Eur J Cancer Clin Oncol, Vol. 25, No. 7, pp. 1067–1072, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

Diagnostic Value of Mucin-like Carcinoma-associated Antigen (MCA) in Breast Cancer

S. RASOUL-ROCKENSCHAUB, C.C. ZIELINSKI, E. KUBISTA, N. VAVRA, E. POSPISCHIL, A. STAFFEN, K. CZERWENKA, P. AIGINGER and J. SPONA

Departments of Medicine II, Obstetrics and Gynecology I and Surgery II, University Hospital, and Ludwig Boltzmann Institut für pränatale und experimentelle Genomanalytik, Vienna, Austria

Abstract—*The diagnostic value of mucin-like carcinoma-associated antigen (MCA) was compared to that of carcinoembryonic antigen (CEA) and/or CA* 15.3 *in patients with breast cancer. A total of* 368 *patients with breast cancer were studied, of whom* 253 *were free of metastases, whereas* 94 *had either skeletal or visceral metastases or diffuse metastatic disease. The diagnostic sensitivity of MCA proved to be comparable to that of CA* 15.3 *and superior to that of CEA in patients with metastatic breast cancer. In contrast, the specificity of MCA was superior to that of CA* 15.3. *Finally, the diagnostic sensitivity of each of the tested tumour markers, i.e. MCA, CEA and CA* 15.3, *could be improved by their combined use. We conclude that MCA, either alone or in combination with CA* 15.3 *and CEA, can improve the monitoring of disease progression in patients with metastatic breast cancer.*

INTRODUCTION

MARKERS for the growth or recurrence of tumours are a topic of continuing interest in clinical oncology [1–5]. These substances should be specific and their detection sensitive enough to discover smaller quantities of tumour than can be assessed by imaging methods [6–8]. In order to further elaborate on this problem, we have investigated the diagnostic value of the new tumour marker mucin-like carcinoma-associated antigen (MCA,* [9]) and have compared it to that of carcinoembryonic antigen (CEA, [10, 11]) and CA 15.3 [12] in patients with breast cancer with and without metastases. Our study showed that while the sensitivity of MCA was comparable to that of CA 15.3, its specificity was superior to the latter. In addition, a combination of two or more of the employed tumour markers resulted in an even more pronounced gain in diagnostic sensitivity in patients with breast cancer.

MATERIALS AND METHODS

Patients

Studies of serum concentrations of tumour markers were performed in the following groups of patients:

(a) Two hundred and fifty-three patients with a history of breast cancer who were free of tumour at the time of investigation and during a median follow-up period of 5 months (range 3–6 months). The presence of visceral or skeletal lesions was excluded by chest X-ray, sonography of the liver, by bone scintigraphy and blood chemistry. In this group of patients, serum concentrations of each of the tumour markers MCA, CA 15.3 and CEA were assessed at least three times in monthly intervals during the follow-up period starting 4 weeks after tumour removal and axillary lymph node surgery. Data were calculated as a mean of the results of tumour marker estimations. Ninety-seven patients had no lymph node involvement (N0), whereas 156 patients had stage II disease (N1), out of whom 89 patients had 1–3 and 67 patients 4–10 involved axillary lymph nodes.

(b) Ninety-four patients with metastatic breast cancer, of whom 36 had visceral metastases, 37 skeletal metastases and 21 diffuse metastatic disease. In patients with visceral metastases, tumour marker analyses were performed during 24 episodes of progressive disease and 22 episodes of stable disease, whereas patients with skeletal lesions were studied during 21 episodes of progressive disease and 22 episodes of stable disease. In patients with diffuse metastatic disease, tumour marker analysis was performed during 17 episodes of progressive disease and eight episodes of stable disease.

The localization of metastatic lesions was performed by radiographic, sonographic and scintigraphic procedures.

Accepted 1 March 1989.
Address for correspondence: Dr. Christoph C. Zielinski, 2nd Department of Medicine, University Hospital, 13 Garnisongasse, A-1090 Vienna, Austria.

*Abbreviations: MCA—mucin-like carcinoma-associated antigen; CEA—carcinoembryonic antigen; PD—progressive disease; SD—stable disease; S.E.M.—standard error of the mean; TP—true positive; FP—false positive; FN—false negative.

Twenty-six patients with progressive disease and visceral metastases were followed for change in tumour marker levels following three courses of chemotherapy consisting of cyclophosphamide, adriblastin and methotrexate.

Definition of metastatic disease

Episodes of progressive disease (PD) were defined according to previous classifications [13]. Stable disease (SD) was assumed when there was no change of metastatic disease under chemotherapeutic treatment or when a condition of partial remission was maintained up to 9 weeks after the last administration of cytostatic drugs.

