BENEFITS OF MULTIGENE PANEL TESTING IN BREAST CANCER

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Abstract

Introduction : Breast cancer is the most common type of cancer in women and the second cause of death by neoplasia, being known that nowadays 1 in 8 women will develop breast cancer. Genetic screening began to be used on a larger scale since the introduction Next Generation Sequencing (NGS) and the decrease of the costs. Accelerated development of multigene test panels had a positive impact on the medical conduct and individualized treatment for both patients and their relatives.

Material and method: 80 patients diagnosed with breast cancer between January 2015 - July 2016 which met the 2016 NCCN criteria for genetic testing were included in the study. Peripheral blood was collected, and the samples were sent and analyzed in Greece using two test panels: a test panel just for the BRCA 1 and 2, and a multigene panel for 25 genes responsible for breast cancer.

Results: Out of the 80 patients tested, 30 had mutations with high and medium penetrance (25 pathogenic and 5 VUS (Variant of Uncertain Clinical Significance)), 15 had rare mutations with moderate penetrance and 37 were negative (23 using multigene panel test and 14 using BRCA panel). From the 14 patients tested negative using the BRCA test, 6 were retested by multigene panel, and were detected two pathogenic mutations (CHEK2, PALB2) and one VUS mutation (STK11).

Conclusion: Testing only the BRCA 1 and 2 gene mutations in patients with hereditary breast cancer syndrome can lead to a misdiagnose of other mutations, and this can alter the medical conduct of the patients and their relatives. Although multigene test panels costs are slightly increased compared to standard tests, they offer the possibility to detect a broader palate of mutations and the possibility of an accurate treatment.

Rezumat: Beneficiile utilizării panelurilor multigenice în cancerul mamar

Introducere: Cancerul mamar este cel mai frecvent tip de cancer la femei şi a doua cauză de mortalitate prin boală neoplazică, fiind cunoscut faptul că 1 din 8 femei va dezvolta cancer mamar. Screeningul genetic a început să fie folosit pe scară tot mai largă odată cu introducerea Next Generation Sequencing (NGS) şi cu reducerea costurilor. Dezvoltarea accelerată a panelurilor multigenice de testare a avut un impact pozitiv asupra stabilirii unei conduite individualizate de tratament atât pentru pacienţi cât şi pentru rudele acestora.

Material şi metodă: Au fost introduse în studiu 80 de paciente diagnosticate cu cancer mamar în perioada ianuarie 2015 - iulie 2016 şi care îndeplineau criteriile NCCN 2016 de testare genetică. S-a recoltat sânge periferic, probele au fost trimise şi analizate în Grecia prin 2 paneluri de testare: un panel de testare doar pentru BRCA 1 şi 2 şi un panel extins pentru 25 de gene responsabile de apariţia cancerului mamar.

Rezultate: Din cele 80 de paciente testate 30 au prezentat mutaţii cu penetranţă mare şi medie (25 patogene, 5 VUS (Variant of Uncertain Clinical Significance)), 15 au prezentat mutaţii cu penetranţă scăzută şi 37 au fost negative (23 prin panelul multigenic şi 14 prin panelul BRCA). Din cele 14 paciente BRCA test negative, 6 au fost retestate prin panelul multigenic, fiind detectate 2 mutaţii patogene (CHEK2, PALB2) şi 1 cu mutaţie tip VUS (STK11).

Concluzii: Testarea numai a BRCA 1 şi 2 la pacientele cu sindrom ereditar de cancer mamar poate duce la scăparea unor mutaţii care ar putea modifica conduita terapeutică a pacienţilor sau a rudelor acestora. Panelurile multigenice deşi au un cost uşor crescut faţă de testele standard oferă posibilitatea detectării unei palate mai largi de mutaţii, şi posibilitatea unui tratament optim.

Cuvinte cheie: NGS, panel multigenic, cancer mamar

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Introduction

Breast cancer is the most common type of cancer in women and the second cause of death by neoplasia, being known that 1 in 8 women will develop breast cancer **[1]**.

