Report

Comparison of breast cancer mucin (BCM) and CA 15-3 in human breast cancer

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Summary

The Breast Cancer Mucin (BCM) enzyme immunoassay utilizes two monoclonal antibodies (Mab), M85/34 and F36/22, for the identification of a mucin-like glycoprotein in serum of breast cancer patients. We have compared BCM with CA 15-3, another member of the human mammary epithelial antigen family.

Serum BCM was evaluated in 151 and CA 15-3 in 134 patients with breast cancer, in 30 normal controls, in 9 pregnant women, and in 13 cancer patients (non-breast). Neither the normal controls nor the pregnant women had BCM levels > 25 U/ml. In contrast, 87 of 115 patients (75%) with metastatic breast cancer had BCM levels > 25 U/ml. All control persons had CA 15-3 levels < 25 U/ml, but 2 out of 9 pregnant women (22%) had levels > 25 U/ml. Seventy-four out of 97 patients (76%) with metastatic breast cancer had CA 15-3 levels > 25 U/ml.

A statistically significant correlation was found between BCM and CA 15-3 in the breast cancer patient group (r = 0.883, p < 0.001, n = 134) and in the normal control group (r = 0.743, p < 0.001, n = 30). BCM and CA 15.3 both showed no correlation with CEA in breast cancer patients (r = 0.060, n = 81; and r = 0.146, n = 78, respectively). BCM had a range of sensitivity similar to that of the CA 15-3 RIA.

Our results suggest that BCM may be a useful new marker for monitoring the clinical course of patients with breast cancer. Furthermore, in the evaluation of breast cancer patients, marker pands depending on disease stage may be a better choice than any single parameter in the evaluation of breast cancer patients.

Introduction

An ideal tumour marker should have both high specificity and sensitivity, and thus have a potential role in screening, diagnosis, and staging, and in the follow up of patients during the course of their disease.

In women with breast cancer the measurement of tumour marker serum levels may be useful in the follow up of the disease [1–4]. However, in primary breast cancer the 'classical' tumour marker carcinoembryonic antigen (CEA) is poorly correlated with disease spread and clinical course [3–5]. Therefore new tumour markers have been developed.

Several clinical studies [5, 6] have suggested that CA 15-3 is a more sensitive marker than CEA in the follow-up of patients with metastatic breast cancer. Some authors advise the use of both CEA and CA 15-3 for better monitoring of these patients

(e.g. 7). By contrast, however, in a recent study no advantage was seen by adding CEA to CA 15-3 [5]. Therefore, tumour markers with higher sensitivity and specificity than CA 15-3 are urgently needed in the management of metastatic breast cancer.

The monoclonal antibodies M85/34 and the F36/22 were found to identify a mucin-like glycoprotein, breast cancer mucin (BCM), in serum of breast cancer patients. The protein originates from ductal epithelial tissue. Mab F36/22 reacts with the human breast cancer cell line MCF-7 and with the ductal carcinoma antigen (DCA), another member of the human mammary epithelial (HME) family of glycoproteins [8–14].

The present study was designed to compare CA 15-3 and BCM levels in serum samples from healthy persons and breast cancer patients and to evaluate which of these markers, either alone or in combination with CEA, has the higher sensitivity and specificity.

Materials and methods

Serum samples

Serum samples were obtained from patients attending the out-patient clinics of both hospitals. They were stored at $-20°$ C until assay. Additional samples were retrieved from our serum bank which is kept at $-70°$ C; mean levels of BCM and CA 15-3 in these samples were not different from those in the fresh samples. Therefore the combined use of freshly frozen and long-term frozen samples was considered to be justified. Samples from healthy blood donors were provided by the Utrecht Red Cross blood bank.

Clinical information

Clinical staging was carried out according to the criteria of the International Union Against Cancer (UICC) and the American Joint Commission in Cancer Staging and Results Reporting Classifications (AJC) [15]. On the basis of the available clinical data the patients were grouped as follows: 21 patients in stage I; 3 patients in stage II; 5 patients in stage III; 115 patients in stage IV; 7 patients stage unknown; 30 healthy blood donors (age

34 ± 9 years); 9 pregnant women; 13 non-breast cancer patients.

