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ERBB2 overexpression in breast carcinomas: no positive correlation with complete pathological response to preoperative high-dose anthracycline-based chemotherapy

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Abstract

The predictive value of ERBB2 amplification/expression to doxorubicin use is controversial. Preoperative chemotherapy, followed by the pathological assessment of tumour response to treatment provide optimal conditions for the evaluation of the predictive value of biological parameters. We report here data on the predictive value of ERBB2 in a series of 54 cases of breast cancer treated by preoperative high-dose anthracycline-based chemotherapy. Our series consisted of 26 women presenting an inflammatory breast cancer (IBC) and of 28 women with poor prognosis primary cancer (PPPC). Patients received a total of four cycles with doxorubicin (75 mg/m^2 for IBC or 70 mg/m^2 for PPPC) and cyclophosphamide (6 g/m^2 for IBC or 1400 mg/m^2 for PPPC), every 21 days. ERBB2 expression was determined by immunohistochemistry (clone CB11) performed on a tumour biopsy taken before chemotherapy. All patients underwent surgery as a second step of treatment, and the tumour response was assessed on pathological specimens. A complete pathological response was observed in 24 of the 54 cases (44%) (95% confidence interval (CI), 31–57). Pathological complete response was positively correlated with high histological grade ($P = 0.02$) and with the absence of oestrogen ($P = 0.003$) or progesterone ($P = 0.02$) receptor expression. ERBB2 overexpression was found in 18 of the 54 cases (33%). A complete pathological response was observed in 33% of these cases (6/18). This figure was not significantly different from the 50% rate of complete response observed for tumours with no detectable ERBB2 expression (18/36). In this small series, ERBB2 overexpression was not a significant predictive marker of the pathological response to high-dose doxorubicin-based chemotherapy.

Keywords: ERBB2; Breast cancer; Preoperative chemotherapy; Pathological response to treatment; Doxorubicin

1. Introduction

Survival improvement amongst patients with breast cancer has been obtained by chemotherapy [1]. However, response to chemotherapy is difficult to predict for the individual patient, and no single clinical or biological parameter has proved reliably predictive to date. The rate of tumour cell proliferation has been associated with the response to neoadjuvant chemotherapy [2], but only the expression of the oestrogen receptor is recognised as a clinically useful marker that predicts response to a specific systemic therapy of breast cancers [3]. Some recent reports suggest that ERBB2 overexpression, besides its prognostic value in node positive breast cancers [4–6], could also present a predictive value of tumour cell sensitivity to anthracycline-based chemotherapy. In tumours with high ERBB2 expression, an improved response to high-dose adjuvant

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treatment with doxorubicin, cyclophosphamide and 5-fluoro-uracil has been reported [7]. The identification of drug(s) whose cytotoxic effects would be increased by ERBB2 overexpression represents a crucial step. Recently, Paik and colleagues provided data suggesting that ERBB2 expression was a marker of preferential response to doxorubicin-containing regimens [8]. However, retrospective analysis of a randomised clinical trial conducted by the Cancer and Leukaemia Group B regarding any relationship between ERBB2 expression and the response to doxorubicin [9] failed to unambiguously validate the previously published data [7]. The authors concluded that the hypothesis that patients whose breast cancer exhibits high ERBB2 expression benefit from dose-intensive cyclophosphamide, doxorubicin, 5-fluorouracil (CAF) therapy should be validated further before clinical implementation [9].

Previously, in a large group of breast cancer patients, we found no significant predictive value of ERBB2 expression regarding clinical response to CAF chemotherapy [10]. However, in this study, a trend towards a higher rate of response to chemotherapy was observed between tumours with (31%) and without (20%) ERBB2 overexpression. Such a difference could reflect an increased sensitivity of ERBB2-positive cells to drugs, which may depend on the dose intensity. This trend could thus be revealed to be more significant for tumours treated with the high-dose chemotherapy regimen. To test this hypothesis, we have further analysed a population of patients with breast tumours whose initial presentation required intensive doxorubicin-based preoperative treatment. All these patients underwent surgery after chemotherapy, and the pathological response to therapy could be assessed, and compared with the pretherapeutic ERBB2 status of tumour cells.