Healthy control persons

Tumour marker levels were assessed in a total of 96 healthy age-matched female patients. The mean blood level ± S.E.M. of MCA was 7.4 ± 0.4 IU/ml (range 1.6–17.9 IU/ml), of CEA 1.2 ± 0.1 ng/ml (range 0.3–5.5 ng/ml) and of CA 15.3 12.0 ± 0.4 IU/ml (range 5–24 IU/ml).

Assessment of tumour marker serum concentrations

The assessment of CEA and CA 15.3 was performed by solid phase immunoradiometric assays using materials obtained from Behring-Werke AG, Marburg, F.R.G. and CIS (Company Oris Industrie S.A., France), respectively. Serum levels of MCA were assayed using materials obtained from Hoffmann-La Roche (Hoffmann-La Roche, Basel, Switzerland). The assay system is a two-step solid-phase enzyme immunoassay utilizing the sandwich principle [14, 15], in which monoclonal mouse antibody mAb b-12 directed against MCA [16] was used. This antibody recognizes a repetitive binding site on the MCA molecule. Serum samples of patients and equal amounts of MCA standards were incubated with mAb b-12 coated beads at 37°C for 60 min. Thereafter, the beads were washed and anti-MCA antibody b-12 conjugated with horseradish peroxidase was added. Unbound anti-MCA peroxidase conjugate was removed by washing after another incubation at 37°C for 60 min. After the addition of enzyme substrate, the tubes were incubated at room temperature for 30 min. Finally, the enzyme reaction was stopped by the addition of sulphuric acid, and the optical density was measured at 492 nm.

Statistics

Tumour marker concentrations exceeding mean levels obtained in healthy individuals by two standard deviations were considered as pathologic. For the patient population, tumour marker blood levels are given as mean ± standard error of the mean (S.E.M.). Statistical analysis was performed by Student's *t*-test.

RESULTS

Tumour marker levels in patients with breast cancer in remission (no evidence of tumour)

In this group of patients ($n = 253$), the mean blood level of MCA was 4.5 ± 0.3 IU/ml, of CEA 2.6 ± 0.2 ng/ml, and of CA 15.3 17.5 ± 0.6 IU/ml. These values are well within the normal range found in healthy control individuals. Table 1 shows the results of tumour marker estimations in patients in the disease-free interval in dependence on their lymph node status. No significant difference was found between the levels of each of the three investigated tumour markers when they were analysed according to this variable ($P > 0.05$, respectively).

The tumour marker analysis was performed in the following manner: Elevations in tumour marker levels exceeding the mean serum concentration in healthy control persons by 2 standard deviations (MCA > 14.4 IU/ml, CEA > 5.4 ng/ml, CA 15.3 > 20.4 IU/ml) were considered to be (true or false) positive.

Percentages of patients with advanced breast cancer with increased tumour marker serum levels (true positive)

Table 2 shows that in metastatic breast cancer, skeletal lesions could be detected by CEA alone in 57.6% of patients; in association with MCA and/or CA 15.3, the correct diagnosis of metastatic disease could be increased by a further 15.2% (CEA + MCA), 18.2% (CEA + CA 15.3) or 24.3% (CEA + MCA + CA 15.3). Visceral lesions were detected by CEA in 19.5% of patients; in this group of patients, the best results were obtained by a combined analysis by CEA + MCA + CA 15.3 (72.2%). Table 2 also shows that CEA was positive in only 66.7% of patients with diffuse metastatic disease. All other tumour markers gave positive results in >90% of patients. When either CEA + CA 15.3, CEA + MCA or CEA + MCA + CA 15.3 were combined with each other, a correct diagnosis was possible in 100% of patients. Furthermore, a combination of tumour markers increased the predictive value of negative results from 68.3% for CEA alone, 71.5% for MCA alone and 69.9% for CA 15.3 alone to 80.6% for CEA + CA 15.3, to 84% for CEA + MCA and to 87.88% for CEA + MCA + CA 15.3.

