Perioperative Serum VEGF and Extracellular Domains of EGFR and HER2 in Early Breast Cancer

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Abstract. *Background: The prognostic role of serum levels of molecular biomarkers during the perioperative period in patients with early breast cancer is not clear. Patients and Methods: Serum VEGF and extracellular domains (ECD) of EGFR and HER2 were prospectively determined in 119 consecutive patients with early breast cancer on the day before and after surgery. Results: After a median follow-up of 93 months, the preoperative value and the absolute change from pre- to postoperative serum levels of VEGF and HER2 ECD did not predict disease-free survival (DFS). A decrease after surgery of EGFR ECD correlated with a statistically significant lower DFS; each 1 ng/ml decrease in EGFR ECD serum level was associated with an increase of event risk of 15% on multivariable analysis (hazard ratio 1.15 95% confidence interval 1.04.-1.28, p=0.006). Conclusion: The perioperative absolute change of EGFR ECD significantly correlated with disease outcome of patients with early breast cancer. No correlation was found between preoperative and perioperative absolute change of serum VEGF and HER2 ECD.*

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The epidermal growth factor receptor (EGFR) family and its related ligands and downstream effectors are involved in the development and progression of breast cancer (1, 2). The overexpression and/or gene amplification of HER2, present in about 20% of the cases, is associated with aggressive disease and poor survival (1). EGFR overexpression is frequent in basal-like carcinomas (3) and is also associated with high proliferative activity, lack of estrogen receptors (4) and poor prognosis (2).

Soluble forms of these receptors, embodying only portions of their extracellular domain (ECD), are detectable in the bloodstream, making these molecules appealing as tumor markers. These soluble forms can originate from proteolytic cleavage of the full-length receptor by metalloproteases (5). In addition, alternative splicing of the *EGFR* mRNA encodes different truncated receptor isoforms (6), and tumor-specific EGFR transcripts may result from genomic deletions (7) or chromosomal translocations (8). Few data are available on the association between EGFR ECD levels and breast cancer characteristics and, most importantly, in relation to outcome.

On the other hand, in patients with breast cancer, circulating HER2 ECD levels have been shown to be associated with several tumor features, such as stage, grade, HER2 expression, to be predictive of response to chemotherapy, endocrine therapy and trastuzumab, and to decrease after surgery in early breast cancer (9, 10).

Angiogenesis is an essential process for tumor growth and metastasis (11). In breast cancer, tumor-induced angiogenesis is first evident at the pre-invasive stage of ductal carcinoma *in situ* (12). Vascular endothelial growth factor (VEGF) has been identified as a potent pro-angiogenic protein involved

in modulating tumor growth and progression. Elevated serum VEGF levels have been detected in breast cancer patients and significantly correlated with high intratumoral microvessel density (13), an independent prognostic factor for breast cancer survival (14). Moreover, the equilibrium between pro- and antiangiogenic factors is influenced by the activation of the EGFR-HER2 intracellular pathways; a consequence of this activation is increased VEGF synthesis. (15)

Surgical trauma and the processes of wound healing interfere with the production of growth factors for epithelial and endothelial cells, and have been shown to produce an increase in proliferation index, particularly in those tumors overexpressing HER2, which might translate into tumor growth. The primary tumor may shed angiogenesis inhibitors in the blood, and its removal may shift the angiogenic balance towards neovascularization, inducing the development of micrometastases (15-20).

We therefore evaluated preoperative serum levels of VEGF, EGFR and HER2 ECD and their changes after surgery. The prognostic role of serum VEGF, EGFR and HER2 ECD levels in terms of disease-free survival was also evaluated.

Patients and Methods

Patients. From April 1998 to April 2001, patients with T1-3 and N0-2 clinical classification and no secondary lesions undergoing surgery for early breast cancer at the Division of Senology of the European Institute of Oncology entered a clinical study investigating the role of different biomarkers in blood and bone marrow. All patients signed a written informed consent, and the study was approved by the local Ethics Committee. Patients underwent either quadrantectomy or total mastectomy, and either axillary sentinel node biopsy and/or axillary dissection. All patients received some adjuvant treatment.

