3. Zinser JW, Hortobagyi GN, Buzdar AU, Smith TL, Fraschini G. Clinical course of breast cancer patients with liver metastases. *J Clin Oncol* 1987, **5**, 773–782.
4. Australian and New Zealand Breast Cancer Trials Group. A randomised trial in post-menopausal patients with advanced breast cancer comparing endocrine and cytotoxic therapy given sequentially or in combination. *J Clin Oncol* 1986, **4**, 186.
5. Bagley, CM, Bostick FW, De Vita VT. Clinical pharmacology of cyclophosphamide. *Cancer Res* 1973, **33**, 226.
6. Benjamin RS, Wiernick PH, Wesley M, Bacher NR. Adriamycin® chemotherapy, efficacy, safety and pharmologic basis of an intermittent single high dose schedule. *Cancer* 1974, **33**, 19.
7. Hayward JL, Carbone PP, Heuson J-C, Kumaoka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. *Eur J Cancer* 1977, **13**, 89.
8. Peto R, Pike MC, Armitage P *et al.* Design and analysis of clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 1977, **35**, 1–39.
9. Cox DR. Regression models and life tables. *J R Stat Soc* 1972, **34**, 187–202.
10. Elston CW *et al.* The relationship of oestradiol receptor (ER) and histological tumour differentiation with prognosis in human primary breast carcinoma. In: Mourisden HT, Palshof T, eds. *Breast Cancer: Experimental and Clinical Aspects*. Oxford, Pergamon Press, 1980, 59.
11. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer* 1987, **55**, 61–66.
12. Stewart JF, King RJB, Sexton SA *et al.* Oestrogen receptors, sites of metastatic disease and survival in recurrent breast cancer. *Eur J Cancer* 1981, **17**, 449–453.

Eur J Cancer, Vol. 26, No. 5, pp. 577–580, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

CA-15.3, TPA and MCA as Markers for Breast Cancer

M. Barak, M. Steiner, B. Finkel, J. Abrahamson, S. Antal and N. Gruener

Serum concentrations of CA-15.3, tissue polypeptide antigen (TPA) and mucinous-like carcinoma-associated antigen (MCA) were measured in 327 women: 81 controls, 93 patients with benign breast disease, 46 patients recently diagnosed with breast cancer and 107 patients during breast cancer follow-up. CA-15.3 was elevated in 16% of the controls, in 29% of the patients with benign breast disease, in 65% of the breast cancer patients and in 74% of the follow-up patients. TPA was elevated in 4%, 11%, 36% and 75%, respectively. The corresponding figures for MCA were 10%, 8%, 30% and 64%. The highest sensitivity for cancer detection (74%) was obtained with a combination of CA-15.3 and TPA, while the specificity of this panel was 75%. The negative predictive value of these combined tests was 93%. MCA scored lower values, being only 30% sensitive. The CA-15.3/TPA panel may increase sensitivity compared with single marker tests and provide additional information for clinical evaluation.
Eur J Cancer, Vol. 26, No. 5, pp. 577—580, 1990.

INTRODUCTION

CARCINOMA of the breast is the leading cause of cancer mortality among women in western countries [1]. Early detection will enable prompt treatment and reduce mortality and morbidity. However, in primary breast cancer the 'classic' tumour markers, carcinoembryonic antigen (CEA) [2] and tissue polypeptide antigen (TPA) [3], are neither sensitive nor specific enough to indicate the spread of the disease and its clinical course. Monoclonal antibodies (Mab) techniques are more sensitive and specific than previous assays. Mabs against the milk fat globule membrane (115D8, BC4N154), against enriched fractions of membranes from metastatic breast cancer (DF3), against breast carcinoma cell lines (BC4E 549, b8, b12, b15) and against a high molecular-weight glycoprotein (3×10^5) from human serum (3E 1.2) are used together in several tests. CA15-3 uses 115D8 and DF3 [4]; mucinous-like carcinoma-associated antigen (MCA) uses b12 [5]; MSA uses 3E 1.2 [6]; and CA549 uses BC4E 549 and BC4N154 [7]. With these markers in breast cancer, detection among patients with breast problems sensitivity was at best 45% and specificity 90%.

Correspondence to: M. Barak, Biochemistry Department, Lady Davis Carmel Hospital, 7 Michal Street, Haifa 34362, Israel.
M. Barak, N. Gruener are at the Department of Biochemistry, Carmel Hospital, M. Steiner is at the Northern Oncological Center and Carmel Hospital, B. Finkel, J. Abrahamson are at the Department of Surgery A, Carmel Hospital and S. Antal is at the Department of Surgery B, Carmel Hospital, Haifa, Israel.

