Clinicopathological characteristics of breast cancer patients with *NOD2* mutation according to age

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Introduction: The purpose of the present study was to characterise patients with breast cancer (BC) and *NOD2*-mutation (age ≥ 50 years) according to their clinicopathological factors or family history. Patients aged ≥ 50 years were compared with the control group and with *NOD2*-mutation carriers aged < 50 years.

Material and methods: Prognostic factors were analysed in patients with BC with confirmed *NOD2* c.3016_3017insC (n = 150) mutations. The control group was selected from patients with BC without mutations (n = 376).

Results: There were significant differences between *NOD2*-mutation carriers and the control group aged ≥ 50 years, according to HER2 overexpression (p = 0.0001), ER (–) (p = 0.007), PR (–) (p = 0.003), T1–T2 (p = 0.011), and G3 (p = 0.036). Similarly, significant differences were observed between *NOD2*-mutation carriers and the control group aged < 50 years, according to HER2 overexpression (p = 0.0001), ER (–) (p = 0.049), and N (+) (p = 0.038). In patients aged ≥ 50 years, family history of cancer, including BC, was observed more often in *NOD2*-mutation carriers compared with the control group of patients (OR = 1.66; p = 0.072, for BC in family history: OR = 2.65; p = 0.002). *NOD2*-mutation carriers aged ≥ 50 years had significantly less frequent G3 (p = 0.004) and HER2 overexpression (p = 0.043) compared with patients with *NOD2* mutation aged < 50 years.

Conclusions: The presence of the *NOD2* mutation is not only characteristic of younger patients but also in patients > 50 years of age. In *NOD2*-mutation carriers aged ≥ 50 years, the presence of larger tumour size, G3, or HER2 overexpression were lower compared with younger patients with *NOD2* mutation.

Key words: breast cancer, *NOD2* mutation, family history of cancer.

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Introduction

The NOD2 protein plays an important role in the function of the immune system. It is active in certain 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 [1]. NOD2 also regulates the activity of genes that control immune responses and inflammatory reactions, and plays a role in a process called autophagy [3]. The gene that encodes this protein is located on 16q12.1 [2].

The *NOD2* 3020insC allele is reported in ~7.3% of the Polish population [4]. In the literature, the presence of *NOD2* mutation is associated with increased risk of breast cancer (BC) before the age of 50 years (~1%) [5]. This mutation occurs in ~8% of all BC and increases the risk of DCIS in those aged < 50 years by five times. The presence of the *NOD2* 3020insC allele also increases the lifetime risk of colorectal cancer at the age of over 60 years (by over 2-fold), lung cancer (~2-fold), and ovarian cancer (~1.5-fold) [6, 9]. The 3020insC mutation of the *NOD2/CARD15* gene may also be a genetic predisposing factor for aggregations of breast and lung cancer [11].

In the literature, there are only a few data describing the association between the presence of *NOD2* mutation and clinicopathological factors. The associated factors confirmed in the aforementioned studies were younger age at diagnosis, family history of cancer (especially breast and gastrointestinal cancer) [8], and early-stage disease [6]. In our previous study, the presence of *NOD2* (3020insC) in women with BC was characterised by positive predictive factors such as: lymph nodes without metastasis (N0), lower histological grade (G<3), and negative HER2 receptor status (HER2–) [7].

The purpose of the present study was to characterise BC *NOD2*-mutation carriers at age ≥ 50 years, according to clinicopathological factors or family history. Patients aged ≥ 50 years were compared with the control group and with *NOD2*-mutation carriers at age < 50 years.

Material and methods

The analysed group were divided into two subgroups according to patient age: 1) < 50 years; and 2) ≥ 50 years. The clinicopathological factors were analysed, as well as the presence of cancer in family history in younger (age, < 50 years) (45.3%, n = 68/150) and in older (age, ≥ 50 years) (54.7%, n = 82/150) patients with BC with confirmed *NOD2* (c.3020insC) mutation (GenBank NM _022162.1). Control groups were selected from patients with BC who tested negative for the mutations (37.5% < 50 years, n = 141;

62.5% ≥ 50 years; *n* = 235/376). The presence of the most common mutations in *BRCA1* (c.68_69delAG, c.181T>G, c.4034delA, c.5266dupC, c.3700_3704del5) (GenBank NM_007294.3), *BRCA2* (c.5946delT and c.9403delC) (GenBank NM_000059.3), and CHEK2*1100delC or I157T (GenBank NM_007194.3) genes were excluded. Mutation analysis was carried out by a multiplex allele-specific PCR assay. Genetic diagnostics were conducted between the years 2012 and 2018. All patients gave written, informed consent for genetic examination.

