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Prognostic Value of CEA and Ferritin Assay in Breast Cancer: a Multivariate Analysis

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Abstract—*The prognostic significance of preoperative serum carcinoembryonic antigen (CEA) and ferritin levels was evaluated in 191 women operated for breast cancer. The influence of CEA, ferritin and another 11 clinical and pathological features on the disease-free survival was investigated in a multivariate analysis, using Cox's proportional hazard model. Axillary node status (P = 0.004), CEA level (P = 0.011), and the histological grade of the tumor (P = 0.029) emerged as independent prognostic factors. By contrast, no significant relationship was found between ferritin and disease-free survival.*

These three parameters were used to derive a prognostic index (I) for each patient. Multivariate analysis showed that its prognostic value was better than the value of any single factor (P < 0.0001). The I score was used to divide patients into groups at different risk of recurrence: low, moderate and high (97.5%, 45% and 22.5% of recurrence-free patients at 3 years respectively). The data showed that the prognosis of patients with different combinations of node status and tumor grade was related to the level of CEA. Only women with very good (node-negative with well-differentiated tumors) or very bad prognosis (node-positive with four or more metastatic nodes and poorly differentiated tumors) had a disease-free survival independent of CEA values.

These findings suggest that the preoperative measurement of CEA enhances the possibility of correctly predicting outcome and hence could be of assistance in the planning of adjuvant therapies.

INTRODUCTION

MORE THAN 50% of women with breast cancer without detectable distant metastases have a relapse after surgery. The histological status of the axillary lymph nodes is widely accepted as the most reliable indicator of this risk [1, 2]. The correct planning of adjuvant therapies, however, demands the use of additional prognostic factors [3].

High postoperative serum levels of the biological markers for breast carcinoma, namely carcinoembryonic antigen (CEA) and ferritin, may provide preclinical evidence of metastases [4–8]. Their preoperative value, however, is still questioned [7–12].

This paper describes the result of a multivariate analysis designed to show the association between preoperative CEA and ferritin levels and the disease-free survival (DFS). The relationship between CEA and DFS was such as to allow it to be combined with node status and tumor grade in a prognostic index.

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MATERIALS AND METHODS

One hundred and ninety-one eligible women consecutively operated on for breast cancer at the Institute of Oncology, University of Turin, between September 1983 and July 1986 were studied.

All tumors were staged according to the TNM system. Eligible patients had: (a) histological diagnosis of invasive ductal carcinoma of the breast; (b) no evidence of distant metastases; (c) complete axillary node dissection.

Assays for estrogen and progesterone receptors (ER, PgR) were performed on samples from the primary tumor with the dextran-coated charcoal technique. The histological grade of the tumor was evaluated according to Bloom and Richardson [13]. Vascular invasion was referred to both blood and lymph vessels.

Blood samples for marker assay were taken preoperatively between 8.00 and 9.00 a.m. CEA and ferritin values were determined in serum by a monoclonal double-antibody radioimmunoassay using commercially available kits: CEAK-PR, Sorin Biomedica, Saluggia, Italy and Lisophase ferritin kit, Sclavo, Milan, Italy, respectively.

The CEA values were divided into four categories as previously described [12]: ≤2.5, 2.6–5.0,

5.1–9.9 and ≥10 ng/ml. For ferritin, two cut-off points (120 and 200 ng/ml) were used to discriminate low, intermediate and high values [9].

After mastectomy, 94 patients with pT4 tumors and/or capsular invasion of lymph nodes were irradiated. Twenty node-positive premenopausal patients received chemotherapy (CMF, six courses). One hundred and thirty-five women with ER ≥ 3 tumors were given hormonal therapy (tamoxifen 20 mg daily for 2 years). The adjuvant therapies were variously combined. Follow-up ranged from 9 to 42 months (mean 27).

All data were processed with BMDP series of computer programs elaborated by Health Science Computing Facility, UCLA [14]. The relationship between CEA and ferritin values and pathological features was examined by means of the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) as implemented in BMDP3S. Recurrence curves were calculated from the product-limit estimate of Kaplan and Meier [15] and statistical significance between curves was assessed using the Mantel–Cox test BMDP1L [16].

