Molecular biomarker discordances of breast cancer before and after neoadjuvant chemotherapy: Are they related to neoadjuvant chemotherapy or intratumoral heterogeneity and is there any clinical significance?

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Research Article

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Abstract

PURPOSE: To investigate the frequency, clinical significance, and causes of molecular discordances between tru-cut biopsy and residual tumor in patients with breast cancer who received neoadjuvant chemotherapy (NACTx).

METHODS: Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses; hormone expression levels; Ki-67 index; and tumor grade in tru-cut biopsy before NACTx and in the residual tumor after NACTx were evaluated and compared using the Wilcoxon signed-rank test.

RESULTS: The study included 102 women. Histopathologically, approximately 70% of the patients were partially responsive and 30% were unresponsive. The concordance and discordance rates between tru-cut biopsy and residual tumor were 95.1% vs. 4.9% (p = 0.180) for ER, 97.1% vs. 2.9% (p = 0.083) for PR, and 89.2% vs. 10.8% (p = 0.763) for HER2. Following NACTx, 15% of hormone receptor (HR)-negative patients and 5.7% of HER2(−) patients became positive, requiring adjuvant treatment. In particular, 18% of triple-negative patients became HR(+) and 12% became HER2(+). HER2 loss was detected in 40% HER2(+) patients. Ki-67 and PR expression significantly decreased in pathologically responsive patients (p = 0.001 and p = 0.004) and tumor grade increased in pathologically unresponsive patients (p = 0.034).

CONCLUSION: NACTx alone cannot explain receptor discordance observed in breast cancers**.** HER2 loss and decreased PR expression levels are mainly associated with NACTx; whereas the receptor status turning positive, which has clinical significance for adjuvant treatment, is primarily associated with intratumoral heterogeneity. Therefore, immunohistochemical re-staining for biomarkers should be performed in residual tumors.

Introduction

The pathological diagnosis of breast cancer is usually made by performing a tru-cut biopsy on suspicious lesions [1, 2]. The histological type, molecular subtype, and tumor grade are determined according to the biopsy specimen's morphological examination and immunohistochemical (IHC) staining. The molecular subtyping essential for determining the prognosis and planning the treatment is established by analyzing the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 proliferation index. However, these evaluations are not entirely reliable because tru-cut biopsy may not reflect the entire tumor due to intratumoral heterogeneity [3, 4].

In locally advanced stage and aggressive early-stage breast cancers, neoadjuvant chemotherapy (NACTx) is frequently employed to allow breast-conserving surgery, reduce the risk of postoperative complications, and evaluate tumor chemosensitivity [5]. Receptor status discordances (from positive to negative or vice versa) may be observed between pre-NACTx tru-cut biopsy and postoperative residual tumor in patients without pathological complete response (pCR). IHC re-staining of residual tumor and the causes of discordances are controversial [5-7]. If one of the hormone receptors (HRs) turns positive, adjuvant endocrine therapy can be administered, and if HER2 turns positive, adjuvant trastuzumab therapy can be administered. Not administering adjuvant therapy in these situations may cause a deficiency in treatment because it has been shown that adjuvant endocrine and anti-HER2 therapies prolong the disease-free survival and overall survival [8-11]. Therefore, it seems necessary to examine the residual tumor's receptor status.

It has been shown that pCR, residual tumor burden, and cellularity are associated with disease-free survival and overall survival [12-14]. As a result, the degree of pathological response affects survival. Similarly, pre- and post-treatment molecular changes (grade, Ki-67, and hormone expression levels) may present prognostic and predictive values for chemosensitivity in patients with non-pCR, as in the case of residual tumor burden.

This study aimed to investigate the frequency and clinical importance of molecular biomarker discordances and review their causes in patients with breast cancer operated after NACTx regimens used at present.