Assessment of serum markers. Peripheral blood samples (about 15 ml, 7.5 ml at the Division of Pathology and 7.5 ml at the Unit of Hemato-oncology) were collected the day before and the day after surgery from all patients. Moreover 5 ml of blood sample were collected and submitted to the Division of Pathology for the assessment of VEGF.

Serum HER2 was measured with the Bayer Immuno 1 automated analyzer. The assay is based on two monoclonal antibodies directed against the ECD of the HER2 antigen (21).

VEGF concentrations were determined with a quantitative sandwich enzyme immunoassay (Quantikine Human VEGF; R&D Systems, Inc., Minneapolis, MN, USA), using a solid-phase monoclonal antibody and an enzyme-linked polyclonal antibody raised against recombinant human VEGF165. All samples and standards were measured in duplicate, and concentrations are reported as pg/ml. The optical densities were assessed by a microtiter plate reader at 450 nm. A standard curve was created by plotting the logarithm of the mean absorbance of each standard *versus* the logarithm of the cytokine concentration.

The minimal detectable amount of serum VEGF was 9.0 pg/ml and the maximum undiluted was 2,000 pg/ml.

The intra-assay and inter-assay coefficients of variation were less than 7% and 9% respectively.

Serum EGFR concentrations were determined using the Oncogene Science EGFR Microtiter ELISA (Oncogene Science Diagnostics, Inc. OSDI, Cambridge, MA, USA), a sandwich immunoassay with a mouse monoclonal capture antibody and an alkaline phosphatase-labeled mouse monoclonal antibody as detector. Both capture and detector reagents specifically recognize the extracellular domain of EGFR. The capture antibody recognizes a protein domain on the extracellular portion of EGFR and is immobilized on the interior surface of the microtiter plate well. To perform the test an appropriate volume of specimen is incubated in the well to allow binding of the antigen by the capture antibody. The immobilized antigen is then exposed to the alkaline phosphatase-labelled detector antibody. Addition of the substrate to the wells allows the catalysis of a chromogen into a coloured product, the intensity of which is proportional to the amount of EGFR. The optical densities were assessed by a microtiter plate reader at 650 nm. A standard curve was created by plotting the mean absorbance of each standard *versus* the concentration of EGFR. Results for samples are expressed in nanograms per ml by reading directly from the standard curve values.

Immunohistochemistry. Immunostaining for estrogen receptor (ER), progesterone receptor (PgR), Her2/*neu* protein and Ki-67 antigen was performed on consecutive tumor tissue sections, using the following antibodies: monoclonal antibody (mAb) to ER (Dako, Glostrup, Denmark; at 1/100 dilution), mAb to PgR (Dako; at 1/800 dilution); MIB-1 mAb to the Ki-67 antigen (Immunotech, Marseille, France; at 1/1200 dilution); and polyclonal antiserum (Dako; at 1/3200 dilution) to the Her2/*neu* protein. Samples were considered negative for ER and PgR if staining was observed in <1% of neoplastic cells, and positive otherwise. An intense and complete membrane staining in more than 10% of the neoplastic cells (=score 3+) was taken as evidence of HER2 overexpression. No membrane staining or any membrane staining in 10% or less of tumor cells (=score 0), faint/barely staining only in part of the membrane in more than 10% of the neoplastic cells (=score 1+) and weak-to-moderate complete membrane staining in more than 10% of neoplastic cells (=score 2+) were taken as evidence of HER2 not being overexpressed.

Statistical considerations. Means and standard deviations (SD) of serum HER2, EGFR and VEGF levels, taken pre and post surgery, are reported overall and by clinical and pathological characteristics.

