

Changes in morphological and immunohistochemical parameters of tumors during neoadjuvant hormone therapy in postmenopausal patients with luminal type of breast cancer

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ABSTRACT

Aim: Breast cancer (BC) remains the most common malignant disease among women in Ukraine and worldwide. The indication for neoadjuvant hormone therapy (NAHT) is HR+/HER2- breast cancer of stage II-III. However, there are currently insufficient data on its impact on the IHC biological characteristics of tumors and clinical outcomes.

Materials and Methods: The study was conducted at the Uzhgorod Treatment and Diagnostic Oncology Center during 2015–2023. At the diagnostic stage, morphological verification and IHC profile were determined based on trephine biopsy material. The study included 28 patients with HR+/HER2- BC, aged from 38 to 75 years old. All patients were prescribed NAHT with the use of aromatase inhibitors for 6.10 ± 2.23 months. The expression levels of ER, PR, Ki-67, category G were assessed before and after NAHT using histological and IHC analyses.

Results: After NAHT, we observed a decrease in Ki-67 levels from 25.31% to 10.25%, ER expression levels from 97% to 84%, and PR expression levels from 87% to 11%. There were changes in category G: G1 was observed in 45.83% of patients, G2 in 54.17%. The staging of BC also decreased. A moderate degree of curative tumor pathomorphosis was achieved in 76.47% of patients, and complete regression (pCR) in 5.88%.

Conclusions: Neoadjuvant hormone therapy demonstrated high efficacy in the treatment of patients with luminal HR+/HER2- BC of stage II-III. In most cases, it was possible to reduce the proliferative activity of the tumor, which was accompanied by a decrease in tumor staging. This made it possible to perform organ-preserving surgeries in 60.71%, which confirms the feasibility of using NAHT as an alternative to chemotherapy in certain groups of patients.

KEY WORDS: breast cancer, neoadjuvant hormone therapy, estrogen and progesterone receptors, Ki-67 tumor proliferation index

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LIST OF ABBREVIATIONS

BC - breast cancer
IHC - immunohistochemical study
NAHT - neoadjuvant hormone therapy
HR+ - positive hormone receptors
HER2- human epidermal growth factor receptor 2
ER - estradiol receptors
PR - progesterone receptors
Ki-67 - tumor proliferation index
NACT - neoadjuvant chemotherapy
pCR - pathological complete regression

INTRODUCTION

Breast cancer (BC) is the most common malignant disease and the leading cause of death among women worldwide [1]. In 2020, 2,261,419 cases of BC were diagnosed in the female population worldwide, of which 531,086 were diagnosed in Europe [2]. In 2021, the standardized incidence rate per 100,000

population for women in Ukraine was 62.4 [3]. The indication for prescribing neoadjuvant hormone therapy (NAHT) is HR+/HER2- BC of stage II-III [4]. Despite effective changes after NAHT, it is used quite rarely [5]. Approximately 70-75% of breast malignant tumors are hormone receptor positive (HR+), so the use of NAHT should be considered as a priority for the treatment of patients with such tumors [6]. The low cost and availability of drugs are also a significant advantage of NAHT, since 70% of deaths from breast cancer occur in patients with low or middle incomes [1]. The expression levels of ER, PR and Ki-67 are considered to be prognostic indicators of tumor response to NAHT. As described in studies, expression levels tend to decrease with NAHT [7]. There is no consensus on the duration of NAHT [8,9]. Some believe that 3-6 months before surgery is the gold standard [9], while others believe that longer use is necessary [8]. At the conference "Optimization of Treatment for Patients with Primary Breast Cancer - a Brief Summary