Table 1. Clinicopathological characteristics of all study group breast cancer patients (*n* = 526)

Risk factor	Breast cancer	
	n	%
Age median (range)	52.4 years (25.5–80.7)	
Clinical staging nodes		
N positive	226	43.0
N negative	300	57.0
Tumour size		
T3–4	83	15.8
T1–2	443	84.2
Grade G		
G3	161	30.6
G1+G2	365	69.4
ER		
Negative	159	30.2
Positive	367	69.8
PR		
Negative	196	37.3
Positive	330	62.7
HER2 overexpression		
Positive	215	40.9
Negative	311	59.1
Molecular subtype		
Luminal A & B– type	236	44.9
Others	290	55.1
Triple negative		
Yes	75	14.3
No	451	85.7
Histological type		
Ductal invasive	401	76.2
Lobular invasive ca	52	9.9
Other	73	13.9
Molecular subtype		
Luminal A type	102	19.4
Luminal B– type	134	25.5
Luminal B+ type	143	27.2
Triple negative	75	14.3
Non luminal	72	13.7

All patients were females who were diagnosed, treated, and followed-up at the National Research Institute of Oncology in Gliwice. Patients underwent clinical follow-up examinations every three months in the first two years, every six months afterwards until the fifth year after diagnosis, and every year subsequently. The inclusion criteria were: BC confirmed by microscopic examination; performance status ZUBROD 0–1; age above 18 years; the normal levels of renal and liver function and normal values of bone marrow. The data of age at onset, menopausal status, surgical procedure, disease stage according to TNM classification, histology, oestrogen (ER) and progesterone receptor (PR) status, HER2 status, and contralateral BC were gathered from hospital records and pathology reports. The analysis of patient medical records was performed according to national law regulation.

The median age at diagnosis of all patients was 52.4 years (range, 25.5–80.7 years). Patient clinicopathological characteristics are presented in Table 1. A total of 209 patients were at the age < 50 years and 317 at the age ≥ 50 years. All patients had good performance status (ZUBROD 0–1). The complete characteristics of patients with regards to demographic and clinicopathological features are presented in Tables 2 and 3.

Statistical analysis was carried out using Statistica13 software. The qualitative features were presented as the percentage of their occurrence and evaluated with Fisher's test and the χ^2 test with Yates' correction. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated as measures of association between the analysed factors and the presence of *NOD2* mutation. The differences were considered significant if the *p*-value was < 0.05.

Results

The median age at BC diagnosis for the carriers of the *NOD2* mutation was 51.4 years (range, 25.5–80.7 years) and 53.2 years (range, 26.4–78.3 years) for the control group. A total of 209 patients were aged under 50 years: 68 (45.3%) were *NOD2*-mutation carriers and 141 (37.2%) were in the control group.

Group of patients aged ≥ 50 years

In the group of patients ≥ 50 years old, a family history of cancer, including BC, was observed more often in *NOD2*-mutation carriers compared with the control group of patients (65% vs. 52%; OR = 1.66; *p* = 0.072, with family history of BC vs. no family history of BC; 32% vs. 15%; OR = 2.65; *p* = 0.002). There were no differences between the control group and *NOD2*-mutation carriers at age ≥ 50 years, in those with a family history of other cancer types.

In the group aged ≥ 50 years, lower tumour size (T1–T2) was observed more often in *NOD2*-mutation carriers compared with the control group (94% vs. 82%; OR = 3.35; *p* = 0.011). Similarly, in patients aged ≥ 50 years, lymph nodes without metastases were reported insignificantly more frequently in *NOD2*-mutation carriers (67% vs. 58%, OR = 1.48, *p* = 0.182). Conversely, histological grade G3 tumours (17% vs. 30%, OR = 0.49, *p* = 0.036) and HER overexpression (10% vs. 49%, OR = 0.11, *p* = 0.0001) were present significantly

