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Neoadjuvant Chemotherapy Exerts Selection Pressure Towards Luminal Phenotype Breast Cancer

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Keywords

Breast cancer · Chemotherapy, neoadjuvant · Endocrine therapy · Residual disease

Summary

Background: Breast cancer (BC) phenotype after neoadjuvant chemotherapy (NAC) has not been extensively described and few data exist on whether expression of the primary tumor hormone receptors, HER2 and Ki-67 changes as a result of chemotherapy. **Materials and Methods:** We analyzed specimens from all BC patients treated with anthracycline/taxane-based NAC at our Institution between January 2010 and March 2015 (n = 325). The expression of estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 was determined in pre- and post-NAC specimens. McNemar's test was used to compare paired proportions. **Results:** Among patients with residual disease after NAC, basal phenotype was luminal A, luminal B, HER2 positive and triple negative in 44, 111, 74 and 27 cases, respectively. PR-positive tumors decreased from 68.0% in the initial biopsy sample to 61.7% in the surgical specimen (p = 0.024). A Ki-67 of < 20% increased from 23.6% to 45% (p < 0.001). ER expression changed from positive to negative in 5% and from negative to positive in 16.7% of cases. Overall, 30% of cases underwent subtype changes, 79% of them towards luminal differentiation. **Conclusions:** The switch towards luminal phenotype suggests some kind of endocrine effect of NAC. Our findings raise renewed interest in combinatorial cytotoxic chemotherapy with concomitant or rather sequential endocrine therapy, either alone or with targeted agents.

Introduction

Neoadjuvant chemotherapy (NAC) was originally introduced to reduce tumor size and enable surgery of locally advanced breast cancer (BC) [1]. Subsequent meta-analyses showed no differences in outcome between pre- and post-operative chemotherapy [2]. More recently, NAC has been considered as a unique opportunity to understand BC biology, thanks to the possibility of analyzing paired tumor samples before and after treatment [3]. The agents developed in this setting are similar to those validated in the adjuvant context, anthracyclines and taxanes being the most effective compounds [4]. Previous studies investigated the relation between Ki-67 drop and response to NAC. More recently, scientific interest was raised by the effects of NAC on hormone receptor (HR) and HER2 expression. Nevertheless, most previous works focused on small populations and various cytotoxic regimens. The objective of this study was to address BC phenotype changes in a homogeneous population of patients with localized or metastatic disease, mostly treated with anthracycline and taxane-based NAC within the same institution.

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Materials and Methods

We retrospectively collected information from the institutional database on all consecutive BC patients treated with NAC who underwent surgery at the Istituto Nazionale dei Tumori, Milan, Italy, between 1 January 2010 and 31 March 2015. For the current analysis we excluded all cases treated with chemotherapy in our Institution but undergoing surgery in other Institutions, as well as those without paired basal and surgical histological specimens and/or those treated with experimental therapy within a clinical trial. Data about patients' age, NAC regimen and duration, type and timing of surgery, clinical and pathological BC stage, and disease phenotype before and after NAC were extracted. All cases obtaining pathological complete response (pCR) were excluded from analysis. In absence of contraindications, all patients received anthracycline and taxane-based NAC. All HER2-positive cases were treated with trastuzumab, according to international guidelines. HR and HER2 status, Ki-67 index and disease stage were examined by Institutional pathologists. Diagnostic cut-offs for HR and HER2 positivity were defined according to American Society of Clinical Oncology–College of American Pathologists (ASCO-CAP) guidelines [5, 6]. Tumor subtype classification followed the St. Gallen/Vienna 2015 criteria [7]. All surgical specimens were completely sampled to detect residual disease. pCR was defined as the absence of invasive disease in both breast and lymph nodes. The primary objective of the study was to estimate percentage of changes in HR and HER2 status after NAC. Secondary objective was to compare percentage of cases with a specific tumor receptor status between pre- and post-NAC setting. 2-sided 95% exact confidence intervals (CIs) were calculated to estimate percentage of patients changing BC subtype. The 2-sided exact McNemar's test was used to assess equality of basal and surgical paired percentages. Patients' and tumor characteristics, NAC regimens and disease response were summarized using descriptive statistics (median and range for continuous variables, absolute and percentage frequencies for categorical ones). Median follow-up was estimated using the reverse Kaplan-Meier method. Given the descriptive nature of the study, a quantitative hypothesis testing was applied instead that a formal one (e.g. no thresholds for statistical significance were defined). Statistical analysis was performed using SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Table 1. Patients' characteristics

Age at surgery, years	
Median	50.5
Range	22.7–84.5
ER status at diagnosis, n (%)	
Positive	239 (74.0)
Negative	84 (26.0)
Missing	2 (0.6)
PR status at diagnosis, n (%)	
Positive	198 (62.3)
Negative	120 (37.7)
Missing	7 (2.2)
HER2 status at diagnosis, n (%)	
Positive	90 (28.6)
Negative	225 (71.4)
Missing	10 (3.1)
Ki-67 status at diagnosis, n (%)	
≥20%	228 (79.7)
<20%	58 (20.3)
Missing	39 (12.0)
Tumor stage at diagnosis, n (%)	
X	1 (0.3)
T1	5 (1.5)
T2	184 (56.6)
T3	31 (9.5)
T4	104 (32.0)
Nodal stage at diagnosis, n (%)	
N0	95 (29.2)
N1	199 (61.2)
N2	22 (6.8)
Metastasis at diagnosis, n (%)	
M0	285 (87.7)
M1	40 (12.3)

ER = estrogen receptor, PR = progesterone receptor.