Table 1. ER, PR, Ki67 and Grade data before and after NAHT

	count		mean		median		Std		q25		q75		min		max	
Surgery	After	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before
ER	15	15	84,66667	97,33333	90	100	20,91365	7,98809	80	100	97,5	100	20	70	100	100
PR	15	15	11,66667	74,73333	5	90	15,43033	35,07638	0	65	22,5	100	0	0	40	100
Ki67	16	16	10,25000	25,31250	10	20	9,16151	16,87886	2	15	20	40	1	5	30	60
Grade	24	24	1,54167	1,87500	2	2	0,50898	0,53670	1	2	2	2	1	1	2	3

of the Consensus Discussion" (St. Gallen, 2023), up to 70% of the discussion participants reported that the use of NAHT 2-4 weeks before surgery and monitoring of Ki-67 dynamics can provide valuable information for deciding to administer chemotherapy [10]. In a prospective cohort study of 146 patients with ER+/HER2- BC, NAHT led to effective changes [11]. It has been noted that for patients with HR+ BC, it is preferable to use NAHT rather than NACT, as the pCR rate was lower with NACT [12]. However, other researchers have reported that the frequency of organ-preserving surgeries and clinical response HR+ BC with NAHT are similar to those with NACT [4]. NAHT is also the method of choice for elderly women who have contradictions to or refuse neoadjuvant chemotherapy, as well as in cases of inoperable tumors [6]. It has been reported that 20–30% may have primary resistance to NAHT. Treatment of BC with hormonal drugs, even in cases of resistance, is less toxic [6]. There are opinions that Ki-67 cannot reflect the regression of the primary tumor after NAHT, but instead it is a predictor for assessing the progression-free survival of patients with BC [13]. Approximately 60-80% of patients can undergo organ-preserving surgery after NAHT [6]. Organ-preserving surgeries have a positive effect on the psychosocial and cosmetic outcomes for patients with BC, and they are the main advantage of NAHT [14].

AIM

To study the effect of NAHT on the morphological and IHC parameters of the tumor in luminal types of breast cancer.

MATERIALS AND METHODS

Examination and treatment of 28 patients with HR+ / HER2- BC of stage II-III was carried out at the Uzhhorod Treatment and Diagnostic Oncology Center in 2015-2023. The age group of patients was from 38 to 75 years, with an average age of 56.52 ± 9.83 years. All women had postmenopausal status at the time of NAHT. Those patients who were premenopausal at the time of diagnosis of BC and before the start of NAHT

were transferred to artificial menopause by surgery or medication. Confirmation of the presence of luminal type malignant tumors was performed on trephine biopsy material with histological and IHC studies, which obtained data on the expression levels of ER, PR, Ki-67 and category G. IHC studies were performed using the electrochemiluminescent method in the certified laboratory CSD (Kyiv). After completion of NAHT and surgeries, a postoperative histological and IHC study was performed with the determination of the degree of therapeutic pathomorphosis using the RCB System, which allowed to detect changes in the morphological parameters of tumors and assess their sensitivity to NAHT. 28 patients were prescribed aromatase inhibitors (letrozole), the average duration of administration was 6.10 ± 2.23 months. NAHT was discontinued for patients on the day of surgical interventions for BC or one day before surgery.

STATISTICAL METHODS

The study results were statistically processed in the Google Colab environment using the TableOne, scipy and DescTools libraries. Comparison of results before and after surgery was performed using the Wilcoxon paired test (for ER, PR, Ki-67) and the Stewart-Maxwell test (for comparing the G category and cancer stage before and after treatment). Paired comparisons were used to analyze the pre- and post-surgery indicators. In this case, only patients for whom both indicators were present were taken into account. The expression levels of ER, PR were compared in 15 patients, the Ki-67 level in 16, and category G in 24. Descriptive statistics are given in Table 1.

RESULTS

Expression levels of ER. Before the start of NAHT administration in patients with HR+ BC of stage II-III, the average ER value was within 97.33%. Since the distribution of differences between ER levels before and after surgery was not normal according to statistical methods, as determined by the Shapiro-Wilk test, $p=0.0176$, the nonparametric Wilcoxon test was used

