Changes in ER, PR, HER2 and Ki67 Expression in Breast Cancer after Neoadjuvant Treatment and Comparison with Clinical, Pathological Features and Ethnicity of Patients

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Abstract

Purpose: To determine the discordance between estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and the proliferation marker Ki67 rates in core needle biopsy (CNB) and surgical specimens after neoadjuvant treatment (NAT) of women with invasive breast cancer.

Methods: In this study 100 patients with breast cancer treated with NAT and whose CNB and surgical specimens after definitive surgery were located at Ziv Medical Center between 2007 and 2016, were included. Clinical and pathological data were reviewed from the files of patients. Formalin fixed paraffin embedded or fresh tissue samples were used for evaluation of ER, PR, HER2 and Ki67. Sections were assessed by the same two pathologists, both for pathological response and for predictive biomarkers.

Results: One hundred of 122 patient were suitable for evaluation for this study. Patients not suitable either had a complete pathological response (pCR in 20 patients) or their primary CNB was not found (2 patients). Mean age of patients was 52.3 ± 13.9 years. 54% of the patients were Jewish and 46% Arab. In CNB: ER, PR, HER2 and Ki67 were positive in 76%, 63%, 32% and 45% of patients retrospectively. Discordance was found in 5% in ER (not significant: NS), 13% in PR (P =<0.001), 19% in HER2 (P =<0.001) and 20% in Ki67 (P =<0.001) after NAT. A higher rate of change HER2 was found in Jewish than Arab patients.

Conclusions: To our knowledge this is the first study comparing changes of ER, PR, HER2 and Ki67 rates following NAT against clinical and pathological features, response to treatment and ethnicity in Middle Eastern women. The discordance in biomarker rates after NAT is a guide to response to treatment, prognosis and may affect treatment decisions after surgery.

Keywords: Breast cancer, Biomarkers, Change after neoadjuvant therapy.

Introduction

The large volume of molecular and genetic research which has been done during the last 15 years has emphasised the cardinal importance of the biomarkersestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2) and proliferation-associated antigen (Ki67) for treatment choices and decisions in patients with all stages of breast cancer [1,2]. In addition, traditional prognostic and predictive factors such as tumor size, tumor grading, lymph node status and distant metastases are also still useful [1-3]. During the last few years, other predictive and prognostic factors have also been added to the previous ones, including genetic phenotyping, molecular drivers, PI3K and others, which determine the use of additional therapies in breast cancer [4-6].

In our medical center, core needle biopsy (CNB) is used for the pathological diagnosis of primary breast cancer and lymph nodes. It is used also to detect ER, PR, HER2 and Ki67 status in the primary tumor before surgery and before adjuvant or neoadjuvant treatment [1].

Originally neoadjuvant treatment (NAT) (including chemotherapy, targeted therapy, hormonotherapy and others) was used for patients with locally advanced breast cancer to reduce the tumor volume and to eliminate possible micro metastasis [7]. Today NAT followed by surgery has become the standard of care for patients with locally advanced breast cancer and definitely for all triple negative and HER2 positive tumors [8,9]. NAT provides information on tumor response to specific chemotherapeutic or targeted agents. This may be important as a prognostic factor and for choosing treatment agents in the adjuvant setting [10].

Endocrine therapy is widely used in adjuvant and metastatic breast cancer but is also optional for NAT in women with ER/PR positive breast cancer [1,2]. Trastuzumab, pertuzumab and other tyrosine kinase inhibitors (TKIs) are used in treating HER2 positive breast cancer. Recently TKIs were used in combination with chemotherapy for NAT, adjuvant and metastatic disease [11].

Immunohistochemistry (IHC) assays are the basis for testing the ER, PR, HER-2, and Ki67 status of breast cancers. Detection of these prognostic and predictive factors is done from both fresh tissue and paraffin block tissue samples [12]. Ki-67, a cell-cycle and a mitosis-related marker, is a non-histone nuclear protein that is closely linked with cell proliferative activity [13]. HER2 positive +2 tumors were reexamined with chromogenic in-situ hybridization (CISH) test for positivity.