2. Patients and methods

2.1. Patients

The population analysed consisted of 26 patients with an inflammatory breast carcinoma (IBC) and 28 patients with poor prognosis primary carcinoma (PPPC), treated at the Institut Curie between 1988 and 1993. IBC was defined according to the classical clinical criteria and documented in all cases by histological analysis of a biopsy of breast skin. PPPC corresponded to tumours occurring in patients under 55 years of age, larger than 2 cm in diameter, with no significant hormone receptor expression and an S-phase growth fraction $>4\%$. When the S-phase fraction was not determined, inclusion criteria in this group were age under 35 years, and/or a high tumour histological grade and/or histological evidence of tumour vascular invasion observed on a tumour biopsy sample. Initial metastatic patients were excluded. The median follow-up was 18.5 months (range: 5–63).

2.2. Treatments

The first step of treatment was the administration of four cycles of chemotherapy, every 21 days. Patients with IBC received doxorubicin 75 mg/m^2 day 1; cyclophosphamide 3 g/m^2 days 1 and 2; 5-fluorouracil 500 mg/m^2, days 1–5 during the third and fourth cycles. Patients with PPPC received doxorubicin 70 mg/m^2 day 1; cyclophosphamide 700 mg/m^2 days 1 and 8; 5-fluorouracil 700 mg/m^2, days 1 and 5. Following chemotherapy, all 54 patients underwent a surgical procedure. Breast conserving surgery was carried out in 16/54 patients (30%): a wide surgical resection of the residual mass was done as well as axillary node clearance. Whole breast irradiation was then delivered, using either Cobalt60 or 5–6 MV photons to a median dose of 52 Gy (range: 50–54). An additional radiation dose (boost) was given to the tumour bed in 13/16 (81%) patients, to a median total dose of 65 Gy (range: 60–75 Gy). In addition, 10/16 (63%) patients had radiotherapy to the internal mammary and supraclavicular nodes. 38 patients (70%) underwent a mastectomy after chemotherapy. This was followed by chest wall irradiation in 33 patients (61%) (median dose: 48 Gy, range: 45–52 Gy) and node irradiation in 34 patients (63%).

2.3. Evaluation of the response to treatment

For all patients, the response to therapy was determined clinically and pathologically. Clinical response was considered as complete when no residual tumour mass was palpable in the breast or, in cases of IBC, when a disappearance of the inflammatory signs was observed. The response was scored as major when the residual mass was less than 50% of the initial tumour volume.

For the pathological analysis of the tumour response, the rate of residual epithelial neoplastic cells in the tumour mass, the location of this malignant component (invasive versus intraductal), the mitotic index in malignant epithelial cells and the aspect of the metastatic axillary nodes were taken into account. The response was considered as pathologically complete when there was no residual invasive malignant epithelial cells. Tumours with epithelial malignant residual component strictly *in situ* or representing less than 5% of the breast and/or axillary tumour mass and without any mitosis were also classified in the group of pathological complete response. The response was considered as absent when no histological modification of the tumour tissue could be related to therapy. The response was classified as partial in the remaining cases. Tumour characteristics are summarised in Table 1.

2.4. Immunohistochemistry

ERBB2 immunostainings were performed on histological sections prepared from a biopsy sample taken before treatment. Tissue sections were incubated for 1 h with the CB11 anti $p185^{HER/neu}$ monoclonal antibody (Novocastra, Newcastle, U.K.) at a 1/400 dilution, without antigenic retrieval. The development of stain was performed using the Vectastain Elite ABC peroxidase mouse IgG kit (Vector Burlingame, CA, U.S.A.) and diamino-benzidine (Dako A/S, Glostrup, Denmark), as a chromogen. In these conditions, normal epithelial cells were not stained and represented an internal negative control. Immunostainings were scored as strong, weak or negative according to the rate of labelled tumour cells and the membrane staining intensity. The ERBB2 status was further classified as overexpressed (strong or weak staining) or not significantly expressed (no detectable staining). The level of ERBB2 gene expression was then compared with the value of classical prognostic factors of breast cancers (histological grade, tumour size, hormone receptor status) and with the clinical and histological data concerning the response to treatment.