Paired *t*-test was used to evaluate the change from pre to post surgery in serum levels of HER2, EGFR ECD and VEGF within patients. The mean absolute changes, along with the 95% confidence intervals (CI) are reported; *t*-test and *f*-test were used to test for differences in the change in serum levels between two groups and more than two groups, respectively. Parametric tests were preferred because of the relatively large sample size. However, nonparametric analyses and/or log-transformations in the case of data non-normally distributed were also performed, in order to check for agreement with the parametric analysis results. Disease-free survival (DFS) was defined as the length of time from the date of surgery to any relapse (including ipsilateral breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer) or death, whichever occurred first.

Univariable Cox proportional hazard regression models were used to evaluate the impact on DFS of preoperative HER2, EGFR and VEGF levels and of their absolute changes after surgery. Multivariable models were used to adjust the impact of absolute serum level changes both for the preoperative levels and other

potential prognostic factors. Prognostic factors were included in the final model only if they were significantly associated on univariable analysis ($p<0.10$) with the absolute changes and/or DFS.

Results from models were expressed in terms of hazard ratios (HRs), with 95% CIs. The variables considered for the analysis, apart from pre and postoperative serum VEGF, EGFR and HER2 levels, were: age, tumor (T1, T2, T3) and nodal (node-negative, node-positive) classification, histological grade (1, well-differentiated; 2, moderately differentiated; 3, poorly differentiated), peritumoral vascular invasion (present or absent), tumor expression of hormone receptors (hormone receptor-negative: estrogen and progesterone receptor in fewer than 1% of tumor cells; hormone receptor-positive: estrogen and/or progesterone receptors in ≥1% of tumor cells), tumor expression of HER2 (HER2 not overexpressed: 0, 1+ or 2+ staining; HER2 overexpressed: 3+ staining), and Ki-67 proliferative index (low: ≤20%, high: >20%, according to the percentage of cells exhibiting definite nuclear staining over at least 2,000 neoplastic cells examined).

Departure from linearity in the relationship between serum HER2, EGFR and VEGF level changes and DFS was evaluated fitting restricted cubic splines models (22). Schoenfeld residuals were used to check if the effect of serum level changes on DFS was constant in time (23).

Survival curves were estimated using the Kaplan-Meier method and the log-rank test was used to assess survival differences between groups.

All analyses were performed with SAS software version 9.1 and R software with the Design and Hmisc libraries (http://cran.r-project.org/) (Cary, NC, USA). All *p*-values were two sided.

Results

Serum samples were available for 124 patients; 5 patients were excluded from the analysis because they received preoperative chemotherapy. Serum VEGF, EGFR ECD and HER2 ECD measurements before and after surgery were thus available in 119 patients. Patients and tumor characteristics are reported in Table I. The median age was 49 years (range 30 to 70). Most patients had T1 (55%) or T2 (40%) tumors and node positive (58%) disease. Eighty-five patients (71%) underwent quadrantectomy and 34 (29%) total mastectomy; all patients had sentinel node biopsy or axillary dissection. Eighty-four received adjuvant chemotherapy, 37 with cyclophosphamide, methotrexate and fluorouracil (CMF) for 3 to 6 cycles, 23 with doxorubicin/epirubicin and cyclophosphamide (AC/EC) for 4 cycles, 17 with AC/EC for 4 cycles followed by CMF for 3 cycles, and 7 with other regimens. Ninety-two patients received adjuvant endocrine therapy: tamoxifen alone, 70 patients; tamoxifen plus gonadotrophin-releasing hormone analogue, 17 patients; letrozole, one patient; 4 patients were treated within a double-blind, randomized clinical trial comparing tamoxifen *versus* letrozole.