There is no consensus in the oncology literature regarding the value of repeating ER, PR, HER2 and Ki67 testing in tumor tissue after surgery, in patients who have been treated with any form of NAT before surgery. Neither their importance nor the effect on treatment decisions after surgery are known [14,15]. Several retrospective studies have reported that NAT significantly altered ER and/or PR status [16]. Others concluded that a switch of hormone receptor status after NAT is unique in breast cancer. Hormone receptor switching may identify patients who would benefit from adjuvant endocrine therapy and impact long-term outcome [15]. Updating reevaluation of ER, PR, HER2, and Ki67 in tumor tissue after surgery in patients treated with NAT is not a routine procedure [2].

The aim of this retrospective study was to compare concordance and discordance rates of IHC expression of ER, PR, HER2 and Ki67 in CNB samples before any cancer treatment with that in the surgical excision specimens after NAT in the same patients. Results were compared with pathological, clinical and ethnical characteristics of the patients.

Patients and Methods

Patients and data collection

In this retrospective study the relevant data for 122 patients with breast cancer who received NAT was collected from their personal records and hospital files from 2007 to 2016. All were treated at the Oncology Division at Ziv Medical Center, in northern Israel. Of the whole population of 100 patients suitable for evaluation, who had residual disease in the operated breast after NAT, 20 patients had pCR in surgical specimens and in 2 patients the primary biopsy before NAT was missing. All had primarily undergone CNB or open biopsy confirming invasive breast cancer. ER, PR, HER2 and Ki67 were tested. Patients received NAT mostly chemotherapy (taxanes, epirubicin, cyclophosphamide), trastuzumab, pertuzumab and hormonal therapy followed by surgery of the breast either lumpectomy or mastectomy, sentinel lymph node biopsy (SLNB) or axillary lymph nodes dissection (LND) (Table 1). After surgery, patients were treated with adjuvant biological targeted therapy, chemotherapy or hormonal therapy. Patients undergoing lumpectomy or advanced local disease before NAT were treated also with radiation therapy (RT). The study was conducted in compliance with Good Clinical Practice guidelines and approved by the Clinical Ethics Helsinki committee in our medical center.

Inclusion criteria

- Patients with invasive breast cancer confirmed by histological examination, and treated with NAT, followed by surgery of the breast. Primary biopsy and tumor tissue after surgery were available.

- Patients with no age limitation at the diagnosis.

- Patients who were followed for 3 years or more after the primary diagnosis of the disease.

Exclusion criteria

- Patients who were followed for less than 3 years from diagnosis of the disease.

- Patients with other malignancies diagnosed simultaneously or within 5 years before the diagnosis of breast cancer.

- Pregnant women at diagnosis

- Patients with metastatic disease at diagnosis of the primary tumor.

Samples

Formalin fixed paraffin embedded samples or fresh tissue samples from primary biopsy and tumor after surgery were used. Sections 4 microns thin were cut into and mounted on special charged slides that are suitable for immunohistochemical (IHC) staining.

Evaluation

All histopathological preparations were prepared by the same 2 experienced technologists. Each slide was read by the same two special pathologists.

Staining

Immunohistochemical markers. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded breast cancer specimens or on fresh tissue as described previously [17]. ER and PR status were considered as positive if more than 10% of tumor cells showed staining. Immunohistochemical score of 3+ for HER2 was accepted as HER2 positivity. The immunohistochemical detection of Ki-67 (clone MIB-1, DAKO M7240, Dako Corporation, Carpinteria, CA, USA; dilution 1:70) was carried out as

Characteristic	Number (%)
Mean age (years ± SD)	52.3 ± 13.9
No of children (± SD)	4.0 ± 2.9
Ethnicity	
Jews	54 (54)
Arabs	46 (46)
Menopausal status	
Pre	56 (56)
Post	44 (44)
Pathology of carcinoma	
Invasive ductal	90 (90)
Invasive lobular	7 (7)
Invasive papillary	2 (2)
Mucinous	1 (1)
Grade of histology	
Grade 1	8 (8)
Grade 2	25 (25)
Grade 3	36 (36)
Grade x	31 (31)
Primary tumor status	
T1	26 (26)
T2	56 (56)
T3	16 (16)
T4	2 (2)
Primary axillary LN status	
N0	64 (64)
N1	24 (24)
N2	12 (12)
Surgery	
Lumpectomy	75 (75)
Mastectomy	25 (25)
Latest status	
Alive without disease	94 (94)
Alive with disease	4 (4)
Dead with disease	2 (2)
G: grade, N: lymph nodes, T: tumor, SD: standard deviation	

Table 1: Clinical and pathological characteristics of 100 women with breast cancer included in this study.

previously reported [17]. Ki-67 positivity was defined in presence of more than 15% positively stained cells. CISH test was also used for HER2 positive +2 in IHC.

