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Relationship Between Tumor Markers CEA and CA 15-3, TNM Staging, Estrogen Receptor Rate and MIB-1 Index in Patients with pT1-2 Breast Cancer*

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Abstract. *Background: The aim of this study was to analyze whether a correlation exists between preoperative serum tumor markers (STM) CEA and CA 15-3, age of the patients, TNM staging, hormone receptor (ER, PgR) status, and MIB-1 proliferation index in patients who underwent surgery for primary breast cancer (BC). Patients and Methods: Data regarding a series of 255 consecutive women (median age 60 years, range 30-85) with pT1-2 BC were reviewed, while patients with confirmed pT3-4 BC were excluded. All patients underwent preoperative CEA and CA 15-3 serum levels measurement, and the removed tissue was routinely processed for the detection of ER, PgR, and MIB1 index. Results: Serum CEA and CA 15-3 measurements were above the cut-off in 44 (17.2%) and 75 (29.0%) patients, respectively, and the overall sensitivity of STM was 37.6%. A strong correlation between ER and PgR rate (R=0.77) was found. There was no relationship (p=NS) between age of the patients, size (R=0.08), MIB-1 index (R=0.11), and both ER (R=0.01) and PgR (R=0.03) rate. No linear correlation was found between both CEA and CA15-3 and the other variables, except for CA 15-3 vs. tumor size, which showed a mild (R=0.57) linear relationship. Tumor size, ER rate, and the number of positive nodes were significantly (p<0.01) different between patients with CA 15-3 values normal and above the cut-off. Comparing the subgroup of patients with CA 15-3 above the cut-off and CEA within normal values (Group 1) versus patients with CA 15-3 within normal values and CEA above the cut-off (Group 2), a significant difference was found in the tumor size (Group 1: 28.3 ± 9 mm; Group 2: 17.9 ± 7.5 mm; p<0.0001) and in the number of positive nodes (Group 1: 2.2 ± 3.3; Group 2: 0.5 ± 1.5; p<0.01). Finally, CA 15-3, but not CEA, showed a significant correlation with the tumor grading (p<0.0001). Conclusion: In patients with BC, STM correlate exclusively with the size of the tumor. Both have low sensitivity and no significant relationship with other prognostic factors. Thus, preoperative serum tumor markers measurements are of little value, especially in patients with early-stage BC, and are not useful in the therapeutic decision-making of patients with BC.*

Several serum tumor markers (STM) have been proposed to indicate the presence and future behavior of breast cancer (BC). Moreover, tumor marker measurement can be used to help make treatment decisions, especially in patients without axillary node involvement (1). Unfortunately, the sensitivity of STM is usually considered low, especially in patients with early-stage tumors, and subsequently their clinical usefulness is still controversial (2, 3). The aim of this study was to analyze whether a correlation exists between preoperative STM CEA and CA 15-3, age of the patients, TNM staging, hormone receptor (ER, PgR) status and MIB-1 proliferation index in patients who underwent surgery for primary BC.

Patients and Methods

Data regarding a series of 255 consecutive women (median age 60 years, range 30-85) with pT1-2 BC were reviewed, whilst patients with confirmed pT3-4 BC were excluded. There were 71 (27.8%) premenopausal and 184 (72.2%) postmenopausal women. Conditions for entry in the study were: (1) no other or previous

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Table I. *Parameters considered in the overall population, differences between the two groups and relative p value.*