Materials And Methods

Women operated in our center for breast cancer following NACTx were retrospectively analyzed. The patients were divided into four groups according to their pathological response status: patients with pCR (no invasive tumor observed in the operated material), patients with minimal residual disease (≤10% residual invasive carcinoma), partially responsive patients, and unresponsive patients. Those with pCR or minimal residual disease were excluded from the study. Patients in whom tumor regression findings were observed in the histopathological evaluation and also who had a reduction in pathological tumor size of ≥30% from the clinical tumor size or had at least 30% reduction in tumor cellularity were accepted as partially responsive. Those who did not exhibit signs of histopathological regression or did not exhibit sufficient decrease in tumor size, or had increased tumor size were considered pathologically unresponsive. Demographic data, tumor characteristics, tru-cut and postoperative residual tumor histopathology, NACTx regimens, and adjuvant therapies of partially responsive and unresponsive patients were examined. ER, PR, HER2 receptor statuses, hormone expression levels, Ki-67 proliferation index, and histological grade of the tumor were evaluated in pre-NACTx tru-cut biopsy and postoperative residual tumor material. In ER or PR, ≥1% IHC staining was evaluated as ER(+) or PR(+). Any HR≥1% positivity was considered HR(+) disease. A HER2 score of 3 or a score of 2 with fluorescence in situ hybridization (FISH) positivity was considered HER2(+) disease. Triple negativity of ER, PR, and HER2 was considered triple-negative (TN) disease. Breast cancer was divided into five groups according to the molecular subtypes: Luminal A (LA; ER strongly positive, PR ≥20% positive, HER2 negative, and Ki-67 <14%), luminal B-HER2 negative (other HER2 negative luminal cancers), LB-HER2(+), HER2 enriched, and TN. Pre- and post-treatment ER, PR, HR, and HER2 receptor statuses; ER and PR expression levels; tumor grades; and Ki-67 proliferation indices were compared. In order to evaluate chemosensitivity, pre-and post-treatment HR expression levels (%ER and %PR), tumor grade, and Ki-67 index were compared individually in the patient groups with and without pathological response. If pre- and post-treatment receptor statuses were the same, it was referred to as “concordance,” and if they were different, it was referred to as “discordance.”

Statistics: IBM SPSS statistics software version 21.0 was used for statistical analyses. Pre- and post-treatment receptor statuses, hormone expression levels, tumor grades, and Ki-67 indices were compared using the Wilcoxon signed-rank test. A p-value of ≤0.05 was considered statistically significant.

Results

Altogether, 102 women operated after NACTx between January 2014 and September 2021 were included in the study. The mean age of the patients was 50.3 ± 9.6 (28–69) years. Histopathologically, approximately 70% of the patients were partially responsive, whereas 30% were unresponsive. Distribution of the patients according to receptor statuses and molecular subtypes were as follows; 79.4% ER(+), 74.5% PR(+), 14.7% HER2(+), and 16% LA, 53% LB-HER2(−), 12% LB-HER2(+), 3% HER2 enriched, 17% TN. Approximately 90% of the patients received NACTx containing anthracycline and taxane, and 10% were given anthracycline or taxane-based NACTx. Except for one, all HER2-positive patients received anti-HER2 drug(s). Clinicopathological features, NACTx regimens, and pathological response statuses of the patients are shown in Table-1.

Estrogen Receptor: One (1.2%) of the 81 ER(+) patients became ER(−), and 4 (19%) of the 21 ER(−) patients became ER(+) after NACTx. The total rate of ER status change was 4.9% (5 patients) (p = 0.180). ER status did not change in 95.1% of the patients. The rate of the effect of ER discordance on adjuvant therapy in ER(−) patients was 19% (4/21 patients) (Table-2). The ER expression levels of four patients who became ER(+) were 70%, 10%, 10%, and 5%. The ER expression level of the single patient who became ER(−) was 20% before treatment.

Progesterone Receptor: Three (3.9%) of the 76 PR(+) patients became PR(−), and none (0%) of the 26 PR(−) patients became positive after NACTx. The total rate of PR status change was 2.9% (3 patients) (p = 0.083). The PR status did not change in 97.1% of the patients, and PR discordance did not affect the adjuvant treatment (Table-2). The PR expression levels of the three patients who became negative were 80%, 1%, and 1% before treatment.