Statistical analysis

The Chi-squared test was used to measure the statistical association between ER, PR, HER2 and Ki67 detection and the time of measurement. Demographic, clinical and pathological characteristics and all analyses, were carried out using SPSS, version 25 (SPSS Inc., Chicago, IL, USA) using the SPSS Data Analysis Program (ver. 17.1). P<0.05 were considered statistically significant.

Results

One hundred patients were identified for this study All patients had both CNB from the primary tumor and tumor tissue from surgical specimen after NAT. Mean age of patients at diagnosis was 52.3±13.9 years (range 25-88). Invasive ductal carcinoma was detected in 90 (90%) of patients at primary diagnosis, invasive lobular carcinoma in 7(7%) patients, 2(2%) invasive papillary and one (1%) mucinous carcinoma. The characteristics of patients, and their clinical and pathological features are presented in table 1. Fifty four percentage (54%) patients were Jewish and 46 (46%) were Arab. The majority of patients (56%) were postmenopausal and 44% were premenopausal at the time of diagnosis. T1 and T2 tumors at presentation were diagnosed in 82% of patients (Table 1). Clinically 36% of patients presented with involved axillary lymph nodes. Following NAT 75% of patients underwent lumpectomy and 25% mastectomy, both with sentinel lymph node biopsy or ALND.

Estrogen receptor status

In the primary biopsy ER was positive in 76 (76%) and negative in 24% of patients. In surgical specimens ER was positive in 71% and negative in 29% of patients (Table 2). ER did not change in any of the 100 tumors from negative to positive after NAT while the number of patients with ER negative status rose from 24% to 29% (P =<0.20).

Progesterone receptor status

PR was found to be positive in 63 (63%) and negative in 37 (37%) of tumors in the primary biopsy. In surgical biopsy specimens it was positive in 50% of tumors and negative in 50% of the tumors (Table 2). PR positive status decreased from 63% to 50% following NAT (P =<0.001).

Human epidermal receptor 2 (HER2) status

HER2 detected by a IHC or CISH test was positive in 32 (32%) and negative in 68 (68%) of patients in the primary biopsy (Table 3). In surgical specimens HER2 was positive in 19 (19%) of patients. HER2 positivity decreased from 32% to 19%. The number of patients with HER2 negative status increased from 68% to 81% after NAT (P =<0.001).

Ki67 status

In primary biopsies Ki67 was high (presence of more than 15% positively stained cells) in 45 (45%) patients and low in 55 (55%) of the patients. Ki67 was found to be high in 25 (25%) and low in 75 (75%) of patients in surgical

Status	Primary biopsy number		Surgical specimen		P value
			Positive (%)	Negative (%)	
ER	Positive	76	71 (71)	5 (5)	NS
	Negative	24	0 (0)	24 (24)	
PR	Positive	63	51 (51)	11 (11)	<0.001
	Negative	37	2 (2)	53 (53)	
ER: estrogen receptor, PR: progesterone receptor, NS; non significant					

Table 2: Comparison of ER and PR Status in core needle biopsy and surgical specimen after neoadjuvant treatment.

Status	Primary biopsy number		Surgical specimen		P value
			Positive (%)	Negative (%)	
HER2	Positive	32	29 (29)	13 (13)	<0.001
	Negative	68	0 (0)	68 (68)	
HER2	Positive Total	19	19 (19)	81 (81)	<0.001
HER2 human epidermal growth factor receptor 2					

Table 3: Comparison of HER2 status in core needle biopsy and surgical specimen.

Status	Primary biopsy number		Surgical specimen		P value
			Positive (%)	Negative (%)	
Ki67	High	45	24 (24)	21 (21)	<0.001
	Low	55	4 (4)	51 (51)	
Ki67	Total	100	28 (28)	72 (72)	<0.001
Ki-67 mean ± SD: 34.8 ± 26.1 before and 17.8 ± 22.9 after surgery <0.001					

Table 4: Comparison of Ki67 status in core needle biopsy and surgical specimen.

specimens (P =<0.001). Mean value ± SD was 34.8 ± 26.1 in CNB of primary tumors decreasing to 17.8 ± 22.9 after NAT (Table 4).

