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ORIGINAL ARTICLE

Expression of ER, PgR, HER-2, and Ki-67 in core biopsies and in definitive histological specimens in patients with locally advanced breast cancer treated with neoadjuvant chemotherapy

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Abstract

Purpose Many studies have indicated that the response to therapy and the prognostic impact of a pathologic complete response after neoadjuvant treatment differ among breast cancer subtypes.

Methods The aim of our study is to evaluate the effect of this treatment on the expression of estrogen and progesterone receptors, human epidermal growth hormone receptor 2 and Ki67 in breast cancer. We identified 125 patients.

Results The estrogen receptor modified its expression from positive to negative in 8% patients and from negative to positive in 22%; progesterone in 21% and in 37% cases. Median Ki-67 value was 20.9% at biopsy and 18% after, HER-2 status did not show a remarkable change before or after neoadjuvant chemotherapy (NACT). We have identified a significant reduction in Ki-67 expression levels after chemotherapy in patients with a pathologic response. Detection of pretreatment Ki-67 could identify patients most likely to benefit from NACT.

Conclusions NACT can change the status of ER, PgR, and Ki-67 expression in patients with breast adenocarcinoma, but it did not exert a significant effect on HER-2 status; HER-2 amplification appears to be more stable. We have identified a prognostic role for a decreased expression of PgR and Ki-67 after preoperative chemotherapy in breast cancer patients.

Keywords Breast cancer · Neoadjuvant chemotherapy · Estrogen receptor · Progesterone receptor · Human epidermal growth factor receptor 2 · Ki67

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Introduction

Breast cancer is the most frequent tumor in women living in industrialized countries and the second cause of death for cancer among women worldwide [1]. Our knowledge about biological characteristics of breast cancer largely improved in the last years and now we know different molecular profiles according to endocrine parameters, proliferation indicators and growth factor receptor expression. Approximately 30% of invasive breast cancer patients have nodal involvement at the time of diagnosis [2]. These women and particularly those with locally advanced breast cancer (LABC) require multidisciplinary clinical approach and personalized therapy to optimize patient care. From many years NACT has shown to be an attractive alternative to adjuvant chemotherapy for patients with early stage and operable breast cancer. According to many randomized trials, NACT could be considered today a standard therapeutic strategy in patients with locally advanced and

operable breast cancer [3]. Primary chemotherapy before surgery has many advantages, including increased rates of conservative surgery and administration of chemotherapy through an intact vascular system [4]. Moreover, this strategy allows to evaluate in vivo clinical response to treatment, improving the rates of breast conserving surgery too. Another advantage could be a less extensive axillary lymph node dissection, if an important downstaging occurs. Although NACT is a well-established clinical approach in early breast cancer, there is today an extensive scientific discussion about its role in the management of this neoplastic disease. Furthermore, patients with pathological complete response (pCR) after NACT are most likely to experience improved DFS and OS [5]. Unfortunately, too many patients have residual disease in the breast or axilla after NACT and several studies reported that the extent of residual disease (including the primary tumor and the involved lymph nodes) is an independent predictor of disease-free survival (DFS) and overall survival [4, 6, 7]. Expression profiles for hormone receptor (HR), human epidermal growth factor receptor-2 (HER-2), and Ki-67 have significant implications in the prognosis and choice of treatment for breast adenocarcinoma [8]. Little is known about the influence of NACT on those receptors and the impact on subsequent adjuvant systemic therapy. Previous studies have reported changes in the expression of estrogen receptor (ER), progesterone receptor (PgR), HER-2 and Ki-67 following NACT, suggesting that these modifications could be an additive important prognostic factor [8–10].

Other studies evaluated the prognostic and predictive value of Ki-67 expression in neoadjuvant settings.

Ki-67 is a nuclear protein expressed during all phases of the cell cycle, except G0 phase, and it is a specific marker for tumor proliferation [11]. In breast cancer high levels of Ki-67 are associated with poorer survival. Bottini et al. [12] and Makris et al. [13] reported that Ki-67 expression decreased after chemotherapy and that this reduction correlated significantly with clinical tumor response. Similarly, also the levels of estrogen receptor (ER) and progesterone receptor (PgR) could change after chemotherapy [14]. Several studies have shown higher levels of Ki-67 in surgical resected tumors than in core biopsy samples [15, 16]. Chen et al. found that Ki-67 value significantly increased after core needle biopsy (CNB), an accurate technique in evaluating ER, PgR, HER-2, and molecular subtype status. Ki-67 value significantly increased after CNB and was associated with surgery time interval (STI) and molecular subtype [16]. Further translational research needs to consider Ki-67 changes following CNB among different breast cancer molecular subtypes.

