Research Article

Effect of neutrophil-to-lymphocyte ratio and vitamin D3 levels on the pathological complete response after neoadjuvant treatment in TNBC and HER2-positive early breast cancer – results of a prospective study

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Background: The neutrophil-to-lymphocyte ratio (NLR) is an inexpensive and readily available blood test that may predict response to neoadjuvant chemotherapy (NACT) in breast cancer patients. However, its utility as a biomarker remains unclear.

Objective: To evaluate NLR as a predictive biomarker for pathological complete response (pCR) to NACT in triple-negative breast cancer (TNBC) and HER2-positive breast cancer patients.

Methods: In this prospective analysis, 96 patients with early stage TNBC (n=51) or HER2-positive (n=45) breast cancer receiving NACT were assessed. NLR and other variables were analyzed for correlation with pCR.

Results: pCR rates were 49% for TNBC and 46.7% for HER2-positive patients. NLR did not correlate with pCR in either subset. Only chemotherapy regimen predicted pCR. Mean NLR was lower in pCR groups but did not reach statistical significance.

Conclusion: Pretreatment NLR did not predict pCR after NACT in this cohort of early stage TNBC and HER2-positive breast cancer patients. NLR should be further studied in combination with other biomarkers to determine its utility as a universal predictive biomarker for NACT response.

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Introduction

Breast cancer has been a global health concern for many years. In Poland, breast cancer was the most frequently diagnosed malignancy in women in 2019 (19,620 plus cases) and was the second leading cause of cancer deaths after lung cancer (6,951 deaths) [1]. For this type of cancer, the prognosis depends on many factors. These include patient characteristics (such as performance status, biological age, and/or race), tumour stage and subset, and the response to systemic therapy [2].

Many studies emphasize that prognosis may be affected not only by the characteristics of the tumour itself, but also by the host response, including the inflammatory response. As such, inflammatory biomarkers may provide important information regarding the prognosis of breast cancer patients [3]. It was demonstrated as early as 10+ years ago that cytokines and chemokines secreted by both tumour and host cells (e.g., leukocytes) can contribute to metastasis [4]. Classifying inflammation as a factor promoting tumour transformation and disease progression has initiated research on immune cells as predictive and prognostic factors. Particular attention was paid to the readily available pool of cells determined by blood counts. The evaluation of their clinical utility focuses primarily on the correlation between different cell fractions.

The most common inflammatory biomarkers used in daily clinical practice include leukocyte count, lymphocyte count, neutrophil count, C-reactive protein (CRP) level, and such indicators as a neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR). They may be used to evaluate the inflammatory response of the patient's body [4].

A high level of neutrophils is associated with poor prognosis as it inhibits the immune system by suppressing the cytotoxic activity of T cells [4][5]. On the other hand, the presence of tumour-infiltrating lymphocytes (TILs) has been associated with a better response to chemotherapy and prognosis [6].

NLR has been found to be an independent prognostic factor for survival in most adjuvant treatment studies [7][8][9][10][11]. However, no significant correlation was found between the survival and NLR for early breast cancer patients receiving neoadjuvant chemotherapy (NACT) and advanced BC patients [10][11][12][13]. Some data showed that IBM could be predictive of chemotherapy-related toxicity [12]

Several publications have shown that pre-NACT NLR is correlated with pCR. [13]. Chae suggests in her study that this correlation is particularly pronounced in higher malignancy tumours like TNBC and HER2-positive cancers due to their higher chemosensitivity compared to luminal cancers. The TNBC patients with a lower NLR had a significantly higher pCR (42.1% vs 18.4%, p = 0.018) [14].

Aim

A prospective analysis was performed to evaluate NLR as a potential biomarker for achieving pCR for patients with triple-negative and HER2-positive cancers receiving NAC at the Reconstructive Surgery Department, National Research Institute of Oncology Breast Cancer. In addition, the data obtained were also analysed against the menopausal status, BMI, and vitamin D3 levels for two age groups (namely patients below the age of 65 and 65+ years old) to see if the correlations in elderly patients, if any, might be different.