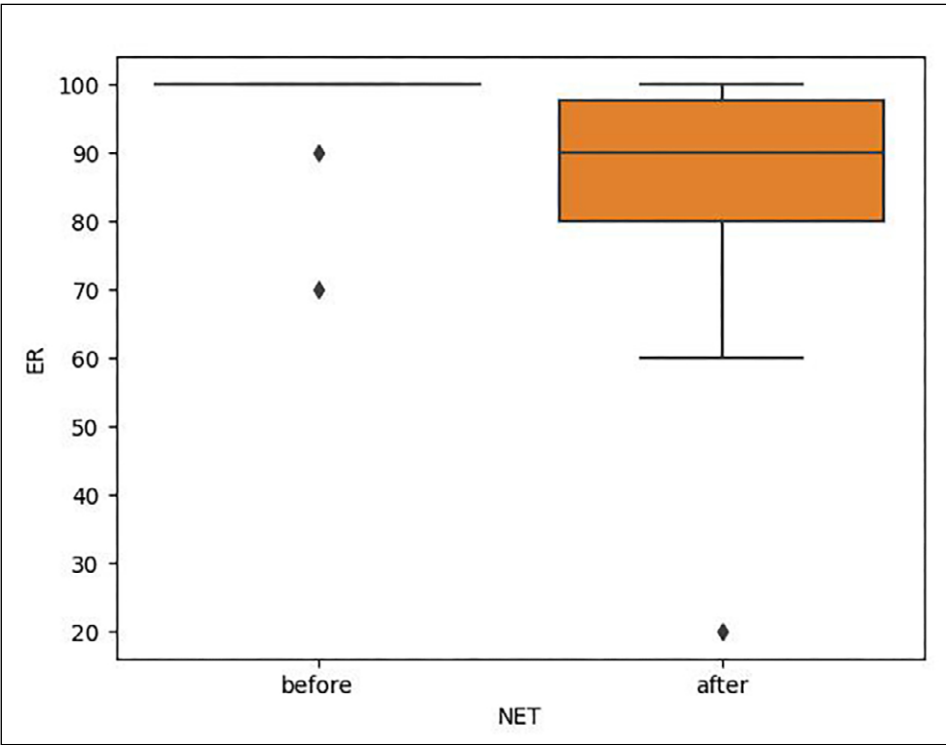


Fig. 1. Comparison of ER expression levels before and after NAHT.

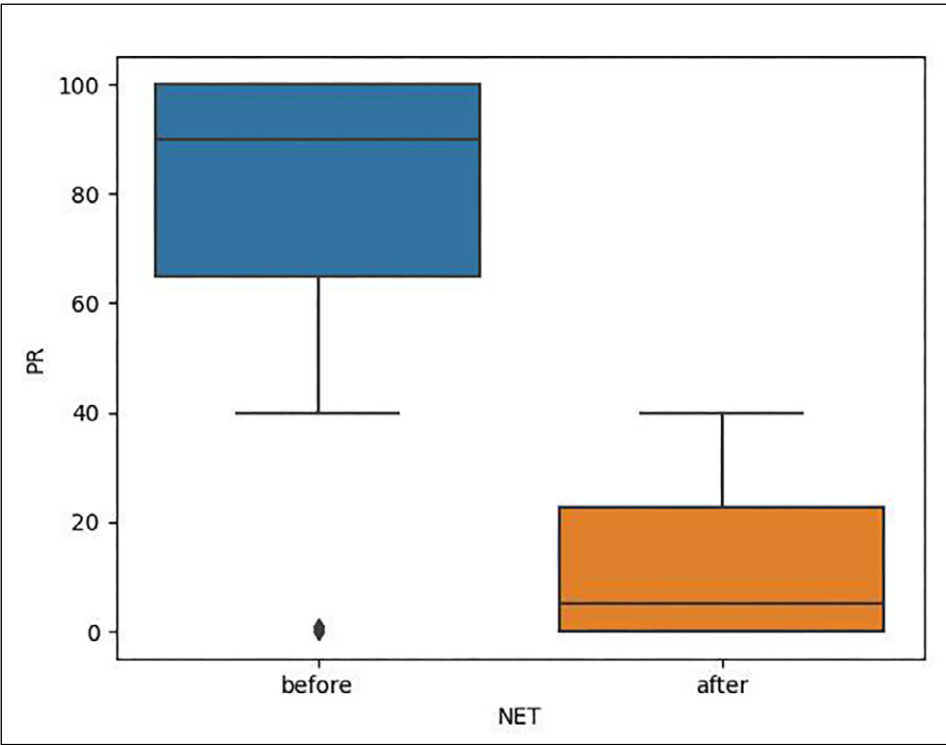


Fig. 2. Comparison of PR expression levels before and after NAHT.

to determine the difference between the groups. The value of $p=0.02236$ was obtained, so we reject the hypothesis of equality of medians before and after surgery. After completion of NAHT, based on the results of the postoperative IHC study, the levels of ER expression were reassessed. The average value after NAHT was 84.66%, therefore, we can conclude that the expression levels of ER decreased. Fig. 1. presents changes in the expression levels of ER.

