Original Article

CHANGES IN HER2 AND HORMONAL RECEPTORS BIOMARKERS AFTER NEOADJUVANT CHEMOTHERAPY FOR BREAST CANCER AND IMPACT ON ADJUVANT THERAPY SELECTION

ALTERAÇÕES NA EXPRESSÃO DO RECETOR HER2 E RECETORES HORMONAIS APÓS QUIMIOTERAPIA NEOADJUVANTE NO CANCRO DA MAMA E SEU IMPACTO NA SELEÇÃO DA TERAPÊUTICA ADJUVANTE

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Received: 28/01/2024 *Accepted:* 12/02/2024 *Published online:* 27/07/2024

ABSTRACT

Introduction: Breast cancer biological subtype has an important impact in the definition of the treatment strategy and the prognosis. However, the influence of neoadjuvant chemotherapy in the immunohistochemistry profile of the tumor is not well clarified. This study aims to evaluate the incidence of immunohistochemistry profile changes after neoadjuvant chemotherapy and the impact of these changes in adjuvant therapeutic decisions. **Methods:** Retrospective review of all breast cancer patients consecutively treated with neoadjuvant therapy followed by surgery between January 2013 and July 2019. Only patients with complete information on hormone receptors (HR) and human epidermal growth factor 2 (HER2) status on both pre-chemotherapy biopsy and post-chemotherapy surgical specimen were included. **Results:** During the study period, a total of 655 patients with 662 carcinomas were submitted to neoadjuvant therapy and surgical treatment. From this original cohort, 37.9% didn't have a complete immunohistochemistry profile, 22.7% had a complete pathological response and one patient had only neoadjuvant hormonal therapy, and was excluded from the study. From the 260 analyzed tumors, 99.2% of the patients were female, with a median age of 50 years. The majority of tumors were cT2 (38.8%) and cT3 (39.2%), as well as cN+ (71.5%). The most common biological subtype at diagnosis was HR-positive/HER2 negative in 50.8% of cases, followed by HR negative/HER2 negative in 27.3%, HR-positive/HER2 positive in 15.4% and HR negative/HER2 positive in 6.5%. There was a change in biological subtype in 10% of patients, namely 5.7% of changes in HER2 profile and 4.2% changes in HR. The changes in progesterone receptors were the only statistically significant between the biopsy and surgical specimen analysis ($p<0.001$). From the group where immunohistochemistry markers changed, in 42.3% there was a change in adjuvant treatment. In all cases in which HER2 or RH status changed from negative to positive, there was a modification in adjuvant treatment. In most cases in which HR and/or HER2 status changed from positive to negative, adjuvant therapy was performed

ISSN: 1646-6918 eISSN: 2183-1165

according to the pre-chemotherapy biopsy findings. **Conclusion:** Immunohistochemistry markers changed in 10% of breast cancer patients after neoadjuvant chemotherapy. The main therapeutic modifications were made when there was a change in receptor status from negative to positive. Therefore, it is important to reconsider the evaluation of biological markers in surgical specimens, mainly in patients with negative receptors at diagnosis, so that adjuvant therapies can be adjusted accordingly.

