Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

MOLECULAR GENETIC MARKERS FOR DIAGNOSING METACHRONOUS ONCOGYNECOLOGICAL TUMORS IN BREAST CANCER PATIENTS

Izrailbekova K.Sh., Kamishov S.V., Qobilov O.R., Niyozova Sh.Kh., Ubaydullaeva M.J.

Abstract

Background: Breast cancer (BC) patients face an increased risk of developing multiple primary malignant tumors, particularly metachronous oncogynecological neoplasms. Early detection and risk stratification of these secondary malignancies are critical for improving patient outcomes.

Materials and Methods: A total of 38 BC patients with multiple primary malignant tumors (MPMT) of the female reproductive system (including both synchronous and metachronous tumors) and 15 BC patients without MPMT were evaluated. Comprehensive clinical assessments, imaging studies, and laboratory tests were conducted. Tumor marker levels (CA 15-3, CA 19-9, CA 125, AFP, and CEA) were measured in peripheral venous blood using ELISA. Histological analysis, tumor ploidy evaluation, and BRCA1/2 mutation screening via next-generation sequencing (Illumina MiSeq platform) were performed on tumor tissue samples.

Results: The findings revealed that BC patients with metachronous tumors exhibited a higher prevalence of aneuploid tumor cells, lower tumor differentiation, and elevated levels of multiple tumor markers compared to controls. BRCA1/2 mutations—particularly the variants 5382insC, 4154del4, and C61G—were significantly more common in the metachronous group. A significant correlation between these molecular markers and adverse histopathological features was observed. Based on these results, an algorithm for risk grouping was proposed to identify BC patients at higher risk for developing metachronous tumors.

Conclusion: Simultaneous assessment of multiple molecular genetic markers, including tumor markers and BRCA1/2 mutations, offers a promising strategy for the early detection and risk stratification of metachronous oncogynecological tumors in BC patients. This integrated diagnostic approach may lead to more timely interventions and ultimately improve clinical outcomes.

Keywords: breast cancer, metachronous tumors, molecular genetic markers, tumor markers, BRCA1/2 mutations, risk stratification.

Introduction

In recent years, oncologists have shown increasing interest in multiple primary malignant tumors (MPMT) due to their rising prevalence. The incidence of malignant polyneoplasia of the breast ranges from 6% to 8.5%, indicating a 2.5-fold increase in MPMT cases. Primary multiple malignant tumors (PMMT) are observed in 1.9–7.1% of breast cancer (BC) patients, most commonly affecting the female reproductive system (33–42%), including ovarian (15–17%), endometrial (12–14%), and cervical cancer (10–12%). Other frequent malignancies

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

include colorectal (12–13%), gastric (14–15%), and thyroid cancer (7.7%) (Imyanitov E.N., 2010; Sidorenko Yu.S. et al., 2010).

A key approach in diagnosing malignant neoplasms is detecting tumor markers in serum. In addition to traditional markers like alpha-fetoprotein and human chorionic gonadotropin, increasing attention is given to carcinoembryonic antigen (CEA), mucin-like cancer-associated antigen (MCA), and high-molecular-weight antigens CA-15-3, CA-19-9, and CA-125. Among these, CA-125 is widely used, detected in over 85% of ovarian cancer cases (Greenlee H. et al., 2017; Helm J.S., Rudel R.A., 2020).

Another significant factor in BC development is BRCA gene mutations, which significantly increase cancer risk. However, BRCA1 can lose its tumor-suppressor function even without hereditary mutations due to epigenetic modifications—reversible genomic changes involving chemical modifications of DNA nucleotides and chromatin histones (Byrski T. et al., 2010; Parkes A. et al., 2017).

Materials and Methods

The study included 38 patients with stage II–III breast cancer (BC) and multiple primary malignant tumors (MPMT) of the female reproductive system, as well as a control group of 15 BC patients without MPMT. All patients underwent examination and treatment in the Oncomammology and Chemotherapy-2 departments of our center between 2015 and 2021.

At the initial stage, clinical assessment involved collecting patient complaints and medical history, performing a general examination, breast evaluation, and gynecological examination, including inspection of the external genitalia, vagina, and cervix using specula, as well as a bimanual rectovaginal examination. Laboratory tests included a complete blood count, biochemical analysis, coagulation studies, and molecular genetic marker evaluation.

A comprehensive clinical and instrumental assessment was conducted to determine tumor spread and identify complications, including ultrasound (US), radiography, and computed tomography (CT).

