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The 3020insc Allele of the NOD2 Gene in Breast Cancer Patients – A Clinicopathological Analysis

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ABSTRACT

Introduction: *The 3020insC mutation of NOD2 predisposes to many types of common cancers, e.g. breast cancer. In this report we present our preliminary clinicopathological analysis of the NOD2 3020insC allele in breast cancer patients from the Silesia region in Poland.*

Material and Methods: *We reviewed the medical records of 27 early breast cancer patients with 3020insC mutation of NOD2, who were diagnosed and treated in Maria Skłodowska – Curie Memorial Cancer Center and Institute of Oncology (COI) in Gliwice. NOD2 mutation was assessed by PCR technique.*

Results: *The mean age of the mutation carriers at breast cancer diagnosis was 46 years (range 27- 64 years). 16 (60%) of NOD2 mutation carriers had history of cancer in family, including breast cancer in 9 (34%) and gastrointestinal cancer in 6 (22%). Co-morbid conditions was observed in 34% of patients. Most of NOD2 carriers had tumors without HER2 overexpression (82%) and with positive steroid receptor status (71%). TNBC (triple negative breast cancer)was detected in 26% of mutation carriers. Lymph node metastases was observed in 26% of patients. Most of NOD2 mutation carriers had lower tumor size (T1-T2) – 89%.*

Conclusions: *NOD2 mutation carriers was associated with prognostic clinicopathological factors such as: positive steroid receptor status, HER2 negative tumors, lower tumor size (pT< 2cm), lymph nodes without metastases and younger age (<50 years).*

Key words: *Breast cancer, NOD2 mutation carriers, clinicopathological factors.*

Abbreviations:

COI – Maria Skłodowska – Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch.

TNBC – triple negative breast cancer

ER – estrogen receptor status

PR – progesterone receptor status

HER2 - Human epidermal growth factor receptor 2

INTRODUCTION

The *NOD2* gene has been identified and mapped to chromosome 16q12 byHugot et al. It consists of 12 exons and its product, a cytosolic protein, consists of 1,040 amino acids [1]. The *NOD2* protein plays an important role in immune system function. It is active in some types of immune system cells (including monocytes, macrophages, and dendritic cells), which help to protect the body against foreign invaders such as viruses and bacteria. The protein is also active in several types of epithelial cells, including Paneth cells, which are found in the lining of the

intestine. These cells help to defend the intestinal wall against bacterial infection [2]. *NOD2* is involved in the inflammatory response and the activation of the NFkB pathway.

At least 17 mutations in the *NOD2* gene have been found to cause Blau syndrome, an inflammatory disorder that primarily affects the skin, joints, and eyes.*NOD2* gene mutations can also cause early-onset sarcoidosis. The mutation predisposes to Crohn's disease, a common chronic inflammatory bowel disease that is known to favor colorectal cancer development [3, 4]. Approximately 40 variations in the NOD2 gene have been associated with an increased risk of Crohn disease.

The 3020insC mutation of *NOD2* also predisposes to many types of common cancers, e.g. breast cancer. The mutant allele is more frequently found in women with early- onset breast cancer and in women with ductal breast cancer with an in situ component. The *NOD2* 3020insC allele is relatively common (7.3%) in the Polish population. The aim of this study was to determine the risk of cancer in family of patients with *NOD2* gene mutation and clinicopathological features of breast cancer in *NOD2* gene mutation carriers. To our knowledge, this is the first publication describing the clinicopathological characteristics of breast cancer patients with *NOD2* mutations.

MATERIAL AND METHODS

This retrospective study was conducted in Clinical and Experimental Oncology Department, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice Branch in Poland. The analysis included 27 early breast cancer patients treated between 2006 – 2012. All patients were women diagnosed, treated and followed at COI in Gliwice. Inclusion criteria were: breast cancer confirmed by microscopic examination, performance status ZUBROD 0-1, age over 18, lack of distant metastases, the correct value of renal and liver function and normal values of bone marrow. The data on the age at onset, menopausal status, surgical procedure, disease stage according to TNM classification, histology, estrogen and progesterone receptor status, HER2 status and contralateral breast cancer were gathered from hospital records and pathology reports.