Keywords: *breast cancer, receptor alterations, chemotherapy, target treatment.*

RESUMO

Introdução: Os subtipos moleculares do cancro da mama tem um impacto importante na definição da estratégia de tratamento e no prognóstico. No entanto, a influência da quimioterapia neoadjuvante no perfil imuno-histoquímico do tumor não está bem esclarecida. O objetivo deste estudo é avaliar a incidência de alterações no perfil imuno-histoquímico após a quimioterapia neoadjuvante, bem como o impacto dessas alterações na decisão terapêutica. **Métodos:** Revisão retrospetiva de todas as doentes com cancro da mama tratadas consecutivamente com terapêutica neoadjuvante seguida de cirurgia entre janeiro de 2013 e julho de 2019. Apenas foram incluídas doentes com informações completas sobre os recetores hormonais (RH) e o estado do fator de crescimento epidérmico humano 2 (HER2) tanto na biópsia pré-quimioterapia como na amostra cirúrgica pós-quimioterapia. **Resultados:** Durante o período de estudo, um total de 655 pacientes com 662 carcinomas foram submetidas a terapêutica neoadjuvante e tratamento cirúrgico. Deste grupo original, 37,9% não tinham um perfil imuno-histoquímico completo, 22,7% tiveram uma resposta patológica completa e uma doente realizou apenas tratamento hormonal neoadjuvante, sendo por esse motivo sido excluídas do estudo. Dos 260 tumores analisados, 99,2% das doentes eram do sexo feminino, com uma idade mediana de 50 anos. A maioria dos tumores eram cT2 (38,8%) e cT3 (39,2%), assim como cN+ (71,5%). O subtipo molecular mais comum ao diagnóstico foi RH positivo/HER2 negativo em 50,8% dos casos, seguido de RH negativo/HER2 negativo em 27,3%, RH positivo/HER2 positivo em 15,4% e RH negativo/HER2 positivo em 6,5%. Houve uma alteração no subtipo molecular em 10% das doentes, nomeadamente 5,7% de alterações no perfil HER2 e 4,2% com alterações nos RH. As diferenças nos recetores de progesterona foram estatisticamente significativas entre a análise da biópsia e da amostra cirúrgica (p<0,001). No grupo onde os marcadores imuno-histoquímicos mudaram, em 42,3% houve uma alteração no tratamento adjuvante. Em todos os casos em que o estado HER2 ou RH mudou de negativo para positivo, houve uma modificação no tratamento adjuvante. Na maioria dos casos em que o estado RH e/ou HER2 mudou de positivo para negativo, a terapêutica adjuvante foi realizada de acordo com os resultados da biópsia pré-quimioterapia. **Conclusão:** Os marcadores imuno-histoquímicos mudaram em 10% das doentes com cancro da mama após a quimioterapia neoadjuvante. As principais modificações terapêuticas foram feitas quando houve uma alteração no estado dos recetores de negativo para positivo. Portanto, é importante reconsiderar a avaliação dos marcadores biológicos nas amostras cirúrgicas, principalmente nas doentes com recetores negativos no diagnóstico, para que as terapêuticas adjuvantes possam ser ajustadas adequadamente.

Palavras chave: *cancro da mama, alterações dos recetores, quimioterapia, terapêutica alvo.*

INTRODUCTION

Neoadjuvant chemotherapy (NAC) is a well-established treatment for locally advanced breast cancer with the intent of downsizing to allow for higher rates of breast-conserving surgery[1-3] and it has been shown to achieve these results, without compromising distant recurrence and overall survival[4]. A pathological complete response to neoadjuvant chemotherapy has been recognized as a predictor of improved disease-free survival[5].

Based on immunohistochemistry and molecular profile, several breast cancer biological subtypes have been defined[6]. The treatment strategy decision in breast cancer is highly dependent on the expression of these biomarkers, namely the evaluation of the

ISSN: 1646-6918

eISSN: 2183-1165

Human Epidermal growth factor Receptor 2 (HER2) and the estrogen and progesterone receptors, designated together as hormonal receptors (HR). Tumor response to chemotherapy varies according to biologic subtype which, in turn, impacts recurrence-free and overall survival[7].

However, the effects of neoadjuvant chemotherapy on tumor biology are not completely clarified[8-10]. On the other hand, there is no specific recommendation for routine evaluation of immunohistochemistry analysis of the surgical specimen after NAC, making it difficult to evaluate the real extent of biological change in the tumor, as well as the potential impact of these alterations on adjuvant treatments and even in survival.

The primary goal of this study was to evaluate the incidence of immunohistochemistry profile changes after neoadjuvant chemotherapy. The secondary goal was to evaluate the impact of these changes to establish an adequate adjuvant treatment.