Table 2. Clinical characteristics of breast cancer patients according to age

Risk factor	*NOD2* < 50 years (*n* = 68)		Control group < 50 years (*n* = 141)		OR	*p*	*NOD2* ≥ 50 years (*n* = 82)		Control group ≥ 50 years (*n* = 235)		OR	*p*
	n	%	*n*	%			*n*	%	*n*	%		
Age median (range)	42.8 years (25.5–49.9)		43.8 years (26.4–50.0)			0.331*	58.2 years (50.0–80.7)		59.3 years (50.0–78.3)			0.928*
Menopausal status												
Premenopausal	61	89.7	129	91.5	0.81	0.798	13	15.9	31	13.2	1.24	0.678
Postmenopausal	7	10.3	12	8.5	1.0		69	83.1	204	86.8	1.0	
Co-morbid condition												
Yes	17	25.0	46	32.6	0.69	0.335	53	64.6	139	59.1	1.26	0.457
No	51	75.0	95	67.4	1.0		29	35.4	96	40.9	1.0	
Diabetes												
Yes	2	2.9	2	1.4	2.11	0.597	4	4.9	13	5.5	0.88	1.00
No	66	97.1	139	98.6	1.0		78	95.1	222	94.5	1.0	
Cardiovascular diseases												
Yes	2	2.9	1	0.7	4.24	0.248	9	11.0	38	16.2	0.64	0.284
No	66	97.1	140	99.3	1.0		73	89.0	197	83.8	1.0	
Viral diseases												
Yes	0	0.0	5	3.5	0.0	0.176	3	3.7	13	5.5	0.65	0.770
No	68	100	136	96.5	1.0		79	96.3	222	94.5	1.0	
Cancer in family history												
Yes	41	60.3	83	58.9	1.06	0.963	53	64.6	123	52.3	1.66	0.072
No	27	39.7	58	41.1	1.0		29	35.4	112	47.7	1.0	
Breast cancer in family history												
Yes	17	25.0	27	19.1	1.41	0.429	26	31.7	35	14.9	2.65	0.002
No	51	75.0	114	80.9	1.0		56	68.3	200	85.1	1.0	
Lung cancer in family history												
Yes	6	8.8	13	9.2	0.95	0.870	6	7.3	21	8.9	0.80	0.819
No	62	91.2	128	90.8	1.0		76	92.7	214	91.1	1.0	
Gynaecological cancer in family history												
Yes	7	10.3	12	8.5	1.23	0.870	10	12.2	25	10.6	1.17	0.855
No	61	89.7	129	91.5	1.0		72	87.8	210	89.4	1.0	
Stomach cancer in family history												
Yes	6	8.8	12	8.5	1.04	0.851	8	9.8	14	6.0	1.71	0.361
No	62	91.2	129	91.5	1.0		74	90.2	221	94.0	1.0	
Pancreas cancer in family history												
Yes	4	5.9	2	1.4	4.34	0.089	2	2.4	4	1.7	1.44	0.651
No	64	94.1	139	98.6	1.0		80	97.6	231	98.3	1.0	
Bowel cancer												
Yes	10	14.7	8	5.7	2.87	0.037	9	11.0	25	10.6	1.04	0.903
No	58	85.3	133	94.3	1.0		73	89.0	210	89.4	1.0	

* *Mann-Whitney U test*

less often in *NOD2*-mutation carriers. There was also a significant difference between *NOD2*-mutation carriers and the control group according to ER– (16% vs. 32%; OR = 0.39; $p = 0.007$) and PR– (23% vs. 43%; OR = 0.41; $p = 0.003$) negative steroid receptor status in the group of ≥ 50 years. In the study group, there was no notable difference between *NOD2*-mutation carriers and the control group, according to the BC histological type (Table 3).