Assay of tumour markers

BCM was measured in serum with a solid phase enzyme immunoassay (EIA) provided by Abbott Laboratories (Chicago, IL, USA). Polystyrene beads coated with Mab M85/34 [12] were incubated with standards, controls, and 1 : 11 diluted serum specimens for 2 h at 37° C. The incubation buffer contained neuraminidase to liberate sialic acid residues and to expose the carbohydrate epitope. Unbound material was removed by aspiration of fluid followed by two washings of the beads with distilled water by the Pentawash device. After washing, Mab F36/22 [8] conjugated with horse radish peroxidase was incubated with the beads for 2 h at 37° C. Unbound conjugate was removed by aspiration and the beads were washed again. The beads were next incubated with hydrogen peroxide and orthophenylene diamine-HCl at room temperature for 30 minutes. The reaction was stopped by the addition of 1N sulfuric acid, and absorbance was read at 492 nm using the Abbott Quantum spectrophotometer which also performed the calculations. The intra-assay coefficient of variation was 9% at 20 U/ml (n = 2), and 7% at 150 U/ml (n = 8); the inter-assay coefficient of variation was 10% at 200 U/ml (n = 4). All samples were tested in duplicate. The variation in duplicates was 5.7, 6.2, 6.2, 5.0, and 4.6% for samples containing < 25, 25–50, 50–100, 100–150, and 150–200 U/ml respectively.

CA 15-3 was estimated in serum with an immunoradiometric assay (IRMA), using reagents purchased from Centocor (Malvern, USA). The IRMA utilizes two monoclonal antibodies (115D8, DF3) which probably recognize different epitopes of the same antigen. Inter-assay variation determined from control samples supplied with the kits was 8% at 38 U/ml (n = 39). BCM and CA 15-3 determinations were performed simultaneously.

CEA was assayed by enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA). Inter-assay variation determined from control samples supplied with the kits was 10% at 2.6 ng/ml (n = 14) and 4% at 15.3 ng/ml (n = 14).

Statistical evaluation

Data were evaluated with the coefficient of correlation and Wilcoxon's rank-sum test [16]. P-values below 0.05 were considered to reflect statistical significance.

Results

Serum tumour marker concentrations obtained are summarized in Table 1.

Breast cancer mucin

There was no statistically significant difference between men and women of the normal control group. The 97.5th percentile value (25 U/ml) was chosen as positive/negative cut-off. None of the normal controls had values above 25 U/ml (Table 2, Fig. 1). Similarly none of the pregnant women had BCM levels > 25 U/ml (Table 2) which further illustrates the specificity of BCM-EIA. Differences between pregnant women and non-pregnant controls were not statistically significant.

Patients with breast cancer exhibited a wide range of circulating levels of BCM (Table 1, Fig. 1). The incidence of elevated values for this group was 65%. Only 33.5% of values observed in patients with Stage I breast cancer were elevated, whereas in patients with stage IV, BCM levels were elevat-

ed in 75% (Table 2). In patients with bone metastases 66% of BCM values were > 50 U/ml (Table 2), whereas 15% of the patients had normal levels. In patients with hepatic metastases BCM levels of 888 ± 423 U/ml (mean ± SEM) were found. None of these patients had normal BCM values. The sera from 13 patients with other malignancies were evaluated. Results are given in Table 3. Only two patients (15%) had elevated values (one ovarian cancer 81 U/ml and one lung cancer 50 U/ml).

CA 15-3

The mean serum levels (± SEM) of CA 15-3 in the healthy blood donor group were 14 ± 1 U/ml. All CA 15-3 values were below 25 U/ml. The 97.5th percentile of CA 15-3 values in control samples was 24 U/ml. For practical purposes, therefore, 25 U/ml was chosen as a cut-off value between normal and elevated CA 15-3.

The CA 15-3 values in pregnant women were 20 ± 2 U/ml (mean ± SEM). Two of the 9 pregnant women had elevated CA 15-3 levels.

CA 15-3 was determined in sera from 134 breast cancer patients. Elevated levels were found in 65% of these patients. In 50.5% of these samples levels even exceeded 50 U/ml (Tables 1 and 2). Similar to BCM, the percentage of elevated values increased with stage of the disease (Table 2). In patients with bone metastases CA 15-3 values of 324 ± 80 U/ml