Ethics Statement

The study protocol was approved by the Ethics Committee of Maria Sklodowska-Curie National Research Institute of Oncology (No 21/2017). The study was performed in accordance with Good Clinical Practice standards and the ethical principles that have their origin in the Declaration of Helsinki. All patients provided their informed consent for the use of their data for research purposes.

Materials and Methods

The analysis comprised early or locally advanced breast cancer patients eligible for neoadjuvant chemotherapy between 01/03/2017 and 30/06/2019 who gave their written consent to participate in the study.

All patients met the following criteria: performance status ECOG 0-1, histopathological diagnosis of HER2-positive or three-negative invasive breast cancer, breast cancer staging (cT1-2, cN0-1, M0), neoadjuvant therapy with a TCbH-P regimen, or the sequential 4xAC regimen followed by 12xPCL +/- Carboplatin and baseline left ventricular ejection fraction (LVEF) of ≥50%.

Initially, a core needle biopsy was used to diagnose a breast tumour. Suspicious axillary lymph nodes were evaluated using an ultrasound-guided fine needle aspiration biopsy. The presence of ERs and PRs was identified when ≥1% of nuclei stained positive whereas if <1% of nuclei stained positive, it was

considered a negative result. The HER2 status was deemed positive based on an immunohistochemistry score of 3+, or 2+, which was confirmed with a FISH test.

All patients underwent surgery when neoadjuvant therapy was completed.

All hormone receptor-positive patients also received hormone replacement therapy after the surgery, as per the current guidelines, for at least 5 years.

Prior to the neoadjuvant therapy, the patients were given mammography, breast and regional lymph nodes ultrasound, abdominal ultrasound or CT, chest X-ray or CT, bone scintigraphy, ECG, echocardiography, and blood tests.

All patients were routinely given peg-GCSF after each course of TCH and AC chemotherapy, but no patients received GCSF before they were commenced on systemic therapy.

Adverse events were evaluated according to the Common Terminology Criteria for Adverse Events, version 4.0 [15].

Statistical Analysis

Descriptive statistics were used to describe the characteristics of the study group: mean, median, first and third quartile (IQR) values, and range. The normality of the distribution of the individual parameters evaluated in the study was verified using the Shapiro-Wilk test. In the case of normal distribution, the Student's t-distribution test was used to compare the mean values of independent variables. For the other parameters without normal distributions, appropriate methods of statistical analysis were selected based on non-parametric tests. The Mann-Whitney U test was used to compare numerical variables between the two groups observed. Spearman's rank correlation coefficient (monotonic relationships, linear or not) and Pearson correlation coefficient (linear monotonic relationships) were used to examine the existence of monotonic relationships between two variables. All calculations and graphs were performed using the R stats package, version 4.0.2.

Results

96 patients met the inclusion criteria and gave their consent to participate in the study. TNBC was diagnosed in 51 patients, whereas HER2-positive cancer was diagnosed in 45 patients. All subjects were Caucasian. The group characteristics are shown in Table 1.