Group of patients aged < 50 years

In the group of patients < 50 years old, there was no difference observed between *NOD2*-mutation carriers and

Table 3. Pathological characteristics of the tumours in breast cancer patients according to age

Risk factor	*NOD2* < 50 years (*n* = 68)		Control group < 50 years (*n* = 141)		OR	*p*	*NOD2* ≥ 50 years (*n* = 82)		Control group ≥ 50 years (*n* = 235)		OR	*p*
	n	%	*n*	%			*n*	%	*n*	%		
Clinical staging nodes												
N positive	25	36.8	75	53.2	1	0.038	27	32.9	99	42.1	1	0.182
N negative	43	63.2	66	46.8	1.95		55	67.1	136	57.9	1.48	
Tumour size												
T3–4	11	16.2	25	17.7	1	0.934	5	6.1	42	17.9	1	0.011
T1–2	57	83.8	116	82.3	1.12		77	93.9	193	82.1	3.35	
Grade G												
G3	27	39.7	50	35.5	1.20	0.658	14	17.1	70	29.8	0.49	0.036
G1 + G2	41	60.3	91	64.5	1		68	82.9	165	70.2	1	
ER												
Negative	16	23.5	54	38.3	0.50	0.049	13	15.9	76	32.3	0.39	0.007
Positive	52	76.5	87	61.7	1		69	84.1	159	67.7	1	
PR												
Negative	21	30.9	56	39.7	0.68	0.277	19	23.2	100	42.6	0.41	0.003
Positive	47	69.1	85	60.3	1		63	76.8	135	57.4	1	
HER2 overexpression												
Positive	15	22.1	76	53.9	0.24	0.0001	8	9.8	116	49.4	0.11	0.0001
Negative	53	77.9	65	46.1	1		74	90.2	119	50.6	1	
Molecular subtype												
Luminal A & B– type	41	60.3	42	29.8	3.58	0.0001	64	78.0	89	37.9	5.83	0.0001
Others	27	39.7	99	70.2	1		18	22.0	146	62.1	1	
Triple negative												
Yes	12	17.6	23	16.3	1.10	0.965	10	12.2	30	12.8	0.95	0.953
No	56	82.4	118	83.7	1		72	87.8	205	87.2	1	
Histological type												
Ductal invasive	54	79.4	110	78.0	1		61	74.4	176	74.9	1	
Lobular invasive ca	7	10.3	12	8.5	1.19	0.932	8	9.8	25	10.6	0.92	0.977
Other	7	10.3	19	13.5	0.75	0.702	13	15.8	34	14.5	1.10	0.926
Molecular subtype												
Luminal A type	18	26.5	10	7.1	1		40	48.8	34	14.5	1	
Luminal B– type	23	33.8	32	22.7	0.40	0.0885	24	29.3	55	23.4	0.37	0.005
Luminal B+ type	11	16.2	48	34.0	0.13	0.0001	5	6.1	79	33.6	0.05	0.0001
Triple negative	12	17.6	23	16.3	0.29	0.034	10	12.2	30	12.8	0.28	0.005
Non luminal	4	5.9	28	19.9	0.08	0.0001	3	3.6	37	15.7	0.07	0.0001

the control group, according to those with a family history of cancer (60% vs. 59%; OR = 1.06; $p = 0.963$), particularly those with a family history of BC (25% vs. 19%; OR = 1.41; $p = 0.429$). Conversely, history of colorectal cancer was observed significantly more often in younger *NOD2*-mutation carriers compared with the control group (15% vs. 6%; OR = 2.87; $p = 0.037$). A similar tendency was reported in those with a family history of pancreatic cancer (5.9% vs. 1.4%; OR = 4.34; $p = 0.089$). There were no differences between younger patients and the control group, based on a family history of stomach cancer (8.8% vs. 8.5%; OR = 1.04; $p = 0.851$). Similar results were reported in those with a family history of lung cancer (8.8% vs. 9.2%; OR = 0.95; $p = 0.870$) or gynaecological cancer (10.3% vs. 8.5%; OR = 1.23; $p = 0.870$) (Table 2).

There was no difference between younger *NOD2*-mutation carriers and the control group, according to tumour size (T1–T2) (84% vs. 82%; OR = 1.12; $p = 0.934$). Lymph nodes without metastases (63% vs. 47%; OR = 1.95; $p = 0.038$) were significantly more frequently observed in *NOD2*-mutation carriers compared with the control group at this age. In the group of individuals of < 50 years old, histological grade G3 was non-significantly more often observed in *NOD2*-mutation carriers ($p = 0.658$). Conversely,