This result is supported by Kim et al. too, who further demonstrated that a substantial discordance in Ki-67 after biopsy was significantly associated with different variables including tumor size, negative PR expression, G3 and age less than 35 years [17].

Criscitiello et al. reported that Ki-67 expression can identify a subset of patients among Luminal B and node positive breast cancer cases who could benefit from addition of adjuvant chemotherapy to hormone therapy [18]. On the contrary, Andre F. et al. reported that in the adjuvant setting Ki-67 staining lacks analytical validity; moreover, no robust evidence indicates that Ki-67 staining predicts the efficacy of adjuvant chemotherapy [19]. The post-chemotherapy Ki-67 value is a strong predictor of outcome for patients not achieving a pCR [20–22].

HER-2 is overexpressed in 10–25% of breast cancers and is associated with a more aggressive form of breast cancer. Patients with HER-2 positive disease have better responses and higher pCR rates are reported when trastuzumab is added to NACT [23–25]. Mittendorf et al. reported that one-third of patients with significant residual disease loses HER-2 amplification, and this change is associated with poor outcome [26]. Therefore, to improve our knowledge in this attractive field, we retrospectively examined with immunochemistry the expression of ER, PgR, HER-2, and Ki-67 in core biopsies and in definite histological specimens in patients with Stage I–III breast cancer who were treated with anthracycline and taxane based neoadjuvant chemotherapy.

Materials and methods

From December 2008 to January 2018, 125 patients diagnosed with invasive breast cancer (BC) were retrospectively evaluated in the Department of Medical Oncology of Sapienza University of Rome, Polo Pontino.

All patients were treated with neoadjuvant chemotherapy for operable or locally advanced breast cancer. Only histological confirmed locally advanced BC stages IIB, IIIA, and IIIB were enrolled in this study. Patients with inflammatory breast cancer were excluded. The patients were considered eligible if they were 18 years of age and older and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1. Pregnant or nursing women were excluded. Moreover, patients with multifocal or bilateral breast cancer with metastatic disease and pulmonary/cardiovascular disease were excluded too. Core biopsies were collected after local anesthetic infiltration with a 14-gauge needle while definite surgical samples were collected with surgical resection. All histological specimens were formalin-fixed and paraffin-embedded. After hematoxylin and eosin staining analysis, they were evaluated by a skilled breast pathologist.

Data on medical history, concurrent diseases, histopathological features, age, menopausal status, tumor size,

lymph node status, hormone receptorial status, HER-2 expression, Ki-67 index, tumor phenotype, NACT regimens, cardiac/renal/liver function were reviewed and recorded. All cases were discussed during the weekly multidisciplinary disease meetings. ER, PgR, HER-2 and Ki-67 status were evaluated by immunohistochemistry (IHC) in the pre-treatment core biopsy specimens and in the definite surgical tissue. The cut-off value for ER and PgR positivity was 1% positive tumor cells with nuclear staining. Hormone receptorial (HR) status was defined as being negative for both ER and PgR. HER-2 IHC positivity was defined when a strong membrane staining in > 10% of tumor cells occurred. Tumors with a score of 2 + were tested for gene amplification by Fluorescence in situ Hybridization (FISH) analysis. Tumors were considered HER2-positive if IHC staining was 3 + or FISH positive. Ki67 score was defined as the percentage of total number of tumor cells (at least 1000) with nuclear staining over 10 high powered fields (× 40). The median age of patients was 54.6 years (range 37–77 years). Complete response, partial response, and progressive disease were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST 2000). The maximum diameter of tumor was measured also with ultrasounds, before and after chemotherapy. Tumor size was also measured with magnetic resonance imaging in several patients to confirm the clinical responses evaluated with ultrasounds. Local ethical review board approved the protocol; patients provided written informed consent before starting therapy.

Before chemotherapy, patients were required to have an adequate bone marrow reserve, regular neutrophils/platelets count and hemoglobin levels, good renal/hepatic function. A left ventricular ejection fraction (LVEF) ≥ 50 was requested.

