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Original Paper

Plasma c-erbB2 Concentrations in Relation to Chemotherapy in Breast Cancer Patients

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Amplification and overexpression of the *C-ERBB2* oncogene have been associated with a poor prognosis and a lower response to chemotherapy in human breast cancer. In this study, plasma c-erbB2 concentrations were determined using an enzyme immunoassay in patients with breast cancer. The links between c-erbB2 concentration and tumoral response to chemotherapy were established. The patients with a c-erbB2 concentration higher than the cut-off value (27 U/ml) were considered as c-erbB2+. Ten of the 33 metastatic breast cancers were c-erbB2+. No statistically significant difference in response to chemotherapy was noted between c-erbB2+ and c-erbB2– patients (4/10 objective responses versus 10/23). Variations in c-erbB2 concentrations during treatment were not related to response to treatment.

Key words: *C-ERBB2* oncogene, plasma detection, breast cancer, chemotherapy, clinical response
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INTRODUCTION

THE *C-ERBB2* oncogene, also referred to as *HER-2/neu*, has been independently identified by several groups [1–3]. It is the human homologue of the *neu* oncogene which was identified in DNA from rat neuroglioblastomas induced by ethylnitrosourea [4]. The *C-ERBB2* oncogene encodes for a 185 Kd transmembrane receptor with tyrosine kinase activity which is closely related to epidermal growth factor receptor (EGFR) [5].

C-ERBB2 gene amplification and protein overexpression have been reported in mammary tumour cell lines [6, 7]. In human breast cancer, amplification and overexpression of *C-ERBB2* are associated with a poor prognosis [8–15]. Recently, several studies have demonstrated that these *C-ERBB2* modifications are related to a lower response to endocrine therapy and chemotherapy [16–21].

The development of antibodies to the c-erbB2 oncoprotein allows the detection by enzyme immunoassay (EIA) of a shed c-erbB2 fragment in the plasma of breast cancer patients [22–24].

In the present retrospective study, we used this plasma assay to establish tentatively the relationship between plasma c-erbB2 concentrations before and during treatment and clinical response to chemotherapy in metastatic breast cancer patients.

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PATIENTS AND METHODS

Patients

In this retrospective study, 33 patients were treated for advanced breast cancer: 20 received a standard FEC regimen (epirubicin 50 mg/m^2, cyclophosphamide and 5-fluorouracil 500 mg/m^2 every 3 weeks); 8 patients received a modified FEC regimen (5-fluorouracil administered by continuous intravenous infusion 750 mg/m^2/day from day 1 to day 5); 2 patients received a CMF regimen: methotrexate 40 mg/m^2, cyclophosphamide and 5-fluorouracil 600 mg/m^2 every 3 weeks; 3 patients received vinorelbine 25 mg/m^2/week.

Evaluation of responses

All patients had a physical and radiological evaluation every three courses of chemotherapy (four courses for vinorelbine). Patients were considered to have achieved a complete response if no residual evidence of malignancy was found at re-evaluation (i.e. normalisation of all abnormal physical or radiographical findings). Partial response was defined as an objective decrease of 50% or more in tumour size (product of perpendicular diameters). Stable disease was defined as a less than 50% decrease or a less than 25% increase in tumour size. Progression was defined as a greater than 25% increase in

tumour size. Only patients with complete or partial response were considered to have an objective response.

Chemotherapy was continued in all patients showing any evidence of response after three courses (four courses for vinorelbine). Chemotherapy was stopped in cases exhibiting progression or in cases manifesting a stable disease throughout six courses (eight courses for vinorelbine).

Blood specimen

During chemotherapy, blood samples were collected in tubes containing ethylene-diaminetetraacetic acid (EDTA) via an indwelling forearm catheter; before the onset of the first course and then after subsequent courses.

Plasma analyses

The quantitative measurements of c-erbB2 protein in plasma were performed by an enzyme immunoassay (Triton Diagnostics, California, U.S.A.). The within-run reproducibility of the assay was 5% and the between-run reproductibility was 7% (coefficient of variation). In a healthy subpopulation, a tentative cut-off value had been set at 25 U/ml for the c-erbB2 plasma EIA kit by Triton Diagnostics. In our laboratory, the c-erbB2 plasma assay in healthy women ($n = 30$) resulted in a mean concentration of 17 ± 10 (± 2 S.D.) U/ml corresponding to a cut-off value of 27 U/ml. One of these healthy women's samples had levels above the cut-off value (31 U/ml). Therefore, all patients with a plasma c-erbB2 concentration higher than 27 U/ml were considered as c-erbB2 positive (c-erbB2+), conversely patients with a c-erbB2 concentration lower than 27 U/ml were considered as c-erbB2 negative (c-erbB2–).

Statistical analyses

Response rates between c-erbB2– and c-erbB2+ patients were compared using the chi-squared test with the Yates' correction when necessary.

RESULTS

Plasma c-erbB2 concentrations in metastatic breast cancer

Among the 33 patients with metastatic breast cancer, 30.3% ($n = 10$) were c-erbB2+; the median concentration of positive values was 63.8 U/ml (range: 34–2460 U/ml) (Figure 1).