The age of the examined BC patients ranged from 23 to 79 years, with a mean age of 46.8 years. According to the data, stages IIB and IIA were the most common among patients in the main groups (Table 1).

Table 1. Distribution of Breast Cancer Patients According to the TNM Classification (7th Edition, 2010)

TNM	Stage	Main Group (n=38)		Control Group (n=15)		Total (n=53)	
		n	%	n	%	n	%
$T_1N_1M_0$	IIA	3	7,9	2	13,3	5	9,4
$T_2N_0M_0$	IIA	4	10,5	3	20,0	7	13,2
$T_2N_2M_0$	IIB	6	15,8	4	26,7	10	18,9
$T_3N_0M_0$	IIB	6	15,8	2	13,3	8	15,1

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

$T_1N_2M_0$	IIIA	7	18,4	2	13,3	9	17,0
$T_2N_2M_0$	IIIA	5	13,2	1	6,7	6	11,3
$T_3N_1M_0$	IIIA	4	10,5	1	6,7	5	9,4
$T_2N_3M_0$	IIIC	3	7,9	–	–	3	5,7

According to the presented data, synchronous tumors were detected in 12 (31.6%) BC patients. Among them, synchronous contralateral breast cancer was found in 9 (23.7%) cases, while ovarian cancer (OC) was diagnosed in 3 (7.9%) cases (Table 2).

Metachronous tumors, developing six months or later, were observed more frequently. A total of 26 (68.4%) BC patients were later diagnosed with metachronous malignancies, including contralateral breast cancer in 14 (36.8%) cases, endometrial cancer (EC) in 7 (18.4%) cases, and OC in 5 (13.2%) cases.

Table 2. Distribution of Patients in the Main Group with Primary Multiple Tumors

First Detected Tumor	Synchronous Tumor (n=12)		
		n	%
BC (n=38)	BC	9	23,7
	OC	3	7,9
	Metachronous Tumor (n=26)		
		n	%
	BC	14	36,8
	EC	7	18,4
	OC	5	13,2

Histological examination of tumor tissue samples revealed that infiltrative ductal carcinoma was the most common type, diagnosed in 21 (55.3%) patients in the main group and 8 (53.3%) in the control group. The mixed ductal-lobular carcinoma followed, observed in 10 (26.3%) and 5 (33.3%) patients, respectively. Infiltrative lobular carcinoma was found in 4 (10.5%) and 2 (13.3%) cases. Additionally, infiltrating carcinoma was identified in 3 (7.9%) patients in the main group, while this histological type was not observed in the control group (Table 3).

Table 3. Distribution of Breast Cancer Patients by Histological Tumor Type

Histological Type	Main Group (n=38)		Control Group (n=15)		Total (n=53)	
	n	%	n	%	n	%
Infiltrating carcinoma	3	7,9	–	–	3	5,7
Infiltrative ductal carcinoma	21	55,3	8	53,3	29	54,7
Infiltrative lobular carcinoma	4	10,5	2	13,3	6	11,3

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

Mixed infiltrative ductal and lobular carcinoma	10	26,3	5	33,3	15	28,3

As previously mentioned, metachronous tumors included contralateral breast cancer, endometrial cancer, and ovarian cancer.

Analysis of tumor ploidy in BC patients revealed that metachronous tumors were more frequently associated with aneuploid cells, characterized by chromosomal material loss and the presence of multiclonal (aneuploid) cell populations (Table 4). Notably, aneuploid tumors were predominantly observed in patients under 50 years of age, while diploid tumors were more common in those over 50.

Table 4. Tumor Ploidy in Patients with Primary Multiple Breast Cancer

Ploidy	Synchronous BC (n=12)	Metachronous BC (n=26)	Control Group (n=15)
Diploid	1 (8,3%)	3 (11,5%)	1 (6,7%)
Aneuploid with chromosomal material loss	4 (33,3%)	11 (42,3%)	3 (20,0%)
Aneuploid with DNA index within the mitotic cycle	4 (33,3%)	8 (34,6%)	4 (26,7%)
Tetraploid	1 (8,3%)	2 (7,7%)	–
Multiclonal (aneuploid)	2 (16,7%)	6 (23,1%)	2 (13,3%)

Tumor markers were determined in the peripheral venous blood of patients, collected on an empty stomach, using enzyme-linked immunosorbent assay reagent kits from Hema LLC.