In all patients NCT schedule consisted of four cycles of anthracycline and 12 weekly paclitaxel courses. In cases of HER-2 amplification, trastuzumab was added to NACT during paclitaxel period. Patients with breast related cancer antigens (BRCA) mutation were candidates for platinum containing chemotherapy.

Patients were directed to surgery after 16 cycles of chemotherapy. Following surgery, pCR was defined as the complete absence of viable tumor cells (in breast and axillary nodes) by hematoxylin and eosin staining; Patients were considered in complete response if the final histological examination showed a complete response or ypTis with negative lymph nodes. Partial response (PR) was defined as tumor cells detected in < 10% or 10−50% specimens; when final histological examination showed ypT1 with negative lymph nodes. Progressive disease (PD), were defined for patients with no evidence of response to therapy; they were considered non-responders if they progressed during treatment or whose final histological examination had positive lymph nodes or yT > 1.

Molecular profile subtypes were defined according to 2013 St. Gallen Consensus Conference indications: Luminal A, Luminal B/HER2-, Luminal B/HER2+, HER2 + and triple negative categories [27, 28].

Patients were considered evaluable if they had completed neoadjuvant chemotherapy, and patients who had not completed all cycles were excluded. During or after NACT no patients developed intolerable adverse effects and no patients discontinued therapy because of any reaction from chemotherapy. All women treated with breast conserving surgery (BCS) received adjuvant radiotherapy (RT). Radiotherapy was administered also to mastectomy patients with extensive axillary node involvement.

For patients with endocrine-responsive disease, adjuvant endocrine therapy, according to menopausal status, was used (tamoxifen or aromatase inhibitor) for a duration of 5 years.

Patients were followed every 4 months for the first 2 years and after 2 years every 6 months for other 3 years.

Results

We identified and evaluated a total of 125 patients who received NACT in our Oncology Unit between 2008 and 2018. They met all the inclusion criteria and were retrospectively evaluated in this study. We analyzed expression of ER, PgR, ki-67 and HER-2 in pre and post-chemotherapy.

Thirty-two patients were not currently evaluable, because of lack of histological diagnosis or still in chemotherapy treatment.

Of the 93 remaining patients evaluable, 26 (28%) were classified as Luminal A (ER+, HER2− and Ki-67 < 20%); 32 (35%) were Luminal B (ER+, HER2−, Ki-67 > 20%); 14 (15%) were Luminal B HER + (ER+, HER +); 8 (8%) were HER+ (ER−, HER+); 13 (14%) were triple negative (ER−, HER2−).

Twenty-seven patients (29%) achieved a pathologic complete response (pCR), 18 ypT0 and 9 ypT in situ. The rate of pCR after NACT has been analyzed according to different subtypes (Table 1).

In patients with luminal A disease pCR occurred in 15% of patients; in luminal B disease (HER2–) in 28%; in luminal

Table 1 Pathologic complete response (pCR) after NACT according to subtype

	N	%
pCR	27/93	29
Luminal A	4/26	15
Luminal B HER−	9/32	28
Luminal B HER+	4/14	28
HER+	4/8	50
TNBC	6/13	46

B (HER2+) in 28%; in HER2 + in 50% and in triple negative in 46%.

Table 2 shows the clinical pathological characteristics of the patients at the time of diagnostic core biopsy.

Tumors were ER-positive in 63 patients (67.7%) of cases and expressed a PgR positivity in 60 patients (64.5%) of the analyzed samples. In 28 patients (30%) HER-2 positivity was detected. Fourty eight patients (51.6%) were in menopause. Histologically, 67 (72%) were ductal carcinomas and 14 (15%) lobular carcinoma: the other 12 patients (13%) expressed other subtypes.

Sixty-seven patients (72%) had axillary positive lymph nodes. Ki-67 was high (> 20%) in 53 cases (57%).

