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ORIGINAL ARTICLE

Evaluation of changes of biologic markers ER, PR, HER 2 and Ki-67 in breast cancer with administration of neoadjuvant dose-dense doxorubicin, cyclophosphamide followed by paclitaxel

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Summary

Purpose: To assess the changes of biologic markers estrogen receptors (ER), progesterone receptors (PR), HER 2 and Ki-67 in locally advanced breast cancer patients after neoadjuvant chemotherapy.

Methods: Data from 63 locally advanced breast cancer patients (stage II or III), whose histological diagnosis was made by core biopsies were retrospectively evaluated. The patients were given 4 cycles of 600 mg/m^2 cyclophosphamide, 60 mg/m^2 doxorubicin every 15 days, followed by 4 cycles of paclitaxel 175 mg/m^2, followed by mastectomy within 2 weeks after the last chemotherapy cycle. The changes in ER, PR, HER 2 and Ki-67 status of the operated tumor tissue were compared with the material obtained by initial core biopsies.

Results: The patient mean age was 49.2±10.7 years. Most (57.1%) were premenopausal. Clinical disease stages ranged between T2N1 and T3N2. Pathological complete response (pCR) rate was 14.9 % (n=9). Two (5.7%) patients who were ER positive prior to treatment showed ER negativity after treatment. In 7 (21.1%) patients PR became negative and in 3 (9.0%) became positive after neoadjuvant chemotherapy. Changes in ER and PR receptors were not statistically significant (p=0.500 and PR p=0.549, respectively), whereas in 2 (5.8%) patients hormonal status changed significantly when compared to initial biopsies (p=0.003).

In addition, the median value of PR intensity decreased from 20 to 10% (p=0.003) and Ki-67 decreased from 10 to 1% (p<0.001) following neoadjuvant therapy. Five (14.1%) patients exhibited some changes in HER 2 expression: HER 2 expression became 2+ in 3 patients previously being HER 2 negative, and in 2 patients HER 2 became negative whilst it was 1+ and 2+ prior to neoadjuvant chemotherapy.

Conclusion: It was observed that the biologic markers ER, PR, HER 2 and Ki-67, from the same tumor material demonstrated differences after neoadjuvant treatment in breast cancer patients. These changes may affect the treatment decision.

Key words: breast cancer, ER, HER 2, Ki-67, neoadjuvant chemotherapy, PR

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Introduction

Treatment modalities in breast cancer are modified according to tumor grade, disease stage, and molecular properties of the tumor and characteristics of the patient. These parameters are all taken into account in order to choose the treatment modality most appropriate for each patient. Neoadjuvant chemotherapy is one of these treatment modalities, once used only in locally advanced breast cancer, but nowadays it is frequently used as an initial treatment option for operable patients [1].

It is known that chemotherapeutic agents cause some changes in the cell leading to cell death [2-5]. However, very few studies investigated if molecular changes beyond the lethal process are initiated with chemotherapy in cancer cells. However, it is critical to know if a neoadjuvant chemotherapy regimen changes some of the biologic markers of the tumor or causes selection of a tumor fraction which is biologically different from the chemotherapy-naive tumor.

In this study, we aimed to evaluate the changes in the biologic markers ER, PR, HER 2 and Ki-67 in locally advanced breast cancer patients after neoadjuvant chemotherapy.

Methods

From December 2005 to December 2010, 63 patients with breast cancer, clinical stage II or III, planned for surgery were retrospectively analyzed. Patients with early stage or metastatic breast cancer were excluded from study. These 63 patients whose initial histologic diagnosis was performed by core biopsy were included into this study. Patients received dose-dense chemotherapy consisting of 4 cycles of 600 mg/m^2 cyclophosphamide and 60 mg/m^2 doxorubicin every 15 days, followed by 4 cycles of paclitaxel 175 mg/m^2 every 15 days. Surgery was performed within 2 weeks after the last chemotherapy cycle. ER, PR and Ki-67 estimations were performed using standard immunohistochemical techniques. For ER and PR, nuclear expression in >1% of tumor cells was accepted as positive. Ki-67 proliferation index was defined as the percent of Ki-67 positive cells measured in 1000 cancer cells. ER, PR, HER 2 and Ki-67 expression was compared between pre-therapy tumor core biopsies and post-neoadjuvant chemotherapy surgical tumor biopsies. Evaluation of HER 2 status was performed according to the ASCO/CAP guidelines by immunohistochemistry as 4-graded system (0-3+). Cases with grade 2+ were further evaluated with fluorescence *in situ* hybridization (FISH) method. To determine the HER 2 positivity rate using FISH, the FDA-approved cut-off ratio (HER 2 signals/chromosome 17 signals) of 2.0 was used. All specimens were reviewed by two pathologists at the same time.