Tumor marker determination:
• MUC1 (CA 15-3): Sensitivity – 1.5 U/mL; analysis time – 80 min; incubation temperature – +18…+25 °C.
• CA 19-9: Sensitivity – 2 U/mL; analysis time – 80 min; incubation temperature – +37 °C.
• Carcinoembryonic antigen (CEA): Detection range – 0.2–50,000 ng/mL.
• Carbohydrate antigen 125 (CA 125): Detection range – 0.6–25,000 U/mL.
• Alpha-fetoprotein (AFP): Detection range – 0.2–50,000 IU/mL.

The presence of BRCA1 or BRCA2 gene mutations is associated with an increased predisposition to hereditary breast and ovarian cancer syndrome (HBOC). These mutations are inherited, meaning that every cell in the body carries the genetic alteration.

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

Hereditary BRCA-associated breast cancer accounts for 5–10% of all breast cancer cases. Among these cases:
• 35% are attributed to defects in the BRCA1 gene.
• 25% are linked to mutations in the BRCA2 gene.

Carrying a BRCA1 mutation is also strongly associated with an increased risk of ovarian cancer, which rises with age. However, the differences in BRCA1/2 mutation frequency among breast cancer patients depending on the timing of the development of synchronous or metachronous tumors remain insufficiently studied (Fedorov V.E. et al., 2011; Parkes A. et al., 2017; Graeser M.K. et al., 2009).

The study of BRCA1/2 mutations was conducted using next-generation sequencing (NGS) on the Illumina MiSeq platform. Tumor samples were obtained from paraffin-embedded histological tissue blocks.

The sequencing process included:
• Library preparation using the MiSeq Reagent Kit v2.
• Sequencing on the Illumina MiSeq platform following the manufacturer's instructions.
Analysis of the Association Between Molecular Genetic Markers and Multiple Primary Tumors in Breast Cancer

Results
Enzyme-linked immunosorbent assay of tumor markers in blood serum showed that patients with metachronous breast cancer had higher levels of the studied markers compared to those in the control group (Table 5).

Table 5. Tumor Marker Levels in Patients with Multiple Primary Tumors in Breast Cancer

Tumor Marker	Expression	Patients with Synchronous BC (n=12)	Patients with Metachronous BC (n=26)	Control Group (n=15)
CA-125	Positive	7 (58,3%)	17 (65,4%)	4 (26,7%)
	Negative	5 (41,7%)	9 (34,6%)	11 (73,3%)
CA-15-3	Positive	5 (41,7%)	14 (53,4%)	2 (13,3%)
	Negative	7 (58,3%)	12 (46,2%)	13 (86,7%)
CA-19-9	Positive	4 (33,3%)	12 (46,2%)	1 (6,7%)
	Negative	8 (66,7%)	14 (53,4%)	14 (93,3%)
АФП	Positive	3 (25,0%)	8 (30,8%)	2 (13,3%)
	Negative	9 (75,0%)	18 (69,2%)	13 (86,7%)
РЭА	Positive	3 (25,0%)	7 (26,9%)	1 (6,7%)
	Negative	9 (75,0%)	19 (73,1%)	14 (93,3%)

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

In the group with synchronous tumors, germline and somatic variants in the BRCA1/2 genes were detected in 5 out of 12 patients (41.7%), while in the group with metachronous tumors, these variants were found in 17 out of 26 patients (65.4%). In the control group of breast cancer (BC) patients, the frequency of germline and somatic mutations was 2 out of 15 (13.3%). The most common variants were 5382insC, 4154del4, and C61G, which have an increased frequency in the general population.

We also analyzed the relationship between the presence of oncological diseases of the female reproductive system among close relatives of BC patients and mutations in the BRCA1/2 genes (Table 6). The results showed that oncological diseases in close relatives were most frequently observed in the group of BC patients with metachronous tumors (70.6% of cases), followed by the group with synchronous tumors (60.0%), and the control group (50.0%).

Table 6. Presence of Oncological Diseases Among Close Relatives in Breast Cancer Patients with BRCA1/2 Mutations

Presence of Oncological Diseases in Close Relatives	Breast Cancer Patients with BRCA1/2 Mutations		
	Patients with Synchronous BC (n=5)	Patients with Metachronous BC (n=17)	Control Group (n=2)
Present	3 (60,0%)	12 (70,6%)	1 (50,0%)
Absent	2 (40,0%)	5 (29,4%)	1 (50,0%)

Our study demonstrated a certain association between the histological features of breast cancer in patients with metachronous tumors. BRCA1/2 mutations were most frequently detected in infiltrating ductal carcinoma (Table 7).

