Monitoring serum CEA in women with primary breast tumours positive for oestrogen receptor and with spread to lymph nodes

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SUMMARY Serum carcinoembryonic antigen concentrations (serum CEA) in 80 patients with primary breast cancer were measured preoperatively, one month after operation, and thereafter serially every third month. These data were related to histological and morphometric features of the primary breast carcinoma and the lymph node metastases and to clinical follow up data.

Analysis of the serum CEA values showed significant correlations with size of tumour, the presence of lymph node metastases, oestrogen receptor, and occurrence of distant metastases. Furthermore, the results indicated that serial determination of serum CEA in the first two years after operation may be useful in monitoring for the occurrence of distant metastases in patients with metastatic spread to lymph nodes and with large (≥ 2 cm) primary breast tumours positive for oestrogen receptor. In agreement with other studies, however, it was found that the predictive value of serum CEA concentrations in general is weak and costs may prohibit the implementation of the routine assessment of CEA concentrations.

Determination of carcinoembryonic antigen (CEA) in the serum of patients with breast cancer has been the subject of many investigations,¹⁻¹⁷ most of which had different aims, varying from defining CEA as a prognostic factor at or before operation^{10 16 17} to serial estimations of serum CEA to monitor for metastatic disease or reaction to treatment.^{3 8}

The results of these studies were rather negative as they showed that CEA is not a specific tumour marker for breast carcinoma and that high serum CEA concentrations at or before operation have no prognostic importance. Although studies showed that serial estimations of serum CEA were useful in monitoring patients operated on for breast cancer, the sensitivity for discovering recurrence was not very high (varying from 30 to 71%).^{6 13}

It might be, however, that evaluation of serum CEA would be useful in a particular subgroup of patients. In this study, therefore, we investigated the relation of serial estimations of serum CEA concentrations to clinical follow up and to the histologi-

cal and morphometric features of the primary tumour. The main purpose of our study was to find criteria for selecting patients who would benefit from the monitoring of serum CEA concentration.

Material and methods

PATIENTS AND CLINICAL DATA

All of the female patients ($n = 80$) who had undergone surgery for primary breast carcinoma without other overt diseases, who presented at the Reinier de Graaf Hospital, Delft, The Netherlands, from 1 January 1981 until 31 December 1982 were included in this study.

The mean age of the patients was 59.1 years (range 26-91 (SD) 13.8). We were able to assess the size of the tumour in 73 patients: the mean diameter was 2.5 cm (range 0.5-6.5 cm (1.4)). In 70 patients lymph nodes were available for study. Forty (51%) of these patients had lymph node metastases and 29 (36%) did not. The mean duration of follow up was 36 months (minimum 25, maximum 49 months).

The data collected from the clinical records were:

organs affected by metastases, date of appearance of distant metastases, type of treatment, and cause of death. All patients studied were examined every three months postoperatively during the first two years and every six months thereafter. At each control visit measurement of serum CEA formed part of the routine haematological, biochemical, and physical examinations. X ray films of the thorax were obtained every six months, in addition to those made because of symptoms verbalised by the patient or physical findings, or both. Distant metastasis was diagnosed if a combination of clinical, radiographic, scintigraphic, and biochemical data (excluding CEA values) were conclusive, or if metastasis was diagnosed histologically. Both local and distant metastases were considered. Patients with local recurrence (in the skin, axilla, and mastectomy scar) and one patient with contralateral breast disease were separately considered in the study; histological examination did not determine whether this last patient had a second primary tumour or a metastasis.

HISTOLOGICAL METHODS

The size of the primary tumour was assessed on the excision specimen. The primary breast tumours and all the available lymph nodes were fixed in 5% buffered formalin and subsequently embedded in paraplast. From this material tissue sections 4 μ m thick were stained with haematoxylin and eosin. The condition of the lymph nodes and the following histological features of the primary carcinoma were assessed.

HISTOLOGICAL TUMOUR TYPE

All 80 cases of breast carcinoma were classified according to the World Health Organisation recommendations¹⁸: ductal (n = 68); lobular (n = 1); medullary (n = 4); and others (n = 7). Nuclear grading was performed according to Black *et al.*¹⁹ Histological grading was done according to Bloom and Richardson.²⁰ Only ductal carcinomas were graded (n = 68).