Ethnicity (Arab versus Jewish patients)

Arab women presented with more aggressive tumors than Jewish women. T1 and T2 tumors were diagnosed in 76.1% of Jewish women as against 68.5% in Arab women (P =<0. 01). N1 and N2 at presentation was detected in 20 of 46 (43.5%) Arab compared to 16 of 54 (29.6%) in Jewish patients (P =<0. 001). HER2 overexpression was higher in Arab breast cancer patients; 18 of 46 (39%) patients. In Jewish patients HER2 was positive in 14 out of 54 (26%) patients in their primary biopsy. 13 patients showed a change of HER2 from positive to negative after NAT, 7 were Arab patients (39% of 18 patients) and 6 (43% of 14 patients) were Jewish patients (NS). Change in Ki67 levels after NAT was similar in both groups.

Discordance and concordance (before and after NAT)

Concordance in ER and PR levels before and after NAT was 95% and 87% retrospectively. All ER negative cases were also negative after NAT. Five of 76 (6.5 %) tumors with ER positive in the primary CNB became negative in surgical specimen. In PR positive tumors 11 of 63 (17.5%) patients became negative following NAT while 2 of 37 PR negative (5.4%) became positive. HER2 was negative and continued to be negative after NAT in 68 (68%) of women. Among 32 patients with a positive HER2 in the primary biopsy, 13 of 32 (40.1%) tumors became negative in surgical specimens following NAT. In the whole population of patients there was an 87% concordance of HER2 expression before and after NAT. Discordance was 13% (P =<0.01). Among 19 patients with no change in HER2 status pathological partial response (pPR) was recorded in 10 (53%) and minimal response or

no change in tumor diameter was found in 9 (47%) patients following NAT. In 13 patients who had a pPR, HER2 changed from positive to negative after NAT 6 (46%). No significant difference was found between the 2 groups although a trend was evident.

A noticeable change was seen in the expression of Ki67 levels following NAT. In 21 of 45 (46.7%) patients Ki67 was high and became low after NAT (P =<0.001), while it was low and became high in 4 of 55 (7.3%) patients. Concordance of Ki67 level was found in 75% in primary versus post-surgery levels. Discordance was 25% (P =<0.001). No correlation was found between pathological response rate to NAT and changes in Ki67 levels.

Discussion

NAT is widely used in breast cancer leading to high rates of disease free survival and overall survival compared with adjuvant therapy [7,8]. NAT has became a standard in locally advanced, operable, HER2 positive and triple negative breast cancer. It also increases the success of breast-conserving surgery. There is no consensus in the literature about the concordance and discordance of ER, PR, HER2 and Ki67 expression levels before in CNB and after in surgical specimens NAT and its effect on treatment decisions. No known publication has reported all the 4 markers in one study and its comparison with clinical, pathological, ethnicity and tumor response to NAT [14,18-20]. Arens et al. and Kinsella et al. reported NS changes in ER, PR, HER2 and Ki67 expression rates before and after NAT [14,19]. Wen-Kai Ge et al. in their publication in 2015 found no change in ER, PR, HER2 and Ki67 before and after NAT and surgery [20].

This single study has tested all the markers in 100 patients and compared their expression and changes in their levels after NAT with all clinical, pathological, ethnic and treatment response data. The discordance rate of HER2 and

Ki67 in our study was 13% and 25% retrospectively after NAT. Robertson et al. reported a 7.4% discordance in HER2 expression after surgery. In their study discordance in HER2 and Ki67 after NAT was not significant [21]. In this study discordance in both was statistically significant (P =<0.01). As in other studies, we report only minor changes in ER and PR levels following NAT [22,23]. In our study discordance in ER status was 6.5% while in PR it was higher, 13%.

Changes in predictive markers may happen not only as a result of NAT. Discordance of HER2 overexpression, ER, PR in breast cancer metastases compared to primary tumor has been reported in several studies. HER2 levels may change from positive to negative or visa versa [24,25]. Changes have also been seen in ER, PR levels [26]. Clinical metastases may grow from micro metastases resistant to adjuvant therapy and may represent one particularly aggressive clone from among many clones of primary breast cancer [27].