Table 2. Treatment characteristics

Cytotoxic regimen, n (%)	
Anthracycline and taxane	292 (91.0)
Anthracycline alone	9 (2.8)
Taxane alone	12 (3.7)
Other	8 (2.5)
Missing	4 (1.2)
Cycles administered	
Median, n	6
Range, n	2–18
Missing, n (%)	9 (2.8)
Type of surgery, n (%)	
Modified radical mastectomy	221 (68.0)
Conservative surgery	104 (32.0)

Results

We identified 325 case with a median age of 51 years (range 23–85 years). Details about patients' and tumor characteristics are summarized in table 1 and table 2. 31 patients achieved pCR and were therefore excluded from analysis. Among the 294 patients with residual disease at surgery, paired tissue samples for determination estrogen receptor (ER) status at diagnostic biopsy and surgery were available in 274 cases (93.2%). 11 of 220 (5.0%, CI 2.5–8.8%) patients showed a change from ER-positive to ER-negative disease. 9 of 54 (16.7%, CI 7.9–29.3) patients converted from ER-negative to ER-positive disease. 34 of 183 (18.6%, CI 13.2–25.0%) cases with progesterone receptor (PR)-positive primary tumor had PR-negative residual disease at surgery. 17 of 86 (19.8%, CI 12.0–29.8%) cases with PR-negative disease showed PR-positivity at surgery. Among the 239 patients tested for HER2 in initial biopsy and surgical specimen, HER2 changed from positive to negative in 9 of 49 (18.4%, CI 8.8–32.0%) patients and from negative to positive in 7 of 190 (3.7%, CI 1.5–7.4%). Among the 229 cases with paired values of Ki-67, its expression decreased from ≥ 20% to < 20% in 63 of 175 (36.0%, CI 28.9–43.6%) patients and increased from < 20% to ≥ 20% in 14 of 54 (25.9%, CI 15.0–39.7%). The expression of PR and Ki-67 significantly differed between basal and surgical samples. Indeed, PR positivity was reported in 68.0% (CI 62.1–73.6%) of diagnostic biopsy samples and in 61.7% (CI 55.6–67.5%) of surgical specimens (McNemar's test p = 0.024). Ki-67 was ≥ 20% in 76.4% (CI 70.4–81.8%) of pre-treatment biopsies and in 55.0% (CI 48.3–61.6%) of residual diseases (McNemar's test p < 0.001). Overall, 62 of 206 (30.1%) cases presented a phenotype modification from basal biopsy to surgical specimen. In particular, of these 206

Table 3. Immunohistochemistry in initial biopsy and surgical specimens

Initial biopsy	Final surgery, n		Prevalence, % (95%CI)		p-value
	positive (≥20%)	negative (<20%)	initial biopsy	final surgery	
ER status					
Positive	209	11	80.3 (75.1–84.8)	79.6 (74.3–84.2)	0.824
Negative	9	45	19.7 (15.2–24.9)	20.4 (15.6–25.7)	
PR status					
Positive	149	34	68.0 (62.1–73.6)	61.7 (55.6–67.5)	0.024
Negative	17	69	32.0 (26.4–37.9)	38.3 (32.5–44.4)	
HER2 status					
Positive	40	9	20.5 (15.6–26.2)	19.7 (14.8–25.3)	0.804
Negative	7	183	79.5 (73.8–84.4)	80.3 (74.7–85.3)	
Ki-67					
≥ 20%	112	63	76.4 (70.4–81.8)	55.0 (48.3–61.6)	< 0.001
< 20%	14	40	23.6 (18.2–29.6)	45.0 (38.4–51.7)	

Table 4. Subtype changes after NAC

Basal histotype, n		Post-treatment histotype, n (%)			
		unchanged	changed		missing data
Triple negative	27	23 (95.8)	luminal B	1 (4.2)	3 (11.1)
			triple negative	2 (4.2)	26 (35.1)
HER2 positive	74	40 (83.3)	luminal A	4 (8.3)	
			luminal B*	2 (4.2)	
Luminal A	44	31 (77.5)	luminal B	9 (22.5)	4 (9.1)
Luminal B	111	50 (53.2)	triple negative	5 (5.3)	17 (15.3)
			HER2 positive	6 (6.4)	
			luminal A	33 (35.1)	