The analysis of patient's medical records was performed according to national law regulation. The mean age of the mutation carriers at breast cancer diagnosis was 46 years (range 27- 64 years). 63% of women were diagnosed under the age of 50, and 19% women under the age of 35. No one of them was older than 65 years. The patient's characteristics are shown in Table I. The surgical treatment was performed in 23 (85%) women including mastectomy and breast conserving treatment. 63% of patients received anthracycline based chemotherapy in Clinical and Experimental Oncology Department. 19 (70%) women with steroid positive receptor breast cancer were treated with anti-estrogen therapy. Trastuzumab was used in 35% women, 5 (19%) had been identified with HER2 overexpression via immunohistochemistry or gene amplification using FISH. Radiotherapy was given to 19 (70%) patients. The total radiotherapy dose was 50 Gy in 25 fractions. If indicated, a boost was delivered.

TableI. *Patient's characteristics and therapy strategies*

Risk factor		N=27; %
Age <65 years		27 (100%)
Cigarette smoking	Yes No	6 (22%) 21 (78%)
Co morbid condition	Yes No	16 (59%) 11 (41%)
Diabetes	Yes No	1 (4%) 26 (96%)
Cardiovascular diseases	Yes No	5 (19%) 21 (81%)
Radiotherapy	Yes No	19 (70%) 8 (30%)
Hormonotherapy	Yes No	20 (74%) 7 (26%)
Chemotherapy	Yes No	17 (63%) 10 (37%)

Table II. *Pathological characteristics of the tumors.*

Risk factor		**N=27; %**
Clinical staging nodes	N0 N positive	20 (74%) 7 (26%)
Tumor size	T3-4 T1-2	3 (11%) 24 (89%)
Grade G	G1+2 G3	15 (55%) 5 (19%)
ER	Negative Positive	7 (26%) 20 (74%)
PR	Negative Positive	7 (26%) 20 (74%)
HER2 overexpression	Negative Positive	22 (82%) 5 (19%)
Triple negative	Yes No	7 (26%) 20 (74%)

All patients were tested for the presence of 3020insC mutation of *NOD2*. Mutation analysis was carried by a multiplex allele-specific polymerase chain reaction assay.Each patient signed informed consent before venous blood collection for a genetic test. Genomic DNA was isolated from peripheral blood leucocytes.

Statistical analysis was carried out using STATISTICA 7 software. The frequency of side effects appearance was counted. The qualitative features were presented as the percentage of their occurrence and evaluated with *Fisher* test and Chi 2 test with Yates correction. The differences were considered as significant if the p value was ≤ 0.05.

RESULTS

We analyzed the group of 27 breast cancer patients. The mean age at breast cancer diagnosis was 46 years (range 27-64 years) for the carriers of the mutation. 63% of women were diagnosed under the age of 50, and 19% women under the age of 35. Co-morbid conditions were observed in 34% of patients, including cardiovascular diseases (19%), diabetes (4%), viral disease (4%) and hypothyroidisms (11%).

Triple-negative breast cancer tumors were detected in 26% of mutation carriers. Luminal A and luminal B type were detected in 44% and 16%, respectively. Most of *NOD2* carriers had tumors without HER2 over expression (82%) and with positive steroid receptor status (ER/PR +) (71%). Lymph node metastases were observed in 26% of patients. Most of *NOD2* mutation carriers had lower tumor size (T1-T2) – 89%. The lobular type of breast cancer was observed only in 7% of patients with *NOD2* mutations. The remaining patients (93%) had invasive ductale carcinoma. Higher histological grade>G2 was observed in 19% of *NOD2* mutation carriers. Grade 1 and Grade 2 were detected in 19% and 45% of patients, respectively.