We have assessed the individual and combined value of CA-15.3, MCA and TPA measurement in patients with benign and malignant breast diseases.

PATIENTS AND METHODS

Serum samples were collected with the approval of the Carmel Hospital Human Experimentation Committee and informed consent was received from the women. Normal samples were collected from 81 age-matched, apparently healthy women on the staff, from 131 inpatients with benign or malignant breast disease before any surgical manipulation and from 107 women undergoing breast cancer follow-up at the Northern Oncological Center. The controls had no evidence of any type of cancer or inflammatory disease, nor a family history of breast cancer. The group with benign breast disease underwent operative biopsy. The following lesions were revealed: fibrocystic disease (51), lipoma (9), fibroadenoma (16), benign papillomatosis (7), benign epitheliosis (4) and fat necrosis (6). Among the 46 patients with recently diagnosed and previously untreated breast cancer (36 ductal, 7 lobular, 3 medullar), 6 had lymph-node involvement, 7 had bone metastases, and 7 also had benign diseases of the breast.

107 patients in the follow-up group were clinically and biochemically evaluated when no therapy was being administered. The clinical evaluation of the status of each patient consisted of chest X-ray, sonography of the liver, bone scintigraphy, and blood chemistry measurement. The patients were classified as in progression, in remission, clinically stable or with no evidence of disease. Progression was defined by any increase in existing tumour or by the appearance of any new lesions. Regression was defined as a 50% or greater reduction of the clearly measurable known malignant tumour together with no increase in size or appearance of other lesions. Stable disease was defined as a decrease in tumour size of less than 50%, with no appearance of new lesions. Among these patients, 57 had no evidence of disease, 13 had stable disease, 7 were in regression and 30 were in progression.

Serum samples were stored at −80°C until analysis. We used the CA-15.3 ENZELSA kit (International CIS, France) according to the manufacturer's instructions. The prediluted sera samples were incubated for 1 h in antibody-coated tubes, washed and incubated for an additional hour with horseradish-peroxidase-labelled second antibody. After another wash, the colour development after addition of hydrogen peroxide-ortho-phenylene-diamine acid was recorded at 492 nm in an ELISA reader. Serum MCA levels were measured with the Hoffman-La Roche MCA-ELISA kit. This test is similar to the CA-15.3 kit, except that it uses Mab-coated beads. Serum TPA was assayed with the Sangtec Medical (Bromma, Sweden) TPA-IRMA kit. Diluted samples were preincubated for 4 h with antibody-coated beads, washed and incubated for 20 h with the second radioiodinated antibody. After additional washings, the tubes were counted for radioactivity.

RESULTS

CA-15.3, TPA and MCA tests had intra-assay variabilities of 7.6, 11.2 and 8.4%, respectively, and inter-assay variability was 9.1, 12.4 and 7.9%, respectively. The mean (S.D.) levels of these markers in apparently healthy females were 16.65 (5.09) ng/ml for CA-15.3, 30.38 (15.64) U/l for TPA and 10.77 (4.77) U/ml for MCA (Fig. 1). The cut-off levels of these markers corresponded to the 90th centile of the controls: 22 ng/ml for CA-15.3, 82 U/l for TPA, and 16 U/ml for MCA. Of the 81 controls, 16%, 4% and 11% had elevated CA-15.3, TPA and MCA, respectively. 4 controls with elevated CA-15.3 and MCA had no clinical nor mammographic evidence of breast cancer. Of the 93 patients with biologically proven benign breast disease, 29%, 8% and 11% had elevated CA-15.3, TPA and MCA, respectively. The mean levels were 19.2 (7.5) ng/ml for CA-15.3, 43.3 (51) U/l for TPA and 9.3 (5.1) U/ml for MCA. The mean CA-15.3 and TPA levels in these patients were slightly higher than the control means.