METHODS

The data from all patients with breast cancer consecutively treated with neoadjuvant therapy followed by surgery at Instituto Português de Oncologia do Porto, between January 2013 and July 2019 were retrospectively reviewed. All adult patients (≥18 years old) with histologic confirmation of invasive breast cancer, submitted to neoadjuvant chemotherapy who had complete information on HR and HER2 status on both pre-NAC biopsy specimen and post-NAC surgical specimen were included. Exclusion criteria were stage IV at diagnosis, recurrent breast cancer, pathologic complete response and patients who were submitted only to neoadjuvant hormone therapy.

Patients received neoadjuvant chemotherapy according to the standard protocols at our institution based on guidelines. All patients with HER2 positive receptors also received anti-HER2 therapy either with trastuzumab alone or trastuzumab and pertuzumab combined. Pathologic complete response was defined as absence of invasive carcinoma in both the breast and lymph nodes. Flourescence *in situ* hibridization (FISH) testing was performed in patients in whom the HER2 immunohistochemistry result was 2+.

Data regarding demographic features, prechemotherapy biopsy, treatment and anatomopathology analysis of surgical specimen were recorded. Statistical analysis was performed with the 24th version of SPSS® software and the McNemar's test was used to compare the biomarkers before and after NAC, with a $p<0.05$ considered statistically significant. Continuous variables were presented as median and interquartile range [IQR] and categorical variables as absolute (n) and relative (%) frequency.

RESULTS

A total of 655 patients with 662 carcinomas were submitted to neoadjuvant therapy during the study period. In 251 (37.9%) carcinomas the immunohistochemistry study in the surgical specimen was incomplete and 1 patient was treated with neoadjuvant hormone therapy alone and these cases were excluded from the study. Additionally, 150 (22.7%) cases had a pathologic complete response and were also excluded (Figure 1).

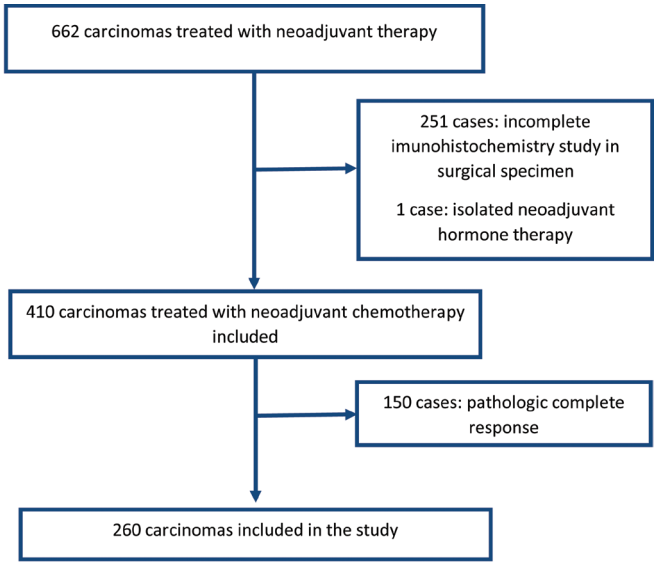

Figure 1 – **Study flowchart.**

From the 260 carcinomas included, 99.2% (n=258) corresponded to female patients, with a median age of 50[43-58] years old.

The characteristics of the patients before chemotherapy are described in Table 1. The majority of tumors were cT2 (38.8%; n=101) and cT3 (39.2%; n=102), as well as cN+ (71.5%; n=186). The most common histological subtype was ductal (78.1%; n=200) and the most common histologic grade was 3 (52.2%; n=128).