Expression levels of PR. Before the start of NAHT, the average value of PR expression was 74.73% (Table 1, Fig. 2). In this case, the distribution of differences between the level of PR expression before and after surgery is not normal, as determined by the Shapiro-Wilk test ($p=0.00596$), therefore, the non-parametric Wilcoxon test was used to determine the difference between the groups. The value of $p=0.00182$ was obtained, so we reject the hypothesis of equality of medians before and

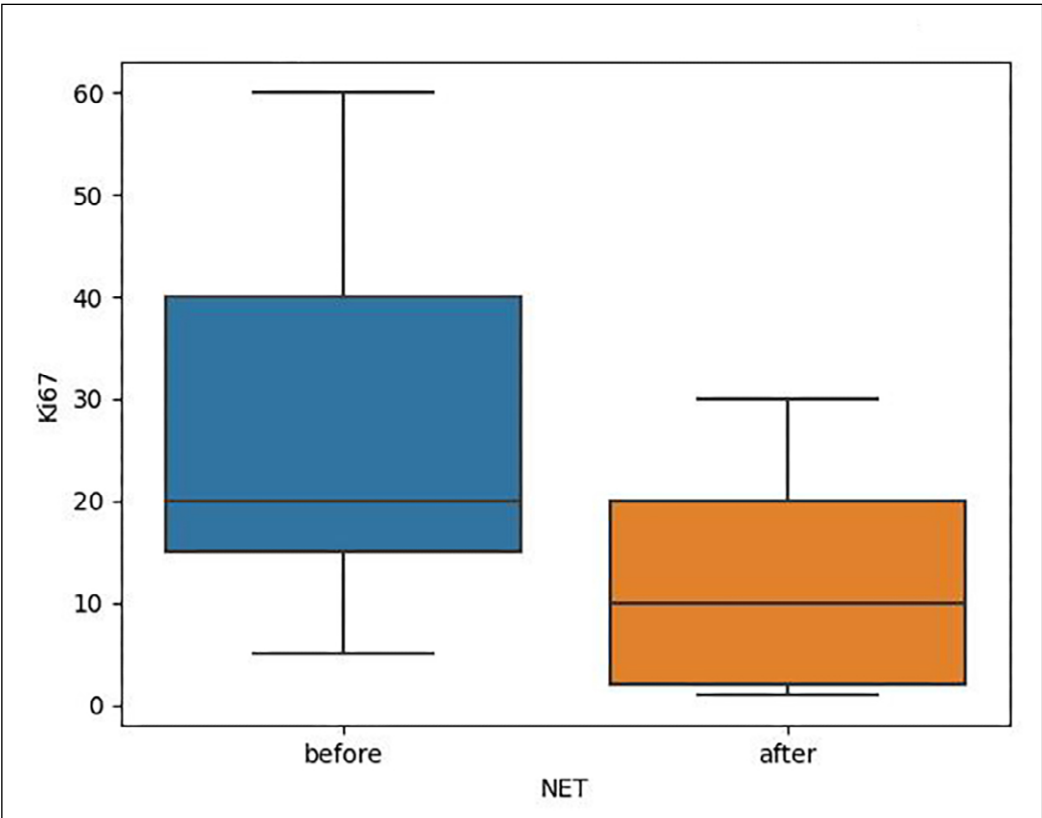


Fig. 3. Comparison of Ki-67 levels before and after NAHT.