Hormone Receptor: No change was observed in 96.1% of the patients in terms of their HR status. The HR status of four patients (3.9%) changed after NACTx (p = 0.317). The proportion of HR(+) patients was 80.4% (82 patients) before chemotherapy, which increased to 82.4% (84 patients) after treatment. One patient who was HR(+) before treatment became negative after treatment. In contrast, 3 (15%) of the 20 patients who were HR(−) became HR(+) after treatment. HR(−) patients who became HR(+) in their residual tumors were administered adjuvant endocrine therapy (tamoxifen or aromatase inhibitor) (Table-2).

Human epidermal growth factor receptor 2: Six (40%) of the 15 HER2(+) patients became negative, and 5 (5.7%) of the 87 HER2(−) patients became HER2(+) after NACTx. The total rate of change in HER2 status was 10.8% (11 patients) (p = 0.763). There was no change in the HER2 status in 89.2% of the patients. The rate of the effect of HER2 discordance on adjuvant therapy in HER2(−) patients was 5.7% (Table-2). Two of the five patients who became HER2(+) after NACTx had an IHC score of 3; the remaining had an IHC score of 2 plus FISH(+). Adjuvant trastuzumab was started in these patients. Of the six patients who became HER2(−) after NACTx, five had an IHC score of 3, and one had an IHC score of 2 and was FISH(+). Trastuzumab treatment was continued in these patients.

Molecular subtype changes: One of 16 LA patients and 2 of 54 LB-HER2(−) patients became HER2(+) postoperatively. Consequently, 4.3% of the patients with HER2(−) luminal tumors became HER2(+) postoperatively. Of the 17 TN patients, 2 (11.8%) became HER2(+) and 3 (17.6%) became HR(+) after NACTx. Of the 12 patients who were LB-HER2(+), 50% became HER2(−) postoperatively. All three HER2-enriched patients remained the same postoperatively (Table-3).

Tumor grade, Ki-67, %ER, and %PR: In general, no significant difference was observed in the tumor grade of patients before and after NACTx. However, a statistically significant decrease was observed in the mean Ki-67 proliferation index (27.6 vs. 22.2, p = 0.001). A statistically insignificant increase was observed in the ER(+) cell percentage (62.4% vs. 66.1%, p = 0.062). In contrast, a statistically significant decrease was found in the PR(+) cell percentage after NACTx (51.2% vs. 42.3%, p = 0.001) (Table-4).

Patients with pathological partial response and no response: No significant change was observed in the mean tumor grade and ER(+) cell percentage of patients with partial response. However, Ki-67 index and PR(+) cell percentage of these patients decreased significantly after NACTx (Ki-67 index %; 27.5 vs. 20.6, p = 0.001; PR%; 49.9 vs. 40.5, p = 0.004). In pathologically unresponsive patients, the ER%, PR%, and Ki-67 indices did not change significantly, whereas the mean tumor grade increased significantly (mean tumor grade 2.1 vs. 2.3, p = 0.034) (Table-5).

Discussion

ER, PR, and HER2 discordance without NACTx

Many studies have reported that there may be discordances in ER, PR, and HER2 receptor statuses between tru-cut biopsy and surgical resection material in patients with breast cancer who have not undergone NACTx. Concordance rates have been reported as 62%–99% for ER, 69%–89% for PR, and 54%–100% for HER2 [4, 15-25]. Seferina et al. reported the concordance rates as 89.5% for ER, 82.5% for PR, and 80.6% for HER2. The false-negative rates in their study were 26.5% for ER, 29.6% for PR, and 5.4% for HER2 [4]. If the overall rate of HR(+) breast cancer is accepted as 70%, nearly 20%–30% of the remaining 30% HR(−) patients would be considered HR(−) based on tru-cut biopsy even though they are HR(+). Therefore, approximately 5%–10% of the patients may be deprived of adjuvant endocrine therapy. In contrast, if

the rate of HER2(+) breast cancer is accepted as 20%, 5.4% of the remaining 80% patient group will be considered HER2(−) even though they are HER2(+) and approximately 4%−5% of the patients will be devoid of anti-HER2 treatments. *Consequently, receptor discordance, which is important for adjuvant therapy, may be observed frequently even in patients with breast cancers who have not undergone NACTx*.