Table 1 – **Tumor characteristics before neoadjuvant chemotherapy (HER2: human epidermal growth factor 2; HR: hormone receptors)**

Pre-Neoadjuvant chemotherapy	
Characteristics	**Values**
Clinical T staging, n (%)	
cT1	10 (3.8%)
cT2	101 (38.8%)
cT3	102 (39.2%)
cT4	47 (18.1%)
Clinical N staging, n (%)	
cN0	74 (28.5%)
cN+	186 (71.5%)
Histologic subtype (n=256), n (%)	
Ductal	200 (78.1%)
Lobular	32 (12.5%)
Others	24 (9.4%)
Histologic grade (n=196), n (%)	
Grade 1	6 (2.4%)
Grade 2	111 (45.3%)
Grade 3	128 (52.2%)
Estrogen receptors, n (%)	
Negative	87 (33.5%)
Positive	173 (66.5%)
Progesterone receptors, n (%)	
Negative	121 (46.5%)
Positive	139 (53.5%)
HER2, n (%)	
0/1+	173 (66.5%)
2+	41 (15.8%)
3+	46 (17.7%)
Biologic subtype, n (%)	
HR positive/HER2 negative	132 (50.8%)
HR positive/HER2 positive	40 (15.4%)
HR negative/HER2 positive	17 (6.5%)
HR negative/HER2 negative	71 (27.3%)

The most common neoadjuvant chemotherapy scheme used was AC-D (adriamycine, cyclophosphamide, and docetaxel) (81.9%; n=213), and the majority of patients had total mastectomy (75%; n=195) and axillary dissection (82.7%; n=215) as their definitive surgery.

After neoadjuvant chemotherapy, the majority of tumors were pT1 (46.9%; n=122) and pN0 (34.6%; n=90). The most common histological subtype remained ductal (81.5%; n=203), but grade 2 was the most common histologic grade (67.9%; n=167), (Table 2).

Regarding the biological subtype, before chemotherapy the distribution was: HR positive/HER2 negative in 50.8% (n=132), HR negative/HER2 negative in 27.3% (n=71); HR positive/HER2 positive in 15.4% (n=40) and HR negative/HER2 positive in 6.5% (n=17). After neoadjuvant chemotherapy, the most common biological subtype remained HR positive/HER2 negative (51.5%; n=134), followed by HR negative/HER2 negative in 28.5% (n=74), HR positive/HER2 positive in 12.7% (n=33) and HR negative/HER2 positive in 7.3% (n=19).

There was a change in biological subtype in 10% (n=26) of patients, namely 5.7% (n=15) of changes in HER2 profile and 4.2% (n=11) changes in HR. (Figure 2). Only the changes in progesterone receptors were statistically significant between the biopsy and surgical specimen analysis ($p<0.001$), the changes in estrogen receptors and HER2 status were not statistically significant ($p=0.092$ and $p=0.238$, respectively).

Specifically, there were 9 cases in which HER2 status changed from positive to negative, 8 cases in which HR status changed from positive to negative, 6 cases in which HER2 status changed from negative to positive and 3 cases in which HR status changed from negative to positive. From the group where immunohistochemistry markers changed, in 42.3% (n=11) there was a modification in adjuvant treatment. In all cases in which HER2 or RH status

ISSN: 1646-6918

eISSN: 2183-1165

Table 2 – **Tumor characteristics after neoadjuvant chemotherapy (HER2: human epidermal growth factor 2; HR: hormone receptors)**

Post-Neoadjuvant chemotherapy	
Characteristics	**Values**
Pathological T staging, n (%)	
pT0	4 (1.5%)
pT1	122 (46.9%)
pT2	80 (30.8%)
pT3	47 (18.1%)
pT4	7 (2.7%)
Pathological N staging, n (%)	
pN0	90 (34.6%)
pN1	85 (32.7%)
pN2	56 (21.5%)
pN3	29 (11.2%)
Histologic subtype (n=249), n (%)	
Ductal	203 (81.5%)
Lobular	25 (10%)
Others	21 (8.4%)
Histologic grade (n=246), n (%)	
Grade 1	15 (6.1%)
Grade 2	167 (67.9%)
Grade 3	64 (26%)
Estrogen receptors, n (%)	
Negative	94 (36.2%)
Positive	166 (63.8%)
Progesterone receptors, n (%)	
Negative	147 (56.5%)
Positive	113 (43.5%)
HER2, n (%)	
0/ 1+	191 (73.5%)
2+	33 (12.7%)
3+	36 (13.8%)
Biologic subtype, n (%)	
HR positive/HER2 negative	134 (51.5%)
HR positive/HER2 positive	33 (12.7%)
HR negative/HER2 positive	19 (7.3%)
HR negative/HER2 negative	74 (28.5%)

changed from negative to positive, there was a modification in therapeutic strategy, with patients undergoing adjuvant treatment with trastuzumab or endocrine therapy, as appropriate. In most cases in which HR and/or HER2 status changed from positive to negative, there was no therapeutic change and adjuvant therapy was performed according to