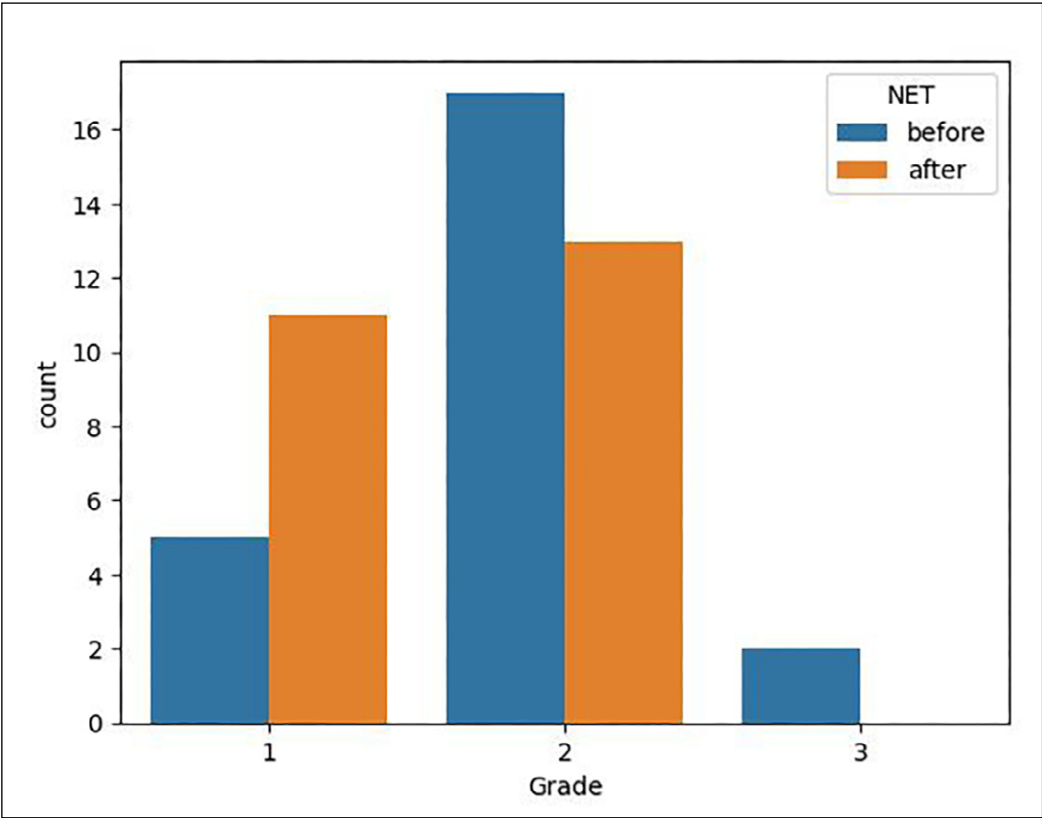


Fig. 4. Distribution by category G before and after NAHT.

after surgery. After the completion of the NAHT course, the average value of PR expression levels was 11.66%.
Ki-67 level. Before the start of the NAHT course, the Ki-67 level averaged 25.31%, and after NAHT this indicator decreased to an average value of 10.25% (Table

1, Fig. 3). The Shapiro-Wilk test revealed a normal distribution of the differences in Ki-67 before and after NAHT ($p=0.62454$), but the IQR method revealed an outlier (a difference of 40 units). Therefore, the non-parametric Wilcoxon test was used to assess the differ-