A multivariate analysis was carried out to assess the relative importance of 13 possible prognostic factors, using Cox proportional hazard survival regression model BMDP2L [17]. This is a stepwise regression technique which allows each variable (covariate) to be evaluated independently, taking into account the effects of all other variables. The coefficients (β-values) produced by the analysis reveal how each covariate is related to hazard and survival. A positive coefficient indicates a poorer DFS as the code of the corresponding covariate increases, and vice versa for negative coefficients. The absolute β-value indicates the magnitude of the influence of each variable on DFS. The coefficients of significant prognostic factors obtained

Table 1. Variables examined by multivariate analysis (No. of cases in parentheses) and their codes

Variable		Codes used in Cox analysis
Age	range 27–92 years; mean 58.9 years	years
Menopausal status	premenopausal = last period <2 years (60)	1
	postmenopausal = last period ≥ 2 years (131)	2
Tumor size (pT)	pT1 (80)	1
	pT2 (82)	2
	pT4 (29)	4
Node status (pN)	pN0 (92)	1
	pN1, 1–3 positive nodes (44)	2
	pN1, ≥ 4 positive nodes (55)	3
ER	0–2 fmol/mg (46)	1
	3–9 fmol/mg (47)	2
	≥ 10 fmol/mg (98)	3
PgR	0–2 fmol/mg (127)	1
	3–9 fmol/mg (27)	2
	≥10 fmol/mg (37)	3
Histological grade	well differentiated, G I (10)	1
	moderately differentiated, G II (112)	2
	poorly differentiated, G III (69)	3
Vascular invasion	present (56)	1
	absent (135)	2
CEA	≤ 2.5 ng/ml (80)	2.5
	2.6–5.0 ng/ml (58)	5
	5.1–9.9 ng/ml (24)	7.5
	≥ 10 ng/ml (29)	10
Ferritin	< 120 ng/ml (120)	100
	120–200 ng/ml (38)	200
	> 200 ng/ml (33)	300
Adjuvant radiotherapy	administered (94)	1
	not administered (97)	2
Adjuvant chemotherapy	CMF, six courses (20)	1
	not administered (171)	2
Adjuvant hormonal therapy	Tamoxifen, 20 mg daily (135)	1
	not administered (56)	2

by multivariate analysis can be used to create a prognostic index (I) for each patient in a simplified risk-score equation [18, 19]:

$$I = (\beta a \times Ca) + (\beta b \times Cb) + (\beta c \times Cc) + \ldots$$

where $\beta a, \beta b, \beta c, \ldots$ represent the coefficients and $Ca, Cb, Cc, \ldots$ the codes of covariates $a, b, c, \ldots$ The higher is the I value, the poorer is the prognosis.

RESULTS

Table 1 lists the distribution of the 13 clinical and pathological factors examined and their codes.

As shown in Table 2, the Kruskal–Wallis analysis showed a direct relationship between CEA and ferritin levels. The levels of both markers were significantly higher in cases with skin involvement (pT4). No relationship existed between CEA or ferritin levels and any of the other pathological features examined.

The multivariate analysis data are summarized in Table 3. Axillary node status ($P = 0.004$), CEA level ($P = 0.011$), and histological grade ($P = 0.029$) emerged as independent prognostic factors. All the coefficients were positive.

Table 2. Relationship between CEA, ferritin and pathological features

	CEA (ng/ml) median	*P* value (Kruskal–Wallis)	Ferritin (ng/ml) median	*P* value (Kruskal–Wallis)
Tumor size				
pT1	2.90		81.5	
pT2	2.80	0.046	75.5	<0.001
pT4	5.00		200.5	
Node status				
pN0	3.00		78.0	
pN1 (1–3)	2.20	N.S.	75.0	N.S.
pN1 (≥ 4)	4.80		94.0	
ER				
0–2	4.00		77.5	
3–9	3.20	N.S.	66.5	N.S.
≥ 10	2.70		93.0	
PgR				
0–2	3.55		91.5	
3–9	3.15	N.S.	82.5	N.S.
≥ 10	2.70		69.0	
Histological grade				
G I	2.95		81.5	
G II	2.90	N.S.	78.0	N.S.
G III	3.00		93.0	
Vascular invasion				
present	3.15	N.S.	93.0	N.S.
absent	3.00		78.0	
Ferritin				
< 120	2.10			
120–200	2.70	0.004		
> 200	5.10			

N.S. = not significant ($P > 0.05$).