Parameters	Overall	Group A (N1)	Group B (N0)	*P*
No. of patients	255 (100%)	70 (27.5%)	185 (72.5%)	-
Age (years)	60.6 ± 13.2	56.5 ± 12.1	62.2 ± 13.3	< 0.01
Size (mm)	19.8 ± 9.5	23.9 ± 9.0	18.2 ± 9.3	< 0.01
PT1a	9 (3.5%)	0	9 (4.9%)	-
PT1b	38 (14.9%)	1 (1.5%)	37 (20.0%)	< 0.01*
PT1c	107 (42.0%)	30 (42.8%)	77 (41.6%)	0.98*
PT2	101 (39.6%)	39 (55.7%)	62 (33.5%)	0.05*
G1	67 (26.3%	15 (21.4%)	52 (28.1%)	0.49*
G2	122 (47.8%)	32 (45.7%)	90 (48.6%)	0.90*
G3	66 (25.9%)	23 (32.9%)	43 (23.3%)	0.30*
No. of removed nodes	15 ± 5	16 ± 4.6	15 ± 5.1	0.15
No. of positive nodes	4.5 ± 3.6	4.5 ± 3.6	0	-
CEA (ng/mL)	3.7 ± 3.4	4.3 ± 4.8	3.4 ± 2.7	0.06
CA 15-3 (U/mL)	20.6 ±15.9	26.9 ± 16.3	18.2 ± 15.1	< 0.01
CEA>10 ng/mL	44 (17.2%)	16 (22.9%)	28 (15.1%)	0.30*
CA 15-3>30 U/mL	75 (29.4%)	33 (47.1%)	42 (22.7%)	0.01*
CEA>10 ng/mL and/or CA 15-3>30 U/mL	96 (37.6%)	37 (52.9%)	59 (31.9%)	0.06*
ER	59.9 ± 33.0	51.3 ± 37.7	60.4 ±30.6	0.048
PgR	52.4 ± 32.3	52.1 ± 35.2	52.3 ± 31.1	0.96
MIB-1	21.2 ± 23.2	30.1 ± 26.3	17.6 ± 21.0	< 0.01

* χ^2 test

Table II. *Differences between patients with CEA and CA 15-3 serum levels within normal values and above the cut-off.*

CEA	Normal	>10 ng/mL	*P*
No. of patients	211	44	-
Age (years)	60±13	63.5±13.6	0.11
Size (mm)	19.1±9.3	23.1±10	0.009
ER (%)	60.2±32	46.6±35.5	0.013
PgR (%)	53.9±31.9	44.9±33.4	0.094
MIB-1 (%)	21.2±23.3	21.5±22.8	0.922
No. of positive nodes	1.03±2.4	2.2±4	0.013
CA 15-3	**Normal**	**>30 U/mL**	***P***
No. of patients	180	75	-
Age (years)	60.9±13.3	60±13	0.60
Size (mm)	16.3±7.2	28.2±9.2	< 0.001
ER (%)	62.8±30.6	46.2±35.5	0.002
PgR (%)	55.5±31.2	45±33.7	0.02
MIB-1 (%)	19.2±22.7	25.9±23.7	0.04
No. of positive nodes	0.64±1.8	2.6±3.9	< 0.001

cancer, (2) no evidence of distant metastases or multicentric BC at the moment of the diagnosis of cancer (M0), and (3) no presence of multifocal BC at final pathology.

No patients had undergone preoperative chemotherapy. All women underwent curative surgery followed by radiation therapy and/or chemotherapy, according to the tumor staging and the age of the patients. Breast-conserving surgery was performed in 156 (61.2%) women, whilst 99 (38.8%) underwent modified radical mastectomy. According to the American Joint Committee on Cancer (AJCC), tumor size (pT) was defined as the maximum diameter measured by the pathologist and the lymph nodes involvement (pN1) was histologically confirmed (4). Prior to surgery, the presence of distant metastases was excluded by routine laboratory tests, liver ultrasound, standard chest X-ray and bone scanning.

CEA and CA 15-3 levels were determined by automated test systems using a two-site enzyme-linked immunosorbent assay (ELISA, monoclonal antibody). A cut-off limit of 10 ng/mL (CEA) and 30 U/mL (CA 15-3) was taken as recommended by the manufacturer, as previously described (5, 6). Estrogen (ER) and progesterone (PgR) receptors were assayed using a quantitative standard immunoenzymatic method, and results were expressed as percentage of positivity in the overall cell population. Immunostaining of Ki-67 antigen was performed using the monoclonal antibody MIB-1 by a microwave antigen retrieval technique, and the MIB-1 labelling index was expressed in percentage (6). The histological grade was defined according to the Scarff-Bloom-Richardson classification.

Two groups of patients were considered according to the axillary lymph node status: *Group A*, 70 (27.5%) cases (pN1), and *Group B*, 185 (72.5%) cases (pN0). All patients underwent preoperative CEA and CA 15-3 serum levels measurement, and the removed tissue was routinely processed for the detection of ER, PgR and the MIB-1 index.