Statistics

Normally distributed continuous variables were presented as means ± standard deviation (SD) and skew-distributed continuous variables as median with range. Categorical variables were shown as percentages. To make comparisons between groups, the Mann-Whitney U test was used for skew-distributed continuous variables and the McNemar test was used for dependent categorical variables. The Statistical Package for Social Science (SPSS), version 17,0 was used. Statistical significance was put at <0.05.

Results

The mean age of the patients was 49.2 ±10.7 years. Thirty-six (57.1%) of them were premenopausal, 23 (36.5%) postmenopausal and 4 (6.3%) perimenopausal. Histologic classification of the tumors was as follows: 49 (77.8%) patients had infiltrative ductal carcinoma, 9 (14.3%) mixed type carcinoma (infiltrative ductal carcinoma +lobular carcinoma), and 5 (8.0%) patients had other subtypes.

The initial clinical stages assessed clinically and radiologically ranged between T2N1 and T3N2. Most patients (87.3%) had radical mastectomy after neoadjuvant chemotherapy. Three of the tumors were grade 1 (4.8%), 28 (44.4%) grade 2 and 24 (38.1%) grade 3 and in 8 (12.7%) patients tumor grade was undetermined.

The overall clinical response rate obtained with neoadjuvant therapy was 88.8 % (n=56). Forty-six percent (n=29) of the patients had a complete clinical response (CR) and 42.8% (n=27) had a partial clinical response (PR). In 6 (9.5%) patients disease remained stable (SD) after neoadjuvant therapy. The pathological complete response (pCR) rate was 14.9 % (n=9; Table 1).

Due to technical reasons it was not possible to study tumor biologic markers after neoadjuvant therapy in all patients with incomplete pathological response. Quali-

Table 1. Clinical characteristics of the patients and response to neoadjuvant therapy

Characteristics	*N (%)*
Age, years	
mean±SD	49.2 ±10.7
Menopausal status	
Premenopausal	40 (63.4)
Postmenopausal	23 (36.5)
Comorbid disease	
None	37 (58,7)
Hypertension	12 (19.4)
Diabetes mellitus	5 (7.9)
Hyperlipidemia + coronary artery disease	8 (12.6)
Thyroid disease	3 (4.7)
Histologic cancer type	
Infiltrative ductal	49 (77.8)
Mixed (infiltrative +lobular)	9 (14.3)
Metaplastic	3 (4.8)
Others	2 (3.2)
Tumor grade in initial biopsy	
1	3 (4.8)
2	28 (44.4)
3	24 (38.1)
Unknown (could not be assessed)	8 (12.7)
Response to neoadjuvant treatment	
pCR	9 (14.9)
cCR	29 (46.0)
cPR	27 (42.8)
cSD	6 (9.5)
cPD	1 (1.6)

p: pathological, c: clinical, SD: standard deviation

tative changes in ER and PR receptors before and after therapy are shown in detail in Table 2.

Of the 35 patients with evaluated ER change, 33 (94.2%) conserved the same ER status. Two (5.7%) patients who were ER positive prior to neoadjuvant treatment showed ER negativity after treatment. Thirty-three patients were assessed for the PR status and 23 (69.2%) patients conserved the same PR status. In 7 (21.1%) patients PR became negative after neoadjuvant chemotherapy and in 3 (9.0%) PR became positive. Changes in ER and PR receptors were not statistically significant (p=0.500 and PR p=0.549, respectively).

In addition to assessing the changes in ER and PR status separately, changes in ER and PR status were also evaluated together as hormone positive and hormone negative (Table 3). According to this grouping the hormonal status remained unchanged in 32 (94.1%) patients, whereas in 2 (5.8%) patients the hormonal status changed when compared to initial biopsies (one from hormone negative to positive and the second one from hormone positive to negative). The difference in hormonal status pre- and post treatment was statistically significant (p=0.003).

