Semi-quantitative evaluation of estrogen receptor expression is a strong predictive factor of pathological complete response after anthracycline-based neo-adjuvant chemotherapy in hormonal-sensitive breast cancer

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Abstract

Background : Absence of hormonal receptors (HR) expression is a predictive factor of high pathologic complete response (pCR) rate after neo-adjuvant chemotherapy. However, HR-positive tumors are less chemosensitive. In the present study, we evaluated the predictive value of estrogen (ER) and progesterone (PgR) semi-quantitative expression in patients with HR-positive tumors treated uniformly with antracycline-based neoadjuvant chemotherapy without hormonal treatment. Value of HR expression as a predictive factor was then evaluated in a multivariate analysis with tumor grade, Ki67 index and HER2 expression.

Methods : From January 2000 and December 2006, 177 patients with HR-positive breast ductal invasive carcinoma ≥ 2cm in its largest diameter were treated with 6 cycles of an anthracycline-based neo-adjuvant chemotherapy. Tumor grade, ER, PgR, HER2 status and Ki67 index were determined on microbiopsy performed before chemotherapy. A semi-quantitative evaluation of ER and PgR expression by IHC was performed using the Barnes'score.

Results : pCR rate was significantly different ($P < 0.001$) according to the ER expression score. pCR rate was 28 % for low score, 9 % for medium score and 3 % for high score. On the contrary, pCR rate was not significantly different ($P = 0.49$) according to the PgR expression score. In the multivariate analysis, ER expression score ($P = 0,0002$) and Ki67 index ($P = 0,02$) were the only predictive factors of response for HR-positive tumors.

Conclusion : pCR after anthracycline-based chemotherapy is significantly correlated with the ER expression score.

Introduction

Hormonal receptors (HR) are the most important factors used today to tailor adjuvant therapies [1]. Endocrine therapies provide benefit only in the presence of HR [2]. Adjuvant chemotherapy in early breast cancer improves patient survival, but not all groups of patients benefit equally from chemotherapy [3]. Several adjuvant trials indicated that patients with HR- negative tumors benefit more from chemotherapy than patients with HR-positive tumors [4].

HR-positive tumors are a heterogeneous subgroup with different levels of estrogen (ER) and progesterone (PgR) receptors expression [5]. The St Gallen consensus recognized highly endocrine responsive tumors with high expression of both ER and PgR and incompletely endocrine responsive tumors with lower expression of ER and/or PgR [1]. It was also suggested that response to chemotherapy decreased as HR expression increased by retrospective analysis in adjuvant trials [6-7].

The neoadjuvant chemotherapy setting provides further evidence. Absence of HR expression is a classical predictive factor of high pathologic complete response (pCR) rate after neo-adjuvant chemotherapy [8-9]. However, well-differentiated tumors with HR expression are less chemosensitive [10].

The aim of the present study was to evaluate whether level of ER and PgR expression had any predictive value of pCR in 177 patients with HR-positive tumors treated uniformly with antracycline-based neoadjuvant chemotherapy, according to the REMARK recommendations [11]. Value of HR expression as predictive factor was then evaluated in a multivariate analysis with tumor grade, Ki67 index (percentage of Ki67-positive cancer cells) and HER2 expression [12].

Materials and methods

Patients and treatment

Three hundred and fifteen patients with breast invasive ductal carcinoma ≥ 2cm in its largest diameter by ultrasound evaluation were treated with 6 cycles of an anthracycline-based neo-adjuvant chemotherapy (FEC 100 regimen: 5FU 500 mg/m², Epirubicine 100 mg/m², Cyclophosphamide 500 mg/m², d1 = d22) between January 2000 and December 2006 in our institution. Median age at diagnosis was 53 years.

A core-needle biopsy with 14G needles was performed before chemotherapy to allow pathological diagnosis and biological parameters evaluation. Patients were scheduled to undergo surgery 4 weeks after the 6th cycle with tumor excision and axillary node dissection. pCR was defined as absence of invasive tumor cells or persistence of in situ disease in the breast and negative axillary lymph nodes.

Two hundred and sixty nine patient were assessable for both ER and PgR. Out of these 269 patients, 177 patients (66 %) had a HR-positive invasive ductal carcinoma (figure 1). None of these patients had endocrine neo-adjuvant treatment.

Pathological Methods

All slides were analysed in a single laboratory (CLCC Paul Strauss) by two pathologists in a blinded fashion without knowledge of the treatment response.

The tumor grade was assessed according to the Elston-Ellis grade on a scale of 1 to 3 [13].