Table 1
Patients and tumour characteristics[a]

Characteristics	No (%)
Age (years)	
⩽40	18 (33)
40–50	22 (41)
>50	14 (26)
Tumour size (mm)	
⩽20	4 (7)
20–40	12 (22)
>40	38 (70)
Histological grade	
Grade I	2 (4)
Grade II	20 (37)
Grade III	32 (59)
Histological type	
Ductal invasive	52 (96)
Lobular invasive	1 (2)
Apocrine invasive	1 (2)
Node status	
0	20 (37)
1–3	11 (20)
⩾4	22 (41)
ND	1 (2)
Hormonal status	
ER+	21 (39)
PR+	27 (50)
ND	4 (7)

[a] ND, not determined; No, number of cases; ER, oestrogen receptor; PR, progesterone receptor.

2.5. Statistical analysis

Frequency distributions were tested using the Chi-square test and Yate's correction was applied when appropriate. *P* values greater than 0.05 were reported as non-significant (ns).

3. Results

3.1. Clinical and pathological characteristics

The characteristics of patients and tumours are summarised in Table 1. Most of the tumours corresponded to invasive ductal carcinoma (96% of the cases), with a size larger than 40 mm (70%) and a high histological grade (59%). Oestrogen receptors (ER) and progesterone receptors (PR) were expressed in 39% and 50% of the cases, respectively. The postoperative axillary lymph node status analysis showed more than four invaded nodes in 22 of the 53 patients evaluated.

3.2. Response to treatment

Clinical assessment showed a major response to therapy in 40 of 54 cases (74%). In 8 of these 40 cases (20%), there was no residual palpable mass in the breast (complete response), whereas the clinical size of the tumour decreased by 50% or more in the remaining 32 cases (80%) (major response).

Pathological analysis of the surgical specimens showed a complete response in 24 of the 54 cases (44%) (95% CI, 31–57). The rate of clinical and pathological responses to therapy did not depend on the clinical presentation of the disease (PPPC versus IBC): 50% of the cases with a major or complete clinical response (20/40) or with a complete pathological response (12/24) corresponded to IBC, whereas the remaining 50% of cases corresponded to PPPC.

Table 2
Comparison between clinical and pathological response to chemotherapy

Pathological response	*n* (%)	Clinical response		
		Complete *n* (%)	⩾50% *n* (%)	<50% *n* (%)
		8[a] (15)	32[a] (59)	14 (26)
Complete	24 (44)	6	16	2
Partial	12 (22)	1	8	3
Absent	18 (33)	1	8	9

n, number of cases.

[a] Half of the cases corresponded to inflammatory breast tumours and half to high risk breast carcinoma.

Table 3
Comparison between ERBB2 expression and prognostic factors[a]

Prognostic factor	ERBB2 expression		*P* value
	Overexpression *n* (%)	No expression *n* (%)	
	18 (33)	36 (67)	
Age (years)			
⩽40	8 (44)	10 (56)	
40–50	5 (23)	17 (77)	ns
>50	5 (36)	9 (64)	
Tumour size			
⩽40 mm	4 (25)	12 (75)	
>40 mm	14 (37)	24 (63)	ns
Histological grade			
I/II	10 (45)	12 (55)	
III	8 (25)	24 (75)	ns
Node status			
N−	6 (30)	14 (70)	
N+	11 (33)	22 (67)	ns
ND	1		
Hormonal status			
ER+	7 (33)	14 (67)	
ER−	9 (31)	20 (69)	ns
ND	2	2	
PR+	9 (33)	18 (67)	
PR−	7 (31)	16 (69)	ns
ND	2	2	

[a] ns, not significant; ND, not determined.