	TNBC		HER2-positive	
Variable	**Parameter**	**Value**	**Parameter**	**Value**
Age [years]	N	51	N	45
	Mean (SD)	44.02	Mean (SD)	47.16
	Median (IQR)	42	Median (IQR)	44
	Range	26 - 67	Range	23 - 70
Age [years] - breakdown	< 65 years old	88.2% (N=45)	< 65 years old	86.7% (N=39)
	≥ 65 years old	11.8% (N=6)	≥ 65 years old	13.3% (N=6)
BMI	Mean (SD)	26	Mean (SD)	26.09 (4.75)
	Median (IQR)	25.8 (22.45 - 29.8)	Median (IQR)	25.5 (22.7 - 30.2)
	Range	17.5 - 37.8	Range	16.1 - 36.2
Neutrophils [g/dL]	N	51	N	45
	Mean (SD)	6.06 (5.04)	Mean (SD)	4.91 (3.17)
	Median (IQR)	4.82 (2.96 - 7.8)	Median (IQR)	4.22 (2.64 - 6.13)
	Range	1.27 - 26.24	Range	0.95 - 14.99
Neutrophils [g/dL]	N	51	N	45
	Mean (SD)	1.83 (0.84)	Mean (SD)	1.7 (1.34)
	Median (IQR)	1.71 (1.4 - 2.01)	Median (IQR)	1.31 (0.82 - 2)
	Range	0.33 - 6.19	Range	0.51 - 8.32
NLR [1]	N	51	N	45
	Mean (SD)	4.44 (6.34)	Mean (SD)	4.82 (5.34)
	Median (IQR)	2.51 (1.71 - 4.36)	Median (IQR)	2.4 (1.6 - 6.7)
	Range	0.54 - 40.91	Range	0.51 - 26.3
Vitamin D3 [IU]	N	51	N	45
	Mean (SD)	22.97 (10.64)	Mean (SD)	25.49 (12.19)
	Median (IQR)	21 (16 - 31)	Median (IQR)	23 (15 - 33)

	TNBC		HER2-positive	
Variable	**Parameter**	**Value**	**Parameter**	**Value**
	Range	7 - 52.3	Range	9 - 61
Menopause	Premenopausal	76.5% (N=39)	Premenopausal	64.4% (N=29)
	Post-menopausal	23.5% (N=12)	Post-menopausal	35.6% (N=16)
Response to treatment	pCR	49.0% (N=25)	pCR	46.7% (N=21)
	Non-pCR	51.0% (N=26)	Non-pCR	53.3% (N=24)
Chemotherapy	AC + PCL	72.5% (N=37)	TCH	86.7% (N=39)
	AC + PCL + carbo	27.5% (N=14)	AC + PCL + carbo	13.3% (N=6)
RCB [2]	I	46.2% (N=12)	I	45.8% (N=11)
	II	34.6% (N=9)	II	45.8% (N=11)
	III	19.2% (N=5)	III	8.3% (N=2)

Table 1. Patient characteristics

[1] *NLR: neutrophil-to-lymphocyte ratio*

[2] *This applies only to patients with a response to non-pCR treatment*

The subjects in both groups were predominantly under 65 years of age (88.2%, mean age 44.02 years for the TNBC group; 86.7%, mean age 47.16 years). The neutrophil and leukocyte counts were 6.06 and 1.83 in the TNBC group and 4.91 and 1.7 in the HER2-positive cancer group, respectively. The neutrophil-to-lymphocyte ratio (NLR) was 4.44 and 4.82, respectively.

Vitamin D3 levels were 22.97 ng/mL in the TNBC group and 25.49 ng/mL in the HER2-positive cancer group, respectively. The mean BMIs for both groups were 26 and 26.9.

In both groups, most subjects were premenopausal (76.5% and 64.4%, respectively).

In the TNBC group, the majority of patients were given neoadjuvant therapy according to the 4xAC+12PCL regimen (72.5%, N=37), while the other patients received a 4xAC+12PCL+ carboplatin

regimen (27.5%, N=14). A pathological complete response (pCR) was achieved by 49.0% of subjects (N=25). The mean NLR for patients who achieved pCR was 3.69 (median 2.85; range 1.84-5.35). For non-PCR patients, this value was 4.9 (median 2.32; range 1.7-3.1). No statistical correlations were found between the parameters under evaluation (i.e., age, menopausal status, BMI, neutrocyte count, lymphocyte count, NLR, vitamin D3 level) and the probability of pCR, except for the type of chemotherapy used. Significantly more pCRs were observed in the group of patients receiving the carboplatin-containing regimen (p = 0.0128). The correlations between the parameters evaluated in the study and the response to neoadjuvant treatment in the TNBC patient group are shown in Table 2.