The mean preoperative serum HER2 ECD level was 8.13 (SD 2.46) ng/ml, which decreased to 6.78 (SD 2.23) ng/ml at day 1 after surgery ($p<0.0001$) (Figure 1). Preoperative serum HER2 ECD was significantly higher (mean 9.43 ng/ml) in patients with hormone receptor-negative if compared with the cohort with hormone receptor-positive tumors (mean 7.81 ng/ml, $p=0.004$) (Table II). Preoperative serum HER2 ECD was not significantly associated with other clinical and pathological characteristics (Table II). No clinical or pathological factors predicted the change in serum HER2 ECD levels, except for age, with a significant decrease after surgery for patients aged less than 50 years (Table III).

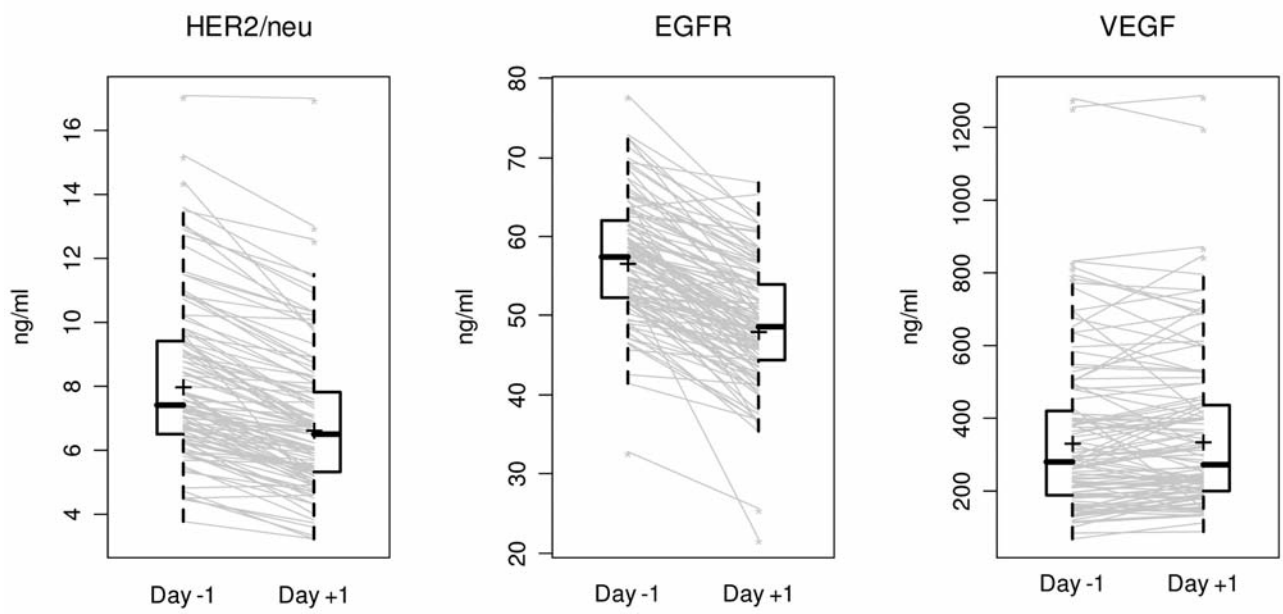
Tumor expression of HER2 was available for 87 patients, 12 of whom (14%) showed an overexpression (3+). Preoperative serum HER2 ECD levels were somewhat higher in patients with HER2 overexpressing tumors (mean 8.67 ng/ml) than in those without HER2 overexpression (median 7.59 ng/ml), but the difference was not statistically significant ($p=0.14$) (Table II). On the other hand, a non statistically significant trend towards a greater absolute change between pre-and postoperative serum HER2 ECD was observed in patients without tumor HER2 overexpression ($p=0.54$).