Besides qualitative changes, we also evaluated the quantitative changes of hormone receptors. The change in the intensity of hormone receptors before and after neoadjuvant therapy is shown in Table 4. The median ER intensity in the core biopsy material prior to chemotherapy was 50 (range 0-90) and after treatment it was 70 (range 0-90) (p=0.75) The median PR intensity prior to neoadjuvant chemotherapy was 20 (range 0-90), and

Table 2. Assessment of qualitative changes in ER, PR after neoadjuvant chemotherapy

	Initial tumor (core biopsy)	*Operated tumor (surgical biopsy)*	*N (%)*
ER (N=35)	Positive	Positive	29 (82.8)
	Positive	Negative	2 (5.7)
	Negative	Positive	-
	Negative	Negative	4 (11.4)
PR(N=33)	Positive	Positive	17 (51.1)
	Positive	Negative	7 (21.1)
	Negative	Positive	3 (9.0)
	Negative	Negative	6 (18.1)

Table 3. The changes in hormonal status after neoadjuvant chemotherapy

	After treatment		
	Hormone negative (ER and PR neg) N (%)	*Hormone positive (ER or PR pos) N (%)*	*p-value*
Before treatment			
Hormone negative (ER and PR negative)	3 (8.8)	1 (2.9)	0.003
Hormone positive (ER or PR positive)	1 (2.9)	29 (85.3)	

Table 4. Changes in median values of hormone receptor intensity and Ki -67 before and after neoadjuvant chemotherapy

	Before neoadj.chemotherapy Median (range)	*After neoadj.chemotherapy Median (range)*	*p-value*
Hormone receptor intensity			
ER	50 (0-90)	70 (0-90)	0.753
PR	20 (0-90)	10 (0-90)	0.003
Ki-67	10 (1-60)	1 (1-1)	<0.001

Table 5. Changes in HER 2 expression assessed by immunohistochemistry

Changes in HER 2 expression	*N (%)*	*p-value*
Increase	3 (8.4)	
Decrease	3 (8.4)	
No change	36 (83)	< 0.001

after treatment it was 10 (range 0-90) (p=0.003). When we looked at the Ki-67 values we found a statistically significant decrease from 10 to 1% following neoadjuvant therapy (p<0.001).

Thirty-six patients were evaluated for change in HER 2 status. In 30 of them (83%) HER 2 expression remained unchanged, whereas 6 (17%) patients exhibited some changes : HER 2 expression became 2 + in 3 patients who had negative HER 2 prior to treatment (0 before treatment and 2+ after treatment), and HER 2 expression became negative in 2 patients who had 1+ and 2+ score following neoadjuvant therapy. When the specimens which converted to 2+ after therapy were further evaluated by FISH no gene amplification was found (Table 5).

Discussion

The fact that chemotherapeutic agents cause some changes on some components of the tumor cells is known since 1960s. First, Waller demonstrated changes such as enlargement of the nucleus, swelling of the cytoplasm, vacuolization of the cytoplasm/nucleus in tumor cells following systemic administration of busulphan [2]. Since the changes in the molecular properties of the cancer cell may affect the tumor behavior and therefore the treatment plan, the number of studies investigating how the chemotherapeutic agents affect tumor grade, receptor properties of tumor cells and tumor proliferation rate have been increasing recently. In this study we examined the qualitative and quantitative changes in ER, PR, HER 2

and Ki-67 in breast cancer patients receiving neoadjuvant dose-dense chemotherapy with doxorubicin and cyclophosphamide followed by paclitaxel.

Several studies looked at hormone receptor changes with neoadjuvant chemotherapy in tumor cells. Taucher et al. studied the effect of neoadjuvant therapy in a group of 214 patients and reported that 14% of the tumors which were ER positive and 51% of the tumors which were PR positive initially, became hormone receptor negative and both changes were statistically significant (p=0.02 for ER and p=0.0005 for PR) [6]. In a study by Makris et al. 11 patients showed a statistically significant change in ER status (p=0.04) and 15 patients showed a change in PR status which was not statistically significant [7]. There are some other studies which demonstrated that neoadjuvant therapy results in changes in hormone receptor status in breast cancer [8,9]. On the other hand, some investigators support the idea that possible changes caused by neoadjuvant therapy in hormone receptor status of a tumor do not show a significant importance [10-15]. In our study 2 (5.7 %) patients who were ER positive before neoadjuvant treatment became ER negative and the change was statistically significant (p<0.001). Ten patients (30.1%) showed a change in PR status: 7(21.1%) of them converted from positive PR to negative and 3 (9.0%) patients converted from negative PR status to positive. However, these changes were not statistically significant (p=0.160).