ER, PR, and HER2 discordance after NACTx

It has been reported that chemotherapy affects tumor biology directly or indirectly and causes receptor discordance in breast cancer. *Van de Ven* et al. evaluated studies conducted between 1996 and 2009 on receptor discordance after NACTx. The discordance in ER status was between 2.5% and 17% in eight studies, and there was no discordance in seven studies; discordance in PR status was between 5.9% and 51.7% in four studies, and there was no discordance in five studies [26]. However, in all these studies, the cutoff values for ER and PR positivity were accepted as 10% or 5%. In addition, usually only anthracycline-based and taxane-free NACTx regimens, which are rarely used today, were administered to the patients. In our study, the cutoff value for ER and PR positivity was accepted as ≥1% according to the recommendations of the American Society of Clinical Oncology [27]. NACTx regimens containing anthracycline and taxane, which are commonly used today, were administered, and anti-HER2 targeted drug(s) were added to the treatment in case of HER2(+) disease [28]. In the same meta-analysis, 19 studies were examined for HER2 status, and only three studies that used trastuzumab in addition to NACTx were evaluated. According to the results of these three studies, 12%–43% of the patients who were HER2(+) before treatment became HER2(−) in post-treatment residual disease, but none of the HER2(−) patients became positive at the end of treatment [29-31]. According to the results of our study, 40% of the HER2(+) patients lost their HER2 status. Unlike previous studies, 5 (5.7%) of 87 HER2(−) patients became HER2(+). Two of these patients had a score of 3, and three patients had a score of 2 and were FISH(+). The fact that FISH was not performed in all patients is a limitation of our study. However, our study is significantly distinct from other studies because of the use of current chemotherapy regimens and cutoff values. *According to the results of our research, which is believed to represent the current situation more accurately, the concordance and discordance rates between tru-cut biopsy before NACTx and residual tumor after NACTx were 95.1% vs. 4.9% for ER, 97.1% vs. 2.9% for PR, 96.1% vs. 3.9% for HR, and 89.2% vs. 10.8% for HER2.*

The effect of NACTx on discordances in ER, PR, HR, and HER2 statuses was tested, and no statistical significance was found (p values: 0.18, 0.08, 0.32, and 0.76 respectively). It should be noted that pre-NACTx tru-cut biopsy may not represent the entire tumor; thus, it may not be possible to reach a definite conclusion. Moreover, the high rate of HER2 loss after NACTx in HER2(+) patients and the statistically significant decrease in PR expression levels in partially responsive patients can be explained by the effect of NACTx. Thus, receptor discordance is caused by a combination of tumor heterogeneity, NACTx effect, and other factors (interventional radiologist experience and tru-cut biopsy procedure, tissue preparation, fixation, IHC stains, and interpretation differences between pathologists). *The change in receptor status from positive to negative can be explained mainly by the effect of NACTx, and the change from negative to positive can be explained by intratumoral heterogeneity rather than chemotherapy.* The impact of other factors is considered less significant.

Discordance with clinical significance

The decision for adjuvant treatment is usually made by considering the receptor statuses in the tru-cut biopsy performed before NACTx. Patients who are HR(+) according to tru-cut biopsy results are administered adjuvant endocrine therapy regardless of HR status in the residual tumor. Similarly, trastuzumab is administered during the neoadjuvant and adjuvant periods in HER2(+) disease. A negative receptor status in the tru-cut biopsy, which turns positive in the residual tumor, is challenging in terms of clinical significance and would affect the adjuvant treatment decision. Adjuvant hormonal therapy and/or trastuzumab should be initiated in patients who are HR(−) and/or HER2(−) in pre-NACTx tru-cut biopsy and become positive after treatment. *In our study, the receptor status of 15% of HR(−) patients and 5.7% of*

HER2(−) patients before treatment changed to positive in the residual tumors. These patients were started on adjuvant endocrine and/or trastuzumab therapy. Initiating adjuvant treatment with trastuzumab would be more appropriate in patients who become IHC HER2(+) in the residual tumors after confirming the same using the FISH test.