The mean preoperative serum VEGF level was 341.73 (SD, 224.7) pg/ml, which increased to 346.84 (SD, 215.66) pg/ml at day 1 after surgery ($p=0.46$) (Figure 1). Neither

Table I. *Patient and tumor baseline characteristics.*

	%
Age (years)	
≤50	53%
>50	47%
pT	
1	55%
2	40%
3	4%
Positive nodes at surgery	
0	41%
1-3	34%
4+	24%
Grade	
Unknown	3%
1	18%
2	41%
3	38%
ER/PgR	
Both negative	19%
ER- and/or PgR-positive	81%
Ki-67	
Unknown	3%
<20%	45%
≥20%	53%
HER2	
Unknown	27%
Not overexpressed	63%
Overexpressed	10%
Perivascular invasion	
Absent	49%
Present/focal/diffuse	39%
Unknown	12%

ER, Estrogen receptor; PgR, progesterone receptor; pT, pathological tumour.



	Day –1	Day +1	Absolute change	*p*-Value
	Mean (SD)	Mean (SD)	Mean (95% CI)	
HER2/neu	8.13 (2.46)	6.78 (2.23)	–1.34 (–1.5, –1.18)	< 0.0001
EGFR	57.63 (7.52)	48.97 (7.69)	–8.65 (–9.6, –7.69)	< 0.001
VEGF	341.73 (224.70)	346.84 (215.66)	5.11 (–3.58, –18.80)	0.46

Figure 1. *Matched box plots showing changes between pre- and postoperative values of serum HER2/neu, EGFR and VEGF levels in each patient. Means are indicated with + symbol.*

preoperative level nor the absolute change of serum VEGF were associated with any of the clinicopathological features studied (Tables II and III).

The mean preoperative serum EGFR ECD level was 57.63 (SD, 7.52) ng/ml, which decreased to 48.97 (SD, 7.69). Preoperative levels of serum EGFR ECD were not associated with any of the clinicopathological variables studied (Table II). There was a significant difference in the change in serum EGFR ECD levels from pre to post surgery between T1 tumors and T2 and T3 combined (p=0.002), and between patients aged more and less than 50 years respectively [(p=0.04) (Table III)]. None of the other clinical or pathological factors predicted the change in serum EGFR ECD level.

The median time of follow-up was 93 months (range 20-122). First observed events were 25, of which 13 were distant metastases, 6 locoregional relapses, 3 cancer-related deaths, 2 deaths from unknown causes and 1 second primary.

To evaluate the prognostic role of serum VEGF, EGFR and HER2 ECD, we considered preoperative values and absolute changes and their impact on DFS. Preoperative levels for all serum parameters were not significantly associated with DFS (data not shown). Tables IV shows the HRs for the absolute change from pre- to postoperative, adjusted both for preoperative values and other prognostic factors. There was a statistically significant 15% linear increase of event risk relative to 1 ng/ml decrease of EGFR ECD (HR adjusted for preoperative level, age, pT, grade and Ki-67: 1.15, 95% CI 1.04-1.28, p=0.006). Test based on Schoenfeld residuals showed no evidence of there being a time-homogeneous effect of EGFR decrease.

As shown in Figure 2, patients with an EGFR ECD decrease greater than or equal to the observed mean had a worse prognosis (log-rank test p=0.05) as compared to patients with a decrease lower than the average.

Discussion

The results of the present study show a significant impact of surgery on the expression of serum growth factors in patients with early breast cancer.

A significant drop of serum HER2 ECD levels from pre- to postoperative samples was observed. This result confirms

Table II. *Association between baseline clinical and pathological characteristics and HER2, VEGF and EGF values at day -1.*