Most of the studies about Ki-67 reported a decrease in this index after neoadjuvant therapy. Studies by Makris et al. and Yin et al. showed a statistically significant decrease in Ki-67 proliferation index following neoadjuvant chemotherapy (p=0.001 and p= 0.01, respectively) [7,16]. Bottini et al. and Pohl et al. reported similar results confirming decrease in Ki-67 index [10,17]. In a study by Koda et al. Ki-67 indices significantly decreased after neoadjuvant chemotherapy in the primary breast tumor when compared to pre-treatment values, although no change was observed in metastatic lymph nodes [18]. In concert with other similar studies, in our study we observed a significant decrease in Ki-67 with neoadjuvant chemotherapy from median 10% prior to therapy to 1% after therapy (p<0.001). This finding may be related with the conversion of the whole tumor cell population to a less proliferative status or may be the selection of the less proliferative cells by means of neoadjuvant treatment. But in both cases the results of our study confirm the results of other investigators who report decrease in Ki-67 index as a result of neoadjuvant treatment.

Another important issue in the treatment of breast cancer is the changes in the HER 2 status. The variations of this biological marker in the primary tumor and its synchronous or metachronous metastases and in HER 2 status after neoadjuvant treatment have been assessed in several studies [19,20].

Studies dealing with the effect of neoadjuvant chemotherapy on HER 2 expression showed conflicting results. Adams et al. reported increased expression of HER 2 after neoadjuvant chemotherapy in breast cancer patients in whom the hormone receptor status remained unchanged [13].

Taucher et al. evaluated HER 2 changes with immunohistochemical methods in addition to hormone receptor changes in their study and showed that HER 2 positivity changed after neoadjuvant chemotherapy but the results were not statistically significant. They also reported that confirmation of the results with FISH method revealed that the difference was much smaller compared with the immunohistochemical method [6]. Another study by Burcombe et al. [21] showed a change in HER 2 expression with neoadjuvant chemotherapy in 9 of 118 patients (from 3+ to 2+ in 3 patients and from 2+ to 3+ in 5 patients) and the authors suggested reevaluation of HER 2 in the residual tumor in patients whose initial HER 2 showed mild to high positivity after neoadjuvant therapy. Additionally Neubauer et al. [22] reported that 13% of the tumors switched from HER 2 positive to negative after neoadjuvant therapy. On the other hand, there are studies which report no change in HER 2 expression with neoadjuvant therapy [23-25].

In our study we observed that HER 2 expression remained unchanged in 30 (83%) of the 36 patients, while some degree of change occurred in 6 (16.6%) patients. In 3 patients HER 2 converted to positive (2+) from negative and in one patient HER 2 converted to negative from 1 +. However, when we reevaluated the specimen which showed a conversion from negative to 1+ with the FISH method we found that none of the 3 samples showed HER 2 amplification. So our results are supporting the studies that report no change in HER 2 expression with

neoadjuvant chemotherapy.

The present study has some limitations. The number of cases included was less than expected due to technical problems, e.g. problems in the conservation of tissue samples and technical problems during specimen staining procedures. Immunohistochemical methods used in the evaluation of HER 2 and hormone receptors may have been affected from different factors. For example, improper tissue sampling and conservation under inappropriate conditions until fixation, insufficient amount of tissue specimen or sampling made from an area which did not represent the heterogeneity within the tumor could all have affected the results. Direct effects of the chemotherapy itself on immunohistochemical staining and factors related to the observing pathologist are other important factors. However, in our study the histological evaluation was made by consensus of two different pathologists who were specialized in this area.

The biological effects of chemotherapeutic agents on cancer cell other than cell death are the issues that should be investigated. Understanding the chemotherapy-induced biological conversion in the tumor cell is strategically important in planning adjuvant therapy and for disease follow up. Studies on different cancer types and on larger populations may help understand the effects of chemotherapeutic agents on tumor biology.