Variable	Parameter	pCR	non pCR	p-value
Age [years]	N	25	26	0.7917
	Mean (SD)	43.64	44.38	
	Median (IQR)	39	43.5	
	Range	29 - 67	26 - 67	
Age [years] - breakdown	< 65 years old	92% (N=23)	84.6% (N=22)	0.668
	≥ 65 years old	8% (N=2)	15.4% (N=4)	
BMI				0.6154
	Mean (SD)	25.63 (4.69)	26.35 (5.44)	
	Median (IQR)	25.7 (22.4 - 29.7)	26.65 (23.5 - 29.65)	
	Range	17.5 - 32.7	17.5 - 37.8	
Neutrophils [G/dL]				0.5341
	Mean (SD)	6.29 (5.04)	5.84 (5.12)	
	Median (IQR)	5.55 (2.92 - 8.11)	4.27 (3.01 - 5.69)	
	Range	1.27 - 26.24	1.49 - 25.83	
Neutrophils [/dL]				0.9925
	Mean (SD)	1.91 (1.08)	1.76 (0.52)	
	Median (IQR)	1.68 (1.4 - 2.27)	1.71 (1.55 - 1.98)	
	Range	0.69 - 6.19	0.33 - 2.97	
NLR [1]				0.4682
	Mean (SD)	3.96 (3.28)	4.9 (8.34)	
	Median (IQR)	2.85 (1.84 - 5.35)	2.32 (1.7 - 3.1)	
	Range	0.54 - 14.34	0.89 - 40.91	
Vitamin D3 [IU]				0.1835
	Mean (SD)	24.81 (11.29)	21.19 (9.86)	
	Median (IQR)	23 (17 - 33)	17.5 (15 - 22.75)	

Variable	Parameter	pCR	non pCR	p-value
	Range	7 - 52.3	8 - 44	
Menopause	Premenopausal	76% (N=19)	76.9% (N=20)	1
	Post-menopausal	24% (N=6)	23.1% (N=6)	
Chemotherapy	AC + PCL	56% (N=14)	88.5% (N=23)	0.0128
	AC + PCL + carbo	44% (N=11)	11.5% (N=3)	

Table 2. Comparison of variables in relation to pCR achieved in TNBC patients

[1] *NLR: neutrophil-to-lymphocyte ratio*

Using the ROC curve the optimal cut-off point for the NLR was determined, which was 2.8. For this value of this parameter, the specificity of the method was 0.52 and the sensitivity - 0.69. The positive predictive value of NLR over 2.8 was 60%, and the negative predictive value was 62%.

The area under the ROC curve (AUC parameter) was 0.55. The calculated 95% confidence interval of this parameter ranged from 0.40 to 0.72.

In this case, it means that the breakdown of patients against the designated cut-off point for NLR cannot be used to distinguish between the pCR and non-pCR groups. Thus, the classifier under consideration cannot be considered better than the random selection of individuals into both groups.

In the TNBC group, there were not statistically significant ($p>0.05$) correlations between the evaluated parameters, namely the age, NLR, vitamin D3 level, BMI, and the probability of pCR.

The women from the HER2-positive breast cancer group received the TCH regimen (docetaxel, carboplatin, trastuzumab) therapy (86.7%) (N=39) or the sequential therapy (4xAC+12 PCL with trastuzumab). In this group, the pCR was achieved in 46.7% of patients (N=21), whereas 53.3% had residual breast cancer (RBC). In pCR patients, the neutrophil-to-leukocyte ratio (NLR) averaged 5.87 (median 3.46; range 0.51-26.3), while for non-PCR patients it averaged 3.9 (median 3.9; range 0.67-19.47). Likewise, no statistical correlations were found in this group between the evaluated parameters (i.e., the age, menopausal status, BMI, neutrocyte count, lymphocyte count, NLR, vitamin

D3 level) and the probability of pCR, except for the type of chemotherapy used ($p = 0.0232$). The correlations between the parameters under evaluation and the response to neoadjuvant treatment in the group of HER2-positive cancer patients are shown in Table 3.