Table 2 Patients characteristics at biopsy

Characteristic	Level	*N* (%)
Age at diagnosis	< 35	0
	35–50	43 (46%)
	51–65	31 (33%)
	> 65	19 (21%)
Menopausal status	Premenopausal	45 (48.4%)
	Postmenopausal	48 (51.6%)
Axillary lymph node	Negative	23 (24.7%)
	Positive	67 (72%)
	Unknown	3 (3.3%)
Histology	Ductal	67 (72%)
	Lobular	14 (15%)
	Mixed/other	12 (13%)
Molecular subtype	Luminal A	26 (28%)
	Luminal B	32 (35%)
	Luminal B HER2 pos	14 (15%)
	HER2 positive	8 (8%)
	Triple negative	13 (14%)
ER	Positive	63 (67.7%)
	Negative	30 (32.3%)
PgR	Positive	60 (64.5%)
	Negative	33 (35.5%)
Ki 67	< 20	40 (43%)
	≥ 20	53 (57%)
HER 2	Negative	65 (70%)
	Positive	28 (30%)
	Triple negative	13 (14%)
ER	Positive	63 (67.7%)
	Negative	30 (32.3%)
PgR	Positive	60 (64.5%)
	Negative	33 (35.5%)
Ki 67	< 20	40 (43%)
	≥ 20	53 (57%)
HER 2	Negative	65 (70%)
	Positive	28 (30%)

We evaluated changes in hormone receptors, ki-67 and HER2 in 56 patients in correlation with pathological response, after excluding patients with a pCR and without complete molecular assessment in pre and post-chemotherapy.

- Changes in hormone receptors

  The estrogen receptor modified its expression from positive to negative in 4/48 patients (8%) and from negative to positive in 2/9 patients (22%).

  The mean value of estrogen receptor positivity remained unchanged before and after chemotherapy (54.5% vs 54.7%). According to a sub-analysis, in the clinical responders group a median value of 43,9% was observed before CHT and 43% after CHT; in non-responders group a median value of 62% was observed before CHT and 63% after CHT.

  Progesterone receptor changed its expression from positive to negative in 10/47 cases (21%) and from negative to positive in 3/8 cases (37%).

  The mean value of progesterone receptor positivity was different before and after chemotherapy (36.7% vs 28%). In patients with pathological response, the mean value was 28% before vs 16.5% after chemotherapy; in patients non-responders the mean value was 42.9% before vs 36.3% after chemotherapy.
- Changes in ki-67 value

  As for the receptorial expression we subsequently evaluated the variation of ki-67 too.

  Median Ki-67 value was 20.9% at the time of diagnostic core biopsy and 18% after the neoadjuvant treatment. Performing an analysis in the different subgroups, we detected the following results: in the responders group ki-67 median value was 25% at biopsy time and 12.8% after NACT. In non-responders group median ki-67 was 18% at biopsy time and 21.8% after. Considering a cut-off of 20%, in 16/29 patients (55%) a ki-67 value ≥ 20% became < 20% after NACT (12 responders and 4 non-responders). In 8/27 patients (30%) a ki-67 value < 20% became ≥ 20% (2 responders and 6 non-responders).
- Changes in HER2 expression

  HER-2 expression changed in 6/13 patients (46%). 4/13 (30.7%) patients with HER-2 positive tumor at biopsy had HER-2 negative residual at surgery (1 in responders, 3 in the non-responders). Conversely, 2/13 (15.3%) patients HER-2 negative at biopsy time became HER-2 positive at surgery (1 in responders, 1 in non-responders).

Discussion

Neoadjuvant systemic chemotherapy is being increasingly used in the treatment of early stage breast cancer [29, 30].

The response to this treatment may be utilized as a prognostic marker and the achievement of pCR has been adopted as the primary end-point in neoadjuvant trials and in current clinical practice [5, 31].

Many studies focused attention on pCR cases [32], unfortunately occurring in 20–40% of patients; therefore, it is crucial to identify the clinical pathological features at final surgery that might play a prognostic role in patients with residual disease [33].

We analyzed expression of ER, PgR, HER-2, and Ki-67 in pre and post-treatment samples to investigate their prognostic and predictive factors in women with Stage II–III breast cancer, who had been treated with anthracycline and taxane based NACT.

In our patients a pCR was observed in 28.4% of cases, the highest occurring in HER2 + breast cancer patients (50%). So, this one which might reflect that NACT containing trastuzumab could bring higher pCR rate in addition to traditional NACT for HER2 + breast cancer patients [34]. It was confirmed by a meta-analysis of five trials which showed that inclusion of trastuzumab in NACT schedule could give higher pCR probability [35].

In our study triple negative patients (TNBC) showed a high percentage of complete response (46%). Those TNBC patients showed high Ki-67 expression and high pCR probability [36].