References

1. Berruti A, Generali D, Kaufmann M et al. International expert consensus on primary systemic therapy in the management of early breast cancer: highlights of the Fourth Symposium on Primary Systemic Therapy in the Management of Operable Breast Cancer, Cremona, Italy (2010). J Natl Cancer Inst Monogr 2011; 43:147-151.
2. Waller U. Giant nuclei after myleran therapy and splenic irradiation in chronic myeloid leukemia. Pathol Microbiol (Basel) 1960; 23: 283-290.
3. Rasbridge SA, Gillett CE, Seymour AM et al. The effects of chemotherapy on morphology, cellular proliferation, apoptosis and oncoprotein expression in primary breast carcinoma. Br J Cancer 1994; 70; 335-341.
4. Honkoop AH, Pinedo HM, De Jong JS et al. Effects of chemotherapy on pathologic and biologic characteristics of locally advanced breast cancer. Am J Clin Pathol 1997; 107: 211-218.
5. Moll UM, Chumas J. Morphologic effects of neoadjuvant chemotherapy in locally advanced breast cancer. Pathol Res Pract 1997;193:187–196.
6. Taucher S, Rudas M, Gnant M et al. Sequential steroid hormone receptor measurements in primary breast cancer with and without intervening primary chemotherapy. Endocr Relat Cancer 2003; 10: 91-98.
7. Makris A, Powles TJ, Allred DC et al. Quantitative changes in cytological molecular markers during primary medical treatment of breast cancer: A pilot study Breast Can Res Treat 1999; 53: 51-59.
8. Jain V, Landry M, Levine EA. The stability of estrogen and progesterone receptors in patients receiving preoperative chemotherapy for locally advanced breast carcinoma. Am Surg 1996; 62: 162-165.
9. Lo SS, Wang HC, Shyr YM, Lui WY. Can the hormonal receptor status of primary breast cancer be altered by neoadjuvant chemotherapy? J Surg Oncol 1994; 57: 94-96.
10. Bottini A, Berruti A, Bersiga A et al. Relationship between tumour shrinkage and reduction in Ki-67 expression after primary chemotherapy in human breast cancer. Br J Cancer 2001; 85: 1106-1112.
11. Lee SH, Chung MA, Quddus MR, Steinhoff MM, Cady B. The effect of neoadjuvant chemotherapy on estrogen and progesterone receptor expression and hormone receptor status in breast cancer. Am J Surg 2003;186:348-350.
12. Hawkins RA, Tesdale AL, Anderson ED, Levack PA, Chetty U, Forrest AP. Does the oestrogen receptor concentration of a breast cancer change during systemic therapy? Br J Cancer 1990; 61:877-880.
13. Adams AL, Eltoum I, Krontiras H. The Effect of Neoadjuvant Chemotherapy on Histologic Grade, Hormone Receptor Status, and Her2/neu Status in Breast Carcinoma. Breast J 2008;14: 141-146.
14. Arens N, Bleyl U, Hildenbrand R. HER2/neu, p53, Ki67, and hormone receptors do not change during neoadjuvant chemotherapy in breast cancer. Virchows Arch 2005 ;446:489-496.
15. Schneider J, Lucas R, Sánchez J et al. Modulation of molecular marker expression by induction chemotherapy in locally advanced breast cancer: correlation with the response to therapy and the expression of MDR1 and LRP. Anticancer Res 2000; 20:4373-4377.
16. Yin HF, Wang YH, Qin XQ et al. Effect of neoadjuvant chemotherapy on histologic grade and expression of biological

markers in breast cancer. Zhonghua Zhong Liu Za Zhi 2009; 31: 858-862.

17. Pohl G, Rudas M, Taucher S et al. Expression of cell cycle regulatory proteins in breast carcinomas before and after preoperative chemotherapy. Breast Cancer Res Treat 2003; 78: 97-103.
18. Koda M, Sulkowska M, Koda KL et al. The effect of chemotherapy on Ki-67, Bcl-2 and Bak expression in primary tumors and lymph node metastases of breast cancer. Oncol Rep 2007; 18: 113-119.
19. Arapantoni-Dadioti P, Valavanis C, Gavressea T et al. Discordant expression of hormone receptors and HER2 in breast cancer. A retrospective comparison of primary tumors with paired metachronous recurrences or metastases. J BUON 2012 ;17:277-283.
20. Aitken SJ, Thomas JS, Langdon SP, Harrison DJ, Faratian D. Quantitative analysis of changes in ER, PR and HER2 expression in primary breast cancer and paired nodal metastases. Ann Oncol 2010 ;21:1254-1261.
21. Burcombe RJ, Makris A, Richman PI et al. Evaluation of Ki67 proliferation index before and during neoadjuvant chemotherapy for primary breast cancer. Breast Cancer Res Treat 2002; 76: abstr no. 229.
22. Neubauer H, Gall C, Vogel U et al. Changes in tumour biological markers during primary systemic chemotherapy. Anticancer Res 2008; 28: 1797-1804.
23. Herbst RS, Khuri FR. Mode of action of docetaxel basis for combination with novel anticancer agents. Cancer Treat Rev 2003; 29: 407-415.
24. Faneyte IF, Schrama JG, Petersen JL et al. Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer 2003; 88: 406-412.
25. Billgren AM, Rutqvist LE, Tani E et al. Proliferating fraction during neoadjuvant chemotherapy of primary breast cancer in relation to objective local response and relapse-free survival. Acta Oncol 1999; 38: 597-601.