Table 4. Univariate and multivariate logistic regression

Characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age ≥ 50 years vs. < 50 years	0.72 (0.49–1.06)	0.098	0.61 (0.40–0.93)	0.020
Status: post- vs. premenopausal	0.76 (0.52–1.11)	0.158		
T3–T4 vs. T1–T2	0.55 (0.31–0.99)	0.044		
N+ vs. N0	0.62 (0.42–0.91)	0.016		
G3 vs. G1–G2	0.80 (0.53–1.22)	0.304		
ER+ vs. ER0	2.20 (1.40–3.48)	0.001	2.10 (1.30–3.41)	0.003
PR+ vs. PR0	1.95 (1.29–2.96)	0.002		
HER2 positive vs. HER2 negative	0.17 (0.11–0.28)	0.0001	0.17 (0.11–0.28)	0.0001
Breast cancer patients age < 50 years				
T3–T4 vs. T1–T2	0.90 (0.41–1.95)	0.934		
N+ vs. N0	0.51 (0.28–0.93)	0.038		
G3 vs. G1–G2	1.20 (0.66–2.17)	0.658		
ER+ vs. ER0	2.02 (1.05–3.88)	0.049	1.94 (0.98–3.83)	0.058
PR+ vs. PR0	1.47 (0.80–2.73)	0.277		
HER2 positive vs. HER2 negative	0.24 (0.12–0.47)	0.0001	0.25 (0.13–0.48)	0.0001
Breast cancer patients: age ≥ 50 years				
T3–T4 vs. T1–T2	0.30 (0.11–0.78)	0.011	0.35 (0.13–0.96)	0.041
N+ vs. N0	0.67 (0.40–1.14)	0.182		
G3 vs. G1–G2	0.49 (0.26–0.92)	0.036		
ER+ vs. ER0	2.54 (1.32–4.87)	0.007	2.03 (1.01–4.08)	0.046
PR+ vs. PR0	2.46 (1.38–4.36)	0.003		
HER2 positive vs. HER2 negative	0.11 (0.05–0.24)	0.0001	0.12 (0.05–0.26)	0.0001

HER2 overexpression was reported significantly less often in *NOD2*-mutation carriers (22% vs. 54%; OR = 0.24; p = 0.0001) at this age. There was a significant difference between *NOD2*-mutation carriers and the control group, according to ER– (24% vs. 38%; OR = 0.50; p = 0.049) negative steroid receptor status, while there was no differences for PR– (31% vs. 40%; OR = 0.68; p = 0.277) negative steroid receptor status. Conversely, there was no difference between *NOD2*-mutation carriers and the control group, according to BC histological type – ductal invasive carcinoma (79% vs. 78%) in the group of individuals < 50 years (Table 3).

Breast cancer molecular subtypes

The presence of BC molecular subtypes in *NOD2*-mutation carriers and in the control group of patients differ from each other significantly (p = 0.0001). This is due to the differences in the presence of HER2 overexpression, positive oestrogen (ER+), and progesterone (PR+) steroid receptor status in both groups (*NOD2*-mutation carriers and the control group). The most commonly observed BC subtypes in the present study among *NOD2*-mutation carriers were luminal A and luminal B HER2(–). These subtypes were observed in 60% of younger *NOD2*-mutation carriers (< 50 years) and in 78% of *NOD2*-mutation carriers over 50 years of age (p = 0.018). In the group < 50 years of age, luminal A (26% vs. 7%) and luminal B HER2(–) (34% vs. 23%) BC subtypes were reported more frequently in patients with *NOD2* mutation compared with the control group (Fig. 1). Similarly, in the group of individuals ≥ 50 years old, luminal A (49% vs. 15%) and luminal B HER2(–) (29% vs. 23%) subtypes were reported more often in patients with *NOD2* mutation (Fig. 2). The risk of the presence of other BC subtypes than luminal A among *NOD2*-mutation carriers is lower compared with the control group, independently of patients' age (Table 3).

Logistic regression analysis results

Factors that were significantly associated with the presence of *NOD2* mutation among BC subgroups, in patients aged ≥ 50 years and < 50 years, were detected. Patients ≥ 50 years of age were characterised by lower tumour size (T1–T2), lower histological grade (G1–G2), positive steroid receptor status (ER+ and PR+), and tumours without HER2 overexpression. Conversely, younger (< 50 years) *NOD2*-mutation carriers were characterised by the deficiency of lymph node metastases (N0), ER+ status, and tumours without HER2 overexpression (Table 3).

