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Prognostic Value of Epidermal Growth Factor Receptor in a Series of 303 Breast Cancers

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^{125}I-EGF (epidermal growth factor) binding assay was used in tumoral specimens concerning 303 clinical T1-T2, N0-N1 breast carcinoma diagnosed between May 1987 and October 1989. Binding assay for epidermal growth factor receptor (EGFR) was performed using single saturating concentration of ^{125}I-EGF incubated with membrane preparations in the presence or absence of unlabelled EGF. A median value of 3 fmol EGF binding capacity per mg of membrane was obtained and then selected as the threshold value to define positive and negative EGFR tumour samples. According to this definition, 50.8% of the samples were EGFR positive. We noted an inverse relationship between the expression of EGFR and that of oestrogen receptor, and a decreased EGFR expression with tumour differentiation. With a rather short median follow-up (16 months), the multivariate analysis shows that progesterone receptor appears as the only powerful predictor of disease-free survival ($P = 0.002$), taking into account that 70% of the patients received an adjuvant medical treatment.
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INTRODUCTION

In T1–T2, N0–N1, and M0 (UICC 1987) breast cancer the present trend is to modulate the therapy as a function of the multivariate prognostic factors available: axillary lymph node invasion, Scarff-Bloom grade, and presence of progesterone receptors. Nevertheless, when there is no bad prognostic factor, the probability of metastatic relapse is near 20%. Additional parameters have thus been examined, including epidermal growth factor receptors (EGFR) [1–5]. In the present updated analysis our aim was to evaluate the potential prognostic value of EGFR expression on disease-free survival for the whole population of 303 clinical T1–T2, N0–N1 breast tumours.

MATERIALS AND METHODS

From May 1987 to October 1989, 303 cases of primary non-metastatic breast cancer were collected for this study. Breast tumour patients were from the University Hospital, other regional hospitals and private clinics as well. They were distributed as follows: 120 T1 (39.6%), 183 T2 (60.4%), 212 N0 (70%) and 91 N1 (30%) according to the clinical TNM classification of the UICC 1987. Median age was 59 years (range, 28–88).

165 patients had a negative axillary clearance (54.5%) and 138 a positive one (45.5%). Scarff-Bloom grade I was encountered in 36.5% of the cases, grade II in 41.3% and grade III in 22.1%. Loco-regional treatment consisted of radio-surgical combination either conservative or not. Patients with at least one poor prognostic factors (either N^+, Scarff-Bloom grade III or negative oestradiol receptor) underwent medical treatment with hormonotherapy and/or chemotherapy. Among 272 patients evaluable for survival 30% received no adjuvant treatment.

Due to the small size of the tumours and the low concentration of membrane proteins, EGF binding assay was usually performed using a single saturating concentration of ^{125}I-labelled EGF. Briefly, tumour membrane components (about 100 μg of protein) were suspended in 50 μl of 20 mmol/l Hepes buffer supplemented with 0.1% bovine serum albumen (BSA) and 10 mM $MgCl_2$ and incubated in triplicate with 1 nM ^{125}I-labelled EGF for 1 h at 25°C in the presence or absence of unlabelled EGF (100 nM, duplicate samples). The reaction was stopped by filtration through GF/C glass fibre filters followed by washings with 2 × 5 ml of chilled PBS (phosphate-buffered saline) pH 7.2; 0.1% BSA. The radioactivity retained on the filter was counted and specific binding was calculated as the difference between bound radioactivity in the absence minus that in the presence of an excess of unlabelled EGF, and expressed as fmol EGF bound/mg of membrane protein. In this study we selected a positivity threshold of 3 fmol. Oestrogen receptors (ER) and progesterone receptors (PR) were assayed on the corresponding cytosolic fractions. Tumours with ER and PR levels higher than 10 fmol/mg of protein were considered as positive [6].

Statistical analysis

The correlation between EGFR and the other prognostic factors was tested using a maximum likelihood ratio, with the Yates correction [7] when necessary. Survival data were analysed using the log rank method and the Cox proportional hazards regression model [7].

RESULTS

EGFR values from 0 to 41.2 fmol/mg were found for the 303 individual tumour samples assayed in the present study. The corresponding mean value was 4.6 (0.6) [mean (S.D.)] and the median value was 3.1 (0.4). We selected 3 fmol/mg protein as a threshold value, which corresponds with the median value. According to this criteria, 50.8% of the samples were EGFR positive.

Analysis of the ER/PR pairing revealed that 61.1% of the tumours were ER^+ PR^+, 15.3% ER^+ PR^-, 22.3% ER^- PR^- and 1.3% ER^- PR^+. A greater expression of EGFR was observed

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Table 1. ER and EGFR distribution

		ER	
	%	−26.1	+73.9
EGFR	−49.2	29.1	56.2
	+50.8	70.9	43.7

$\chi^2 = 16.13$; d.f. = 1; $P = 0.0001$.

Table 2. PR and EGFR distribution

		PR	
	%	−38.3	+61.7
EGFR	−49.2	42.2	53.5
	+50.8	57.8	46.5

$\chi^2 = 3.18$; d.f. = 1; $P = 0.07$.

in ER^-PR^- tumours ($P = 0.0000$). 73.9% of the tumours were ER^+ and 26.1% were ER^-; as illustrated in Table 1, there was an inverse relationship between the expression of EGFR and ER ($P = 0.0001$). On the other hand, 61.7% of the tumours were PR^+, and there was a trend for an increased expression of EGFR in the absence of PR and vice versa ($P = 0.07$) (Table 2).