Table 1. Breast Cancer Mucin, CA 15-3, and CEA in serum

| Group	BCM (U/ml)			CA 15-3 (U/ml)			CEA (ng/ml)		
	n	Mean ± SEM	Range	n	Mean ± SEM	Range	n	Mean ± SEM	Range
Normal control group	30	13 ± 1	5– 23	30	14 ± 1	6– 24	30	1.7 ± 0.3	0.01– 5.5
Pregnant women	9	14 ± 3	2– 23	9	20 ± 2	14– 29	9	0.6 ± 0.1	0.23– 1.2
Patients with other malignancies	13	23 ± 6	4– 81	13	26 ± 5	8– 76			
Patients with breast cancer	151	190 ± 46	0–3564	134	214 ± 42	0–2960	81	28 ± 11	0 –600
Stage I	21	28 ± 9	4– 174	21	34 ± 12	7– 270	12	1.3 ± 0.25	0.1 – 3
Stage IV	115	243 ± 59	0–3564	97	259 ± 54	0–2960	61	25 ± 10	0 –600
bone metastases	52	297 ± 92	1–3564	46	324 ± 80	5–2960	42	27 ± 14	0 –600
hepatic metastases	10	888 ± 423	28–3564	10	864 ± 305	19–2960	10	20 ± 11	1 –229
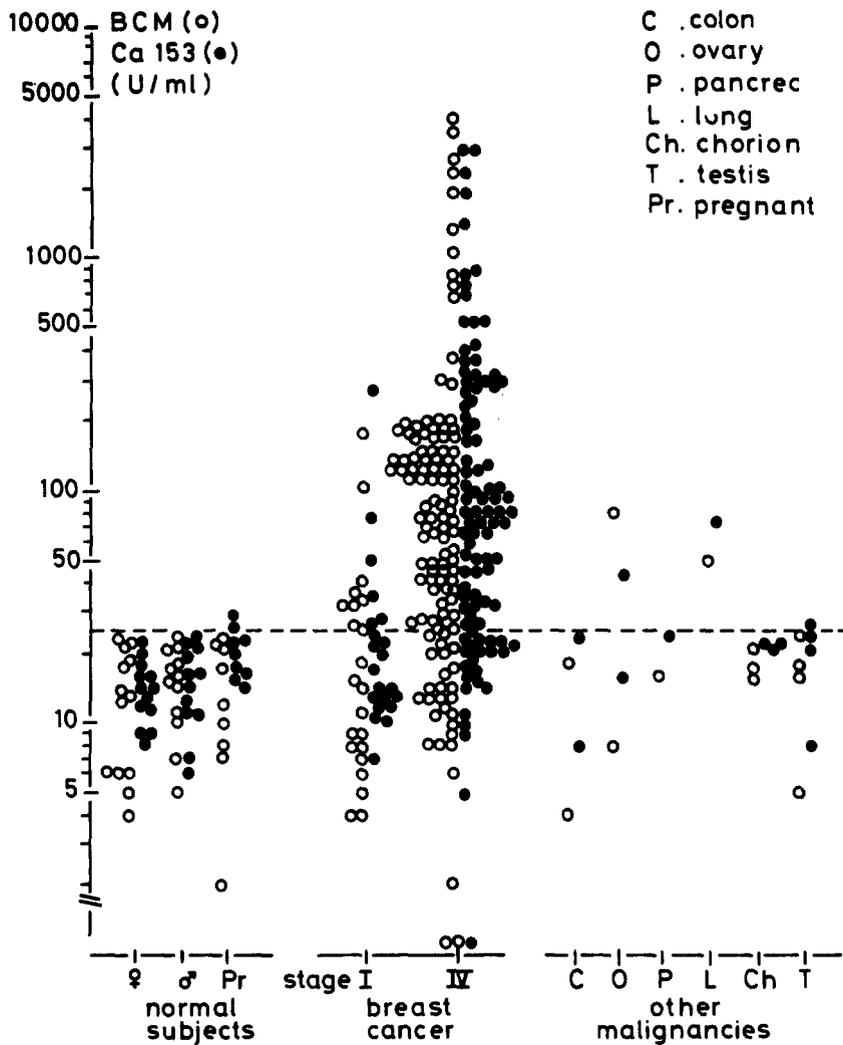
Fig. 1. Serum levels of Breast Cancer Mucin (BCM) and Ca 15-3 in individual samples from healthy persons and patients with different malignancies.

were found. Values of more than 50 U/ml were found in 69% of these patients. Only 8 patients had normal serum levels. Remarkably, four of these patients were in clinical remission. In patients with hepatic metastases the CA 15-3 values were even higher (864 ± 305). Ninety per cent of these patients had levels > 50 U/ml (Tables 1 and 2).

Three out of 13 patients with malignancies of other organs (23%) had elevated CA 15-3 values (one ovarian cancer 42 U/ml, one testicular cancer 26 U/ml, and one lung cancer 76 U/ml).