Table 3. Multivariate analysis: factors affecting DFS (Cox models—final step of stepwise regression)

Factors	*P* value	Coefficient
Node status	0.004	0.8206
CEA	0.011	0.2780
Histological grade	0.029	1.3540
Each of the other 10 factors	>0.05	—

The coefficients were used to derive a prognostic index with the equation described in the Materials and Methods section. Only the three factors found to be independent were employed. The prognostic index (I) for each patient was then:

$$I = (0.8206 \times \text{node status}) + (0.2780 \times \text{CEA}) + (1.3540 \times \text{grade}).$$

The numbers used in the equation for each patient were the codes for the respective pathological feature (Table 1). Of the 36 possible values of I, corresponding to different combinations of the variables, only 25 were represented in our series (Table 4). The larger the value of I, the poorer the prognosis of the women of that group.

Life table analysis was performed on these I groups to identify three subsets of women at different risks of recurrence: low-risk ($I \leq 5$), moderate-risk ($5 < I \leq 6.5$), and high-risk ($I > 6.5$), whose recurrence curves are drawn in Fig. 1. In the low-risk subset 97.5% of patients were disease-free 3 years after surgery, 45% in the moderate-risk subset and only 22.5% in the high-risk subset.

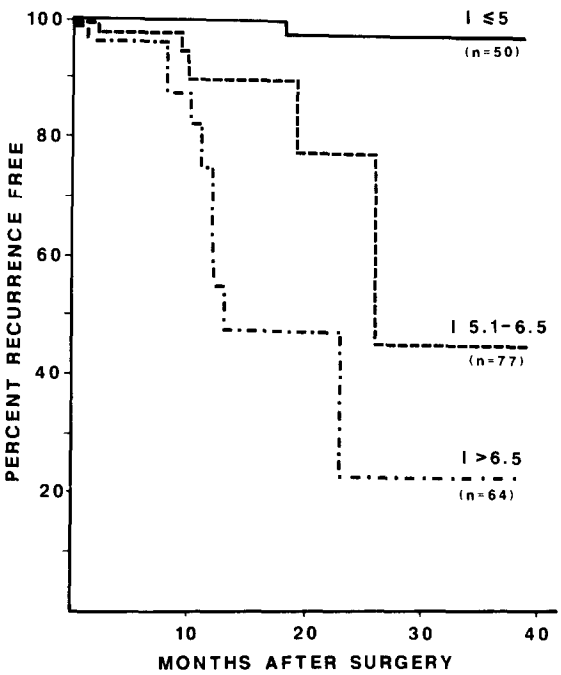

Fig. 1. Recurrence curves of patients divided according to the prognostic index: $I \leq 5$, $I = 5.1$–6.5, $I > 6.5$. The Mantel–Cox test indicates a significant difference between each pair of curves ($P < 0.02$).

Table 4. Subsets of patients identified by prognostic factors and their prognostic index (I)

Node status	Histological grade	CEA (ng/ml)	I	No. of patients	Risk of recurrence
pN0	G I	≤ 2.5	2.870	5	
pN0	G I	2.6–5.0	3.565	3	
pN0	G I	5.1–9.9	4.260	2	Low
pN0	G II	≤ 2.5	4.224	19	
pN0	G II	2.6–5.0	4.919	21	
pN0	G II	5.1–9.9	5.614	5	
pN0	G II	≥ 10	6.309	9	
pN0	G III	≤ 2.5	5.578	14	
pN0	G III	2.6–5.0	6.273	7	Moderate
pN1 (1–3)	G II	≤ 2.5	5.044	16	
pN1 (1–3)	G II	2.6–5.0	5.739	10	
pN1 (1–3)	G III	≤ 2.5	6.398	8	
pN1 (≥ 4)	G II	≤ 2.5	5.865	8	
pN0	G III	5.1–9.9	6.968	4	
pN0	G III	≥ 10	7.663	3	
pN1 (1–3)	G II	≥ 10	7.129	4	
pN1 (1–3)	G III	2.6–5.0	7.093	3	
pN1 (1–3)	G III	5.1–9.9	7.788	3	
pN1 (≥ 4)	G II	2.6–5.0	6.560	8	High
pN1 (≥ 4)	G II	5.1–9.9	7.255	5	
pN1 (≥ 4)	G II	≥ 10	7.950	7	
pN1 (≥ 4)	G III	≤ 2.5	7.219	10	
pN1 (≥ 4)	G III	2.6–5.0	7.914	6	
pN1 (≥ 4)	G III	5.1–9.9	8.609	5	
pN1 (≥ 4)	G III	≥ 10	9.304	6	