Discordance in the expression of biomarkers may affect the choice of postoperative adjuvant treatment in clinical practice and may be a predictive factor for prognosis and resistance to treatment. Pathological complete response after NAT is one of the most important predictive prognostic markers in breast cancer [23].

The reasons for changes in ER, PR, HER2 and Ki67 specifically following NAT may varies. Tumor heterogeneity may be one of the main reasons for changes in these factors. Breast cancer is a multi-clonal tumor and genetically unstable disease [28,29]. NAT may not cause tumor killing in all clones' Other clones resistant to treatment, found in surgical specimens, may have different levels of tumor markers. External reasons can also effect the results of tumor marker testing. Equipment and reagents used in IHC can differ in different pathology departments and medical centers. Differences in technical preparation of the IHC staining, inadequate fixation of the central tumor epithelium, fixation times, problems of delayed fixation, underfixation and overfixation with formalin before IHC have been reported. Inter- and intra-observer variability may occur [30,31]. Other reasons for differences in IHC is the dilution effect, which refers to a decrease of biomarker expression with increasing number of evaluated tumor cells in the surgical sample [32].

In a previous study we reported different pathological, clinical and biological characteristics of breast cancer in Arab as versus Jewish women in northern Israel [33]. HER2 overexpression, poorly differentiated breast cancer cell rate and lymph node metastases rates were significantly higher in Arab women. In the same study HER-2 overexpression was found in 35.4% of Arab compared to 22% of Jewish women (p<0.0001). Discordance in breast features between different ethnicities is reported in the oncology literature [34-36]. Changes in tumor markers after NAT may be not equal in different ethnical groups [35]. In the recent study HER2 overexpression was higher in Arab than in Jewish women; 39% versus 26% in the primary biopsy (P =<0. 01). Although not statistically significant, the rate of change of HER2 from positive to negative after NAT was higher in Jewish than in Arab women; 6 of 14 positive patients (43%) and 7 of 18 positive patients (39%) respectively became negative.

There is no consensus in the literature regarding the effect of chemotherapy agents and targeted agents used in NAT and the change in ER, PR, HER2 and Ki67 expression rate in surgical specimen compared to primary biopsy. Guarneri et al. and Shuai et al. found important changes in HER2 levels after NAT which were related to specific therapeutic agents [37,38]. In this study no significant changes were found in tumor markers after the use of different agents (Epirubicin, docetaxel, paclitaxel, trastuzumab and pertuzumab) used in NAT. In their publication Rey-Vargas et al. also found no relation between the type of agents used and changes in tumor markers after NAT [39].

Niikura et al. found no relationship between changes in HER2 expression after NAT and pathological response rates [23]. Similar to their study, in this study no relation was found between changes in ER, PR and Ki67 following NAT and pathological response rate. On the other hand we found a trend between changes in HER2 positivity and pPR; 53% of HER2 positive patients had no change after NAT compared to 46% of HER2 positive patients who became negative.

A potential bias in this study concerns the exclusion of patients who achieved a complete pathological response after NAT; no tumor cells were obtained to test ER, PR, HER2 and Ki67 in surgical specimens. Secondly, we have not discussed endocrine therapy, which is rarely used in NAT and only in a very few patients in this study.

Tsai et al, Pochler et al. and others have reported similar changes to our results in ER, PR, HER2 and Ki67 following NAT [40]. They concluded that re-testing of predictive biomarkers in surgical specimens is valuable as a prognostic factor and could assist in deciding on adjuvant treatment following surgery.

Conclusion

To our knowledge this is the first study comparing changes of ER, PR, HER2 and Ki67 after NAT with all clinical, pathological, treatment response and ethnic factors (Jewish and Arab women in Israel), in one study. The significant discordance of predictive biomarkers between CNB and surgical specimens assists in a better understanding of the pharmaco-biology of breast cancer and clarifies the importance of testing these biomarkers after NAT in breast cancer patients. Retesting results may affect the choice of adjuvant treatment and understanding resistance to treatment.

Acknowledgment

Sources of Support

Funding

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