In 2012 the estimated annual incidence of breast cancer in Europe was 94.2 / 100 000 and the mortality was 23,1 deaths/100 000 **[2].** The same study published in 2013 estimated an incidence of breast cancer in Romania in 2012 of 25, 22 %, a mortality rate of 16,74% deaths/100 000 and an increase in the estimated 5 year prevalence up to 35% **[2].**

In about 30% of breast cancer cases genetic factors are held responsible, but it has been proven that only 10% of cases are due to the inheritance of a mutation on a major gene involved in the development of this disease **[3].**

Genetic screening for some familial cancers is used on a larger scale worldwide, especially after the introduction of Next Generation Sequencing (NGS**) [4].** This has become possible especially after the process from 2013, when Myriad Genetics lost the right to patent the BRCA1 and 2 genes, and the US Supreme Court ruled that DNA and genes cannot be considered a patent **[4].**

The use of NGS technology provides superior results and lower costs per base compared to classical Sanger sequencing **[5].** The use of NGS technology also resulted in a significant reduction of false-positive results and obtain the results in a shorter time compared with conventional sequencing method **[6,7].**

The purpose of this genetic testing is to create the opportunity for an individualized and a multidisciplinary treatment for each patient, depending on the mutation that she has **[3, 8]**. These genetic tests are also useful for counseling the relatives of these patients about the risk of developing breast cancer or another type of cancer which may be susceptible **[3, 8].**

Genetic factors predisposing to breast cancer can be divided into three categories based on relative risk that the mutation or that the genetic variant confers to a person to develop the disease compared with the general population (Table 1).

Although more genes are nowadays considered to be responsible for breast cancer occurrence, not all mutations that are diagnosed in these genes are pathological (deleterious mutation), some are "benign" (Benign polymorphism) and others are Variants of unknown significance –VUS **[13]**.

Counseling and treatment of patients with breast cancer and pathogenic mutation are difficult and requires a good collaboration between clinicians and geneticists **[14].** This cooperation should be even stronger for VUS mutations, this "gray area" requiring geneticists highly trained in the field of genetic testing using multigene panels **[15].**

More and more articles and studies published recently argue that genetic testing only for BRCA1 / BRCA 2 mutations may result in a misdiagnose of other mutations associated with familial breast cancer. This will result in the impossibility of these patients to benefit from a complete and correct treatment and their relatives cannot benefit from a screening or a prevention strategy.

The most important benefits of multigene test panels are:

1. the possibility to use targeted therapies or to avoid certain treatments, which may not be allowed in some particular mutations **[16, 17];**

2. chemoprophylaxis with Tamoxifen **[18, 19];**

3. the opportunity to benefit from prophylactic surgery: Risk reduction mastectomy (RRM) and Risk Reduction Salpingo-oophorectomy (RRSO). This will lead to a risk reduction of the breast and / or ovarian cancer and a decrease in the mortality from cancer in carriers of pathogenic mutations **[20-22]**;

4. patients with higher risk for developing breast cancer due to the presence of a pathogenic or VUS mutation, will benefit from a more intensive screening (mammography and breast MRI annually), at a younger age, usually after 30 years according to current guidelines **[23];**

5. possibility of genetic counseling of the patients and their relatives.

Material and method

Our study included 80 patients diagnosed with breast cancer between January 2015 - July 2016 and

Table 1. Classification of breast cancer predisposing genes **[9-12].**

Clasification	Relative risk	Genes
High penetrance mutations	RR >5	BRCA1, BRCA2, TP53, PTEN, STK11, CDH1
Moderate penetrance mutations	RR =1,5-5	ATM, CHEK2, PALB2
Rare mutations with mild penetration	RR<1,5	FGFR2, MAP3K1, LSP1, TNRC1

met the 2016 NCCN criteria for genetic testing. Peripheral blood was collected and the samples were sent and analyzed in Greece by two test panels: a test panel just for the BRCA 1 and 2, and a multigene panel for 25 genes responsible for breast cancer.