Molecular subtype changes

Some LA tumors appeared to differentiate into more aggressive subtypes after NACTx such as LB-HER2(−) at a rate of 37.5% and LB-HER2(+) at a rate of 6.3%. In addition, two patients (3.7%) with LB-HER2(−) tumors became HER2(+), and the subtype in one patient (1.9%) differentiated into the TN molecular subtype. Of the TN patients, 11.8% became HER2(+) and 17.7% became HR(+). However, 50% of the LB-HER2(+) patient group lost their HER2 expression. Under NACTx, which usually comprises anthracyclines and taxanes, LA tumors are not expected to differentiate into other aggressive molecular subtypes, and also for LB-HER2(−) tumors to acquire HER2(+) status or for TN tumors to become HR(+) or HER2(+). *This discordance, which turns positive from negative, can mainly be explained because the tru-cut biopsy does not reflect the entire tumor structure. On the contrary, LB-HER2(+) tumors becoming HER2(−) can be easily explained by NACTx containing anti-HER2 targeted therapy.*

Changes in ER and PR expression levels, grade, and Ki-67 proliferation index before and after NACTx

In the meta-analysis of *Van de Ven* et al., 10 studies were evaluated for ER expression, and it was reported that the expression level changed in 4 and remained unchanged in 6 of these studies. PR expression decreased considerably in four of the seven studies evaluated [26]. In our study, the mean ER expression levels increased after NACTx, although it was not statistically significant. However, post-NACTx PR expression levels decreased considerably. Our study differs from previous studies in that it individually evaluated patients with and without pathological response. PR expression levels were considerably decreased in pathologically responsive patients. They also tended to decrease in pathologically unresponsive patients, although this finding was not statistically significant. *When evaluated together with previous studies, the loss of PR expression can be a marker for chemosensitivity.*

There was no significant change in the mean tumor grade of the patients. However, the mean tumor grade of unresponsive patients was considerably higher after NACTx. *Increased tumor grade under NACTx requires investigating aggressive molecular subtypes with high tumor grades unaffected by chemotherapy.* Tumors with a high Ki-67 proliferation index are more prone to NACTx and have a higher pathological complete response rate [32]. In our study, no difference was noted between the mean pre-treatment Ki-67 indices of patients with and without pathological response (27.5 vs. 27.8). However, this could not be confirmed because patients with pCR were not included in our study. Although there was a significant decrease in the Ki-67 index of pathologically responding patients, there was no substantial change in unresponsive patients. *Consequently, the reduction in Ki-67 proliferation index and PR expression levels may indicate chemosensitivity in patients with breast cancer operated after NACTx. An increase in tumor grade may be an indicator of chemoresistance.*

Conclusion

The receptor status in patients with breast cancer may vary before and after NACTx. The frequent inconsistency between tru-cut biopsy and surgical resection material and the NACTx effect can cause discordance. Therefore, IHC staining should be repeated, and the receptor statuses should be examined in the residual tumor after NACTx. The change in receptor status from positive to negative is mainly associated with the NACTx effect, whereas the change from negative to positive is primarily associated with intratumoral heterogeneity. Cases with negative receptors becoming positive after treatment have greater clinical significance and should be administered adjuvant therapy. The status of patients who are HER2(−) before treatment and have an IHC score of 2 or 3 after treatment should be confirmed using a FISH test. An

increase in tumor grade after treatment may indicate chemotherapy resistance, whereas a decrease in PR expression level and Ki-67 index may indicate chemosensitivity. Further randomized clinical trials are warranted.