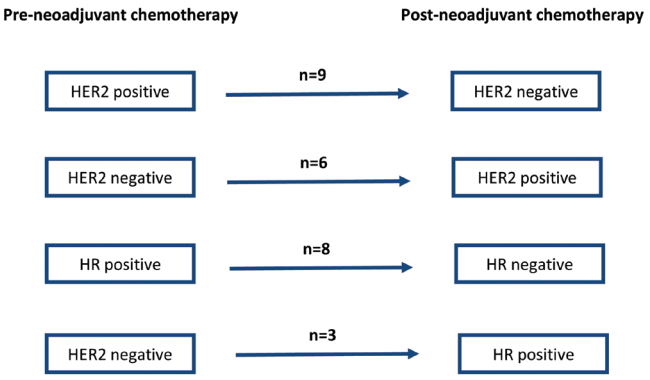

Figure 2 – **Tumors with changes after neoadjuvant chemotherapy (HER2: human epidermal growth factor 2; HR: hormone receptors)**

the pre-chemotherapy biopsy findings. The only exception was one case in which HER2 changed from positive to negative and adjuvant trastuzumab was suspended and another case in which HR changed from positive to negative and the patient did not have adjuvant endocrine therapy done.

DISCUSSION

With the increasing inclusion of neoadjuvant chemotherapy in breast cancer treatment strategy, concerns about the effects of this treatment in biological cancer subtypes have been raised. The main goal of our study was to evaluate the incidence of immunohistochemistry profile change after NAC. In our series, there was a change in 10% of the patients, namely 5.7% of changes in HER2 and 4.2% in HR, although only the changes in progesterone receptor status were statistically significant.

Previous series have reported on the rate of changes in biological subtype after NAC, with varying rates from 16-23%[11,12]. The majority of the available evidence concerns the analysis of HR, with discordance rates between the biopsy and surgical specimen of 7–46% for estrogen receptor and 5.9–58% for progesterone receptor[13-17]. A systematic review of 32 studies reporting on this subject found

a change in HR status of 8-33% and concluded that, although the studies reviewed were heterogeneous, the discordances encountered could only be partly explained by these factors and were much more likely due to the direct effect of the neoadjuvant chemotherapy[18]. In our series, the rate of change was much lower, which might be related to a possible selection bias, since only 60% of patients submitted to NAC had a complete immunohistochemistry analysis in the surgical specimen.

Fewer series have reported on changes in HER2 status after NAC, with changes in HER2 expression varying from 15-25%[19,20], although studies suggest that the addition of trastuzumab to neoadjuvant chemotherapy scheme may correlate with the discordance rate[21] and may lead to loss of HER2 amplification in up to 43% of cases[22]. This loss of amplification has been demonstrated to have a negative impact on survival[23]. In our series, all of the patients with HER2 positive biopsy specimen were given trastuzumab and/or pertuzumab as part of their neoadjuvant regimen and, from this pool of HER2 positive patients (n=57), 16% (n=9) had loss of HER2 amplification. It is not clear whether this change in HER2 status reflects a truthful response to therapy or instead reflects tumor heterogeneity regarding HER2 expression, with the trastuzumab eliminating the HER2-overexpressing clones and leaving only the HER2-negative cells.