Variable	Parameter	pCR (N=21)	Non-pCR (N=24)	p-value
Age [years]	N	21	24	0.1707
	Mean (SD)	44.43 (11.57)	49.54 (13.05)	
	Median (IQR)	44 (38 - 51)	46 (41 - 61)	
	Range	23 - 67	25 - 70	
Age [years] - breakdown	< 65 years old	90.5% (N=19)	83.3% (N=20)	0.6695
	≥ 65 years old	9.5% (N=2)	16.7% (N=4)	
BMI	N	21	24	0.1178
	Mean (SD)	24.89 (4.96)	27.14 (4.39)	
	Median (IQR)	24.4 (20.6 - 29.2)	25.7 (24.2 - 30.3)	
	Range	16.1 - 33.1	20.9 - 36.2	
Neutrophils [g/dL]	N	21	24	0.2601
	Mean (SD)	5.54 (3.75)	4.37 (2.51)	
	Median (IQR)	4.63 (2.82 - 6.63)	3.85 (2.53 - 5.35)	
	Range	0.95 - 14.99	1.78 - 12.07	
Neutrophils [g/dL]	N	21	24	0.5315
	Mean (SD)	1.6 (1.08)	1.79 (1.56)	
	Median (IQR)	1.27 (0.81 - 1.87)	1.46 (0.93 - 2)	
	Range	0.51 - 4.4	0.62 - 8.32	
NLR [1]	N	21	24	0.5021
	Mean (SD)	5.87 (6.39)	3.9 (4.13)	
	Median (IQR)	3.46 (1.6 - 9.38)	2.02 (1.58 - 5.05)	
	Range	0.51 - 26.3	0.67 - 19.47	
Vitamin D3 [IU]	N	21	24	0.6048
	Mean (SD)	24.48 (11.43)	26.38 (13)	
	Median (IQR)	23 (16 - 32)	26 (14.75 - 34.25)	

Variable	Parameter	pCR (N=21)	Non-pCR (N=24)	p-value
	Range	9 - 49	9 - 61	
Menopause	Premenopausal	71.4% (N=15)	58.3% (N=14)	0.5462
	Post-menopausal	28.6% (N=6)	41.7% (N=10)	
Chemotherapy	TCH	100% (N=21)	75% (N=18)	0.0232
	Sequential	0% (N=0)	25% (N=6)	

Table 3. Comparison of variables relative to response achieved in HER2-positive cancer patients

[1] *NLR: neutrophil-to-lymphocyte ratio*

Accordingly, to plot the ROC curve and provide its parameters for TNBC patients, the group of non-pCR patients was assigned a rank – 1, and the group of pCR patients - 0.

Using the ROC curve the optimal cut-off point for the NLR was determined, which was 3.1. For this value of this parameter, the specificity of the method was 0.52 and the sensitivity - 0.62. The positive predictive value of NLR over 3.1 was 60%, and the negative predictive value was 55%.

The area under the ROC curve (AUC parameter) was 0.56. The calculated 95% confidence interval of this parameter ranged from 0.38 to 0.74.

In this case, it means that the breakdown of patients against the designated cut-off point for NLR cannot be used to distinguish between the pCR and non-pCR groups. Thus, the classifier under consideration cannot be considered better than the random selection of individuals into both groups.

In the HER-2 positive cancer group, there was a statistically significant negative correlation was found (Spearman's rank correlation coefficient) between the evaluated parameters, namely the age, NLR, vitamin D3 level, BMI, and the probability of pCR.

In addition, a statistically significant NLR and BMI correlation was also found here, which was positive ($p = 0.029$).

