

Diagnostic usefulness of serum carcinoembryonic antigen determinations in breast cancer patients

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Abstract

Background and purpose: Carcinoembryonic antigen (CEA) is used as a tumour marker in breast cancer (BC). In order to assess diagnostic value of CEA in BC we examined its serum levels and frequencies of its increase in breast cancer patients (BCP), and compared them to those in controls. We also determined CEA in patients with metastatic and non-metastatic BC, and calculated sensitivity and specificity of CEA in BC. **Patients and methods:** The main experimental group consisted of 47 female patients with histologically proved diagnosis of BC. There were two control groups: clinically healthy women, and female patients with other locations of cancer. Circulating levels of CEA were measured by means of immunoradiometric assay. Results were processed by means of t-test and two-way analysis of variance. **Results:** Circulating levels of CEA, before treatment in BCP, were significantly higher ($p < 0.0001$) than in healthy women, and in patients with other cancers ($p < 0.007$), while serum CEA in other cancer patients was significantly higher ($p < 0.01$) than in healthy control. There was a difference between frequencies of CEA increase in BCP and healthy women, while such a difference did not exist between BCP and other cancer patients. The circulating levels of CEA in metastatic BCP were significantly higher ($p < 0.03$) in comparison to non-metastatic patients. Sensitivity and specificity of CEA in

BCP was 65.0%, and 57.1%, respectively. **Conclusions:** CEA does not have high tumour specificity for BC, since its circulating levels as well as frequencies of its increase may be elevated in patients with other types and locations of cancer, different from breast cancer. CEA can be detected in the serum of majority of patients with metastatic BC. CEA may be used as prognostic tumour marker in advanced BC.

Key words: carcinoembryonic antigen, sensitivity of CEA, specificity of CEA, localised breast cancer, metastatic breast cancer.

Introduction

Tumour markers are substances that can be detected in higher than normal amounts in the blood, urine, or body tissues of some people with certain types of cancer. A tumour marker may be produced by tumour itself or to a lesser extent by the body in response to cancer presence. Measuring their circulating levels may be very useful in clinical detection (diagnosis, screening), and management (monitoring, prognosis) of cancer patients. Carcinoembryonic antigen (CEA) is currently used among others as a tumour marker for breast cancer patients. It is a special protein that is actually produced by embryonic and regenerating cells, as well as, cancer cells. This protein belongs to the family of cell-surface

Table 1 The average circulating levels of CEA before treatment in healthy women (Control group I), patients with different locations and histologic types of cancer (Control group II) and women with breast cancer.

Group / number of patients	Circulating levels of CEA (ng/mL) mean \pm S.E.	< upper referent value (21 ng/mL)	> upper referent value (21 ng/mL)	p value
		N patients / Total N patients (%)	N patients / Total N Patients (%)	
Control group I 40	10.2 \pm 0.8 ^a	40/40 (100)	0/40 (0)	< 0.0001 (c versus a)
Control group II 33	28.1 \pm 4.4 ^b	16/33 (48.5)	17/33 (51.5)	< 0.007 (c versus b)
Breast cancer patients 47	69.4 \pm 13.8 ^c	18/47 (38.3)	29/47 (61.7)	< 0.01 (b versus a)

glycoproteins and can be measured in blood, as cells shed these proteins. CEA is a circulating antigen expressed by human breast cancer cells but it is also commonly associated with colorectal cancer. Non-cancerous conditions such as stomach ulcers, colon polyps, cigarette smoking, may also cause elevations of this protein.

In order to assess diagnostic value of CEA determination in breast cancer patients we examined CEA circulating levels and frequencies of its increase in breast cancer patients and compared them to those in healthy women and in patients with cancers of different histologic origin and location. Furthermore, we determined CEA serum levels in patients with metastatic breast cancer and compared them to those in patients with localised breast cancer. We also calculated diagnostic sensitivity and specificity of CEA for both breast cancer patients and other cancer patients.

Patients and methods

Patients

There were two control groups of patients designated as I, and II. Group I consisted of forty clinically healthy women with an age interval 30-65 years from whom the following data have been collected: name, age, place of birth, place of residence, and life habits. All of them were non-smokers, non-obese, and were not under any medication including birth control. All of them were occasional coffee drinkers. All of them had normal mammograms. The circulating levels of CEA were measured in all of them at least two times during the observation period.

Group II consisted of 33 female patients having cancer of different histologic origin and location with an age interval 18-77 years. Majority of them (45.7%) had lung cancer, then gastrointestinal (24.4%), urinary bladder (6.7%), skin (6.7%), uterine (6.7%), laryngeal (3.3%), and bone cancer (3.3%), and cancer with unknown primary location (6.7%). The same data were collected as in group I. The circulating levels of CEA at the time of initial diagnosis before any treatment (signed as baseline) were measured in all of them, and later again at least two times during the observation period. According to the presence of metastases this group was further divided into two subgroups: patients without metastases, and those with metastases.