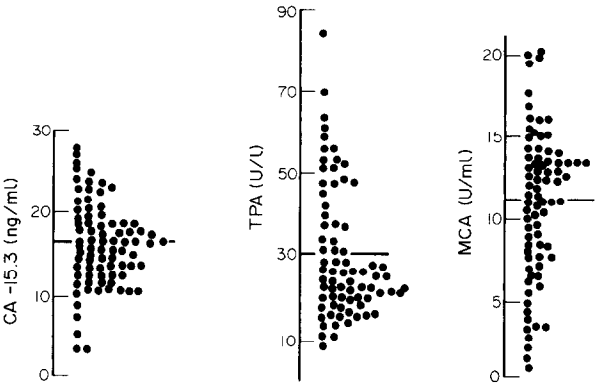

Fig. 1. Serum levels of CA-15.3, TPA and MCA in normal controls. Horizontal line = mean value.

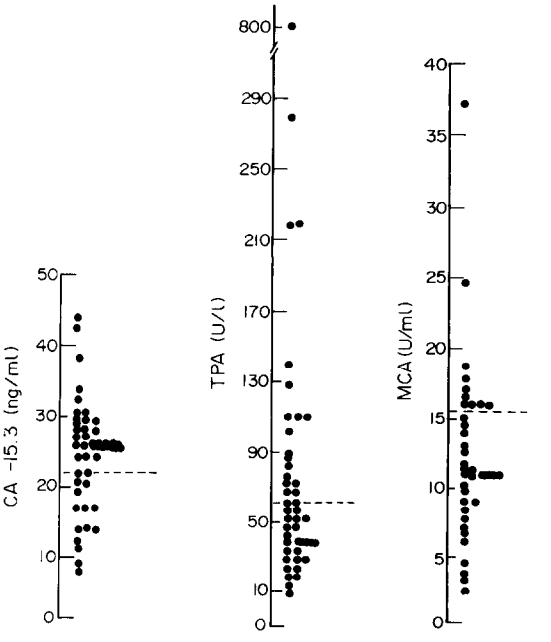

Fig. 2. Serum levels of CA-15.3, TPA and MCA in patients with newly diagnosed breast cancers. Horizontal dashed line = cut-off value.

*Table 1. CA-15.3, TPA and MCA in breast cancer**

Marker	Sensitivity	Specificity	Positive predictive value	Negative predictive value
CA-15.3	59 (59)†	79 (83)	37 (84)	90 (86)
TPA	48 (48)	94 (96)	62 (81)	90 (84)
MCA	30 (30)	90 (90)	38 (50)	86 (79)
CA-15.3 and/or TPA	74 (74)	75 (81)	39 (57)	93 (90)
CA-15.3 and/or MCA	63 (63)	74 (78)	34 (50)	90 (86)
MCA and/or TPA	56 (56)	85 (86)	43 (58)	90 (85)

*Values were calculated with data from patients newly detected as having breast cancer (n = 46) and compared with patients with benign breast diseases plus healthy controls.
†Values for malignant breast disease compared with controls.

Table 2. Mean (S.D.) CA-15.3, TPA and MCA values in breast cancer follow-up patients

Disease status	CA-15.3 (U/ml)	TPA (U/l)	MCA (U/ml)
In progression (n = 30)	57.78 (46.76)*	252.26 (360.12)*	23.11 (17.15)*
In remission (n = 7)	23.82 (12.5)	75.66 (61.42)	10.81 (7.09)
Clinically stable (n = 13)	19.43 (10.3)	52.31 (37.2)	11.08 (7.91)
No evidence of disease (n = 57)	18.72 (7.3)	49.04 (40.9)	10.02 (5.68)

*$P < 0.005$ compared with group with no evidence of disease.

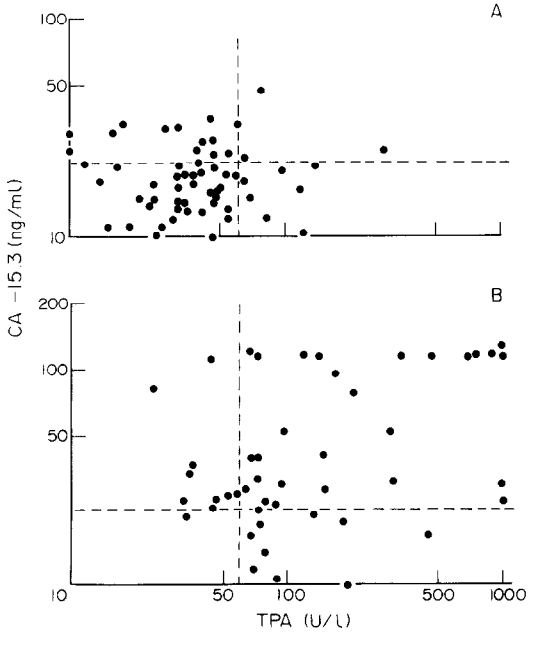

Fig. 3. CA-15.3 and TPA in follow-up group. A = patients with no evidence of disease and B = patients with progressive disease. Dashed lines = cut-off values.