MORPHOMETRY

Morphometry was performed, as described by Baak *et al.*²¹⁻²³ on the stained sections from both the primary tumour and, if present, the lymph node metastases. The following morphometric features were assessed: mitotic activity index; cellularity index; and several nuclear features: perimeter, area, shortest and longest axis, axes ratio, and shape factor as $(4\pi \times \text{area})/\text{perimeter}^2$.

OESTROGEN RECEPTOR CONTENT

The oestrogen receptor content of the primary

breast tumour was determined by biochemical assay as described by the EORTC breast cancer cooperative group.²⁴ According to this procedure values of more than 11 000 fmol bound protein oestradiol were classified as positive. Values were regarded as negative if less than 9000 fmol oestradiol/g protein was found: sufficient frozen material was available from only 51 patients.

CARCINOEMBRYONIC ANTIGEN

The CEA enzyme immunoassay applied was a direct radioimmunoassay (Abbott CEA-EIA monoclonal (mouse), Abbott Diagnostics, United States). According to the manufacturer, this kit contains a mixture of five monoclonal antibodies reactive with all known immunoreactive elements of CEA. The serum CEA concentrations (in serum samples of 100 μ l) were estimated preoperatively; one month after operation; and successively every third month. Likewise, the highest serum carcinoembryonic antigen concentrations (CEA-H) found in each patient during the follow up period were collated and analysed.

ANALYSIS

Statistical analysis of the data was performed using χ^2 contingency tables, linear regression, and the Wilcoxon rank sum test. P values of 0.05 or less were regarded as significant. Special attention was paid to the cut off point of serum CEA concentrations. Different thresholds were separately investigated (2.5, 5, 10, and 20 ng/ml, respectively) and 5 ng/ml was found to be the optimal cut off point for monitoring. In subsequent analyses, therefore, serum CEA concentrations ≤ 5 ng/ml were regarded as negative (-), concentrations > 5 and ≤ 10 ng/ml as positive (+), and concentrations > 10 ng/ml as strongly positive (++) .

Results

CEA AND INVESTIGATED FEATURES

Tables 1 and 2 show the data from serum CEA concentrations (those measured preoperatively and one month after operation, and the highest values) and Table 1 *Distribution of serum carcinoembryonic antigen (serum CEA) preoperatively and one month after operation, and distribution of highest value in each patient*

	Preoperative value	Postoperative value (one month)	Highest value
Serum CEA > 10 ng/ml	4	3	13
Serum CEA > 5 , ≤ 10 ng/ml	8	3	10
Serum CEA ≤ 5 ng/ml	68	74	57
Total No of patients	80	80	80

Table 2 Distributions of serum carcinoembryonic antigen (serum CEA) preoperatively and one month postoperatively and of highest concentration in each patient in relation to lymph node metastases, tumour size, and oestrogen receptor states

	Serum CEA						Total No of patients	
	>10 ng/ml		>5, ≤10 ng/ml		≤5 ng/ml		Negative	Positive
	Negative	Positive	Negative	Positive	Negative	Positive		
Lymph nodes:								
Preoperative value*	0	3	1	5	28	33	29	41
Postoperative value	0	2	0	1	29	38		
Highest value†	1	9	2	5	26	27		
Tumour size‡:								
Preoperative value**‡	0	4	0	7	27	35	27	46
Postoperative value**	0	3	0	2	27	41		
Highest value**	1	9	0	10	26	27		
Oestrogen receptor¶:								
Preoperative value	0	4	0	3	11	33	11	40
Postoperative value§	0	3	0	0	11	37		
Highest value	0	6	0	6	11	28		

*Lymph node positive v negative: $p < 0.05$, Wilcoxon.†Lymph node positive v negative: $p < 0.005$, Wilcoxon.

‡Tumour size: negative <2 cm, positive ≥2 cm.

**Tumour size: positive v negative; $p < 0.05$, Wilcoxon.‡Linear regression: preoperative value v tumour size $p < 0.005$, $r = 0.34$.

¶Oestrogen receptor: negative <9 fmol/mg, positive ≥11 fmol/mg.

§Postoperative value v oestrogen receptor state; $p < 0.01$, $r = 0.37$.

the significantly correlated features—that is, size of tumour and oestrogen receptor content, and presence or absence of lymph node metastases. In six of 12 patients the serum CEA value decreased below 5 ng/ml during the first month after operation.