	HER2			EGFR			VEGF		
	Mean	SD	*p*-Value	Mean	SD	*p*-Value	Mean	SD	*p*-Value
Age (years)									
≤50	7.81	2.48		58.19	8.21		320.47	170.12	
>50	8.50	2.41	0.13	56.99	6.68	0.39	365.67	273.12	0.28
pT									
1	7.99	2.32		57.38	7.58		362.69	255.48	
2-3	8.31	2.64	0.48	57.93	7.51	0.69	315.65	178.13	0.24
Nodal status at surgery									
Negative	8.33	2.23		58.51	7.52		325.95	212.46	
Positive	8.00	2.61	0.47	57.00	7.52	0.28	352.79	233.75	0.52
Nuclear grade									
1	7.74	1.89		57.44	6.06		367.54	228.19	
2	8.12	2.38		57.57	8.36		333.28	240.59	
3	8.31	2.86	0.68	57.77	7.55	0.98	326.91	204.01	0.77
ER/PgR									
Both negative	9.43	3.03		58.05	6.82		378.19	254.73	
ER and/or PgR positive	7.81	2.20	0.004	57.52	7.71	0.76	333.01	217.46	0.39
Ki 67									
<20%	8.12	2.19		56.75	7.01		376.03	244.71	
≥20%	8.21	2.72	0.85	58.46	7.99	0.23	321.03	207.27	0.19
HER2									
Not overexpressed	7.59	2.23		57.51	7.77		343.81	198.84	
Overexpressed	8.67	3.11	0.14	53.80	5.38	0.12	263.14	115.14	0.18
Perivascular invasion									
Absent	8.49	2.30		58.39	7.83		355.57	248.15	
Present/focal/diffuse	7.66	2.67	0.09	56.56	7.51	0.23	334.11	202.35	0.64

ER, Estrogen receptor; PgR, progesterone receptor; pT, pathological tumour.

previous data, described for a small sample by Salvadori *et al*., showing a decrease in circulating HER2 ECD after surgery in patients with early breast cancer (24). Several investigators (25-28) demonstrated that a higher preoperative serum ErbB-2 was associated with a poor prognosis in breast carcinoma patients. On the other hand, in the largest series, by Molina *et al*. (29), preoperative serum ErbB-2 did not have any prognostic significance in patients with locoregional breast carcinoma. In our study, preoperative serum level of HER2 ECD did not predict DFS. Methodological issues, such as the use of different antibodies for c-erbB-2 or different cut-offs for determining c-erbB2 positive sera, may partly explain the different results in patients outcome among studies. In contrast to literature analyses reported (25-28), we also evaluated the impact of surgery on the postoperative serum HER2 ECD level and its correlation with survival outcome. However, in our analysis, neither the preoperative nor the postoperative change of HER2 ECD levels predicted DFS. Therefore our results strengthen the evidence that serum HER2 ECD is not a useful prognostic tool,

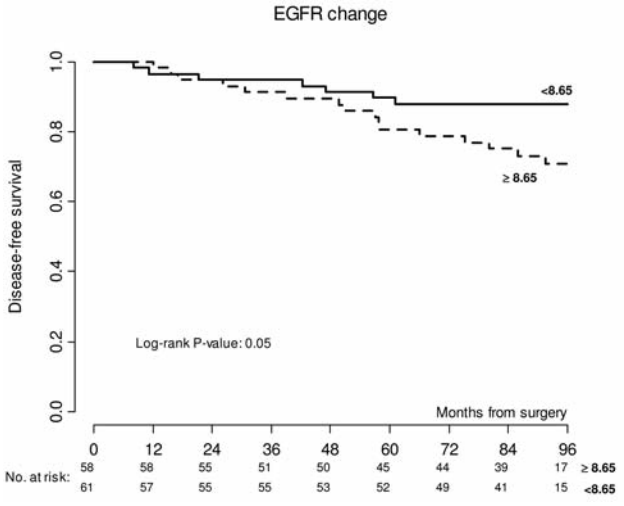


Figure 2. *DFS according to absolute EGFR decrease. Two groups: Dashed line: greater than mean decrease (17 events observed); solid line: lower than mean decrease (8 events observed).*

Table III. *Association between baseline clinical and pathological characteristics and HER2, VEGF and EGFR changes.*