The reported data are expressed as mean ± standard deviation (SD). Differences between means (*i.e.*, age, tumor size) were tested by unpaired Student's *t*-test or, in the case of non-normal distribution (*i.e.*, TNM staging, nuclear grading), the Mann-Whitney *U*-test. The Chi-square (χ^2) test and the Pearson's correlation coefficient (R) calculation were used for comparison of qualitative variables, and to evaluate the linear relationship between pairs of quantitative variables, respectively. A value of $p<0.01$ was considered to be statistically significant. The Bonferroni's correction was used when appropriate.

Results

The size of the tumor ranged between 3 and 48 mm (median 19 mm). Table I presents the parameters considered in the overall population and a comparison between the two Groups. Serum CEA and CA 15-3 were above the cut-off in 44 (17.2%) and 75 (29.0%) patients, respectively, and the overall sensitivity of CEA and CA 15-3 together was 37.6%. A strong correlation between ER and PgR rate (R=0.77) was found. There was no significant relationship between age of the patients, size (R=0.08), MIB-1 index (R=0.11), and both ER (R=0.01) and PgR (R=0.03) rate. No linear correlation was found between both CEA and CA15-3 and the other variables, except for CA 15-3 *vs.* tumor size, which showed a mild (R=0.57) linear relationship (Figure 1). Moreover, no linear correlation was found between the values of CA15-3

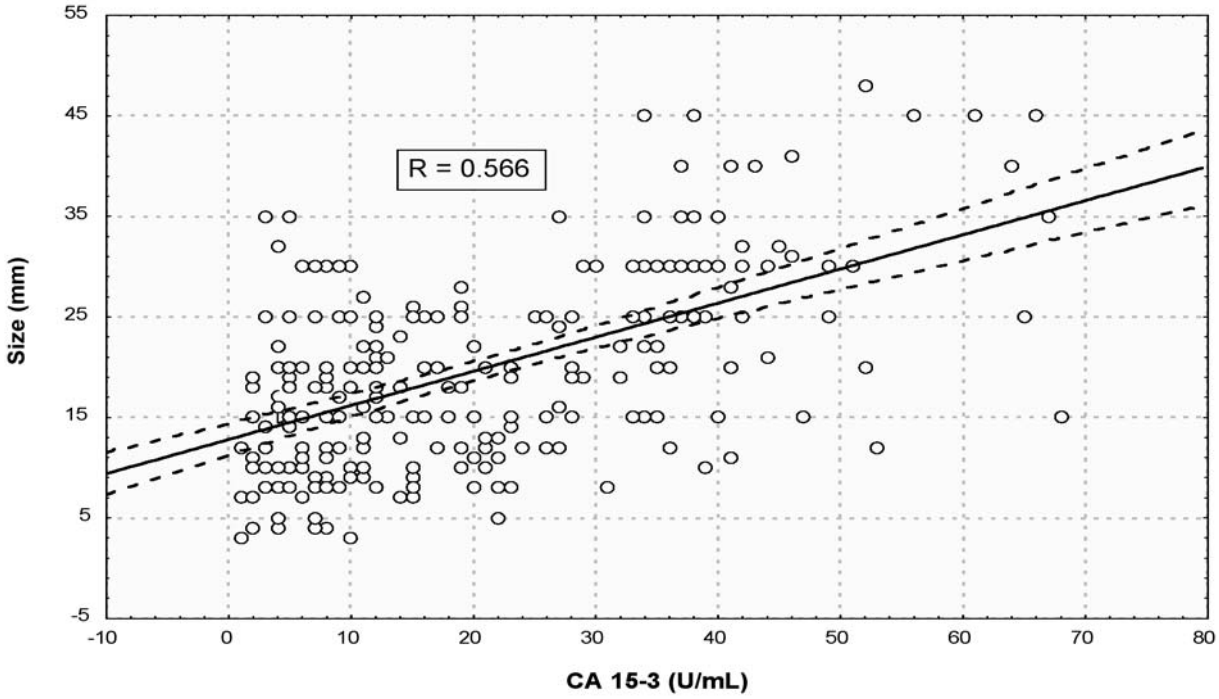

Figure 1. *Relationship between CA 15-3 serum levels and size of the tumor in the overall population.*

and CEA (R=0.29). Patients with negative nodes (Group B) were older ($p<0.01$) than N1 patients (Group A), whilst CA 15-3 serum levels, the MIB-1 proliferation index and tumor size were significantly ($p<0.01$) higher in Group A patients.