Fourteen of the 41 patients (34%) with positive lymph nodes and only three of the 29 patients (10%) with negative lymph nodes achieved serum CEA concentrations >5 ng/ml (Wilcoxon test, $p < 0.01$; Table 2). Nineteen of the 46 patients (41%) with a tumour ≥2 cm had, on more than one occa-

sion, positive (+++) serum CEA values compared with only one patient of the 27 (4%) with a tumour <2 cm (Wilcoxon test, $p < 0.05$; Table 2).

Analysis by linear regression of the data on oestrogen receptor and serum CEA concentrations showed that a significant correlation between these features was present for only the period one month to 18 months after operation (data not shown). Table 2 shows that only patients positive for oestrogen receptor (12 of the 40 (30%)) reach serum

Fig. 1 Histogram of highest serum CEA values in patients with and without recurrence.

Fig. 2 Diagram of patients with recurrence and increased serum CEA values. Only 11 patients with serum CEA values >10 ng/ml are shown. Four patients also had increased serum CEA values between 5–10 ng/ml.

Table 3 Recurrence related to highest serum carcinoembryonic concentrations (serum CEA) and whether absence or presence of lymph node metastases, tumour size, and oestrogen receptor contents were positive or negative

	Serum CEA <5 ng/ml		Serum CEA ≥5 ng/ml		Total
	Negative	Positive	Negative	Positive	
	Lymph node*				
Recurrence	0	4	1	10	15
No recurrence	26	23	2	4	55
Total	26	27	3	14	70
	Tumour size††				
Recurrence	0	4	1	11	16
No recurrence	26	23	0	8	57
Total	26	27	1	19	73
	Oestrogen receptor content§				
Recurrence	3	0	0	6	9
No recurrence	8	28	0	6	42
Total	11	28	0	12	51

* $\chi^2 = 28.8$, $v = 3$, $p < 0.001$.

†Tumour size: negative <2 cm, positive ≥2 cm.

‡ $\chi^2 = 26.0$, $v = 3$, $p < 0.001$.

§Oestrogen receptor content: negative <9 fmol/ng, positive ≥11 fmol/ng.

|| $\chi^2 = 15.3$, $v = 2$, $p < 0.005$.

CEA positivity (+/++) during the follow up period.

No significant correlations were found between the serum CEA values and the age of the patient, the histological grade, the nuclear grade the histological type or the morphometric features of the primary carcinoma.

CEA AND RECURRENCE

Analysis of the clinical data showed a clear correlation between an increase in serum CEA concentrations and the recurrence of the disease (locally or with distant metastases, or both) (Wilcoxon: $p < 0.001$, Fig. 1). During the follow up period recurrences were diagnosed clinically in 19 patients (24%) (three patients had local recurrence). Fifteen of these (79%) had serum CEA values >5 ng/ml (Fig. 1).

Analysis of the 15 patients with serum CEA concentrations >5 ng/ml showed that seven had shown an increase in concentration more than one month before the clinical diagnosis of the recurrence. In four patients it was not completely clear from the clinical data whether the increase in concentration had preceded or succeeded the clinical diagnosis. In four other patients the increase occurred more than one month after the clinical diagnosis of the recurrence (Fig. 2).

CEA AND ASSOCIATED FEATURES RELATED TO RECURRENCE

To find a group of patients who might benefit from serial measurements of serum CEA several analyses were performed, using tumour recurrence as a dependent variable. The highest serum CEA values were combined with one of the three features that proved to be significantly correlated with the serum

CEA values—namely, size of tumour, oestrogen receptor contents, and presence or absence of lymph node metastases. Table 3 shows the data on these combinations. In the analysis (χ^2) highest CEA values of more than 5 ng/ml (CEA-H +) were compared with those 5 ≤ ng/ml (CEA-H -). The combination of (- v +) when related to highest CEA value and presence or absence of lymph node metastases was significant ($\chi^2 = 28.8$ $v = 3$, $p < 0.001$, Table 3). Ten of 14 patients (71%) who were CEA-H positive and lymph node positive developed distant metastases whereas none of the 26 patients who were CEA-H negative/lymph node negative did. Thus CEA-H positive/lymph node positive patients had significantly more recurrences than those who were negative. The combination of CEA-H and size of tumour was significant ($\chi^2 = 26.0$, $v = 3$, $p < 0.001$; Table 3) when related to tumour recurrence. None of the 26 CEA-H negative patients with a tumour <2 cm had recurrence. Eleven out of 19 (58%) of the CEA-H positive group with a tumour ≥2 cm developed recurrent disease.