16 (60%) of *NOD2* mutation carriers had history of cancer in the family, including breast cancer in 9 (34%). Gastrointestinal cancer (gastric or colorectal cancer) in family history was detected in 6 (22%). The other cancer in family history were: renal cancer (4%), gynecological (4%) and lung cancer (7%).

All the patients were followed up in Cancer Center and Institute of Oncology in Gliwice. The median follow up was 3.5 years (range 0.5 to 6.4). The second primary cancers were not found in studied group of patients.

DISCUSSION

The present paper was conducted to evaluate the clinicopathological factors such as hormone status (oestrogen (ER), progesterone receptor (PR)), human epidermal growth factor (HER2), tumor size, the presence of lymph nodes metastases, co-morbid conditions, patients' age and history of cancer in the family in NOD2 mutation carriers.The NOD2 3020insC allele is relatively common (7.3%) in the Polish population [5]. In previous study it had been reported in 8.0% of 462 breast cancer patients from Szczecin [6] and in 8.8% of the 148 breast cancer patients from Bydgoszcz [7]. The population risk of breast cancer before the age of 50, associated with the NOD2 mutation, is approximately 1% [6]. In the present study, the mutation was detected in 27 women, reaching the incidence of 6% (27/485 patients).

In the study conducted by Huzarski et al, the authors found a modest, not statistically

significant, association of the *NOD2* 3020insC mutation with family history of breast cancer [6]. In other studies no such association was demonstrated. Study conducted by Janiszewska et al have suggested that the NOD2 3020insC mutation might predominantly increase the risk of developing digestive tract cancer rather than breast cancer [7]. The presence of the NOD2 3020insC allele increases the lifetime risk of cancer by approximately 25% to 35% [5]. A role for NOD2 mutations has been postulated for several malignant diseases such as gastric and colorectal cancer [9]. In study conducted by Kurzawski et al the frequency of the 3020insC mutation in a consecutive series of 250 non-hereditary nonpolyposis colorectal cancer patients >50 years of age was significantly elevated compared to the control population (odds ratio, 2.23; P = 0.0046). The results indicate that NOD2 may be a predisposing factor to colorectal cancer characterized by an older average age of disease onset in persons who do not harbor any other genetic predisposition to disease [8]. Studies conducted by Teodorczuk et al in the group of 103 patients with a family history of gastric cancer, showed that for NOD2 3020insC carriers being over 50 years of age the risk of cancer more than doubled (OR=2.479, p=0.022) and among women almost was even 3-fold [10]. In our analysis, 60% of NOD2 mutation carriers had history of cancer in family. As in previous studies, the presence of NOD2 mutation increased the risk of gastrointestinal cancer in the family history (gastric, pancreatic and colon cancer) (22%). However, we also found the presence of breast cancer in family history in 9 (34%) of NOD2 mutation carriers.

In some studies significant associations were observed between the presence of the allele and early-onset breast cancer (OR = 1.9; p = 0.01) and between the allele and ductal breast cancer with an in situ component (OR = 2.2; p = 0.006) [6]. Our research confirms the above mentioned results. In our group all patients had early breast cancer. Ducal invasive carcinoma was detected in most patients in our group. The lobular type of breast cancer was observed only in 7% of patients with NOD2 mutations. In our study, the other factors associated with NOD2 mutation are: younger age, lower TNM stage, positive steroid receptor status (ER/PR), HER2 negative tumors (HER2-), lower tumor size (pT< 2cm), lymph nodes without metastases (N-), histological grade G2 and history of cancer in family. As far as we know, this is a first analysis of pathological factors in NOD2 mutation carriers.

Conclusion

NOD2 mutation carriers were characterized by younger age, lower TNM stage, positive steroid receptor status (ER/PR), HER2 negative tumors (HER2-), lower tumor size (pT< 2cm), lymph nodes without metastases (N-), histological grade G2 and history of cancer in family, especially breast cancer and gastrointestinal cancer. The most common type of breast cancer in this group were luminal types (type A and type B, HER negative).

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