Whether NACT can change HR (ER, PgR), HER-2, and Ki-67 expression profiles is controversial. In clinical practice, a change in HR status could strongly modify treatment strategy. Patients with a switch from ER/PgR positive to negative will be less benefited from endocrine therapy, mainly in long time treatment.

We also analyzed the relationship between pre-NACT ER/PgR expression and clinical response. There was no significant association between PgR expression at baseline and clinical response. Considering a cutoff of 20%, the variations of the PgR are evident.

PgR expression was significantly reduced after chemotherapy in responders and in non-responders, while the ER+ rates before and after NACT did not differ significantly.

Cancello et al. reported that PgR loss identifies Luminal B breast cancer subgroups at higher risk of relapse [37].

Gahlaut et al. reported that PgR status changed significantly after NACT. Thirteen cases changed status from PgR positive to negative and only five from negative to positive [38]. In our study in 9 patients PgR status changed from positive to negative and in 7 from negative to positive. Possible mechanisms of molecular transformation include the selection of tumor cells resistant to NACT. Tumor cells in residual disease, by changing the cellular molecular pathway, can develop resistance to a specific therapy [39].

PgR decrease after NACT seemed related to a more favorable outcome in Montagna's study which showed that the decrease in PgR and Ki-67 expression after NACT correlated with improved outcome in terms of DFS [33].

Ki-67 expression is a reflection on the activity of tumor proliferation [40] but the effect of pre-surgical NACT on Ki-67 expression is still controversial.

There are a lot of studies investigating the prognostic role of proliferation marker Ki-67 but, according to many controversies and debated issues,this marker has not been fully integrated into clinical decision making. Some of the questions about the role of Ki- 67 concern the issue of cut-off points for this values in daily clinical practice [41, 42].

In our study a Ki-67 value of 20% has proved to be able to significantly distinguish between the pCR and pNR groups. We observed a Ki-67 variation mainly in the responders group. If we consider a cutoff of 20, in 18% of cases this value became < 20% in responders and only 6% in non-responders.

Faneyte [43] showed that Ki-67+ rate and expression levels declined after NACT.

In addition to its prognostic importance, several authors have also demonstrated that value of Ki-67 has proved to predict the benefit of adjuvant therapy in high-risk luminal B-like and node-negative patients. Penault-Llorca showed that patients whose tumors had Ki-67 levels > 20% benefited from the addition of docetaxel [44]. Criscitiello found a significant benefit with the addition of CHT to endocrine therapy in luminal B-like BC with a Ki-67 > 32% [18]. Viale et al. [45] in the BIG 1-98 trial confirmed the prognostic role of Ki-67 in a population treated with endocrine therapy alone. They also observed a greater benefit with letrozole compared to tamoxifen in BC patients whose tumors had Ki-67 levels of 11%. The significance of Ki-67 in highly proliferative tumors like TNBC is, however, unknown because almost all these BCs have a very proliferative phenotype.

Several studies have demonstrated that the decrease in post-treatment of Ki-67 level is a positive predictor for patient outcome [20, 33] and that the decrease of Ki-67 expression to < 20% of the cells after NACT was associated with better outcome in terms of DFS and overall survival [33].

About HER-2, our study suggested that HER-2 status did not show a remarkable change before or after NACT both in responders and non-responders. This might be related to the latency in the changes in HER-2 overexpression and gene duplication, which, in turn, explain the constancy of HER-2 status after NACT [43].

Conclusions

In conclusion, we have identified a significant reduction in Ki-67 expression levels after NACT in breast cancer patients who demonstrated a pathological response. Ki-67 might

predict pCR and detection of pretreatment Ki-67 could identify patients most likely to benefit from NACT.

Moreover NACT seems able to change ER and PgR receptors expression and status. NACT can change the status of ER, PgR, and Ki-67 expression in patients with breast adenocarcinoma, but it did not exert a significant effect on HER-2 status so that HER-2 amplification appears to be more stable.

In our study, after NACT PgR + expression in tumor cells decreased. Although ER + rate showed a decreasing trend after NACT, the change was not statistically significant. Therefore, ER, PgR, and Ki-67 expression in tumors should be monitored before and after NACT.

In conclusion, we have identified a prognostic role for a decreased expression of PgR and Ki-67 after preoperative chemotherapy in breast cancer patients. Assessment of these changes may be useful to identify subsets of patients with better outcome after NACT even if pCR is not achieved.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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