Dichotomized CEA (normal *vs.* > 10 ng/mL) and CA 15-3 (normal *vs.* >30 U/mL) were studied with respect to the other variables (Table II). Tumor size, ER rate and the number of positive nodes were significantly ($p<0.01$) different between patients with CA 15-3 values normal and above the cut-off. A concordance between CEA and CA15-3 was found in 182 out of 255 patients (71.4%). Comparing the subgroup of patients with CA 15-3 above the cut-off and CEA within normal values (Group 1) versus patients with CA 15-3 within normal values and CEA above the cut-off (Group 2), a significant difference was found in the tumor size (Group 1: 28.3 ± 9 mm; Group 2: 17.9 ± 7.5 mm; $p<0.0001$) and in the number of positive nodes (Group 1: 2.2 ± 3.3; Group 2: 0.5 ± 1.5; $p<0.01$). Finally, CA 15-3, but not CEA, showed a significant correlation with the tumor grading (Mann Whitey *U*-test: $p<0.0001$).

Discussion

CEA and CA 15-3 are the best investigated tumor markers in BC patients, however their sensitivity and specificity are low (7). Data reviewed by the American Society of Clinical Oncology (ASCO) in 1996 showed elevated CEA and CA 15-3 levels in 10-64% and 9-75% of the patients with primary BC, respectively, and studies of the prognostic value of STM are still controversial (5, 8, 9). BC is a progressive disease and small tumors are more likely to have a better prognosis. However, all patients may develop progression or recurrence of the disease, and therefore an effective prognostic evaluation is needed (6, 9). Furthermore, the size of tumor and biological factors, which reflect BC's aggressiveness, determine both the therapeutic approach and survival of patients with BC (10). In node-negative BC, where tumor size, hormone receptor status and proliferation rate are used to design an adequate adjuvant therapy, this is particularly evident (11). The proliferation rate of BC, measured by the MIB-1 index, was found to correlate with risk assessment and to be effective in selecting adjuvant therapies (12, 13). A number of studies reported conflicting data, and none has clearly demonstrated either the clinical usefulness or not of STM (3). However, clinicians generally use STM in the management of patients with BC, but without common and accepted criteria for their application or interpretation.

High levels of CEA in patients with BC were related to poor prognosis in early reports (14). More recently, these results have not been confirmed, and low sensitivity in both early and advanced disease was shown, when compared with other STM (2, 5, 15). Guadagni *et al.* (16) reported elevated CEA and CA 15-3 levels in 16.7% and 33% of patients with

BC, respectively. Cartei *et al.* (17) stated the clinical insufficiency of pre-operative CEA, there being no correlation between pre-operative CEA and tumor burden. Molina *et al.* (18), in a prospective evaluation of STM on 503 patients with BC, found high levels of CEA and CA 15-3 only in 12% and 13% of patients, respectively.

The increase of STM levels is known to be related to the stage of BC. Positive values of CA 15-3 were found in 31% of patients with BC and in 9% of patients with benign diseases, but only in 21% of patients with BC (stage I-III) was CA 15-3 altered (19). The low incidence of CA 15-3 elevation in early stage BC is not significant, and its increase in the follow-up can indicate only a large tumor burden. Furthermore, one-third of patients with recurrence have normal values of CA 15-3, while up to 10% of those without relapse have a false-positive value (20). Routine STM measurements have been discouraged by the American ASCO, an expert panel of oncologists, clinical chemists and patients advocates, which establishes and updates clinical practice guidelines for the clinical use of tumor markers (21).

In conclusion, our data confirm that, in patients with BC, serum markers CEA and CA 15-3 correlate exclusively with the size of the tumor. Both have low sensitivity and no significant relationship with other prognostic factors could be found. Thus, preoperative CEA and CA 15-3 serum levels measurements are of little value, especially in patients with early-stage BC, and are not useful in the therapeutic decision-making for patients with BC.