Abbreviations

ER Estrogen receptor

FISH Fluorescence in situ hybridization

HER2 Human epidermal growth factor receptor 2

HR Hormone receptor

IHC Immunohistochemical staining

LA Luminal A

NACTx Neoadjuvant chemotherapy

pCR Pathological complete response

PR Progesterone receptor

TN Triple-negative

Statements And Declarations

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Competing Interests

The authors declare that they have no conflict of interest.

Author Contributions

CY designed, collected data, analyzed the study, and wrote the manuscript. DKC collected data, conducted the pathological assessments, and aided in writing the manuscript. Both authors were responsible for data collection and interpretation of data.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

This study received approval from the local ethics committee and was conducted in compliance with the Declaration of Helsinki. The study was approved by Health Sciences University İzmir Bozyaka Training and Research Hospital Clinical Research Ethics Committee Date: 15/09/2021 No: 2021/155.

Consent to participate

Not applicable

Consent to publish

Not applicable

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Tables

Table-1. Clinicopathological features, NACTx regimens, and pathological outcomes of the patients (n = 102).

NACTx: Neoadjuvant chemotherapy, cT: Clinical tumor stage, cN: Clinical lymph node stage,

IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, LB: Luminal B, A: Anthracycline, T: Taxane,

trast+pert: trastuzumab + pertuzumab

Table-2. ER, PR, HR, and HER2 status of the patients before and after NACTx and clinical outcomes.

ER: Estrogen receptor, PR: Progesterone receptor, HR: Hormone receptor, NACTx: Neoadjuvant chemotherapy, Bx: Biopsy,

ET: Endocrine treatment, *Additional patients requiring adjuvant treatment after NACTx

Table-3. Molecular subtypes of the patients before and after NACTx.

Variable	Groups	n (%)
Age, years	Mean ± SD (range)	50.3 ± 9.6 (28–69)
	18–30	3 (2.9)
	31–40	13 (12.7)
	41–50	36 (35.3)
	51–60	33 (32.4)
	61–70	17 (16.7)
Menopausal Status	pre-menopausal	45 (44.1)
	peri-menopausal	8 (7.8)
	post-menopausal	49 (48.0)
Clinical Stage	early	54 (52.9)
	locally advanced	37 (36.3)
	inflammatory	5 (4.9)
	oligo-metastatic	6 (5.9)
cT	T1	11 (10.8)
	T2	68 (66.7)
	T3	4 (3.9)
	T4	19 (18.6)
cN	N0	6 (5.9)
	N1	66 (64.7)
	N2	26 (25.5)
	N3	4 (3.9)
Histology	IDC	90 (88.2)
	ILC	6 (5.9)
	mixed	2 (2.0)
	other	4 (3.9)
Molecular Subtype	luminal A	16 (15.7)
	LB-HER2(−)	54 (52.9)
	LB-HER2(+)	12 (11.8)
	HER2 enriched	3 (2.9)
	triple-negative	17 (16.7)
NACTx	A+T	92 (90.2)
	Only A based	6 (5.9)
	Only T based	4 (3.9)

Anti-Her2	not received	88 (86.3)
Drug(s)	trastuzumab	10 (9.8)
	trast + pert	4 (3.9)
Pathological	partial	71 (69.6)
Response	unresponsive	31 (30.4)