Clinically, our series was primarily composed of T2 (60.4%). There appeared to be a tendency toward a greater expression of EGFR in the T1 and lower in the T2 ($P = 0.07$). The analysis of tumour differentiation stage using the Scarff-Bloom grading shows that EGFR expression decreased in parallel with tumour differentiation ($P = 0.038$) (Table 3).

No correlation was detected between EGFR positive status and the number of invaded axillary lymph nodes ($P = 0.28$). The mean follow-up period was too short (16 months), to take into account the overall survival and only 13 deaths occurred. We, therefore, considered disease-free survival. In the univariate analysis (Table 4), four prognostic factors are individualised. The multivariate analysis shows that progesterone receptor appears as the only powerful predictor of disease-free survival ($P = 0.002$) (Table 5).

Table 3. Scarff–Bloom grading and EGFR distribution

		Scarff–Bloom grading		
		I	II	III
	%	36.5	41.3	22.1
EGFR	−49.4	52.5	54.5	35
	+50.6	47.5	45.5	65

$\chi^2 = 6.513$; d.f. = 3; $P = 0.038$.

Table 4. Log rank test results for relapse (272 patients, 22 relapses)

Variable	Log rank χ^2	d.f.	P
Tumour clinical stage	2.804	1	0.094
Axillary lymph node status	0.0001	1	0.972
Scarff-Bloom grading	5.290	1	0.021
ER	6.125	1	0.013
PR	10.437	1	0.0021
EGFR	0.42	1	0.517

Table 5. Regression coefficient (bi) of the significant prognostic variable in the Cox models for relapse-free survival

Variable	Coefficient (bi)	S.E. (bi)	Coeff./ S.E.	P
PR	−1.3688	0.4580	−2.9887	0.002

DISCUSSION

The present updated study examined the EGFR status in 303 breast cancer specimens collected over a 2-year period. Using a ^{125}I-EGF binding assay at a single saturating concentration, the median value of 3 fmol/mg was selected as the threshold above which EGFR was considered as positive. These present data are in agreement with earlier reports describing an inverse relationship between EGFR and ER^- ($P = 0.0001$) [2, 3, 8–10], and a tendency for a greater expression of EGFR in PR negative tumours ($P = 0.07$) [3, 4, 8–10]. We found no relationship between EGFR expression and the invasion of axillary lymph nodes and this is in good agreement with studies by others [4, 11]. Our data also show a significant relationship between EGFR and Scarff-Bloom III grading ($P = 0.04$), which is in keeping with the literature [3]. In addition, a greater expression of EGFR in T1 tumours as compared with the T2 status was observed, that is difficult to explain at the moment.

Considering disease-free survival with a short median follow-up (16 months), the prognostic value of EGFR was assessed by a univariate and a multivariate analysis. PR remains the only powerful factor ($P = 0.002$), despite the fact that 46% of node negative (N^-) patients and 99% of node positive (N^+) patients received adjuvant systemic treatment. The calculated regression coefficient indicates that the disease-free survival increases in parallel with positive PR values. One might notice that the limited number of relapses observed in the present study may explain the discrepancy with series published by others. Sainsbury *et al.* [2] found a prognostic value among ER^- patients receiving no systemic treatment, with a maximum follow-up of 36 months, both for overall and disease-free survival. Nicholson *et al.* [12] stressed that EGFR is a marker of poor prognosis for patients with node negative breast cancer. For Grimaux *et al.* [4] EGFR was the first prognostic parameter at a cut-off date of 40 months for 54 N^+ patients on overall and disease-free survival ($P = 0.01$), invaded node ≥ 4 being the second one. Spyratos *et al.* [5] mentioned EGFR as being the only prognostic factor in a multivariate analysis on disease-free survival of 75 patients (mainly N^-) receiving no adjuvant treatment.

We consider that the potential prognostic value of EGFR in published reports must be confirmed by additional prospective studies and that standardization of EGFR assays is of the utmost importance to obtain uniformity and comparability of the data.

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Inter-observer and Intra-observer Variability of Mammogram Interpretation: a Field Study

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To evaluate the performance of radiologists in mammographic mass screening, seven radiologists read blindly the mammograms of 45 women (two views for each breast). The films included 12 normal, 24 benign disease and 9 cancers. The readings were repeated after 2 years. As expected, variability was higher among radiologists than between the two readings of the same radiologist, but general reproducibility was moderate. Kappa values for a positive/negative classification were 0.45 at the first and 0.44 at the second reading (inter-observer comparisons). For the intra-observer comparisons, Kappa values ranged from 0.35 to 0.67 (mean 0.56). Generally, accuracy was low partly due to the difficulty of the cases. A slight increase in sensitivity was observed at the second reading. The level of agreement is a good indicator of accuracy. Proper training and standardization of criteria are essential before mass breast screening is implemented.
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INTRODUCTION

IT IS well accepted that periodic mammographic screening has the potential to reduce mortality rates for breast cancer by a significant amount, at least in women aged 50 or more [1, 2].

In parallel with the implementation of mass breast screening there is increasing interest in improving the validity of the diagnostic procedures involved [2, 3]. Whereas accuracy has been assessed in previous studies evaluating effectiveness of mass breast screening, less is known about inter- and intra-observer reproducibility on mammographic interpretation. In a large bibliography of publications on observer variability [4], only one out of 51 references included in the section on conventional radiology, considered mammography [5].

Where mass screening has not been implemented, knowledge of the actual accuracy and variability among observers in interpreting the screening test could be of interest. In Turin (a northern Italian city with a population of about 1000000) the local health authority is planning a population screening for breast cancer by mammography. We were asked to describe and evaluate activities that might be involved in the screening programme, including the performance of the radiology units

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