Plasma c-erbB2 status and response to chemotherapy

The objective response rate was 42.4% (14/33): 3 patients (9.1%) achieved a complete response, while 11 patients (33.3%) achieved a partial response. Among the 10 c-erbB2+ patients, 4 had an objective response to chemotherapy while in the 23 c-erbB2–, 10 had an objective response (Table 1). There was no statistically significant difference in response to chemotherapy between c-erbB2+ and c-erbB2– patients ($\chi^2 = 0.033$; $0.5 < P < 0.9$). When considering the chemotherapy given to the c-erbB2+ patients, we observed two partial responses among the 7 FEC treated patients (29% of the cases), one partial response in the 2 modified FEC treated patients (50%) and a partial response for the only patient treated with vinorelbine (100%).

Plasma c-erbB2 concentrations during chemotherapy

In the 10 c-erbB2+ patients, the plasma c-erbB2 variations during chemotherapy were as follows: two increases and eight decreases (Figure 2). Remarkably, we observed in 5 of the 23 c-erbB2– patients an increase in c-erbB2 during the treatment (Figure 3).

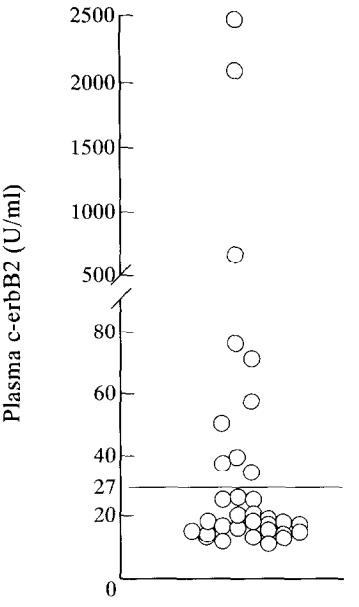

Figure 1. Plasma c-erbB2 concentrations in metastatic breast cancers. Patients with a concentration higher than 27 U/ml before chemotherapy were considered as c-erbB2+.

Table 1. *Response to treatment in relation to pretreatment c-erbB2 status in* 33 *breast cancer patients*

	c-erbB2–	c-erbB2+
Non-responders	13 (9 P+4 SD)	6 (1 P+5 SD)
Responders	10 (7 PR+3 CR)	4 (4 PR)

P, progression; SD, stable disease; PR, partial response; CR, complete response) ($\chi^2 = 0.033$; $0.5 < P < 0.9$)

No objective response was observed in the two c-erbB2+ patients with increasing concentrations while in the 8 patients with decreasing concentrations, 4 had an objective response. One of the 5 patients who became c-erbB2 positive during treatment had an objective response. However, there was no significant relationship between c-erbB2 variations and the clinical response ($\chi^2 = 0.83$; $0.3 < P < 0.5$).

When considering the patients which had a concentration higher than 27 U/ml before and during the treatment, no relationship was observed between c-erbB2 positivity and response to chemotherapy ($\chi^2 = 0.924$; $0.3 < P < 0.5$).

DISCUSSION

The activation of the *C-ERBB2* oncogene has been implicated in a poor prognosis [8–15] and a reduced responsiveness to chemotherapy in patients with breast cancer [18–21]. In the present study, we determined the association between plasma c-erbB2 concentrations and clinical response in patients receiving chemotherapy for metastatic breast cancer.

Our percentage of c-erbB2+ patients was similar to Narita and associates [22], which found that c-erbB2 concentrations were elevated in the plasma of 51% of patients with metastatic breast cancer and 33% of patients with locally recurrent breast cancer. Kynast and colleagues [23] also demonstrated that 34.6% of metastatic breast cancer patients presented high levels of plasma c-erbB2. In agreement with these same