Tumour marker levels in patients with advanced breast cancer

Table 3 shows the results of tumour marker analyses in patients with either progressive or stable metastatic breast cancer. Patients with progressive disease and skeletal lesions had significantly higher tumour marker serum concentrations than those with stable metastatic disease and skeletal lesions (*MCA*: $P < 0.025$; *CEA*: $P < 0.0025$; *CA* 15.3:

Table 1. Tumour marker levels in patients with breast cancer in the disease-free interval according to their lymph node status

No. of involved lymph nodes		CEA (ng/ml)	MCA (U/ml)	CA 15.3 (U/ml)
0	(n = 97)	2.8 ± 0.2	9.3 ± 0.5	17.3 ± 0.7
1–3	(n = 89)	2.6 ± 0.7	9.7 ± 0.8	19.2 ± 0.6
4–10	(n = 67)	2.8 ± 0.4	9.5 ± 0.6	17.3 ± 0.4

Table 2. Percentages of patients with advanced breast cancer with elevations (true positive) of serum tumour marker levels*

	Metastases: skeletal	visceral	diffuse
CEA	57.57%	19.44%	66.66%
MCA	60.60%	58.33%	90.47%
CA 15.3	66.66%	66.66%	95.23%
CEA + MCA	72.72%	61.11%	95.23%
CEA + CA 15.3	75.75%	69.44%	100%
MCA + CA 15.3	63.63%	66.62%	100%
CEA + MCA + CA 15.3	81.81%	72.77%	100%

*For the number of patients in each group see Materials and Methods section.

Table 3. Serum levels of tumour markers in patients with advanced breast cancer*

Patient population	CEA (ng/ml)	MCA (U/ml)	CA 15.3 (U/ml)
Progressive metastatic disease			
Skeletal lesions	41.0 ± 2.8	39.3 ± 1.9	103.2 ± 5.8
Visceral lesions	6.0 ± 0.3	49.4 ± 2.8	72.3 ± 3.7
Diffuse metastases	118.0 ± 13.9	123.8 ± 12.2	556.0 ± 41.4
Stable metastatic disease			
Skeletal lesions	8.0 ± 0.4	21.0 ± 1.0	43.9 ± 2.3
Visceral lesions	3.0 ± 0.1	19.8 ± 0.8	60.7 ± 4.8
Diffuse metastases	48.0 ± 9.7	127.2 ± 22.4	392.0 ± 60.0

*For the number of patients in each group see Materials and Methods section.

$P < 0.025$). In contrast, patients with visceral lesions who had either stable or progressive disease did not differ significantly in their serum concentrations of tumour markers (*MCA*: $P > 0.05$; *CEA*: $P > 0.05$; *CA* 15.3: $P > 0.25$). Similarly, patients with either stable or progressive diffuse metastatic disease did not differ in their circulating tumour marker levels (*MCA*: $P > 0.05$; *CEA*: $P > 0.2$; *CA* 15.3: $P > 0.5$).

An additional analysis of the data shown in Table 3 revealed that in progressive metastatic breast cancer, CEA levels were significantly higher in patients with skeletal, as compared to those with visceral lesions ($P < 0.001$), whereas the concentrations of other tumour markers did not differ significantly (*MCA*: $P > 0.5$; *CA* 15.3: $P > 0.2$). The same result was obtained in patients with stable metastatic breast cancer. Patients with skeletal lesions had a significantly higher value of CEA than patients with visceral lesions ($P < 0.025$). In the case of progressive diffuse metastatic disease, only CA 15.3 ($P < 0.005$) was significantly higher than the maximum value in patients with non-diffuse (i.e. skeletal or visceral) disease (*MCA*: $P > 0.025$; *CEA*: $P > 0.1$). In contrast, patients with stable localized metasatic disease (skeletal or visceral) had significantly lower tumour marker levels than patients with diffuse metastatic involvement (*MCA*: $P < 0.01$; *CEA*: $P < 0.01$; *CA* 15.3: $P < 0.0025$).

False positive and false negative results

(a) *False positive (FP) results.* The number of FP results was assessed in patients with breast cancer without evidence of disease (i.e. during the disease-free interval) in whom an elevation of tumour marker serum levels exceeding the mean level of healthy control individuals by 2 standard deviations

without concomitant morphologic changes during a median follow-up period of 5 months (range 4–6 months) had been found. Thus, FP results were obtained for MCA and CEA in 12 and for CA 15.3 in 30 patients.

(b) *True positive (TP) and false negative (FN) results.* The number of patients with advanced breast cancer with TP and FN results of the investigated tumour markers are shown in Table 4.

Table 4. Numbers of patients with advanced breast cancer with true positive and false negative results in the analysis of tumour markers

Patients		CEA	MCA	CA 15.3
1. *True positive results*				
Skeletal lesions	(n = 37)	28/20*	28/19	30/25
Visceral lesions	(n = 36)	8/3	27/16	27/18
Diffuse metastatic	(n = 21)	16/13	20/21	21/21
2. *False negative results*				
Metastatic breast cancer	(n = 94)	43	21	14
Skeletal lesions	(n = 37)	15	14	12
Visceral lesions	(n = 36)	29	15	12
Diffuse metastatic	(n = 21)	7	2	1

*Episodes of progressive disease/episodes of stable disease.