The rate of pathological complete response was found to be positively correlated with high histological grade ($P=0.02$) and with negative ER ($P=0.003$) and PR ($P=0.02$) status, but not with tumour size, patient's age or histological type.

6 patients with a complete clinical response and 16 patients with a major clinical response achieved a complete response on pathological examination (Table 2). In 2 cases, no clinical response was noted whereas a complete pathological response was obtained. 9 of the 18 cases without pathological response had presented a clinical response (8 major and 1 complete). In 9 cases, neither clinical nor pathological response was obtained.

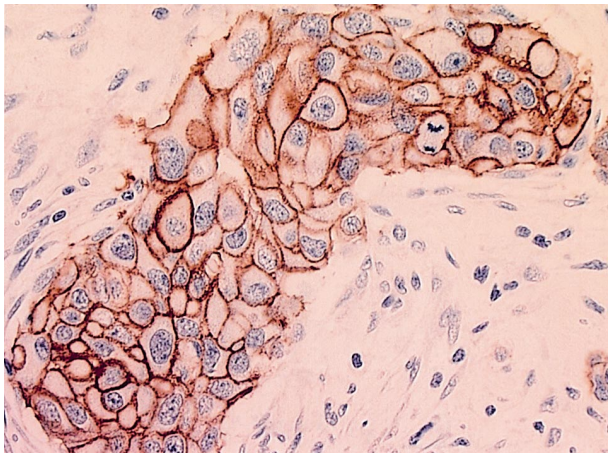

Fig. 1. ERRB2 overexpression in ductal invasive breast carcinoma: strong membrane immunostaining of malignant cells.

3.3. ERBB2 expression

ERBB2 overexpression was detected in 18 of the 54 cases (33%) analysed (Table 3). This rate was 31% (8/26) in the group of IBC and 36% (10/28) in the group of PPBC. The staining, generally strong, was always located on the tumour cell membrane (Fig. 1). No statistically significant link was observed between the ERBB2 status and the classical prognostic factors (age, tumour size, histological grade, node and hormonal status).

In the group of tumours with ERBB2 overexpression, the rate of complete clinical response to treatment was 67% (12/18) (Table 4). This rate was 78% (28/36) in the group of tumours with no detectable ERBB2 expression.

A complete pathological response to chemotherapy was observed in 50% (18/36) (95% CI, 34–66) of the tumours with no ERBB2 expression (Table 4). This rate was higher but not significantly different from the 33% rate (6/18) (95% CI, 11–55) observed for tumours showing ERBB2 overexpression (Table 4). ERBB2 overexpression was observed in only 2 of the 15 cases (13%) with no residual malignant epithelial cells, in 2 of the 5 cases with less than 5% of residual malignant cells and in 2 of the 4 cases with only an *in situ* residual component.

Table 4
Clinical and pathological responses to chemotherapy according to ERBB2 expression

ERBB2	Clinical response		*P* value	Pathological response		*P* value
	⩾50% *n* (%)	<50% *n* (%)		Complete *n* (%)	Incomplete *n* (%)	
	40 (74)	14 (26)		24 (44)	30 (56)	
Overexpression	12 (67)	6 (33)	ns[a]	6 (33)	12 (67)	ns
No expression	28 (78)	8 (22)		18 (50)	18 (50)	

[a] ns, not significant.