Multivariate logistic regression analysis results for *NOD2* mutation among patients with BC is shown in Table 4. Among patients with BC at the age ≥ 50 years, the presence of *NOD2* mutation was significantly associated with lack of HER2 overexpression (OR = 0.12; p = 0.0001), lower tumour size (T; OR = 0.35; p = 0.041), and ER+ status

Table 5. Pathological characteristics of the tumours in breast cancer patients with *NOD2* mutation according to age

Risk factor	*NOD2* < 50 years (*n* = 68)		*NOD2* ≥ 50 years (*n* = 82)		*NOD2* < 50 years vs. *NOD2* ≥ 50 years	*p*
	n	%	*n*	%	OR	
Clinical staging nodes						
N positive	25	36.8	27	32.9	1.18	0.749
N negative	43	63.2	55	67.1	1	
Tumour size						
T3–4	11	16.2	5	6.1	2.97	0.063
T1–2	57	83.8	77	93.9	1	
Grade G						
G3	27	39.7	14	17.1	3.20	0.004
G1 + G2	41	60.3	68	82.9	1	
ER						
Negative	16	23.5	13	15.9	1.63	0.328
Positive	52	76.5	69	84.1	1	
PR						
Negative	21	30.9	19	23.2	1.48	0.380
Positive	47	69.1	63	76.8	1	
HER2 overexpression						
Positive	15	22.1	8	9.8	2.62	0.043
Negative	53	77.9	74	90.2	1	
Molecular subtype						
Luminal A & B– type	41	60.3	64	78.0	0.43	0.029
Others	27	39.7	18	22.0	1	
Triple negative						
Yes	12	17.6	10	12.2	1.54	0.479
No	56	82.4	72	87.8	1	
Molecular subtype						
Luminal A type	18	26.5	40	48.8	1	
Luminal B– type	23	33.8	24	29.3	2.13	0.095
Luminal B+ type	11	16.2	5	6.1	4.89	0.009
Triple negative	12	17.6	10	12.2	2.67	0.093
Non luminal	4	5.9	3	3.7	2.96	0.215

(OR = 2.03; p = 0.046). However, among patients with BC below the age of 50 years the presence of *NOD2* mutation was significantly associated with HER2-negative status (OR = 0.25; p = 0.0001) and insignificantly with ER-positive status (OR = 1.94; p = 0.058).

Multivariate analysis has shown, for all study groups, that the older age (OR = 0.61; p = 0.020), ER+ status (OR = 2.10; p = 0.003), and tumours without HER2 overexpression (OR = 0.17; p = 0.0001) were risk factors for *NOD2* mutation in patients with BC (Table 4).

NOD2-mutation carriers at the age of < 50 years vs. ≥ 50 years

The comparison of patients with *NOD* mutations in age groups < 50 and ≥ 50 years indicated a tendency towards higher incidence of negative factors in the group of younger patients with BC (Table 5). BC tumours with G3 (40% vs. 17%; OR = 3.20; p = 0.004) and with HER2 overexpression (22% vs. 10%; OR = 2.62; p = 0.043) were observed significantly more often among younger patients with *NOD2* mutation. The tendency towards the presence of larger tumour size (T3–T4) in younger *NOD2*-mutation carriers was also observed.

Multivariate analysis of pathological factors in the group of individuals with *NOD2* mutation has shown that only G3 (OR = 3.22; p = 0.003) was significantly associated with the presence of *NOD2* mutation in younger patients.

Discussion

In the present study, *NOD2*-mutation carriers at age ≥ 50 years were compared with the control group and with the younger (< 50 years) subgroup of patients with *NOD2*