Four other pathologic factors, were evaluated by immunochemistry (IHC) using a Dako Autostainer automat (Dako, Glostrup, Denmark) [14]. Tumors were stained for ER (Clone 6F11, Novocastra, Newcastle, United Kingdom; dilution 1/40), PR (Clone PGR 636, Dako, dilution:1/50), Ki67 (Clone MIB1, Dako, dilution 1/50) and HER2 (Ab A0485, Dako, dilution 1/200) according to manufacturer's recommendations. For all markers the slides were subjected to heat-induced epitope retrieval (3 minutes, 0.001M boiling citrate buffer, pH 6, in a pressure cooker).

ER and PgR immunostainings were considered positive when 10% or more tumor cells were immunostained. The tumors were scored as either HR-positive or HR-negative according to the cutoff [15].

A semi-quantitative evaluation of ER and PgR positivity by IHC was performed using the Barnes'score [16]. This score was calculated in two steps. First, staining intensity on a scale of 0-3, proportion on a scale of 0-4 and uniformity on a scale of 0-2 were estimated for HR positive tumors. Then, the Barnes'score was obtained by addition of these three variables. This score ranged from 3 to 9, with a low level (3-5), a median level (6-7) and an high level (8-9).

Tumor cell proliferation was evaluated using Ki 67 staining [17]. Ki67 < 20 % was considered as low level, whereas Ki67 between 20 and 30 % was considered as medium level and KI67 > 30 % high level. These cut-off points were a priori defined to create three groups (similar to ER, HER2, tumor grade distribution) with a median value of Ki67 for the entire cohort of 25.

HER2 IHC was evaluated according to the Dako scoring system on a scale of 0 to 3 +. [18].

Statistical analyses

The Chi-square test was used to analyse the association between response rate to neoadjuvant chemotherapy and the different biological factors. All reported *P* values are two-sided and statistical significance was considered achieved when $P < 0.05$. All variables with a *P* value less than 0.20 in the univariate analysis were included in a stepwise forward logistic regression model, allowing for interaction.

The statistical analysis was performed using SAS Statistical Analysis Software (version 9.1; SAS Institute, Cary, NC).

Results

The pCR rate for the whole group (269 patients) was 9.5 %. Patients with HR-positive tumors (177 patients) had a 6.6% pCR rate whereas patients with HR-negative tumors (92 patients) had a 25% pCR rate. This difference was statistically different ($P < 0.0001$)

HR-positive tumor characteristics are reported in table 1. These tumors were more likely to be grade 1 or 2 (84 %), with a low or medium Ki67 (72 %). Only 11 % of these tumors had an HER2 overexpression. Seventy seven per cent of these tumors were ER and PgR-positive whereas 23 % were ER-positive and PgR-negative. There were no ER-negative and PgR-positive tumors.

In HR-positive tumors, pCR rate was significantly different ($P < 0.001$) according to the ER expression score. pCR rate was 28 % for low score, 9 % for medium score and 3 % for high score (table 2). On the opposite, pCR rate was not significantly different ($P = 0.49$) according to the PgR expression score. pCR rate was 8 % for PgR-negative, 11 % for low score, 8 % for medium score and 4 % for high score (table 2).

Univariate analyses demonstrated that cell proliferation evaluated by Ki67 index, tumor grade and HER2 status were also significant predictive factors of response for HR-positive tumors (respectively, *P* = 0.01; 0.02; 0.02) (table 2).

In the multivariate analysis, ER expression score (*P* = 0,0002) and Ki67 index (*P* = 0,02) were the only predictive factors of response for HR-positive tumors (table 3).

To examine more in detail the relationship between pCR and ER expression or Ki 67, we performed a complementary logistic regression analysis of pathologic complete response using the original Barnes' score as an independent variable. This analysis led to a p = 0.01 (likelihood ratio test), confirming the statistically significant relationship between ER expression expressed by the Barnes' score and pCR.

Similarly, we performed a logistic regression analysis of Ki 67 (values continuously ranging from 1 to 80% in our series). The likelihood ratio test led to p = 0.04, in accordance with our previous categorical analysis. Thus, pCR increased with an increasing level of Ki 67.

Discussion

In the present study, the ER expression score was a significant predictive factor of response to anthracycline-based chemotherapy in HR-positive tumors. pCR rate decreased as ER expression increased. The PgR expression score was not associated with pCR. When the biologic parameters (ER expression score, tumor grade, Ki67 index, and HER2 status) were included in a multivariate logistic regression model, ER expression score (*P* = 0,0002) and Ki67 (*P* = 0,02) were found to be significant predictive factors of response.

The use of immunohistochemical analysis to assess the ER and Ki67 status of breast cancers in paraffin sections is now a routine part of pathology practice but efforts should be

made to develop standard methodology and accepted cut-off points [19-20]. The American Society of Clinical Oncology and College of American Pathologists have recently jointly published guideline recommendations for immunohistochemical testing of HR in breast cancer [19].