The main experimental group consisted of 47 female histologically confirmed breast cancer patients with an age interval 38-82 years. The same type of data has been taken from them as in group II. This group was further divided according to presence of metastases into two subgroups: patients with metastases, and those without metastases. The same group was also divided according to their serum CEA measured before the onset of treatment: hyperCEA with average circulating levels of CEA

of more than 21 ng/mL, and normo and hypo CEA having average circulating levels less than 21 ng/mL.

Methods

Determination of serum levels of CEA

Blood samples were drawn under sterile conditions at eight in the morning each time, centrifuged at 3000 rpm for ten minutes under room temperature and serum was stored in plastic tubes at -200C until processed. The circulating levels of CEA were determined by means of immunoradiometric assay using commercially available kits from Biomedica (Graz, Austria). The major characteristics of this method are: principle - immunoradiometric assay; separation method - coated beans; antibodies - monoclonal on solid phase; labeller - 125-I-labeled monoclonal antibodies; incubation - 2 hours on 370 C; standards - 0, 5, 10, 35 and 70 ng/mL; sensitivity - 0.25 ng/mL; specificity - no cross reactions for hemolysed or lipemic samples; normal values 2-21 ng/mL.

Estimation of range of normal values for CEA

We first estimated the range of normal values in 40 healthy female subjects. Our referral values were mean \pm 2 standard deviations. The values of normal ranges stretched between 2.5- 20.7 ng/mL. There were no age dependent significant differences in CEA concentrations. All cases with their values of CEA above 21 ng/mL were judged as hyperCEA, and all of them with their values of CEA below 2.5 ng/mL were declared as hypoCEA.

Determination of sensitivity and specificity of CEA

All individual values of CEA concentrations were judged based on and according above mentioned lower and upper values. The sensitivity and specificity were calculated according to the following formulas:

Sensitivity = true positive results / (true positive + false negative results)

Specificity = true negative results / (true negative + false positive results)

Statistical analysis of results

The results were evaluated using student's t-test with calculated standard errors and two standard deviations, and analysis of two-way variance in F-test. The nature of distribution and linearity were checked using histogram presentation, and tests of linearity including Kolmogorov-Smirnov Z (with kurtosis and skews), with probability and de-trended probability plots. These methods were done using the Statistical Package for Social Sciences (SPSS) program. The statistical significance of

results was grouped according to the following criteria: $p > 0.05$ (not significant), $p < 0.05-0.01$ (significant), $p < 0.009-0.001$ (very significant), and $p < 0.0009-0.0001$ (highly significant).

Results

The circulating levels of CEA

Table 1 shows the average circulating levels of CEA and frequency of their increase before treatment in breast cancer patients compared to two control groups (healthy women and other cancer patients).

Circulating levels of CEA before treatment in breast cancer patients were highly significantly elevated ($p < 0.0001$) than in healthy women, and very significantly elevated ($p < 0.007$) than in patients with other locations and histologic types of cancer before treatment. Serum CEA levels before treatment in other cancer patients were significantly higher ($p < 0.01$) than in healthy women. None of women from healthy control group was hyperCEA, but 17 (51.5%) from 33 other cancer patients, and 29 (61.7%) from 47 breast cancer patients had hyperCEA levels. There was a difference between frequencies of CEA increase in breast cancer patients and in healthy women while such a difference did not exist between breast cancer and other cancer patients.

The circulating levels of CEA in localised and in metastatic breast cancer

Metastases were not detected in seven (14.8%), and were detected in forty (85.2%) of breast cancer patients during the five-year follow up period (table 2). Elevated levels of CEA were detected in the majority (72.5%) of breast cancer patients with metastases. The average circulating levels of CEA in metastatic breast cancer patients were significantly higher ($p < 0.03$) in comparison with non-metastatic patients (table 2), while in patients with other types and locations of cancer such a difference did not

show up. The sensitivity / specificity for CEA attest to these results.

Table 2 The average serum levels of CEA before treatment in patients with localised and advanced breast cancer.

Group / number of patients	Serum level of CEA (ng/mL) mean \pm S.E.
Breast cancer patients without metastases 7	24.9 \pm 12.5 ^a
Breast cancer patients with metastases 40	66.4 \pm 2.8 ^b
Other cancer patients without metastases 10	21.9 \pm 8.7 ^c
Other cancer patients with metastases 23	33.3 \pm 8.8 ^d

a versus b $p < 0.03$

c versus d $p < 0.36$

Specificity and sensitivity for CEA determination in breast cancer patients

Table 3 shows the results of calculated sensitivity/specificity for CEA in breast cancer patients. Sensitivity for CEA in metastatic breast cancer was 65.0%, and specificity was 57.1%.