Table 7. Morphological Characteristics of BRCA1/2-Associated Breast Cancer

Histological Type			
	Patients with Synchronous BC (n=5)	Patients with Metachronous BC (n=17)	Control Group (n=2)
Infiltrating ductal carcinoma	4 (80,0%)	15 (88,2%)	2 (100%)
Infiltrating lobular carcinoma	1 (20,0%)	1 (5,9%)	–
Mixed infiltrating ductal and lobular carcinoma	–	1 (5,9%)	–

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

A correlation between BRCA gene mutations and the degree of tumor differentiation was also examined. The results indicated that as tumor differentiation decreases and malignancy increases, the number of both germline and somatic mutations rises.

Table 8. Dependence of the Occurrence of Germline BRCA Mutations on Tumor Differentiation

Histological Grade	Patients with Synchronous BC (n=5)	Patients with Metachronous BC (n=17)	Control Group (n=2)
G1	1 (20,0%)	4 (23,5%)	–
G2	1 (20,0%)	6 (35,3%)	2 (100%)
G3	3 (60,0%)	7 (41,2%)	–

Risk Factors for the Development of Metachronous Tumors in Breast Cancer Patients

Based on our studies, we performed a prediction of individual risk factors influencing the development of metachronous tumors in breast cancer patients. For this purpose, the likelihood ratio method was used, which not only accounts for the probability of outcomes resulting from a factor's influence but also identifies the most significant risks.

The significant factors among those considered in our research that may affect the development of metachronous tumors in breast cancer patients include:
• Histological tumor type: Infiltrating ductal carcinoma.
• Tumor ploidy characteristics: The presence of aneuploid cells in the breast tumor characterized by the loss of chromosomal material and a DNA index within the mitotic cycle.
• Tumor differentiation: A low degree of differentiation.
• Tumor marker levels: The simultaneous elevation of several of the following tumor markers—CA-125, CA-15-3, CA-19-9, as well as AFP and CEA.
• Family history: The presence of malignant tumors of the female reproductive system among close relatives.
• BRCA mutations: The presence of germline and somatic mutations in the BRCA1/2 genes, particularly the variants 5382insC, 4154del4, and C61G.
Based on our studies, we have proposed an algorithm for the conditions to form risk groups for the development of metachronous tumors in breast cancer patients with stages IIA–IIIC (Table 9).

Table 9. Risk Factors for the Development of Metachronous Tumors in Breast Cancer Patients with Stages IIA–IIIC

Risk Factors
Infiltrating ductal carcinoma

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

The presence of aneuploid cells in the breast tumor characterized by the loss of chromosomal material and a DNA index within the mitotic cycle
Low tumor differentiation
Simultaneous elevation of several of the following tumor markers: CA-125, CA-15-3, CA-19-9, AFP, CEA
The presence of malignant tumors of the female reproductive system among close relatives
The presence of germline and somatic mutations in the BRCA1/2 genes

Conclusion

Because each tumor marker is associated with tumors of a specific histotype, the simultaneous detection of multiple markers allows the clinician to suspect the existence of several synchronously developing neoplasms. In this situation, tumor markers (TMs) serve as an effective adjunct diagnostic tool, as their diagnostic sensitivity and specificity in extensive tumor processes typically exceed 80–85%. This should influence the sequence of therapeutic interventions in patients treated for hormone-dependent malignancies—such as breast cancer (BC), endometrial (EC) and cervical cancer (CC) of the uterus, and ovarian cancer (OC)—who are at a significantly higher risk of developing a second (metachronous) tumor. Regular monitoring of TMs may facilitate the early detection of these tumors, thereby improving prognosis.

The obtained results allow for a more precise determination of the incidence and diversity of metachronous malignant tumors in BC patients, taking into account factors such as age, disease stage, time of detection, histological structure, tumor ploidy, and the levels of tumor markers. This will, in turn, enable the development of a comprehensive diagnostic strategy for identifying BC patients at risk for developing metachronous tumors, with the aim of further monitoring.

The formation of high-risk groups for the development of multiple primary malignant neoplasms (MPMNs) and the ongoing monitoring of this patient category are of great importance for the early diagnosis of multiple primary malignancies. The results of the planned study will pave the way for new, modern approaches in researching multiple primary malignancies of the female reproductive system in women using clinical-experimental and molecular-genetic markers.

