is 41.9% in tumour tissue samples and 16.2% in peripheral blood samples

Table 5
Frequency of methylation of the *BRCA2* gene promoter in other study groups

No	Country	Method	Type of material		Case		Control		Study
			Case	Control	M	U	M	U	
1	France	QAMA	Peripheral blood ($n = 873$)	Peripheral blood ($n = 980$)	17%	83%	16%	84%	[12]
2	Ukraine	MSP	Tumor tissue ($n = 50$)	–	50%	50%	–	–	[10]
3	Brazil	MSP	Tumor tissue ($n = 50$)	–	44%	56%			[13]
4	Israel	QAMA	Peripheral blood ($n = 100$)	Peripheral blood ($n = 89$)	0%	100%	0%	100%	[14]
5	Nigeria	MSP	Peripheral blood ($n = 14$)	–	57%	43%	–	–	[15]
6	Tunis	MSP	Tumor tissue ($n = 117$)/paired normal tissues ($n = 65$)	Non-tumor tissue ($n = 21$)	69%/5%	31%/95%	0%	100%	[16]
7	Korea	MS-MLPA	Tumor tissue ($n = 60$)/paired normal tissues ($n = 60$)	–	2%/0%	98%/100%	–	–	[17]
8	Turkey	MS-MLPA	Tumor tissue ($n = 77$)/paired normal tissues ($n = 77$)	–	0%/0%	100%/100%	–	–	[18]

MS-MLPA – methylation-specific multiplex ligation-dependent probe amplification; MSP – methylation-specific PCR; QAMA – quantitative analysis of methylated alleles; M – methylated; U – unmethylated.

from breast cancer patients from Ukraine. Given the frequency of detection of *BRCA2* promoter hypermethylation in the peripheral blood of patients with hereditary breast cancer syndrome and ovarian cancer, where it was 0% [19], *BRCA2* promoter hypermethylation cannot be associated with hereditary breast cancer. This was confirmed in our study, where no differences were found in the distribution of gene promoter hypermethylation depending on the family history and heredity. Thus, the frequencies we obtained are comparable to those reported in the current literature. It should be noted that such an analysis in peripheral blood samples of breast cancer patients is less effective than in tumour tissue samples.

We have shown that the frequency of *BRCA2* hypermethylation is higher in a subgroup of women with breast cancer over 50 years of age. Our results are indirectly confirmed by the work of Bosviel et al., who also noted a higher level of *BRCA2* methylation in elderly patients [12]. In Ukraine, the peak incidence of breast cancer and related mortality is rapidly increasing in women over 50 years of age [2]. That is why the study of *BRCA2* promoter hypermethylation, in particular for Ukrainian patients with breast cancer, can potentially be recommended for women in this age subgroup and may be the basis for personalised targeted treatment.

Our study included breast cancer patients with different stages of newly diagnosed disease – from stage I to stage IV. However, no association was found between *BRCA2* promoter hypermethylation and the stage at which breast cancer was diagnosed in women. In contrast to our results, Vos et al in their study showed that hypermethylation of the *BRCA2* promoter is more common in tumours with a high degree of malignancy [20]. However, when evaluating the association of co-hypermethylation of the studied genes with other clinicopathological characteristics, we found that in patients with *BRCA2* promoter hypermethylation or a combination of unmethylated *BRCA1* status and *BRCA2* promoter hypermethylation, the level of Ki67 expression was higher. It is known that this prognostic biomarker in breast cancer has proven clinical reliability – its high levels are associated with a poor prognosis for survival and an increased risk of recurrence [21,22].

Another interesting result of this study is that breast cancer patients with a combination of the unmethylated status of the *BRCA1* and *BRCA2* gene promoter regions were significantly more likely to have positive progesterone receptors. It should be noted that high expression of progesterone receptors, according to some research groups, is more common in tumours with a better prognosis and is associated with better survival [23,24]. On the other hand, we did not find such a relationship with progesterone receptor expression when analysing the hypermethylation of *BRCA1* and *BRCA2* gene promoters separately in this study [5]. This may be due to the small sample size. It should also be noted that we did not stratify breast cancer patients according to the strength of receptor expression and receptor types.

When analysing the relationship between family history of cancer in first- and second-order relatives and *BRCA2* gene hypermethylation status, we did not find any significant differences. Although there are studies that indicate the transmission of epimutations, in particular, hypermethylation of the *BRCA1* promoter, from mother to daughter [25]. And based on this, we hypothesised that in the group of breast cancer patients with a family history, the frequency of *BRCA2* promoter

hypermethylation would be higher. However, taking into account our previous work, we did not find a link between hypermethylation of the *BRCA1* and *BRCA2* gene promoters and either hereditary or sporadic breast cancer [5].

The analysis of *BRCA1* and *BRCA2* promoter regions methylation indicates a certain interaction between them, which has a synergistic effect on the better prognosis in the absence of promoter methylation. Therefore, we assume that further studies of the contribution of hypermethylation of tumour suppressor gene promoters to the development and course of breast cancer, prevention and selection of personalised therapy should be conducted for both *BRCA1* and *BRCA2* genes, and take into account their interaction. In particular, the demethylating effect of such natural compounds as curcumin, genistein, catechin and quercetin has already been proven [26,27,28,29]. The possibility of their use for preventive and therapeutic purposes (as concomitant therapy) in breast cancer patients is being actively studied. In addition, hypermethylation of the *BRCA2* promoter, by analogy with hypermethylation of the *BRCA1* promoter, can be a potential marker for predicting chemosensitivity in patients with breast cancer, especially to drugs such as cyclophosphamide, methotrexate, fluorouracil and platinum drugs [30,31]. Another group of drugs is highly effective targeted drugs – PARP inhibitors. Despite their impressive clinical efficacy, resistance to PARP inhibitors remains a serious problem. The mechanisms of resistance and biological markers that will improve targeting for this type of therapy in patients with breast cancer are being actively studied. Hypermethylation of the *BRCA1* and *BRCA2* promoters is one of the most promising factors in this direction [32].

5. Conclusions

Hypermethylation of the *BRCA2* gene promoter region was significantly more frequently detected in the tumour tissue of women with breast cancer compared to their peripheral blood and peripheral blood of control subjects, thus it is a biological marker of breast cancer risk in women and an early diagnostic marker in case of suspected disease. Hypermethylation of *BRCA2* was more frequently detected in patients with breast cancer over the age of 50 and in patients with higher Ki67 expression levels. An association was found between the absence of hypermethylation of the *BRCA1* and *BRCA2* gene promoter regions and a prognostically better course of the disease, as they also had significantly higher progesterone receptor expression. Our study confirms the hypothesis that *BRCA2* hypermethylation plays an important role in the pathogenesis of breast cancer and the importance of assessing its co-hypermethylation with *BRCA1* in predicting the course of the disease. Determination of this epigenetic alteration of *BRCA2* can be used as a prognostic and predictive marker in breast cancer, as well as for the search/selection of personalised therapy, but further multicentre studies are needed.

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Not applicable.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Commission on Bioethical Expertise and Research Ethics of Bogomolets National Medical University (approval no. 0120U100871). The written informed consent was obtained from each participant included in this study.

Author contributions

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Conflict of interest

The Authors declare that there is no conflict of interest.

Availability of data and materials

The datasets generated and/or analyzed during the

current study are available from the corresponding author upon reasonable request.

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