The predictive value of I and the other factors were tested by a multivariate analysis. The codes for the new covariate I were: 5 ($I \leq 5$), 6 ($5 < I \leq 6.5$), 7 ($I > 6.5$). I was the only factor independently related to DFS ($P<0.0001$) (Table 5).

As shown in Table 4, in the low-risk subset there were node-negative patients with either G I tumors irrespective of CEA level, or G II with CEA ≤ 5.

The moderate-risk subset included: (a) node-negative patients with either G II tumors with CEA > 5, or G III with CEA ≤ 5; (b) node-positive patients with 1–3 metastatic nodes with either G II tumors and CEA ≤ 5, or G III and CEA ≤ 2.5; (c) node-positive patients with ≥ 4 metastatic nodes with G II tumors and CEA ≤ 2.5.

In the high-risk subset there were: (a) node-negative patients with G III tumors and CEA > 5; (b) node-positive patients (1–3 metastatic nodes) with either G II tumors and CEA ≥ 10, or G III and CEA > 2.5; (c) node-positive patients (≥ 4 metastatic nodes) with either G II tumors and CEA > 2.5, or G III irrespective of CEA.

Table 5. Multivariate analysis: factors affecting DFS. Prognostic index (I) was examined with the other 13 factors tested before (Cox models—final step of stepwise regression)

Factors	*P* value	Coefficient
Prognostic index (I)	<0.0001	1.0244
Each of the other 13 factors	>0.05	—

These data showed that the prognosis of 154/191 women (80.6%) with different combinations of node status and tumor grade was related to their CEA value. Only patients with extremely good (node-negative, G I) or bad prognosis (≥ 4 positive nodes, G III) had a DFS unrelated to CEA.

DISCUSSION

The serum assay of CEA and ferritin can be carried out with different aims in early breast cancer: screening and preclinical diagnosis, and prognosis.

There is a general consensus that both CEA and ferritin have no value in the early diagnosis of breast cancer. CEA lacks specificity and sensitivity [7, 8, 12, 20].

In our study high levels of CEA and ferritin were consistently associated with locally advanced tumors. By contrast, no relationship was found with any other pathological feature examined.

Multivariate analysis revealed that in our series the number of positive nodes, the histological grade and the preoperative serum CEA level were independent prognostic factors, whereas neither ferritin nor the other factors examined were significantly associated with DFS. CEA and ferritin values are related to each other but the results of the multivariate analysis showed that the former is more accurate in predicting survival. The combined assay of CEA and ferritin did not improve the accuracy of CEA alone.

An increased recurrence rate in patients with high levels of CEA had been previously demonstrated using a univariate analysis [12]. Its prognostic significance, however, was considered of limited value, since preoperative CEA levels seemed to be associated with prognosis in only 5% of women with early breast cancer. In our study a multivariate analysis was performed, and CEA levels were found to be significantly related to DFS. The use of a prognostic index, by which the influences on DFS of each single parameter are combined, allowed every patient to be included in one of the risk subsets. This could be of great assistance in the planning of adjuvant therapies.

It should be noticed that preoperative serum CEA conditioned the prognosis in all the groups identified by node status and tumor grade, save pN0-G I and pN1 (≥ 4 positive nodes)–G III. The average time of the follow-up (27 months) could probably not be long enough to reveal slight differences, related to CEA, in the DFS of the patients of these two groups, whose risk of early recurrence is extremely low and high respectively. This means that the measurement of CEA gives a clue as to the outcome of treatment in 80% of patients at least.

Our data suggest that CEA should be routinely evaluated preoperatively and the result be taken in account when adjuvant treatments are planned.

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