Specificity and sensitivity for CEA determination in other cancer patients

Table 4 shows the results of calculated sensitivity and specificity for CEA in patients with other types and locations of cancer. Sensitivity for CEA in this group of cancer patients was 60.9%, and specificity was 70.0%.

Table 3 The sensitivity and specificity of CEA determinations in breast cancer patients.

Group of patients	Number of patients with hyperCEA	Number of patients with normo and hypoCEA	Sensitivity (%)	Specificity (%)
Number of patients with metastases	TP (26)	FN (14)	$TP / (TP + FN) \times 100$	$TN / (TN + FP) \times 100$
Number of patients without metastases	FP (3)	TN (4)	$26 / 40 \times 100 = 65$	$4 / 7 \times 100 = 57.1$

TP= True positives, number of patients with metastases correctly classified by the test.

FP= False positives, number of patients without metastases miss-classified by the test.

FN= False negatives, number of patients with metastases miss-classified by the test.

TN= True negatives, number of patients without metastases correctly classified by the test.

Discussion

In spite the fact that there was a significant difference ($p < 0.007$) between the baseline circulating levels of CEA before treatment in breast cancer patients and in patients having cancers of other types and locations, there was not a significant difference when the frequencies of its elevated levels were compared between these two groups of patients. Furthermore majority of patients with cancers of other types and locations (51.5%) had elevated CEA in their blood (table 1 and 4). These findings can be explained by the fact that the most of patients with other cancers had colorectal, gastric or lung cancer. It is well known that CEA is commonly associated with these types of cancer, and, therefore, its high levels does not necessarily indicate that a woman has breast cancer. These data may indicate that CEA doesn't have high tumour specificity for breast cancer, which makes its diagnostic usefulness less valuable.

Diagnostic usefulness of tumour marker CEA was also assessed on the basis of sensitivity and specificity of its determination in breast cancer patients. Sensitivity or true-positive rate was in fact the frequency of CEA elevated levels in breast cancer patients with metastases, and it was 65% (table 3), while specificity or true-negative rate was the frequency of normal CEA levels in non-metastatic breast cancer patients (e.g. possibility for false positive results in all unexpected cases of high circulating levels of CEA) and it was 57.1% (Table 3). Sensitivity and specificity of CEA in control group of patients with other types and locations of cancer were not significantly different in relation to breast cancer patients. These results together with those before about the baseline CEA levels in breast cancer patients indicate that CEA doesn't have high tumour specificity for breast cancer. This in fact means that blood levels of CEA may

be elevated in patients with other types and primary locations of malignant tumours different from breast cancer. Our findings about sensitivity of CEA for metastatic breast cancer are in accordance with other results published before (1, 2, 3, 4, 5, 6, 7).

The circulating levels of CEA in breast cancer patients with metastases were significantly higher than in those without metastases (table 2), while such a difference did not exist in the

control group of patients with other types and locations of cancer. This difference points towards the diagnostic importance of CEA in detection of advanced breast cancer, and in monitoring the results of treatment. Breast cancer patients especially those with metastases had significantly higher serum CEA levels as compared to the controls and those with localised disease, irrespective to the site of metastases (8). The other studies have also shown that circulating CEA tend to be elevated in women with advanced breast cancer (9,10, 11). CEA can be detected in serum of majority of patients with metastatic breast cancer. Since increasing serum levels were shown to be associated with clinical manifestation and progression of metastases, CEA may be used as a marker in metastatic breast cancer. Measurements of circulating CEA may be adequate and useful tool for the follow up and early diagnosis of metastases in breast cancer patients (9, 12). These findings also support the opinion that CEA is a tumour antigen of less differentiated cells. The cells of the metastatic cancer are usually poorly differentiated and size of both primary and metastatic tumour is mainly dependent on the proliferation fraction inside the tumour. Probably the number of metastatic deposits consisting of de-differentiated cells plays here a major role.

Table 3 The sensitivity and specificity of CEA determinations in breast cancer patients.

Group of patients	Number of patients with hyperCEA	Number of patients with normo and hypoCEA	Sensitivity (%)	Specificity (%)
Number of patients with metastases	TP (14)	FN (9)	$TP/TP+FN \times 100$	$TN/TN+FP \times 100$
Number of patients without metastases	FP (3)	TN (7)	$14/23 \times 100 = 60.9$	$7/10 \times 100 = 70.0$

TP, FP, FN, and TN are explained in the legend of Table 3.

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