Table 3 shows the significant correlation between the development of recurrences and the combination of CEA-H level and oestrogen receptor state ($\chi^2 = 15.3$, $v = 2$, $p < 0.001$). Although this correlation when compared with the above correlations was not that significant, it was an interesting factor, for six patients of 12 who were CEA-H positive/oestrogen receptor positive had tumour recurrence.

Discussion

A significant correlation was found between the highest serum CEA concentrations and recurrence of the disease (Fig. 1). This correlation has also been

found in other studies.^{3 6 9 12 13} In our study 15 of 19 (79%) patients with serum CEA values >5 ng/ml developed recurrences. This percentage is somewhat higher than that described in published reports (range 30–71%). This is obviously because most of the studies took 10 ng/ml as the cut off point. If this threshold had been adopted in the present study the sensitivity would have fallen to 57% (11/19), which is more in line with other studies. With 5 ng/ml as a threshold 23 patients had concentrations above that value more than two months after the operation and 13 had concentrations above 10 ng/ml. Therefore, 13 patients had values between 5 and 10 ng/ml. Of these, four developed distant metastasis (and no local recurrence). This high percentage clearly supports the choice of 5 instead of 10 ng/ml as the threshold.

Of the four patients with metastases and without a serum CEA value >5 ng/ml, three had local recurrence. This is in agreement with the findings of Haagensen *et al*⁷ and Lee.⁹ Apparently, local metastases can be easily missed when monitoring serum CEA for recurrence, but physical examination should easily detect these recurrences.

Eight patients had increased serum CEA concentrations without confirmed metastatic disease (Table 1). Two of these had values above 10 ng/ml. One patient developed carcinoma in the contralateral breast. This was not regarded as a metastasis, although the tumour was detected by an increase in serum CEA. The other patient had a large (>6 cm) breast carcinoma (without lymph node metastases) at presentation. The high serum CEA value decreased and became normal within two months after the operation, and no distant metastases were found during the follow up period. In four of the patients with serum CEA values >5 ng/ml but ≤10 ng/ml the values also decreased below 2.5 ng/ml in the first three postoperative months.

This finding may have been due to the fact that it takes time for CEA to be degraded.¹⁵ Our results support the theory that estimations of serum CEA should be started two or three months after operation to remove primary breast carcinoma and that 5 ng/ml is a reasonable threshold value. In this study only two of 65 patients (31%) gave false positive results. This is lower than the value found by Lee, who found falsely increased serum CEA concentrations in his patients.⁹

Meyers *et al* found a correlation between serum CEA values established within three months after operation and the presence of lymph node metastases.¹¹ In that study a quarter of the patients with spread to lymph nodes were also positive for serum CEA compared with 13.9% of the patients without such spread. Values ≥4 ng/ml were regarded as

positive. In our study this difference was also found between patients with positive lymph nodes and those with negative lymph nodes, and the preoperative serum CEA values (19.5% compared with 3.4%; Table 2). In addition, Meyers and other workers found correlations between larger primary tumours and higher serum CEA values.^{4 11 13} In our study this correlation was found, by linear regression analysis, in the serum CEA values established preoperatively (Table 2).

The significant correlations found between the serum CEA values established in a short period (three months) after the operation and the size of tumour and presence of lymph node metastases are, in our opinion, due to tumour load. Therefore, no conclusions can be drawn from these results about monitoring patients with breast cancer for recurrence. During the follow up period, however, larger primary tumours with spread to lymph nodes had significantly higher serum CEA values than their smaller, lymph node negative counterparts (Table 2). This is in keeping with the belief that patients with larger primary tumours and spread to lymph nodes are more likely to develop metastases.

In this study not one patient with an oestrogen receptor negative tumour had a serum CEA value >5 ng/ml. This is strange because oestrogen receptor negative tumours tend to have a worse prognosis. Although the number of patients was small ($n = 51$) and further investigation is required, Table 3 shows that all the patients with a recurrence were either oestrogen receptor negative and CEA negative or oestrogen receptor positive and CEA positive. By monitoring serum CEA in patients positive for oestrogen receptor recurrence can be detected in this group of patients with an apparently better prognosis.

Our results show that the appearance of distant metastases in patients with primary breast cancer can be monitored in the first two years after operation by serial estimations of serum CEA only in patients with tumours ≥2 cm that are positive for oestrogen receptor who have spread to the lymph nodes.

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