Discussion

Our analysis showed that NLR has no predictive value in evaluating the achievement of pathological complete response to neoadjuvant therapy in TNBC- and HER2-positive breast cancer patients. These are results that contradict those of a meta-analysis published in 2021 by Zhu and colleagues [13]. It addressed the importance of NLR as a predictor of response to neoadjuvant treatment in breast cancer patients, involving nearly 6,000 patients from 19 studies published between 2014 and 2020 and it confirmed the importance of NLR as a biomarker of response to neoadjuvant treatment. Most of the studies (74%) were conducted in China, Korea, and Japan. Other studies were conducted in Spain, Italy, Mexico, and Turkey. Seventeen studies analysed the correlation between NLR and pCR, 11 studies - the correlation between NLR and DFS, and 6 studies – the correlation between NLR and OS. It is worth noting that the NLR value indicating a high chance of achieving pCR varied widely, ranging from 1.63 to 3.33. However, it should be highlighted that in most of the publications covered by the meta-analysis, the subjects were heterogeneous in terms of a biological subset. Furthermore, a statistically significant correlation between NLR and pCR was particularly evident in Asian studies (OR 1.726; 95% CI 1.167 - 2.553; p=0.006). They were not confirmed in non-Asian subjects (OR 1.189; 95% CI 0.974-1.451; p=0.089), which may explain why the correlation was not found in our analysis.

It seems that NLR should not be considered a universal biomarker of response to neoadjuvant treatment. In light of the research on the role of other factors that are proven to affect the response to neoadjuvant treatment (TILs, Ki67, and other inflammatory markers), we should focus on evaluating the correlation between multiple biomarkers of NACT response [16][17][18][19].

A study by Yoon and others found a linear inverse correlation between TILs and ANC [18]. It seems that neutrophils may act against the immune system via several mechanisms. Experimental data suggested that neutrophils can suppress the cytolytic activity of lymphocytes, natural killer cells, and activated T-cells [20]. Activated neutrophils have also been reported to secrete myeloperoxidase, which results in the suppression of lymphocyte function [21]. Besides, tumour-associated neutrophils may influence tumour immunity and, indirectly, tumour progression by progression by regulating the microenvironment. The enzymatic activity of neutrophils has been found to promote the remodelling of the extracellular matrix, which results in the release of basic fibroblast growth factors and migration of endothelial or tumour cells [20][21]. Additionally, neutrophil-derived oncostatin M may stimulate cancer cells to grow and increase invasiveness in breast cancer [21]. It has also been reported

that high ANC may negatively impact TILs [18], which has been repeatedly shown to be associated with a good prognosis in the aggressive breast cancer subset [22][23]. To date, the association between ANC and NLR, as well as TILs, has not been conclusively explained, although there are pending studies focusing on this correlation [18].

It is problematic to establish normal NLR values and thus a cut-off point, which – if exceeded, would clearly denote a poorer NACT response. The NLR values in a healthy population (aged 21 to 66) ranged from 0.78 to 3.58. The median value is assumed to be 1.65 (1.2-2.15) [24]. Zahorec makes an interesting suggestion to facilitate the interpretation of the results, namely the NLR values are given by assigning them a clinical status. For a healthy population, the NLR values range between 1 and 2. NLR in the grey zone (2.3-3.0) may serve as a warning of a pathological process present in an organism. Values above 3.0 and below 0.7 are considered pathological. According to this NLR scale, upon diagnosing breast cancer, the median NLR falls in the grey zone. Notably, IQR NLR values in both subsets, particularly in the HER2+ subset, were already pathological. Nonetheless, the author stresses that abnormal NLR values may be caused by co-morbidities [25].

The presence of co-morbidities affecting NLR values is usually not considered in publications on the correlation between NLR and NACT response, but it may ultimately affect the results of statistical analyses, especially for small group sizes, which is the case in this study [26][27].

Our analysis considers only some factors that might result in increasing the NLR values, namely the age of the patients (co-morbidity is statistically more likely in 65+ patients) and BMI. No obvious correlations were found between these parameters and NLR, except for a positive correlation between NLR and BMI in HER2-positive breast cancer patients.

The NLR values determined in our study based on the ROC analysis were 2.8 for TNBC patients and 3.1 for HER2-positive patients. It should be noted that in most studies the NLR below 2.3 was associated with a better response to neoadjuvant treatment and improved survival rates [26][27][28][29].