BRCA test includes testing BRCA1, BRCA2 and CHEK2 mutations (only 1100delC). Genomic DNA was extracted from the sample under investigation and was subjected to PCR using the commercially available CE IVD BRCA MASTR Dx kit (MULTIPLICOM NV, J Mol Diag, 14:623-30, 2012) for the BRCA1 and BRCA2 genes. Sequencing was performed using the Next Generation Sequencing platform MiSeq (Illumina). The presence of the 1100delC mutation in the CHEK2 gene, is investigated using the MLPA method (Multiplex Ligation-dependent Probe Amplification, BRCA1 : P002, BRCA2, CHEK2: P045, MRC Holland; AJHG 67:841-50, 2000).

Multigene panel test includes the analysis of 25 genes: *ATM, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2*, *FAM175A, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2*, *PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2.*

Genomic DNA obtained from the submitted sample is enriched for targeted regions of 25 genes involved in hereditary predisposition to cancer. Sequencing is carried out using Illumina technology.

Reads were aligned to the reference sequence (GRCh37), and sequence changes are identified and interpreted in the context of a single clinically relevant transcript. All clinically significant observations are confirmed by orthogonal technologies. Unless otherwise indicated, all targeted regions were sequenced with e"100x depth.This assay targets all coding regions of the indicated transcript, 10 base pairs of flanking intronic sequence, and specific intronic and intragenic genomic regions demonstrated to be causative of disease. However, for some genes only targeted loci are analyzed. The presence of large genomic rearrangements is investigated using MLPA (Multiplex Ligation-dependent Probe Amplification to Probe Amplification- highly specific chromosome region of interest; AJHG 67: 841-50, 2000).

Patients were considered eligible for our study if they respected NCCN 2016 Genetic Screening for Breast Cancer guidelines **[8]**:

1. Patient diagnosed with breast carcinoma who meets any of the criteria below:

- A known mutation involved in breast cancer susceptibility present in the family;
- Early onset breast cancer;
- Particular histological forms: triple negative breast carcinoma, HER2 overexpression
- Two breast carcinomas in the same individual
- Breast carcinoma diagnosed at any age if:
  - >/= 1 close blood relative diagnosed with breast cancer <50 years or
  - >/=1 close blood relative diagnosed with ovarian cancer at any age
  - >/= 2 close blood relative diagnosed with breast and/or pancreatic cancer at any age
  - patient who comes from a high-risk population

2. Breast carcinoma in males

3. Personal or family history of cancer including three or more of the following:

- Early onset of breast cancer and / or
- multiple primitive cancers in the same individual: breast, pancreatic, prostate, melanoma, sarcoma, brain tumors, leukemia, stomach, colon, endometrial, thyroid, renal and / or

Table 2. Results the testing by the two panels

Mutations with high and moderate penetrance			Rare mutations with mild risk of breast cancer	
BRCA 1	12		BLM	2
BRCA 2	3		RAD 50	2
TP53	1		BARD1	2
PTEN	0		MLH1	1
CDH1	0		MSH2	1
STK	0		MSH6	1
CHEK 2	4		PMS2	2
PALB2	4		MEN1	1
ATM	1		MUTYH	1

- Macrocephaly, intestinal hamartomatous polyps

4. Personal history of ovarian cancer

Results

Out of the 80 patients diagnosed with breast carcinoma and tested by the two panels, 30 had mutations with high and moderate penetrance, 13 had rare mutations with mild risk of breast cancer and 37 were negative (Table 2).

From the high and moderate penetrance mutations 25 were pathogenic, and 5 mutations were Variants of Uncertain clinical Significance - Figures 1 and 2.

Out of the 80 patients, 13 had rare mutations with mild risk for developing breast cancer – Figure3.

From the 37 patients who were tested negative for breast cancer mutations, 23 were tested using the multigene test panel and 14 were tested using the panel for BRCA - Figure 4.

From the 14 patients tested negative using the panel for BRCA, 6 were retested using the multigene panel and were detected two pathogenic mutations (CHEK2, PALB2) and one VUS mutation in the STK11 gene (Figure 5).