Change in tumour marker levels in patients with advanced breast cancer in response to chemotherapy

Twenty-six patients with progressive visceral metastatic breast cancer were followed for the change in tumour marker levels in response to three courses of chemotherapy (see Materials and Methods). In response to treatment, MCA declined from 45.3 ± 2.8 U/ml to 38.9 ± 2.3 U/ml, CEA from 5.9 ± 0.3 ng/ml to 4.1 ± 0.2 ng/ml and CA 15.3 from 73.4 ± 3.5 U/ml to 50.1 ± 4.5 U/ml.

Assessment of the diagnostic value of MCA, as compared to CA 15.3 and CEA

Table 5 shows that the diagnostic sensitivity of MCA was similar to that of CA 15.3. However, MCA or CA 15.3, respectively, had a much higher diagnostic sensitivity than CEA alone. This was found to be true for all groups of patients with metastatic breast cancer. The diagnostic sensitivity could be further improved by the combination of two or more tumour markers (Table 5). In contrast, the specificity of MCA was found to exceed the one of CA 15.3 by 8%, 7.7% and 6.2% in patients with skeletal, visceral and disseminated metastases, respectively.

Table 5. MCA and CA 15.3: gain in diagnostic sensitivity in comparison to CEA

(a) *Skeletal lesions:*	
CEA	57.57%
MCA	+3.03%
CA 15.3	+9.09%
CEA + MCA	+15.15%
CEA + CA 15.3	+18.18%
CEA + MCA + CA 15.3	+24.24%
(b) *Visceral lesions:*	
CEA	19.44%
MCA	+38.99%
CA 15.3	+47.22%
CEA + MCA	+41.67%
CEA + CA 15.3	+50.00%
CEA + MCA + CA 15.3	+52.78%
(c) *Diffuse metastatic breast cancer:*	
CEA	66.66%
MCA	+23.81%
CA 15.3	+28.57%
CEA + MCA	+28.57%
CEA + CA 15.3	+33.34%
CEA + MCA + CA 15.3	+33.34%

DISCUSSION

The present data add the results of a clinical trial to previous immunological and biochemical studies on mucin-like carcinoma-associated antigen (MCA). MCA has been shown to be a member of a polymorphic family of mucin-like glycoproteins expressed on mucinous epithelia [17]; the monoclonal antibody b-12 used in the assay has been raised against four breast carcinoma cell-lines [16] and shown to recognize the corresponding antigen which is released as a secretory product. Additional histological analyses had shown that the b-12 epitope was also present in some normal mucinous epithelia, such as the ductuli of the breast or the distal tubuli of the kidney. Although the b-12 epitope was found to be neither tumour-specific nor oncofoetal, it was noted that all histological types of non-invasive as well as invasive breast carcinomas were reactive, irrespective of the degree of their differentiation [18].

In the present study, the assay for MCA exhibited a sensitivity which was comparable to CA 15.3. This result can be explained by the fact that the monoclonal antibody b-12 detects a glycoprotein molecule which shows some similarity with the CA 15.3 antigen [16]. Differences can be explained on grounds of how the two monoclonal antibodies were raised: thus, the monoclonal antibody used in the assay system for CA 15.3 was produced against a membrane fraction of breast cancer liver metastases [9]. In addition, the antibody mAb-12 does differ immunohistochemically from other antibodies with some specificity for breast carcinomas [19–23].

The sensitivity of MCA as well as CA 15.3 was found greatly to exceed that of CEA, although CEA was noted to be a good marker for progressive disease in patients with skeletal lesions. In contrast, CEA seemed to be of less diagnostic value in patients with visceral metastases. MCA and CA 15.3 on the other hand, exhibited elevated serum levels irrespective of the location of the metastases during progression of disease, but only CA 15.3 was significantly elevated during progression of disease in patients with diffuse disease, as compared to patients with non-disseminated breast cancer. Periods of stable disease were characterized by lower tumour marker serum levels, as compared to periods of disease progression. However, the greater specificity of MCA as compared to CA 15.3 has to be stressed and is of interest for the diagnostic use of this tumour marker. MCA, CA 15.3 and CEA could be increased by the combined use of all three tumour markers. In such cases, the correct diagnosis of metastatic disease was possible in a total of 72% of patients.

In summary, the present data suggest that the monitoring of serum levels of MCA might represent a valuable clinical tool in the monitoring of patients with breast cancer, and that its addition to the tumour marker repertoire can increase the diagnostic sensitivity.