It is important to note that the final outcome of neoadjuvant treatment depends also on the treatment modality. Undoubtedly, more effective modalities guarantee higher pCRs, regardless of the biomarkers. This is clearly noticeable in our study and may ultimately affect statistical calculations. Please note that in our study, 65+ years old subjects achieved pCR less frequently. This is probably due to the less aggressive treatment used in these patients. Only 16.7% of TNBC subjects received

carboplatin in combination with PCL, whereas 33.3% of HER2-positive breast cancer subjects received the TCH regimen.

Recent studies have reported an inverse correlation between vitamin D3 deficiency and breast cancer outcomes. In Poland, more than 90% of the population was found to have vitamin D3 deficits [30]. The results of our study were consistent with those obtained in the Polish population. Similar vitamin D deficiencies are also shown in studies conducted in other European countries [31].

The demonstration of the vitamin D3 receptor (VDR) expression and enzymes involved in vitamin D metabolism on various cells in vitro suggested its pleiotropic actions. In the immune system, not only the presence of VDR in various cells (B and T lymphocytes, monocytes, macrophages, neutrophils) was confirmed but also the presence of 1α-hydroxylase (CYP27B1) enabling the formation of an active form of vitamin 1,25(OH)2D3. Studies have shown that the calcitriol effect depends both on the type of immune cell as well as its activity status [32]. These data suggest that vitamin D plays an important role in the proper functioning of this system, which is supported by clinical observations showing vitamin D deficiency in patients with immune system disorders. Several publications show a correlation between vitamin D3 and NLR levels [33][34]. An inverse correlation between vitamin D3 and NLR values has been observed in patients with inflammatory conditions [32][34].

In light of the literature data, the problem of how to interpret the results of vitamin D levels in patients with malignant neoplasms is complex. The lower values observed may be related to population vitamin D deficiency, inflammation, as well as to cancer accompanied by inflammation. High expression of VDR and CYP27B1 was confirmed in breast cancer cells. Normal breast cells, any line of which can undergo malignant transformation, have high VDR heterogeneity, which makes it difficult to explain the importance of vitamin D in the prevention and treatment of breast cancer [35] [36]. In our study, we observed vitamin D values below the recommended normal values regardless of the cancer biological subset. The findings are consistent with both the results of studies of the Polish population showing common vitamin D deficiency and with publications showing vitamin D deficiency in patients diagnosed with breast cancer [37]. In contrast, our study did not confirm the reports of other authors who showed statistically lower vitamin D levels in TNBC patients [38].

We also analysed the correlation between NLR and vitamin D. Although neither parameter differed between the studied groups, i.e., the TNBC group and the HER2-positive group, a different correlation between vitamin D and NLR values was observed in each group. No significant correlations between

NLR and vitamin D levels were found in the TNBC group. An inverse correlation between vitamin D and NLR values was observed in all HER2-positive subjects. Further analysis, which included the type of response to neoadjuvant treatment, highlighted differences between HER2-positive breast cancer patients with different NACT responses. An inverse correlation between NLR and vitamin D levels was confirmed in pCR patients. No similar analysis has been found in the available literature.

In contrast, in the group of HER2-positive cancer subjects who did not achieve a pCR to NACT, NLR correlated positively with BMI values. In breast cancer, obesity is considered a poor prognostic factor in postmenopausal women. Chronic inflammation has been linked to both obesity and the neoplastic process. Recent studies confirm that elevated NLR combined with obesity are poor prognostic factors [39][40]. In our study, a demonstrated correlation between NLR and BMI was observed only in obese and overweight patients who did not respond to NACT.

Conclusions

The neutrophil-to-lymphocyte ratio (NLR) is an available and easily determined parameter. It may be a good predictor of achieving a pathological complete response to neoadjuvant therapy; however, the complex mechanisms responsible for inflammatory processes, as well as various factors that may modulate the immune response in the course of breast cancer, such as TILs, vitamin D3 levels, changes in the immune system resulting from aging and other co-morbidities may significantly affect the value of this biomarker. Therefore, the introduction of NLR as a biomarker of the NACT response does undoubtedly require further research and consideration of several other parameters, and probably specification of patient groups to which it could be applied.

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