Discussions

The test results had influenced the therapeutic conduct of all the patients with breast cancer and positive high penetrance mutations, who received neoadjuvant systemic therapy followed by radical surgery and prophylactic surgery (RRM and/or RRSO). The exception to this conduct was the patient tested positive for the TP53 mutation, because of the risk of secondary malignancies given by the neoadjuvant treatment (chemo-radiotherapy), so she has been subjected to primary surgical treatment.

Retesting using the multigene panel led to the diagnosis of three mutations (2 pathogenic and 1 VUS), which could have been overlooked and the patients couldn't fully benefit from a curative and prophylactic treatment and also from an appropriate genetic counseling.

The patient tested positive for PALB2 mutation after using the multigene panel received neoadjuvant chemotherapy and after three weeks radical mastectomy and contralateral prophylactic mastectomy. The risk of contralateral breast cancer in these patients is increased, in a study published by Cybulski et al in 2015, on 12 529 patients with breast cancer, the risk to become bilateral in 5 years was 10% in patients with PALB2 mutations, while in the BRCA1mutation group was 17% **[24].**

In the same study the authors demonstrated that the prognosis of patients with breast cancer and positive PALB2 mutation is worse compared to those with BRCA1 mutation, the survival rate at 10 years was 48% (95% CI, 36.5-63.2) compared with 72% in BRCA1 carriers **[24].** Also among the carriers of PALB2 mutations with tumors >2 cm, the 10-year survival rate was only 32.4%, whereas those with tumors < 2 cm, the 10-year survival rate may reach up to 82.4% **[24].**

Regarding RRSO in patients with breast cancer and PALB2 mutation, there are conflicting