Among the 46 women recently detected with breast cancer, 63%, 36% and 30% had elevated CA-15.3, TPA and MCA, respectively (Fig. 2). 74% of the patients had increased CA-15.3 and/or TPA; 63% had increased CA-15.3 and/or MCA and 56% had increased MCA and/or TPA (Table 1).

Mean values for the 107 women undergoing breast cancer follow-up are shown in Table 2. All markers tested differed significantly in those with progressive disease compared with those with no evidence of disease (Fig. 3). All but 1 patient out of 30 with progressive disease had one or both markers above the upper limits. Most of the patients with no evidence of disease had values below the upper limits, although a third (out of 57) had one increased marker and 7% had both markers increased.

DISCUSSION

The prompt detection of breast cancer or the assessment of treated breast cancer progression is critical in management. Besides the most commonly used breast tumour markers, CA-15.3 [4], MCA [5] and CA549 [7, 8], which have similar performance, another marker, MSA [1, 6, 9] has a sensitivity of 73–88% and specificity of 95%. We examined the relative merits of measuring CA-15.3, TPA and MCA levels in the detection and

monitoring of breast cancer. The cut-off values to use are controversial since sensitivity and specificity depend on the cut-off. Eskelinen *et al.* [10], Hayes *et al.* [4] and Pons-Anicet *et al.* [11] plotted receiver-operating characteristics (ROC) curves for CA-15.3. We used low cut-offs that gave good sensitivity with tolerable specificity. However, even with a lower CA-15.3 cut-off level than the one we used, the rates of detection in primary breast cancer were between 0 and 50%, increasing to 75% in advanced breast cancer [12, 13].

In our study, the false-positive rates for CA-15.3, TPA and MCA in normal subjects were 16%, 4% and 10%, respectively. The rate for CA-15.3 was not statistically different from that for MCA. Despite no clinical or mammographic findings, it will be interesting to follow up the 4 controls with elevated CA-15.3 and MCA levels. Not surprisingly, CA-15.3 has been reported as having a 20% false-positive rate in benign breast diseases and 9.9% in healthy women [4]. The clinical significance of these false-positives is not clear; they may stem from benign conditions that produce elevated CA-15.3, and may interfere with proper interpretation, as happened with prostate specific antigen [14].

Patients with benign breast diseases had mean levels of CA-15.3 and TPA that were slightly higher than those of normal controls, but CA-15.3 again showed a high false-positive rate: CA-15.3 was elevated in 29% of the patients with benign breast diseases (compared with 8% for TPA and 11% for MCA).

Our study demonstrated that CA-15.3 was the most sensitive marker correlating with 60% of the cases diagnosed as breast cancers (while only 20% of those patients had lymph node and/or bone metastases). Correlations were lower for TPA (48%) and for MCA (30%). Bombardieri *et al.* [15] showed a 26% sensitivity for MCA, even when its upper limit was 11 U/ml. Bieglmayer *et al.* [5] found MCA and CA-15.3 to be well correlated. However, significant differences in the pattern of these two markers suggest that the antigens measured by both assays are not identical. In our study, only 1 patient with recently diagnosed breast cancer had an elevated level of MCA with a normal level of CA-15.3. However, the greater specificity and the higher positive predictive value of MCA compared with CA-15.3 (as seen by us and others [5, 16]) has to be stressed and is of interest for the diagnostic use of this tumour marker. Surprisingly, TPA, a risk factor rather than a real tumour marker [17], had a good specificity. The use of TPA together with either CA-15.3 or MCA increased the sensitivity of both markers. The sensitivity of TPA is lower than that of CA-15.3 but higher than that of CEA [17], since TPA seems to be a proliferative index (indicating the metabolic state of the tumour) rather than CEA, which seems to correlate with tumour size. Moreover, the combined sensitivity of CA-15.3 and TPA increased to 74%, while combining MCA and TPA resulted in a sensitivity lower than that of CA-15.3 alone. The three marker panel had the same sensitivity as CA-15.3 and TPA (data not shown). Eskelinen *et al.* [10] found that the combination MCA/CA-15.3/CEA gave a sensitivity of 63%. The main problem with CA-15.3/TPA stems from their low combined specificity (also found by Eskelinen *et al.* [10] of 74%, while their individual specificity was 80% for CA-15.3 and 94% for TPA. However, the predictive negative value of both low CA-15.3 and/or TPA was 93%, which can be considered even for screening purposes. In the follow-up group, both elevated CA-15.3 and/or TPA detect the progressive disease, but the markers did not correlate so well in patients with no evidence of disease. These results can be explained by a shorter lead time to disease appearance with the markers compared with clinical, roentgenological, mammographic or other biochemical findings.