Tru-Cut Bx	***Post-NACTx Residual Tumor***			***Concordance***	***Discordance***	***P***	***Clinical Outcome***	
	Positive	***Negative***	***Total***	*%*	*%*		***Positive to (-)***	***Negative to (+)***
	n (%)	*n (%)*	*n (%)*				*%*	*%*
ER Status				***95.1***	***4.9***	***0.180***	***1.2***	***19***
ER(+)	80 (98.8)	1 (1.2)	81 (79.4)				Adjuvant ET	Adjuvant ET
ER(−)	4 (19.0)	17 (81.0)	21 (20.6)					
Total	84 (82.4)	18 (17.6)	102 (100.0)					
PR Status				**97.1**	**2,9**	**0.083**	**3.9**	**0**
PR(+)	73 (96.1)	3 (3.9)	76 (74.5)				Adjuvant ET	Adjuvant ET
PR(−)	0 (0.0)	26 (100.0)	26 (25.5)					
Total	73 (71.6)	29 (28.4)	102 (100.0)					
HR Status				**96.1**	**3.9**	**0.317**	**1.2**	**15**
HR(+)	81 (98.8)	1 (1.2)	82 (80.4)				Adjuvant ET	Adjuvant ET
HR(−)	**3 (15.0) ***	17 (85.0)	20 (19.6)					
Total	84 (82.4)	18 (17.6)	102 (100.0)					
HER2 Status				**89.2**	**10.8**	**0.763**	**40**	**5,7**
HER2(+)	9 (60.0)	6 (40.0)	15 (14.7)				Adjuvant Trastuzumab	Adjuvant Trastuzumab
HER2(−)	**5 (5.7) ***	82 (94.3)	87 (85.3)					
Total	14 (13.7)	88 (86.3)	102 (100.0)					

Tru-Cut Bx	*Post- NACTx Residual Tumor*					*Total, n (%)*
Molecular Subtypes	*LA*	*LB-HER2(−)*	*LB-HER2(+)*	*HER2 enriched*	*TN*	
LA, n (%)	9 (56.3)	6 (37.5)	1 (6.3)	0 (0.0)	0 (0.0)	16 (100.0)
LB-HER2(−), n (%)	19 (35.2)	32 (59.3)	2 (3.7)	0 (0.0)	1 (1.9)	54 (100.0)
LB-HER2(+), n (%)	1 (8.3)	5 (41.7)	6 (50.0)	0 (0.0)	0 (0.0)	12 (100.0)
HER2 enriched, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	3 (100.0)
TN, n (%)	0 (0.0)	2 (11.8)	1 (5.9)	1 (5.9)	13 (76.5)	17 (100.0)
Total, n (%)	29 (28.4)	45 (44.1)	10 (9.8)	4 (3.9)	14 (13.7)	102 (100.0)

NACTx: Neoadjuvant chemotherapy, Bx: Biopsy, LA: Luminal A, LB: Luminal B, TN: Triple-negative.

Table-4. Grade, Ki-67, ER, and PR expression levels before and after NACTx

Category	Tru-cut	Post-op	Stable (n)	Decrease (n)	Increase (n)	Test	P
GRADE, mean	2.24 ± 0.6	2.25 ± 0.6	70	15	17	Wilcoxon SRT	0.724
Ki-67, % mean	27.6 ± 17.5	22.2 ± 18.7	13	59	30	Wilcoxon SRT	0.001
% ER, mean	62.4 ± 36.2	66.1 ± 37.4	47	20	35	Wilcoxon SRT	0.062
% PR, mean	51.2 ± 39.9	42.3 ± 37.0	40	44	18	Wilcoxon SRT	0.001

NACTx: Neoadjuvant chemotherapy, ER: Estrogen receptor, PR: Progesterone receptor.

Table-5. Grade, Ki-67, ER, and PR expression levels of the patients before and after NACTx categorized according to the pathological response.

Category		Partial Response (n=71)	P	Unresponsive (n=31)	P
Grade, mean	Tru-cut	2.3 ± 0.5	0.414	2.1 ± 0.61	0.034
	Post-op	2.2 ± 0.6		2.3 ± 0.6	
Ki-67%, mean	Tru-cut	27.5 ± 15.8	0.001	27.8 ± 21.1	0.446
	Post-op	20.6 ± 15.1		26.1 ± 25.1	
ER%, mean	Tru-cut	64.1 ± 35.8	0.266	58.9 ± 37.5	0.124
	Post-op	67.2 ± 35.9		63.5 ± 41.1	
PR%, mean	Tru-cut	49.9 ± 40.6	0.004	54.2 ± 38.8	0.132
	Post-op	40.5 ± 36.3		46.4 ± 38.7	

NACTx: Neoadjuvant chemotherapy, ER: Estrogen receptor, PR: Progesterone receptor.