*2/2 cases changed from HER2-positive to luminal B HER2-positive (triple positive) breast cancer.

patients, 24 were triple-negative (11.7%) at baseline and 30 (14.6%) after NAC (McNemar's test p = 0.070); 48 were HER2 positive (23.3%) at baseline and 46 (22.3%) after NAC (McNemar's test p = 0.790); 40 were luminal A (19.4%) at baseline and 68 (33.0%) after NAC (McNemar's test p < 0.0001); and 94 luminal B (45.6%) at baseline and 62 (30.1%) after NAC (McNemar's test p < 0.0001). Most cases showing any kind of phenotype change presented locally advanced or metastatic disease as, of the 62, 30 (48.4%) were pT3-pT4, 49 (79.0%) were pN+ and 11 (17.7%) presented synchronous distant metastases. Tumor marker and phenotype changes in paired samples are reported in table 3 and table 4.

Discussion

Change in HR and HER2 status after NAC is a well-known phenomenon in BC, occurring in 10–30% of cases [8, 9]. Although the older results might be affected by pre-analytical and analytical pitfalls [10, 11], a real biological heterogeneity may be implied. It was hypothesized that paired samples from a single lesion may differ, even in absence of therapeutic interventions, as a consequence of malignant subpopulations coexistence. Some studies supported this theory, identifying no differences in phenotype change rates between paired samples from treatment-naïve and chemo-treated patients [12, 13]. An alternative hypothesis supports a drug-induced selection pressure towards malignant cells. Cytotoxic, endocrine and biological agents may specifically target and kill sensitive BC subpopulations, leaving behind the resistant ones. This hypothesis was sustained by some studies, showing higher frequency of BC phenotype change in NAC-treated patients than in naïve controls [14, 15]. The present work shows that most cases undergoing phenotype changes presented with advanced stage (pT3–4, pN+, cM+). This observation is in favor of the hypothesis that treatment might possibly affect part of a rather heterogeneous disease, leading to the emergence of an unresponsive residue. On the other hand, a considerable amount of data suggests that Ki-67 drop after NAC may be an independent positive prognostic factor in patients not achieving pCR [16, 17]. In the present study, we observed a significant decrease in PR and Ki-67 expression after NAC. This finding seems to support a prognostic, rather than an incidental role of BC phenotype change during NAC. Previous works postulated that cytotoxic agents may play an endocrine role, suppressing ovarian and adrenal function with consequent alteration of BC biological profile [18]. Indeed, it was shown that pre-menopausal

women with HR-positive tumors attaining amenorrhea after NAC had better outcome than menstruating women [19]. Post-menopausal patients may also achieve benefit, due to adrenal endocrine suppression induced by cytotoxic agents [20]. If confirmed, these hypotheses may entail important clinical implications, as PR decrease may be viewed as a marker of reduced endocrine stimulation in BC. Another finding of this study concerns the tendency towards luminal differentiation observed after NAC. Even though our data do not allow prognostic or pathogenetic conclusions to be drawn, this previously unreported finding is in line with the hypothesis of an endocrine effect of chemotherapy. This observation potentially raises new interest in the development of sequential cytotoxic and hormonal NAC approaches, or combinations with innovative drugs such as cyclin kinase inhibitors, to target biological modifications of tumor phenotype induced by standard NAC. A plethora of gene expression profiles have been generated during the last decades to discover, develop and validate prognostic and predictive gene signatures. Some of these signatures are commercially available to define the residual risk in ER-positive and HER2-negative tumors after receiving adjuvant endocrine treatment (Mammaprint, Oncotype DX, Breast Cancer Index, PAM50, EndoPredict) [21]. Unfortunately, none of these signatures were aimed at specifically defining the residual risk after standard NAC. Our findings obtained using the St. Gallen criteria for BC classification suggest that gene expression profiling of residual disease could help to further characterize the modifications induced by anthracycline/taxane NAC, with the final goal of defining patients at high recurrence risk despite standard treatment, who could be ideal candidates for trials with investigational drugs in early breast cancer. A significant value of our study consists in the focus on a homogeneous population, treated over a limited period of time. Furthermore, the cytotoxic and biological agents administered were in agreement with recent guidelines on NAC. However, some limitations of this work have to be underlined. First of all, it represents a retrospective case series based on a limited number of patients. Secondly, immature follow-up does not allow outcome conclusions to be drawn. Finally, this study was conceived as an explorative analysis without pre-defined postulates, with a speculative and hypothesis-generating aim.

In conclusion, the reported data suggest that NAC may induce distinct phenotypical changes in BC. We believe this observation deserves further evaluation as it may entail crucial implications in the development of new neoadjuvant therapeutic strategies.

Disclosure Statement

The Authors have no relevant conflicts of interest to disclose.

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