The reasons behind these differences in profile between biopsy and surgical specimens have been largely discussed. Although initially, the suggestion that these differences were related to sampling error, several studies have since revealed a high concordance rate between core needle biopsy and excisional biopsy results[20,24,25], attributing the slight discordance in breast biomarkers to technical preparation of the immunohistochemical stain[26], fixation time[25], inter- and intra-observer variability[27] and intratumoral heterogeneity[18].

In fact, intratumoral heterogeneity seems to be a much more likely explanation than the overly simplistic explanation of sampling error. Studies using large-scale sequencing genetic analysis have established intratumoral heterogeneity as a defining feature of several solid tumors[28-31], and have suggested that this factor may have an important impact on adequate treatment selection since this decision usually relies on single tumor biopsy samples that may not be representative of the entire tumor mutational landscape[32]. *Almendro et al.* analyzed intratumoral genetic and phenotypic diversity in a cohort of breast tumors prior to and after NAC and discovered that, although intratumoral genetic diversity did not change during treatment in tumors with partial or no response, lower pre-treatment genetic diversity was significantly associated with complete pathologic response and that phenotypic diversity was different between pre- and post-NAC samples. They concluded that phenotypic diversity in combination with selection pressure by local microenvironmental signals is the driver of tumor evolution[33].

Some studies have also suggested intrinsic tumor resistance to neoadjuvant chemotherapy to play a role in immunohistochemistry profile changes after neoadjuvant chemotherapy[34]. Several mechanisms of drug resistance (modification in target proteins and intracellular drug concentrations, deregulation of apoptosis and cancer stem cells malignant transformation) have been described[35] and they could sustain the theory that the tumor cells remaining after neoadjuvant chemotherapy contain the cell population intrinsically resistant to chemotherapy, that most likely mirrors the micro-metastatic component of the disease, ultimately responsible for distant metastasis.

Regardless of the mechanisms that could explain the changes in immunohistochemistry profile between pre-NAC biopsy and post-NAC surgical specimen, it becomes clear that a standard of care needs to be established regarding repeated analysis of these biomarkers in surgical specimens after NAC, since this may have a significant impact on adjuvant treatment selection. Endocrine therapy[36,37] and anti-HER2 therapy [38] are firmly established as

ISSN: 1646-6918

eISSN: 2183-1165

effective adjuvant treatments with implications for recurrence-free survival. However, both treatments have associated side effects and impact on patient's quality of life. Therefore, the selection of adjuvant therapy in patients previously treated with NAC must be done carefully to maximize the impact on recurrence and survival, while avoiding potentially unnecessary side effects. Only a few small series have reported the impact of biologic subtype change in adjuvant therapy selection after NAC[12,39], with adjuvant therapy adjustment in up to 100% of patients who had suffered immunohistochemistry profile alterations. In our series, from the group where immunohistochemistry markers changed, in 42% of patients, there was a change in adjuvant treatment and, to our knowledge, this is the largest series to report on adjuvant therapy adjustment after NAC. However, the potential impact of these therapeutic adjustments on survival has not yet been defined.

We also have to address the potential impact of these biomarker changes on survival. In previous series, results were discordant, although some studies suggested that a positive switch in HR status may be correlated with better recurrence-free and overall survival in patients treated with adjuvant endocrine therapy[11,13,14].

Finally, we have to mention the limitations of this study, being a unicentric and retrospective review, with a possible selection bias since we only analyzed patients with complete immunohistochemistry profiles before and after treatment. Besides this, survival analysis is also missing, since the follow-up was short in more recent patients for survival evaluation. Furthermore, this analysis encompasses a long study period, during which the selection criteria for neoadjuvant therapy were adjusted.

CONCLUSION

Immunohistochemistry markers changed in 10% of breast cancer patients after neoadjuvant chemotherapy. The main therapeutic changes were made when there was a change in receptor status from negative to positive. Therefore, it is important to reconsider the evaluation of biological markers in surgical specimens, mainly in patients with negative receptors at diagnosis, so that adjuvant therapies can be adjusted to each case.

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ISSN: 1646-6918

eISSN: 2183-1165

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ISSN: 1646-6918

eISSN: 2183-1165