REFERENCES

1. Newlands ES. Clinical applications of tumor markers. *Med Lab Sci* 1987, **44**, 361–370.
2. Bates SE, Longo DL. Tumor makers: value and limitations in the management of cancer patients. *Cancer Treat Rep* 1985, **12**, 163–207.
3. Beretta E, Malesci A, Zerbi A *et al.* Serum CA 19-9 in the postsurgical follow up of patients with pancreatic cancer. *Cancer* 1987, **60**, 2428–2431.
4. Schlom J. Basic principles and applications of monoclonal antibodies in the management of carcinomas. The Richard and Hinda Rosenthal Foundation Lecture. *Cancer Res* 1986, **46**, 3225–3238.
5. Cohen C, Sharkey FE, Shulman G, Uthman EO, Budgeon LR. Tumor-associated antigens in breast carcinoma: prognostic significance. *Cancer* 1987, **60**, 1294–1305.
6. Lakousen M, Stettner H, Pickel H, Urdl W, Pürstner P. The predictive value of a combination of tumor markers in monitoring patients with ovarian cancer. *Cancer* 1987, **60**, 2228–2232.
7. Steward DM, Nixon D, Zarncheck N, Aisenberg A. Carcinoembryonic antigen in breast carcinoma patients: serial levels and disease progression. *Cancer* 1974, **33**, 1246–1252.
8. Mansour EG, Hastert M, Park CH, Kochler KA, Petrelli M. Tissue and plasma carcinoembryonic antigen in early breast cancer. A prognostic factor. *Cancer* 1983, **51**, 1243–1248.
9. Kufe D, Inghirami G, Abe M, Hayes P, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumor. *Hybridoma* 1984, **3**, 223–232.
10. Wahren B, Lidbrink E, Wolfgren A, Encroth P, Zajicek T. Carcinoembryonic antigen and other tumor markers in tissue and serum or plasma of patients with primary mammary carcinoma. *Cancer* 1978, **42**, 1870–1878.
11. Bast RC, Klug TL, St John E *et al.* A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med* 1983, **309**, 883–887.
12. 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.
13. Miller AB, Hoogstaken B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **41**, 207–215.
14. Staehelin T, Stähli C, Hobbs DS, Pestka S. A rapid quantitative assay of high sensitivity for human leukocyte interferon with monoclonal antibodies. *Methods Enzymol* 1981, **79**, 589–595.
15. Mähr R, Miggiano V, Stähli C *et al.* One-step incubation in enzyme immunoassays (EIA) for tumor markers. *Fresen Z Anal Chem* 1984, **317**, 735–736.
16. Stähli C, Takacs B, Miggiano V, Staehelin T, Carmann H. Monoclonal antibodies against antigens on breast cancer cells. *Experientia* 1985, **41**, 1377–1381.
17. Stähli C, Caravatti M, Aeschbacher M, Kocyba C, Takacs B, Carmann H. A mucinous carcinoma associated antigen (MCA) defined by three monoclonal antibodies against different epitopes. Submitted for publication.
18. Zenklusen HR, v Overbeck J, Stähli C, Gudat F, Rolink H, Heitz PU. The immunohistochemical reactivity of a monoclonal antibody (MAb b-12) against breast carcinoma and other normal and neoplastic human tissues. *Virchows Arch A*, in press.
19. Foster CS, Edwards PAW, Dinsdale EA, Neville AM. Monoclonal antibodies to the human mammary gland. I. Distribution of determinants in non-neoplastic mammary and extra mammary tissues. *Virchows Arch A* 1982, **394**, 279–293.
20. Foster CS, Dinsdale EA, Edwards PAW, Neville AM. Monoclonal antibodies to the human mammary gland. II. Distribution of determinants in breast carcinomas. *Virchows Arch A* 1982, **394**, 295–305.

21. Horand Hand P, Nuti M, Colcher D, Schlom J. Definition of antigenetic heterogeneity and modulation among human mammary carcinoma cell populations using monoclonal antibodies to tumor-associated antigens. *Cancer Res* 1983, **43**, 728–735.
22. Iacobelli S, Natoli V, Scambia G, Santeusanio G, Negrini R, Natoli C. A monoclonal antibody (AB/3) reactive with human breast cancer. *Cancer Res* 1985, **45**, 4434–4438.
23. White CA, Dulbecco R, Allen R, Bowman M, Armstrong B. Two monoclonal antibodies selective for human mammary carcinoma. *Cancer Res* 1985, **45**, 1337–1343.