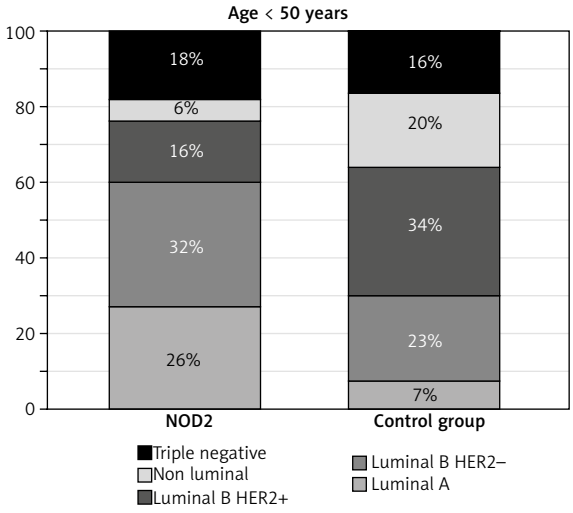

Fig. 1. Breast cancer molecular subtypes in group of patients aged < 50 years

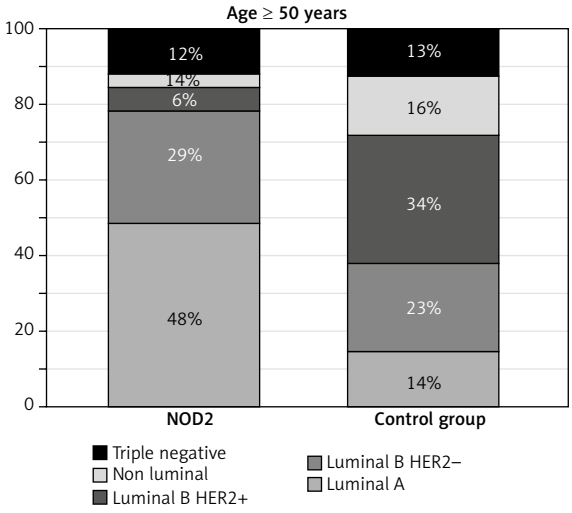

Fig. 2. Breast cancer molecular subtypes in group of patients aged ≥ 50 years

mutation; according to the clinicopathological factors, such as hormone status ER, PR, HER2, tumour size, the presence of lymph node metastases, and history of cancer in the family.

The insignificant association between the *NOD2* 3020insC mutation and a family history of BC was reported by Huzarski *et al.* [5]. The 3020insC mutation of the *NOD2/CARD15* gene may also be a genetic predisposing factor for aggregations of breast and lung cancer. Lener *et al.* reported that the presence of *NOD2* mutation was higher in patients with BC who had a first- or second-degree relative diagnosed with lung cancer compared with patients who had no relatives affected by lung cancer [11]. Janiszewska *et al.* [8] suggested that the *NOD2* 3020insC mutation may increase the risk of developing gastrointestinal cancer rather than BC. Similarly, in a study conducted by Kurzawski *et al.*, the frequency of the 3020insC mutation in 250 patients with non-hereditary nonpolyposis colorectal cancer at age >50 years was significantly higher in comparison to the control group (OR, 2.23; $p = 0.0046$) [9]. In another study, the risk of gastric cancer in *NOD2* 3020insC-mutation carriers at age >50 years was more than doubled (OR = 2.479; $p = 0.022$) and was almost even three-fold greater among women [10]. In a previous analysis, the presence of *NOD2* mutation was associated with an increased risk of a family history of breast, renal, and colorectal cancer [7]. In the present analysis, a family history of cancer, including BC, was observed more often in *NOD2*-mutation compared with the control group; in patients at age ≥ 50 years. In the group of patients at age < 50 years, the presence of cancer in family history was similar between *NOD2*-mutation carriers and the control group. Conversely, a family history of colorectal and pancreatic cancer was observed significantly more often in younger *NOD2*-mutation carriers compared with the control group.

The association between *NOD2* mutation and clinicopathological factors in cancer was described only in a few data [7, 12]. Lakatos *et al.* conducted a study on patients with colorectal cancer and reported no association between clinicopathological factors (such as patient's age or symptoms at diagnosis) and the *CARD15/NOD2* variants carrier status [12]. In a previous analysis, no differences between *NOD2* (3020insC) mutation carriers and non-carriers were reported, according to co-morbid condition, drugs, tumour size, steroid receptor status, and five-year overall survival. *NOD2* mutation in women with BC was characterised by lymph nodes without metastasis (N0), lower histological grade (G<3), and negative HER2 receptor status (HER2–) [7].

In the present analysis, the subgroup of BC *NOD2*-mutation carriers at age ≥ 50 years was characterised by lower tumour size (T1–T2), lower histological grade (G1–G2), positive steroid receptor status (ER+ and PR+), and tumours without HER2 overexpression. Patients at age < 50 years are characterised by lymph nodes without metastases, ER+ status, and tumours without HER2 overexpression. There was no difference between *NOD2*-mutation carriers and the control group observed, according to the BC histological type.