	HER2			EGFR			VEGF		
	Mean	SD	*p*-Value	Mean	SD	*p*-Value	Mean	SD	*p*-Value
Age (years)									
≤50	–1.49	0.97		–9.58	5.82		–4.82	77.17	
>50	–1.17	0.67	0.04	–7.59	4.32	0.04	16.28	72.52	0.13
PT									
1	–1.26	0.73		–7.36	4.03		4.88	72.7	
2-3	–1.44	0.99	0.28	–10.25	6.11	0.002	5.4	79.43	0.97
Nodal status									
Negative	–1.37	0.77		–8.24	4.55		18.02	67.63	
Positive	–1.32	0.91	0.76	–8.93	5.69	0.48	–3.93	79.69	0.12
Grade									
1	–1.16	0.76		–8.16	7.24		–3.9	103.59	
2	–1.47	0.81		–9.09	5.08		–4.54	66.91	
3	–1.25	0.91	0.27	–8.45	4.47	0.75	22.36	65.95	0.18
ER/PgR									
Both negative	–1.31	0.84		–8.11	5.13		7.38	79.98	
ER- and/or PgR-positive	–1.35	0.86	0.86	–8.77	5.29	0.59	4.57	74.75	0.87
Ki-67									
<20%	–1.45	0.98		–8.88	5.90		–1.71	90.13	
≥20%	–1.27	0.73	0.26	–8.66	4.64	0.82	8.82	62.12	0.46
HER2									
Not overexpressed	–1.33	0.97		–8.92	5.73		5.77	84.3	
Overexpressed	–1.15	0.69	0.54	–8.41	4.23	0.77	–13.58	40.74	0.44
Perivascular invasion									
Absent	–1.39	0.97		v8.95	5.47		9.92	63.69	
Present/focal/diffuse	–1.25	0.77	0.42	–7.51	4.14	0.14	1.56	100.64	0.58

ER, Estrogen receptor; PgR, progesterone receptor; pT, pathological tumour.

Table IV. *Impact of serum decrease in HER2, VEGF and EGFR levels on DFS. Effects are expressed in terms of hazard ratio.*

	Unit	HR[1] (95% CI)	*p*-Value	HR[2] (95% CI)	*p*-Value
HER2					
Absolute decrease	1 ng/ml	1.10 (0.65-1.85)	0.71	1.48 (0.80-2.76)	0.21
EGFR					
Absolute decrease	1 ng/ml	1.07 (1.00-1.14)	0.05	1.15 (1.04-1.28)	0.006
VEGF					
Absolute decrease	100 pg/ml	1.09 (0.62-1.93)	0.76	1.41 (0.74-2.65)	0.29

Adjusted for: [1]preoperative level; [2]preoperative level and the following clinical features associated with absolute change and/or DFS: HER2: age, grade and Ki-67; EGFR: age, pT, grade and Ki-67; VEGF: grade and Ki-67.

but is probably more useful as a biological tumour marker for predicting drug response or drug resistance (30-33).

One of the mechanisms which contributes to the poor prognosis of HER2-overexpressing tumors is enhanced intravasation due to increased chemotaxis and invasion, mediated by ErbB3/ErbB2 heterodimers (34). HER2 further contributes to tumor angiogenesis by inducing the expression of angiogenic factors such as VEGF (35).

A slightly though not statistically significant increase of serum level of VEGF from pre- to postoperative evaluation was registered in the present study; this serum VEGF increase was not associated with any baseline clinicopathological characteristics nor with the outcome of patients studied. The prognostic role of serum VEGF has been studied in many types of cancer with contradictory results (36, 37). In lung cancer patients, preoperative serum

VEGF was prognostic only on univariate analysis (36); in hepatocellular carcinoma, in contrast, preoperative serum VEGF was significant predictor of DFS and overall survival (OS) on univariate and multivariate analysis (37). There are few data about the prognostic role of serum VEGF in breast cancer (38) and, above all, there are no studies evaluating the correlation of preoperative and postoperative change with patients outcome. In our study, neither the preoperative or the absolute change from pre- to postoperative serum levels for serum VEGF significantly predicted DFS. However, there are still many issues to be clarified regarding the measurement of blood VEGF in cancer patients, such as the method of sample collection, processing, software manipulation and data interpretation, and as to whether plasma, serum or whole blood will provide the best prognostic information.