The combined use of tumour markers in breast cancer diagnosis may have clinical value, although so far such tests have too low a specificity to be of practical value in screening. However, the markers are useful in cancer follow-up.

1. Tjandra JJ, McLaughlin PJ, Russell IS, Collins JP, McKenzie IFC. Comparison of MSA with beta$_2$-microglobulin and CEA assays in patients with breast cancer. *Eur J Cancer Clin Oncol* 1988, **24**, 1633–1640.
2. De Jong-Bakker M, Hart AAM, Persijn JP, Cleton FJ. Prognostic significance of CEA in breast cancer: a statistical study. *Eur J Cancer* 1981, **17**, 1307–1313.
3. Bates SE, Longo DL. Tumor markers: value and limitations in the management of cancer patients. *Cancer Treat Rev* 1985, **12**, 163–207.
4. Hayes DF, Zurawski VR, Kufe DW. Comparison of circulating CA-15.3 and carcinoembryonic antigen levels in patients with breast cancer. *J Clin Oncol* 1986, **4**, 1542–1550.
5. Bieglmayer C, Szepesi T, Neunteufel W. Follow-up of metastatic breast cancer patients with a mucin-like carcinoma-associated antigen: comparison to CA 15.3 and carcinoembryonic antigen. *Cancer Lett* 1988, **42**, 199–205.
6. Sacks NPM, Stacker JA, Thompson CH *et al.* Comparison of mammary serum antigen (MSA) and CA-15.3 levels in serum of patients with breast cancer. *Br J Cancer* 1987, **56**, 820–825.
7. Chan DW, Beveridge RA, Bruzek DJ *et al.* Monitoring breast cancer with CA 549. *Clin Chem* 1988, **34**, 2000–2004.
8. Bray KR, Gaur PK. Correlation of serum levels of CA-549 to disease status in post-treatment serial samples from breast cancer patients. *J Clin Lab Anal* 1988, **2**, 134–137.
9. Tjandra JJ, Russell IS, Collins JP, Stacker SA, McKenzie IFC. Application of mammary serum antigen assay in the management of breast cancer: a preliminary report. *Br J Surg* 1988, **75**, 811–817.
10. Eskelinen M, Tikanoja S, Collan Y. Use of tumor markers CA-15.3, MCA and CEA in breast cancer diagnostics. *J Tumor Marker Oncol* 1989, **34**, 39–44.
11. Pons-Anicet DMF, Krebbs BP, Mira R, Namer M. Value of CA-15.3 in the follow-up of breast cancer patients. *Br J Cancer* 1987, **55**, 567–569.
12. Gang Y, Adachi I, Okhura H, Yamamoto H, Mizuguchi Y, Abe K. CA-15.3 is present as a novel tumor marker in the sera of patients with breast cancer and other malignancies. *Jpn J Cancer Chemother* 1985, **12**, 2379–2386.
13. Ogawa T, Izuo M, Morita H *et al.* *Gan No Rinsho* (Japan) 1986, **32**, 27–32.
14. Barak M, Mecz Y, Lurie A, Gruner N. Evaluation of prostate-specific antigen as a marker for adenocarcinoma of the prostate. *J Lab Clin Med* 1989, **113**, 598–603.
15. Bombardieri E, Gion M, Mione R, Dittadi R, Bruscagnin G, Buraggi G. A mucinous-like carcinoma-associated antigen in the tissue and blood of patients with primary breast cancer. *Cancer* 1989, **63**, 490–495.
16. Rasoul-Rockenshaub S, Zielinski CC, Kubista E *et al.* Diagnostic value of MCA in breast cancer. *Eur J Cancer Clin Oncol* 1989, **25**, 1067–1072.
17. Nicolini A, Capri A, Di Marco G, Giuliani L, Giordani R, Palla S. A rational postoperative follow-up with CEA, TPA and urinary hydroxyproline in breast cancer patients. *Cancer* 1989, **63**, 2037–2046.