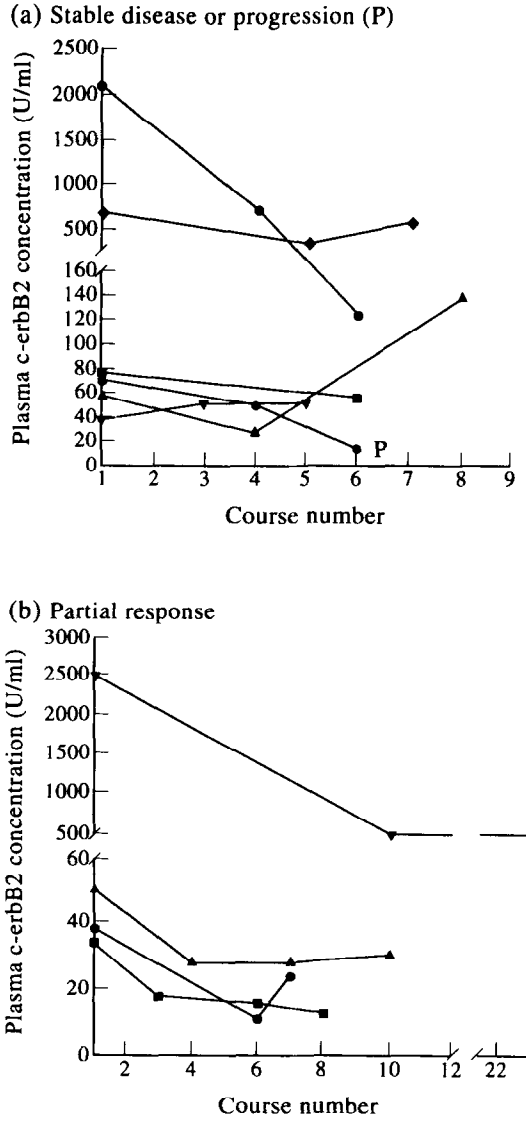

Figure 2. Plasma c-erbB2 concentrations during chemotherapy in c-erbB2+ patients according to response. Each line represents an individual patient. Assay was duplicated.

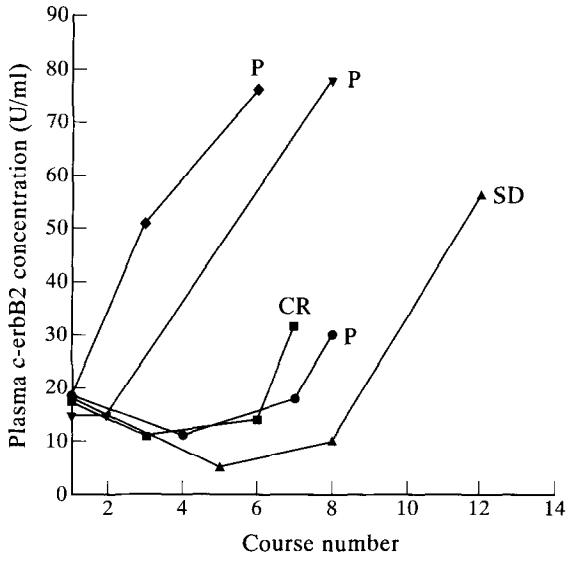

Figure 3. Plasma c-erbB2 concentrations in c-erbB2– patients showing a c-erbB2 increase during chemotherapy. Patients showing a complete response (CR), a stabilisation of the disease (SD), and a progression of the disease (P). Each line represents an individual patient.

authors [22, 23], we have previously observed that none of our patients ($n = 54$) with locoregional non-inflammatory breast cancer were c-erbB2+ (data not shown). All these results suggest that plasma c-erbB2 positivity is most frequently associated with advanced breast cancer.

In the 33 patients in this study, we did not observe any relationship between pretreatment c-erbB2 status and response to chemotherapy. This result is apparently contradictory to those reported in the literature demonstrating that c-erbB2 overexpression in breast tissue is associated with a lower responsiveness to chemotherapy in breast cancer, although the biological basis of this phenomena is not known. Allred and colleagues [18] reported that patients receiving adjuvant chemotherapy for node-negative breast cancer had a lower response when their tumour overexpressed *C-ERBB2*. Gusterson and coworkers [19] demonstrated that node-positive tumours with an overexpression of *C-ERBB2* were less responsive to adjuvant chemotherapy than tumours without an overexpression of *C-ERBB2*. Additionally, in advanced breast cancers previously treated by tamoxifen, patients that overexpressed *C-ERBB2* showed a lower response to mitoxantrone [20]. Interestingly, Muss and associates [21] demonstrated that women with node-positive *C-ERBB2* overexpressing breast cancer were most likely to benefit from high doses of adjuvant chemotherapy.

The lack of a relationship between the plasma c-erbB2 positivity and the response to chemotherapy suggests that tumours which contain a few c-erbB2+ cells probably produce insufficient amounts of c-erbB2 for plasma detection. This hypothesis is supported by the fact that, in 5 cases, plasma c-erbB2 became positive during the treatment. This suggests that it could have been produced by the tumour before treatment but not detected.

C-erbB2 variations during treatment could be related to tumoral evolution. We observed three main types of plasma c-erbB2 variations during the treatment. The c-erbB2 decrease could be explained by a diminution in the size of tumoral tissue, since in 4/8 cases, it was associated with a response to treatment, although in 1 case it was associated with tumoral progression. Conversely, c-erbB2 increase could be the result of tumoral development, with 3/7 cases with a c-erbB2 increase associated with progression of the disease, but in the other 4 cases, it was associated with either stable disease (3 cases) or a complete response (1 case). Finally, in most of the cases ($n = 18$), c-erbB2 remained stable, and was not produced by the tumour. The changes in c-erbB2 during chemotherapy could have alternative causes other than evolution of the tumoral size. Regulation of *C-ERBB2* by hormones has been described with oestradiol inhibiting c-erbB2 production [25–27], while tamoxifen stimulates its synthesis [28]. The effects of chemotherapeutic drugs on c-erbB2 in breast cancer cells are not known. Finally, it may be that drugs induce a selection of c-erbB2+ or c-erbB2– cellular clones leading to an alteration in c-erbB2 plasma concentrations.

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