References

1. Akulenko, L.V. On Hereditary Breast, Ovarian, and Endometrial Cancer (Clinical Lecture). Problems of Reproduction, 2004, No. 6, pp. 20–27.
2. Bit-Sava, E.M., Belogurova, M.B. Hereditary Breast Cancer. Siberian Oncology Journal, 2013, No. 1 (55), pp. 75–81.
3. Diomidova, V.N., Safonova, M.A., Zakharova, O.V., Ionov, A.M., Efimova, O.A. Modern Features of the Diagnosis of Multiple Primary Malignant Neoplasms Involving Gynecological Organs. Practical Medicine, 2019, Vol. 17, No. 2, pp. 117–123.
4. Imyanitov, E.N. Hereditary Breast Cancer. Practical Oncology, 2010, No. 4, pp. 258–266.

Western European Journal of Medicine and Medical Science
Volume 4, Issue 01, January 2026
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

5. Maksimov, S.Ya. Multiple Primary Tumors of the Reproductive System. Practical Oncology, 2009, Issue 10(2), pp. 117–123.
6. Safonova, M.A., Diomidova, V.N., Zakharova, O.V., et al. Involvement of the Female Reproductive Organs in the Structure of Multiple Primary Malignant Neoplasms. Issues in Gynecology, Obstetrics, and Perinatology, 2016, Vol. 15, No. 3, pp. 48–53.
7. Sidorenko, Yu.S., Shelyakina, T.V., Titova, E.V., et al. Problems of Multiple Primary Processes in Breast Cancer Patients. Siberian Oncology Journal, 2010, Vol. 37, No. 1, pp. 18–22.
8. Stepanova, Yu.A., Kalinin, D.V., Vishnevsky, V.A. Multiple Primary Tumors (Literature Review). Medical Imaging, 2015, No. 5, pp. 93–102.
9. Fedorov, V.E., Barsukov, V.Yu., Popova, T.N., Selezneva, T.D. Features of the Course and Difficulties in the Diagnosis of Multiple Malignant Neoplasms. Medical Almanac, 2011, No. 2, pp. 157–160.
10. Brewer, H.R., Jones, M.E., Schoemaker, M.J., et al. Family History and Risk of Breast Cancer: An Analysis Accounting for Family Structure. Breast Cancer Research and Treatment, 2017, Vol. 165, No. 1, pp. 193–200.
11. Byrski, T., Gronwald, J., Huzarski, T., et al. Pathologic Complete Response Rates in Young Women with BRCA1-Positive Breast Cancers after Neoadjuvant Chemotherapy. Journal of Clinical Oncology, 2010, Vol. 28, pp. 375–379.
12. Graeser, M.K., Engel, C., Rhiem, K., et al. Contralateral Breast Cancer Risk in BRCA1 and BRCA2 Mutation Carriers. Journal of Clinical Oncology, 2009, Vol. 27, pp. 5887–5892.
13. Greenlee, H., DuPont-Reyes, M.J., Balneaves, L.G., Carlson, L.E., et al. Clinical Practice Guidelines on the Evidence-Based Use of Integrative Therapies During and Following Breast Cancer Treatment. CA: A Cancer Journal for Clinicians, 2017, Vol. 67, No. 3, pp. 194–232.
14. Helm, J.S., Rudel, R.A. Adverse Outcome Pathways for Ionizing Radiation and Breast Cancer Involve Direct and Indirect DNA Damage, Oxidative Stress, Inflammation, Genomic Instability, and Interaction with Hormonal Regulation of the Breast. Archives of Toxicology, 2020, Vol. 94, No. 5, pp. 1511–1549.
15. Howell, A., Anderson, A.S., Clarke, R.B., Duffy, S.W., et al. Risk Determination and Prevention of Breast Cancer. Breast Cancer Research, 2014, 16: 446.
16. Parkes, A., Arun, B.K., Litton, J.K. Systemic Treatment Strategies for Patients with Hereditary Breast Cancer Syndromes. The Oncologist, 2017, Vol. 22, No. 6, pp. 655.
17. Takatori, E., Shoji, T., Miura, Y., et al. Triple Simultaneous Primary Invasive Gynecological Malignancies: A Case Report. Journal of Obstetrics and Gynaecology Research, 2014, Vol. 40, No. 2, pp. 627–631.