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References

1 McGuire WL, Tandon AK, Allred DC *et al*: Prognosis and treatment decisions in patients with breast cancer without axillary node involvement. Cancer *70 (S1)*: 1775-1781, 1992.

2 Lumachi F, Brandes AA, Ermani M, Bruno G and Boccagni P: Sensitivity of serum tumor markers CEA and CA 15-3 in breast cancer recurrences and correlation with different prognostic factors. Anticancer Res *20*: 4751-4756, 2000.

3 Gion M, Barioli P, Mione R *et al*: Tumor markers in breast cancer follow-up: a potentially useful parameter still awaiting definitive assessment. Ann Oncol *6 (S2)*: S31-S35, 1995.

4 American Joint Committee on Cancer: Breast. *In*: AJCC Cancer Staging Handbook, sixth edition. New York: Springer-Verlag, 2002: 257-281.

5 Lumachi F, Brandes AA, Boccagni P *et al*: Long-term follow-up study in breast cancer patients using serum tumor markers CEA and CA 15-3. Anticancer Res *19*: 4485-4490, 1999.

6 Lumachi F, Ermani M, Brandes AA, Basso SMM, Basso U and Boccagni P: Predictive value of different prognostic factors in breast cancer recurrences: multivariate analysis using a logistic regression model. Anticancer Res *21*: 4105-4108, 2001.

7 Ebeling FC, Schmitt UM, Untch M *et al*: Tumour markers CEA and CA 15-3 as prognostic factors in breast cancer. Univariate and multivariate analysis. Anticancer Res *19*: 2545-2550, 1999.

8 ASCO Report. Recommended breast cancer surveillance guidelines. American Society of Clinical Oncology. J Clin Oncol *15*: 2149-2156, 1997.

9 Tabàr L, Duffy SW, Vitak B *et al*: The natural history of breast carcinoma: what have we learned from screening? Cancer *86*: 449-462, 1999.

10 Bundred NJ: Prognostic and predictive factors in breast cancer. Cancer Treat Rev *27*: 137-142, 2001.

11 Harbeck N, Dettmar P, Thomssen C *et al*: Prognostic impact of tumor biological factors on survival in node-negative breast cancer. Anticancer Res *18*: 2187-2197, 1998.

12 Cooper LS, Gillett CE, Smith P, Fentiman IS and Barnes DM: Cell proliferation measured by MIB1 and timing of surgery for breast cancer. Br J Cancer *77*: 1502-1507, 1998.

13 Jones S, Clark G, Koleszar S *et al*: Low proliferative rate of invasive node-negative breast cancer predicts for a favorable outcome: a prospective evaluation of patients. Clin Breast Cancer *1*: 310-314, 2001.

14 Sikorska H, Schuster J and Gold P: Clinical applications of carcinoembryonic antigen. Cancer Detect Prevent *12*: 321-355, 1988.

15 Given M, Scott M, Mc Grath JP and Given H: The predictive value of tumour markers CA 15-3 and CEA in breast cancer recurrence. Breast *9*: 277-280, 2000.

16 Guadagni F, Ferroni P, Carlini S *et al*: A re-evaluation of carcinoembryonic antigen (CEA) as a serum marker for breast cancer: a prospective longitudinal study. Clin Cancer Res *7*: 2357-2362, 2001.

17 Cartei G, Cartei F, Interlandi G *et al*: Preoperative circulating carcinoembryonal antigen in primary breast cancer: review of the literature and personal experience on 150 cases. Breast *5*: 37-40, 1996.

18 Molina R, Filella X, Zanon G *et al*: Prospective evaluation of tumor markers (c-erbB-2 oncoprotein, CEA and CA 15.3) in patients with locoregional breast cancer. Anticancer Res *23*: 1043-1050, 2003.

19 Safi F, Kohler I, Röttinger E and Beger HG: The value of the tumor marker CA 15-3 in diagnosing and monitoring breast cancer. A comparative study with carcinoembryonic antigen. Cancer *3*: 574-582, 1991.

20 ASCO Expert Panel: Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer: report of the American Society of Clinical Oncology expert panel. J Clin Oncol *14*: 2843-2877, 1996.

21 ASCO Expert Panel: 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol *19*: 1865-1878, 2001.

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