Observations from adjuvant studies suggest that benefit of chemotherapy is limited to HR-negative tumors [4]. Chemotherapy response according to HR status was evaluated in the 3 CALGB/Intergroup trials. While the overall result of each trial showed a significant benefit for the more intensive chemotherapy arm, the difference between the two arms was negligible in patients with HR-positive tumors.

Retrospective analyses of two other adjuvant trials suggest that benefit of chemotherapy in HR-positive tumors is restricted to tumors with low expression of ER. The IBCSG trial IX investigated the role of chemotherapy (CMF regimen) prior to tamoxifen for menopausal women with node-negative breast cancer [6]. A quantitative determination of ER status was performed in this study with a radiolabelled lingand binding assays. Adding CMF to tamoxifen improved disease-free survival. This benefit was observed among the ER-negative tumor group. For the ER-positive tumor group, the benefit of chemotherapy was restricted to tumors with low value of ER expression. Albain et al reported an exploratory analysis of the Intergroup Trial 0100, which compared tamoxifene alone to chemotherapy (CAF regimen) plus tamoxifen for menopausal women with node-positive, HR positive breast cancer [7]. The level of ER expression was determined by the semi-quantitative Allred score. This semi-quantitative evaluation combines scores for intensity of reactivity and for proportion of stained cells [14]. Adding CAF to tamoxifen provided more benefit compared to tamoxifen alone only for the low/intermediate ER expression cohort. There was no additional benefit with the addition of chemotherapy among patients whose cancers had high levels of ER expression.

Gene expression studies have identified five molecularly distinct subtypes of breast cancer with very different prognosis: the luminal A, luminal B, HER2-enriched, basal-like and normal breast-like subtypes [21]. The luminal breast cancers are ER-positive tumors, with luminal B tumors having poorer prognosis than luminal A tumors [22]. Luminal B tumors have a higher Ki67 index and are less hormonal-sensitive compared to luminal A tumors [23]. In the present study, ER expression score and Ki67 index have clearly allowed us to separate these HR-positive tumors between luminal A tumors with low chemosensitivity and luminal B tumors with high chemosensitivity.

In conclusion, this retrospective study strongly suggested that ER expression score is a chemosensitivity predictive factor in HR-positive tumors treated with anthracycline-based neo-adjuvant chemotherapy. In our study, low ER expression and high Ki67 index allowed the identification of patients with HR-positive tumors who did benefit from chemotherapy in the neo-adjuvant setting.

Acknowledgment: We thank Mrs Joy Fest for helping in editing the manuscript

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Figure 1: REMARK diagram, illustrating how patients were selected.

315 patients with breast invasive ductal carcinoma ≥ 2cm treated with an anthracycline-based neo-adjuvant chemotherapy between 1/2000 and 12/2006 in our institution.		
46 patients with unknown HR status	92 patients with HR-negative tumors	177 patients with HR-positive tumors

Table 1 : HR-positive tumor characteristics

Biologic parameters	**Patients (%)**
Grade	
1	48 (27%)
2	101 (57%)
3	28 (16%)
Ki 67	
Low < 20 %	80 (45%)
Medium 20 – 30 %	48 (27%)
High > 30 %	49 (28%)
HER2	
0 – 1 +	126 (71%)
2 +	32 (18%)
3 +	19 (11%)
ER +	

	Low 3-5	22 (12%)
	Medium 6-7	32 (18%)
	High 8-9	123 (70%)
PgR –		40 (23%)
PgR +		
	Low 3-5	36 (20%)
	Medium 6-7	24 (14%)
	High 8-9	77 (43%)

Table 2 : Predictive value of biologic parameters for achieving a pCR in HR-positive tumors : univariate analysis

Biologic parameters	**pCR %**	***P* value**
Grade		
1	2 %	
2	5 %	0,02
3	19 %	
Ki 67		
Low < 20 %	1 %	
Medium 20 – 29 %	8 %	0,01
High ≥ 30 %	14 %	
HER2		
0 + or 1 +	4 %	
2 +	7 %	0,02
3 +	22 %	
ER +		

Low 3-5	28 %	
Medium 6-7	9 %	< 0,001
High 8-9	3 %	
PgR –	8 %	
PgR +		
Low 3-5	11 %	0,49
Medium 6-7	8 %	
High 8-9	4 %	

Table 3: Biological parameters in relation to their predictive value for achieving a pCR in HR-positive tumors: multivariate logistic regression.

Biologic parameters	***P* value**
ER score	0.0002
Ki67	0.02
Tumor grade	0.20
HER2	0.66