4. Discussion

The aim of this study was to evaluate whether the pathological response of breast carcinomas to high doses of doxorubicin was higher in tumours with ERBB2 overexpression than in tumours with no detectable ERBB2 expression. Published data suggested that the enhanced response to treatment reported for patients with ERBB2 overexpressing tumours [7] could be related to a higher sensitivity of tumour cells to doxorubicin [8]. In a previous study, concerning a series of 167 cases treated by primary CAF chemotherapy, we only observed a trend towards a positive association between ERBB2 expression and clinical response to treatment [10]. This partial result could be related to a dose-dependent effect of the response to doxorubicin (50 mg/m^2), or to a lack of accuracy of the clinical evaluation of the effect of treatment. In the present series, patients had been treated with high-dose primary chemotherapy (doxorubicin: 70 or 75 mg/m^2), and pathological response could be assessed by the analysis of tumours removed after treatment. No significant link was observed between the level of ERBB2 expression in the tumour cells and the response to drugs.

The negative result of this study may be related to the methodology used to evaluate the ERBB2 status. For instance, the predictive value of *ERBB2* gene amplification could be higher than that of protein expression. However, in previous works, we found a very strong correlation between ERBB2 overexpression and gene amplification, assessed either by fluorescent *in situ* hybridisation [11] or by semiquantitative polymerase chain reaction (PCR) [12]. This correlation has also been reported by others [13–15] and immunohistochemistry is becoming the standard for the evaluation of ERBB2 status in breast cancers. In addition, the rate of overexpression (33%) detected in our series of cases is in the range reported for breast carcinomas with aggressive clinical or biological parameters [16]. It is thus unlikely that the absence of a predictive value of a ERBB2 in the present cases was related to a defect in methodology. The present series, in spite of its small size, looks valuable regarding its homogeneity in clinical presentation and treatments administered, and regarding its accuracy in the evaluation of the tumour response. Moreover, not only was no positive trend observed between ERBB2 expression and sensitivity to treatment, but the rate of complete histological response was also higher (50%) for tumours with no detectable ERBB2 expression than for those with protein overexpression (33%). The probability would be less than 5% that a true complete response rate would be 50% in tumours with ERBB2 overexpressed versus 33% in tumours with no ERBB2 expression. Therefore, the existence of a significant link between ERBB2 overexpression and a high sensitivity of breast carcinomas to doxorubicin seems unlikely. The trend of association between ERBB2 expression and an incomplete histological response observed in our series, was more obvious when the group of tumours with absolutely no residual malignant epithelial cells (15 cases) was analysed separately, in which only 2 cases (13%) exhibited ERBB2 overexpression on pretherapeutic biopsy. This observation would rather favour the existence of a link between ERBB2 expression and cell resistance to anthracycline and/or cyclophosphamide. Such a result has already been reported for the CMF regimen [4,6,17] and is in agreement with experimental data reporting an increased resistance of ERBB2 transfected cells to cisplatin [18] or to paclitaxel [19] adjunction. Taken together, data suggest that ERBB2-positive tumours are more likely to be resistant to cyclophosphamide than to anthracyline and this should be taken into account when determining the type of chemotherapy based on the ERBB2 status of tumour cells.

In our series of cases, a high rate (44%) of complete pathological response was observed in patients with IBC or with PPPC. This result should be related to the type of therapy and to the criteria used for the selection of patients, in particular S phase and the histoprognostic index. The rates of ER and PR-positive tumours were similar to those reported in the literature [20,21]. It is worth stressing that the pathological assessment of the tumour response to therapy was clearly more accurate than the clinical evaluation. For instance, in 25% of the cases presenting a major clinical response, no pathological response was observed, whereas in 39% of the cases with an incomplete or absent clinical response, no residual tumour cells could be detected at the microscopic level. These data have to be taken into account when assessing the predictive value of ERBB2 status in tumours. Moreover, several studies report a higher prognostic value of the pathological response to treatment than that of the clinical response [22–24].

In conclusion, the analysis of the present series provided no data supporting an enhanced sensitivity of ERBB2 overexpressing breast carcinomas to high doses of cyclophosphamide- and doxorubicin-containing primary chemotherapy. Further analyses of the ERBB2 status in breast cancer are nevertheless necessary to determine the predictive value of this biological trait regarding the response to other drugs and to treatments that associate chemo- and/or radiotherapy with specific anti-ERBB2 immunotherapy.

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