As for EGFR ECD we found a significant drop from pre- to postoperative samples. Considering the absolute EGFR decrease, patients with an EGFR decrease greater than or equal to the observed mean had a worse prognosis as compared to patients with a decrease lower than the average (Figure 2). Interestingly our data show that (at multivariable analysis) every unit decrease of serum level after surgery was associated with an increase of event risk of 15%; this effect was independent of the preoperative value and was homogenous in time. These data are strengthened by long period of follow-up of our analysis.

There are no studies evaluating the correlation of preoperative serum EGFR and its change after surgery with clinical outcome. Serum EGFR levels have been inversely correlated with the extent of tumor burden and with outcome in terms of progression-free survival (PFS) and OS, although the latter findings are not consistent across studies. In a study by Asgeirsson *et al*., serum EGFR was higher in healthy individuals as compared with breast cancer patients. In addition, Asgeirsson analyzed and correlated serum EGFR values measured before primary surgery with values at diagnosis of metastatic disease. The authors reported a median preoperative EGFR ECD value of 56.3 ng/ml, which was significantly decreased at diagnosis of metastatic disease (median 30.9 ng/ml, $p<0.001$) (39). A few reports indicated also the potential prognostic and predictive role of serum EGFR in breast cancer patients. Souder *et al*. showed that metastatic patients with low baseline value of serum EGFR (below 44.1 ng/ml), enrolled in a randomized study of first-line hormonal therapy, had significantly reduced survival compared with patients who had normal serum EGFR levels (median survival, 23.3 months *vs*. 30.9 months; $p=0.007$) (40). Sandri *et al*. showed in metastatic patients treated with metronomic chemotherapy that baseline values of serum EGFR (below 45 ng/ml) were significantly associated with reduced PFS ($p=0.016$) and OS ($p=0.015$) (41). Contrasting results were reported by Lafky *et al*. showing that letrozole-induced decrease of serum EGFR was not associated with clinical response, nor PFS nor OS (42). Moreover, in the study of Witzel *et al*., sEGFR levels at the onset of metastatic disease in 76 patients with breast cancer did not show a significant impact on OS (43). The present study is the first to evaluate the impact of surgery on serum EGFR level, showing the prognostic relevance of the EGFR decrease in patients with early breast cancer. The biological background for this finding is not clear yet.

The reduction of serum EGFR ECD after surgery in patients with early breast cancer supports the hypothesis that at least some tumors are a relevant source of serum EGFR, but the role of this marker cannot be ascertained separately from that of its ligands and perhaps from the levels of EGFR expressed by tumor tissue. As already hypothesized, this result, as the correlation with patient outcome, could be partly explained with a more rapid clearance of EGFR ECD from the blood vessels as a consequence of EGFR binding to its ligands. These ligands could be produced by an aggressive tumor clone as residual after surgery (40), or, more probably, by the wound healing process; the growth factors present in wound fluid, in fact, are known to enhance tumor proliferation, neovascularization and bind EGFR with high affinity (19). Secondly, the decrease in EGFR ECD might be correlated with the high EGFR expression of the tumor removed by surgery with subsequent decrease in EGFR. This hypothesis, if confirmed, could support the exploration of selective EGFR blockade in the preoperative treatment of EGFR-positive tumors.

Conclusion

In the present study, we showed a statistically significant reduction of serum EGFR ECD and HER2 ECD from preoperative to postoperative samples. Moreover, a significant association between the absolute change of EGFR ECD and DFS was observed. Although further research is needed to ascertain the ultimate biological and clinical relevance of this change, our results support a potential role of this molecule as a tumor marker, potentially useful also to tailor selective preoperative and perioperative EGFR/HER2/*neu* blockade in addition to conventional therapies.

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