CEA

In 81 of the patients with breast cancer, the serum levels of CEA were also measured. The results are shown in Table 1. The mean serum level (± SEM) in the healthy blood donor group was 1.70 ng/ml (± 0.26). Three ng/ml was used a cut-off value between normal and elevated values. Thirteen per cent of the donors had elevated levels. This might be related to their smoking habits. These were not known, however. CEA values in pregnant women were 0.62 ng/ml (± 0.12). All patients with Stage I breast cancer had normal values.

Table 2. Distribution of BCM and CA 15-3 in patients with breast cancer and in three control groups [n(%)]

Group	BCM (U/ml)					CA 15-3 (U/ml)				
	n	<25	25–50	50–100	>100	n	<25	25–50	50–100	>100
Normal control group	30	30 (100%)				30	30 (100%)			
Pregnant women	9	9 (100%)				9	7 (78%)	2 (22%)		
Patients with other malignancies	13	11 (85%)		2 (15%)		13	10 (77%)	2 (15%)	1 (8%)	
Patients with breast cancer	151	52 (34%)	32 (21%)	17 (11%)	50 (33%)	134	47 (35%)	19 (14%)	22 (17%)	44 (33%)
Stage I	21	14 (66%)	5 (24%)		2 (9%)	21	15 (71%)	4 (19%)	1 (5%)	1 (5%)
Stage IV	115	28 (24%)	24 (21%)	16 (14%)	47 (41%)	97	23 (24%)	13 (13%)	21 (22%)	40 (41%)
bone metastases	52	8 (15%)	10 (19%)	4 (8%)	30 (58%)	46	8 (17%)	6 (13%)	8 (17%)	24 (52%)
hepatic metastases	10		2 (20%)	2 (20%)	6 (60%)	10	1 (10%)		2 (20%)	7 (70%)

Comparison between tumour markers

In all groups of patients studied a significant correlation between BCM and CA 15-3 levels was found (Table 4). The correlations between BCM and CEA ($r = 0.060$, $p > 0.1$, $n = 81$) and between CA 15-3 and CEA ($r = 0.146$, $p > 0.1$, $n = 78$) were poor.

The sensitivity and specificity of CA 15-3, BCM, and CEA were determined either alone or in combination. Sensitivity is defined as positivity in disease and expressed as per cent ([true positives (true positives + false negatives)] × 100); specificity is defined as negativity in health or absence of a particular disease and is also expressed as per cent ([true negatives (true negatives + false posi-

tives)] × 100). The results are shown in Table 5 and illustrate the superiority of BCM and CA 15-3 over CEA, as well as the similarity of BCM and CA 15-3.

Discussion

Early detection of disease progression may be important in the management of patients with breast cancer. Tumour markers have been widely studied in breast cancer, since a biochemical signal was thought to be the best possibility for detecting any subclinical spread of the disease. Carcino Embryonic Antigen (CEA) was found to be useful only in a limited number of cases in early detection

Table 3. Tumour markers in patients with other malignancies

Tumour site	Remarks	BCM (U/ml)	CA 15-3 (U/ml)	CEA (ng/ml)	CA-125 (U/ml)
Colon	Progression, hepatic meta's	18	24	60	
Colon	Hepatic meta's, after chemotherapy	4	8	6.7	
Ovary	Stage IV	81	42	1.2	
Ovary	Stage III during chemotherapy	8	16		78
Testis	Stage IV, progression	8	22		
Testis	Stage III, progression	18	8		
Testis	Stage I, stable	5	26		
Testis	Stage IIc	24	24		
Chorion		16	22		
Chorion		16	22		
Chorion		22	21		
Pancreas	Progression	16	24		
Lung	Progression	50	76	19	

Table 4. Linear correlation between serum levels of BCM and CA 15-3

Samples	n	r	p-value
Normal controls	30	0.743	$p < 0.001$
Pregnant women	9	0.768	$p < 0.05$
Patients with other malignancies	13	0.745	$p < 0.05$
Patients with breast cancer	134	0.883	$p < 0.001$
Stage I	21	0.847	$p < 0.001$
Stage IV	97	0.932	$p < 0.001$
bone metastases	46	0.954	$p < 0.001$
hepatic metastases	10	0.950	$p < 0.001$

of progressive disease [1]. Using the methodology of cell hybridization, a large number of monoclonal antibodies was raised against breast cancer tissue or breast cancer cells [17, 18].