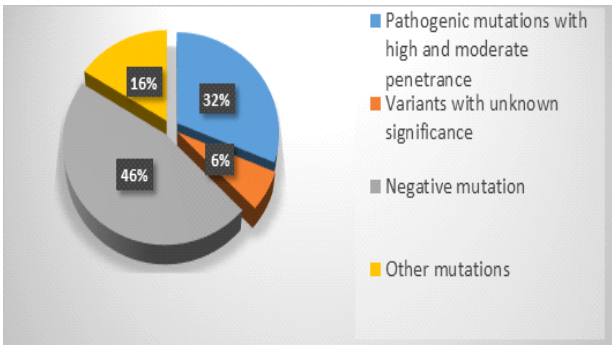

Figure 1 The results of genetic testing

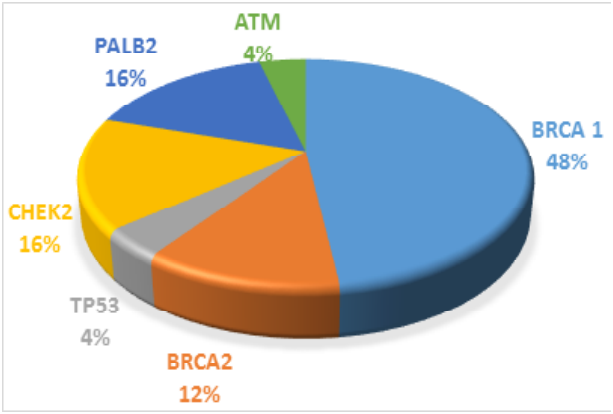

Figure 2. The incidence of pathogenic mutations

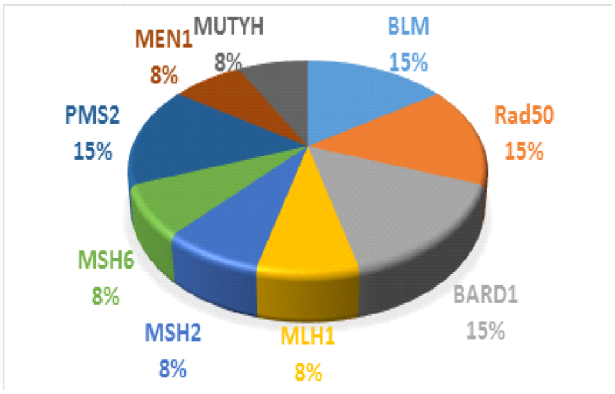

Figure 3. The distribution of rare mutations with mild risk of breast cancer

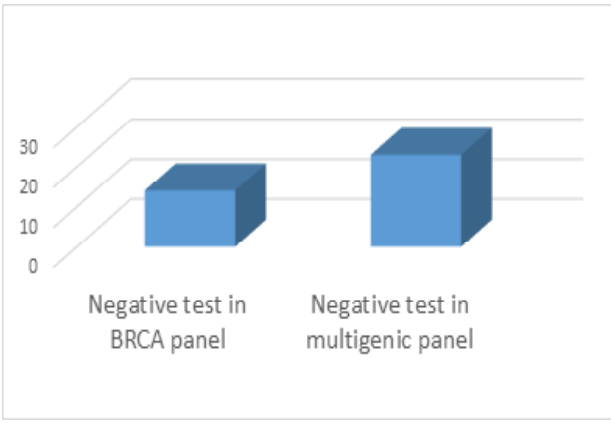

Figure 4. The distribution of the patients with negative test using the two genetic panels

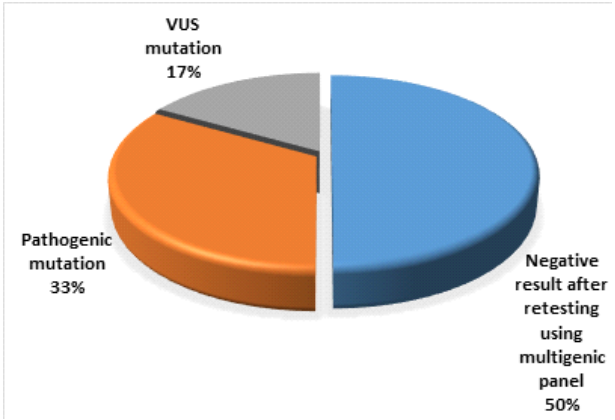

Figure 5. Results of the tests using multigene panel in patients tested negative using BRCA panel.

data and 2016 NCCN guidelines doesn't recommend it **[8]**, arguing that like in the case of BRCA2 mutation carriers only closer monitoring using endovaginal ultrasound and serum CA-125 dosing is enough **[8]**.

In a recent study from 2016 Kwong et al **[25]** recommended routine testing of PALB2 mutations especially in familial forms of breast cancer placing it on the 3rd place after BRCA1, TP53, even before BRCA2.

The patient tested positive for CHEK2 mutation after using the multigene panel, didn't benefit from RRM or RRSO, current guidelines don't support this attitude because of the lack of evidence **[8].** Instead current guidelines recommend continuation and completion of imaging and screening tests using breast MRI in CHEK 2 mutation carriers **[8]**. This led to the diagnosis of a suspicious lesions in the contralateral breast, which was later histologically confirmed as malignant and the patient was subjected to bilateral mastectomy.

Regarding the STK11 gene mutations it is known that they are responsible for Peutz Jeghers syndrome **[26]**. The cumulative risk of breast cancer in patients with STK11 mutations ranges from 32% to 54% **[27, 28]** and for ovarian cancer is 21% **[27].** The risk of breast cancer in women with Peutz-Jeghers syndrome vary widely and depends on the age of the patients, being 8% at 40 years, 13% at 50 years, 31% at 60 years and 45% at 70 years **[28]**.

The benefit of this patient is confined only to intensive screening for colorectal cancer detection because it's the most common malignancy associated with these mutations **[29]**. There are insufficient data

to recommend RRM in patients with breast cancer and positive STK11 mutations, since the risk of bilateral breast cancer is low **[8]**.

Another benefit of this test was that the daughter of the patient was diagnosed also with the same STK11 mutation and she'll benefit from colonoscopy screening and breast imaging (MRI) to minimize cancer risks.

Conclusion

Genetic testing is gradually becoming an integral part of breast cancer treatment, in many countries being already considered a standard. In Romania genetic testing and hereditary breast cancer management are still in the beginning compared to Western countries, but with cost reduction of the tests and increasing awareness among patients, these things will improve.

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