ER+ status and tumours without HER2 overexpression were mostly characteristic of patients with BC with *NOD2* mutation, independently of age, in comparison to the control group. Multivariate logistic regression has improved these results and has showed that patients at age ≥ 50 years have a lower risk of *NOD2* mutation compared with the younger age group (OR = 0.61; $p = 0.020$).

In the group of patients with *NOD2* mutation at age ≥ 50 years, histological grades G3 and HER overexpression were observed significantly less often compared with younger *NOD2*-mutation carriers.

Luminal A and luminal B HER2 BC subtypes were most characteristic of patients with *NOD2* mutation, independently of age. This analysis was conducted on a larger group of patients, in comparison to the previous study.

The present study has potential limitations. The most important limitation is the group size. Due to some differences between the previous and present studies, a larger group of patients is required. HER2-negative status was characteristic of patients with BC with *NOD2* mutation,

independently of age, compared with the control group, in the previous and present studies. The other factors such as N0 and G1–G2 were reported from univariate analysis in the present study, but in different subgroups. N0 is observed significantly more often in *NOD2*-mutation carriers at age < 50 years and G1–G2 in the subgroup of individuals at age ≥ 50 years. In a previous study, patients without *NOD2* mutation and *NOD2*-mutation carriers had similar ages at diagnosis. This can be the cause of the differences.

Conclusions

A family history of BC was characteristic of *NOD2*-mutation carriers at age ≥ 50 years. HER2 overexpression and ER status were significantly associated with the presence of *NOD2* mutation in patients with BC, independently of age. Luminal A or luminal B HER2-negative BC subtypes were most characteristic of patients with *NOD2* mutation, independently of age.

In *NOD2*-mutation carriers at the age ≥ 50 years, the presence of higher tumour size, G3 or HER2 overexpression were lower compared with younger patients. Multivariate analysis has shown that only G significantly differentiates both groups of *NOD2*-mutation carriers.

The authors declare no conflict of interest.

References

1. Inohara N, Nuñez G. NODs intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 2003; 3: 371-382.
2. Hugot JP, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 1996; 29: 821-823.
3. Al Nabhani Z, Dietrich G, Hugot JP, Barreau F. *NOD2*: The intestinal gate keeper. PLoS Pathog 2017; 13: e1006177.
4. Lubiński J, Huzarski T, Kurzawski G et al. The 3020insC allele of NOD2 predisposes to cancer of multiple organs. Hered Cancer Clin Practice 2005; 3: 59-63.
5. Huzarski T, Lener M, Domagala W, et al. The 3020insC allele of NOD2 predisposes to early-onset breast cancer. Breast Cancer Res Treat 2005; 89: 91-93.
6. Kurzawski G, Suchy J, Cybulski C, et al. DNA tests for variants conferring low or moderate increase in the risk of cancer. Szczecin 2017: 1-10.
7. Huszno J, Kołosza Z, Tęcza K, Pamuła-Piłat J, Mazur M, Grzybowska E. Comparison between *NOD2* gene mutation carriers (3020insC) and non-carriers in breast cancer patients: a clinicopathological and survival analysis. Arch Med Sci Civil Dis 2018; 3: e10-e15.
8. Janiszewska H, Haus O, Lauda-Swieciak A, Bak A, Mierzwa T, Sir J, Laskowski R. The *NOD2* 3020insC Mutation in Women with Breast Cancer from the Bydgoszcz Region in Poland. First Results. Hered Cancer Clin Pract 2006; 15: 4: 15-19.
9. Kurzawski G, Suchy J, Kładny J, et al. The NOD2 3020insC mutation and the risk of colorectal cancer. Cancer Res 2004; 64: 1604-1606.
10. Teodorczyk U, Cybulski C, Jakubowska A, et al. Mutations and polymorphisms of genes moderate increase in gastric cancer risk. Hered Cancer Clin Pract 2012; 10 (Suppl 3): A22.
11. Lener MR, Oszutowska D, Castaneda J, et al. Prevalence of the NOD2 3020insC mutation in aggregations of breast and lung cancer. Breast Cancer Res Treat 2006; 95: 141-145.
12. Lakatos PL, Hitre E, Szalay F, et al. Common *NOD2*/CARD15 variants are not associated with susceptibility or the clinicopathological characteristics of sporadic colorectal cancer in Hungarian patients. BMC Cancer 2007; 7: 54.

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