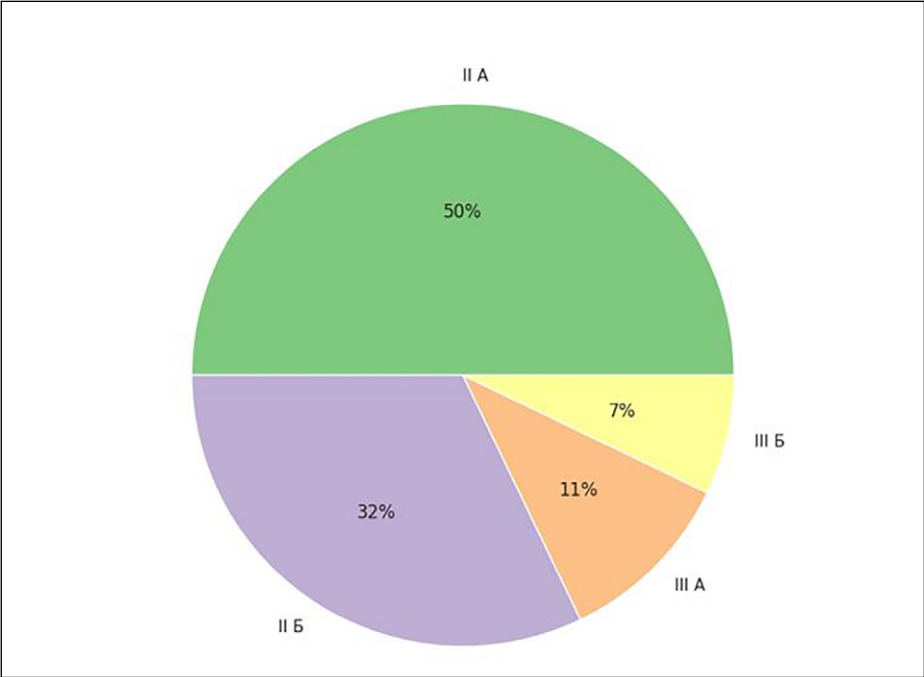


Fig. 5. Distribution by cancer stage before NAHT.

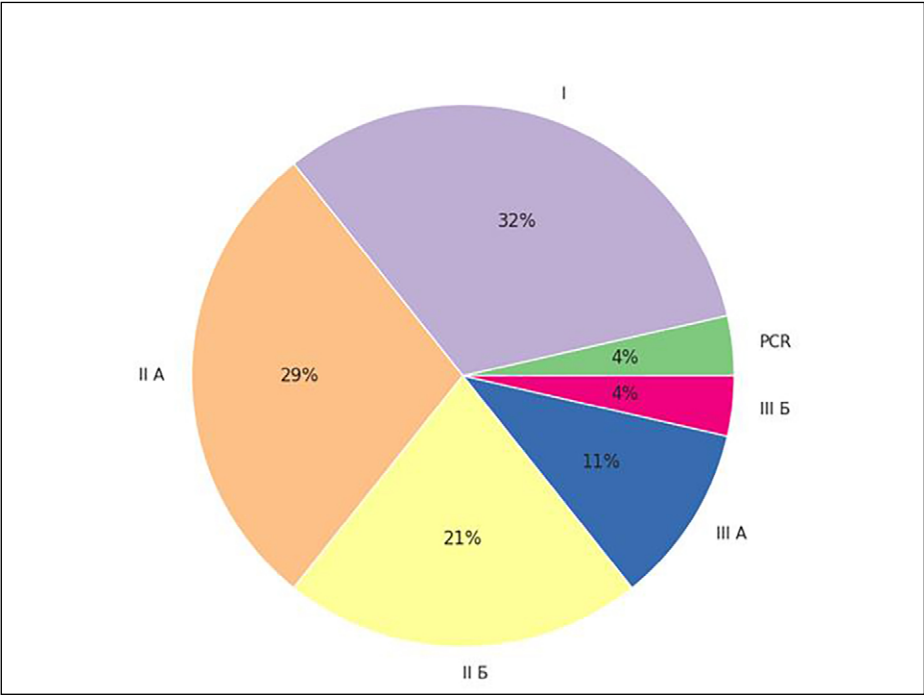


Fig. 6. Distribution by cancer stage after NAHT.

ence between the groups. The value of $p=0.00021$ was obtained, and we rejected the hypothesis of equality of medians before and after surgery. We can conclude that the Ki-67 level decreased after NAHT.

Category G. The Stewart-Maxwell test was used to compare the distribution of category G before and after surgery, and at $p=0.01832$ the hypothesis that the distribution of categories G was in the same proportion in both groups was rejected. The average value for category G before NAHT was 1.87, after NAHT 1.54 (Table 1). Changes in category G are shown in more detail in Fig. 4. When compared in percentage terms, before NAHT 7

of 28 patients had G1 (25%), 19 had G2 (67.86%) and 2 had G3 (7.14%). After NAHT, category G was compared in 24 patients, of which 11 had G1 (45.83%), and 13 had G2 (54.17%), and G3 was absent.