CA 15-3 has already been shown to be more sensitive than CEA in patients with metastatic carcinoma of the breast [5, 6]. Another enzyme immunoassay has been developed by using the Mab M85/34 and the Mab M36/22. These monoclonal antibodies are raised against breast cancer cells and recognize the mucin-like glycoprotein BCM, a member of the HME family of glycoproteins [8–13]. In the present study BCM was detectable in sera of breast cancer patients, patients with other malignancies, normal controls, and pregnant women, suggesting that the antigen is not tumour-specific. In the sera of breast cancer patients BCM levels were found to be significantly higher than in the three other groups (Table 1).

Serum BCM levels appeared not to be related to age or menopausal status. BCM and CA 15-3 serum levels were well correlated. Differences might be expected since the BCM assay detects a carbohydrate epitope exposed following neuraminidase treatment, while the CA 15-3 assay identifies a polypeptide epitope. The high degree of similarity observed suggests that the two epitopes are closely related. The final decision in this matter, where two assays which utilize four different antibodies are compared, must await complete characterization of the epitopes. Therefore several analogies were expected between these two assays. BCM concentrations are directly related to the stage of disease and to some extent to the site of metastasis.

In two of the thirteen patients with malignancies of the other organs, the levels of BCM and CA 15-3 were elevated. One of these patients had an ovarian carcinoma Stage IV (BCM: 81 U/ml; CA 15-3: 42 U/ml), while the other had lung carcinoma in clinical progression (BCM: 50 U/ml; CA 15-3: 76 U/ml). An explanation could be that Mab F36/22 reacts against ductal carcinoma antigens which are also present in other carcinomas [19, 20]. In one other patient with ovarian carcinoma, however, BCM and CA 15-3 were not elevated in spite of high levels of CA 125 (78 U/ml).

The observation that sera from apparently healthy individuals contain immunoreactive BCM and CA 15-3 is remarkable. Burchel *et al.* [21] using Western blotting could not detect mucin epithelial antigens in sera which were positive by immunoassay. Several possibilities appear to be able to ac-

Table 5. Sensitivity and specificity of tumour markers

Markers	Specificity (%)		Sensitivity (%)		
	Contr + Preg	NBC	Tot.BC	Stage I	Stage IV
BCM (cut-off value 25 U/ml)	100	85	64	33	76
CA 15-3 (cut-off value 25 U/ml)	95	77	64	29	76
CEA (cut-off value 3ng/ml)	92	—	52	8	61
BCM + CA 15-3	95	77	70	38	78
BCM + CEA	90	—	74	33	84
CA 15-3 + CEA	85	—	72	33	83
BCM + CA 15-3 + CEA	85	—	77	33	86

Contr = blood donors, Preg = pregnant women, NBC = other malignancies, Tot. BC = all patients with breast cancer.

count for this phenomenon, i.e. background values due to assay conditions, or release of different antigens in malignant states and health [22].

In the control group BCM was shown to have the highest (100%) specificity when compared with CA 15-3 and CEA. In breast cancer patients with stage I BCM + CA 15-3 showed the highest sensitivity (38%). For stage IV breast cancer patients the highest sensitivity (86%) was obtained with a combination of BCM, CA 15-3, and CEA (Table 5).

The following conclusions can be drawn:

- BCM is detectable in sera from breast cancer patients as well as in a normal control group, in pregnant women, and in patients with other malignancies. The values for Stage IV breast cancer patients were found to be higher than in the three other groups as well as in patients with early breast cancer.
- The highest values of BCM are found in patients with bone and hepatic metastases.
- The correlation between BCM and CA 15-3 was statistically significant in all four groups studied.
- The concentrations of BCM and CA 15-3 did not show any correlation with CEA.
- BCM appeared to be as sensitive as CA 15-3 in patients with metastatic breast cancer.
- The optimal combination of tumour markers depended on the stage of the disease (Table 5). The degree of specificity in control persons was as follows: BCM (100%), CA 15-3 (95%), BCM + CA 15-3 (95%), CEA (92%), BCM + CEA (90%), CA 15-3 + CEA (85%), BCM + CA 15-3 + CEA (85%). Sensitivity in breast cancer patients increased as follows: CEA (52%), CA 15-3 = BCM (64%), BCM + CA 15-3 (70%), CA 15-3 + CEA (72%), BCM + CEA (74%), BCM + CA 15-3 + CEA (77%). For stage I breast cancer the highest sensitivity (38%) was obtained with a combination of BCM and CA 15-3; for stage IV the optimal combination was BCM + CA 15-3 + CEA (86%).
- Neither marker is 100% specific for breast cancer, as elevated values were also observed in patients with ovarian and lung cancer.
- BCM and CA 15-3 appeared to have little addi-

tive value. The sensitivity of BCM was not higher than that of CA 15-3.

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