Staging of the malignant process. The Stewart-Maxwell test was used to compare the distribution of cancer stages before and after surgery, and at $p = 0.04038$, the hypothesis that the distribution of cancer stages was in the same proportion in both groups was rejected. At the time of diagnosis and before the start of NAHT, none of the 28 patients had stage I, 50% had IIA, 32% had IIB, 11% had stage III A, and 7% had stage III B (Fig. 5). After

the completion of the NAHT course, restaging revealed significant changes. A complete pathological tumor response (pCR) was achieved in one of the 28 women, stage I was observed in 32% of women, stage II A in 29%, stage II B in 21%, stage III A in 11%, and stage III B in 4% (Fig. 6).

The degree of therapeutic pathomorphosis-RCB. RCB was determined in 17 patients. Complete tumor RCB-0 (5.88%) was achieved in one patient, 13 patients had regressed malignant moderate residual tumors - RCB II (76.47%), and 3 patients had severe residual tumors - RCB III (17.65%).

Types of surgeries performed. After NAHT, 17 patients out of 28 underwent quadrantectomy with axillary lymphadenectomy (60.71%), and 11 patients underwent radical mastectomy with preservation of both pectoral muscles and axillary lymphadenectomy (39.29%).

DISCUSSION

Thus, after NAHT in postmenopausal patients with luminal type BC of stage II-III, significant changes were achieved. Aromatase inhibitors were used for NAHT, which inhibit estrogen biosynthesis and thus block the proliferative effect on hormone-sensitive tumor cells [6]. When comparing the results of IHC studies before and after the completion of NAHT, a decrease in the expression levels of ER, PR, Ki-67 was observed. For ER, the average value before the start of NAHT was 97.33%, and after NAHT 84.66% ($p=0.02236$). For PR, this indicator before NAHT was 74.73%, and after 11.66% ($p=0.00596$). The levels of the Ki-67 proliferation index before NAHT had an average value of 25.31%, after the completion of NAHT 10.25% ($p=0.62454$). Category G also underwent changes, since before the start of therapy the average value was 1.87, after completion of the NAHT course 1.54 ($p=0.01832$). According to large randomized studies, the use of NAHT before surgery in postmenopausal women

reduces tumor size, lowers Ki-67 levels and prevents complete removal of the breast [1, 7, 11]. In our study, at the time of diagnosis, the patients had stage II-III BC. As a result of NAHT, it was possible to achieve changes in staging. Stage I was observed in 32% of women after NAHT, pCR was achieved in one patient ($p=0.04038$). The average duration of NAHT was 6.10 ± 2.23 months. As a result of the use of NAHT in a group of 28 women, in 60.71% of cases we performed organ-preserving interventions. According to some publications, after 3 months of NAHT, organ-preserving surgeries can be performed in 69.8% of cases, and after 2 years, in 83.5% of cases [15]. Therefore, HR+ tumors are sensitive to NAHT, which has improved the immediate treatment results of patients.

CONCLUSIONS

1. After NAHT, there is a decrease in the expression levels of ER and PR. The expression levels of ER and PR decreased from 97% to 84%, and from 87% to 11%, respectively.
2. The use of NAHT leads to a decrease in the level of Ki-67 from 25.31% to 10.25%.
3. As a result of NAHT, changes occur in the degree of tumor differentiation (category G). After the completion of NAHT, its increase was observed in 24 patients - in 11 to G1 (45.83%), and in 13 to G2 (54.17%).
4. The stage of the BC also decreases - at restaging after NAHT, 32% of women had stage I, in 50% stages II A and II B, and only in 15% stages III A and III B.
5. As a result of NAHT, 13 patients out of 17 (76.47%) achieved a moderate degree of therapeutic tumor pathomorphosis, and one (5.88%) had complete tumor regression.
6. The use of NAHT made it possible to perform organ-preserving surgeries in 60.71% of cases.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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