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Original Paper

CEA, MCA, CA 15.3 and CA 549 and Their Combinations in Expressing and Monitoring Metastatic Breast Cancer: a Prospective Comparative Study

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Serum levels of carcinoembryonic antigen (CEA), mucin-like carcinoma-associated antigen (MCA), CA 15.3 and CA 549 were concurrently assayed in patients with metastatic breast cancer. Overall sensitivity in detecting metastatic breast cancer (201 pts) was CEA 45%, MCA 59%, CA 15.3 71% and CA 549 72% ($P < 0.01$). Sensitivity increased by only 6% to 8% when two or more antigens were simultaneously considered. An overall sensitivity of correlation with objective response ($n = 71$) was observed in the range of 53–67% (P = n.s.) in patients with abnormal baseline marker values, and in the range of 42–87% ($P < 0.05$) in patients with normal baseline values. The combination of two or more markers did not improve sensitivity, but decreased specificity of correlation with objective response. In conclusion, CA 15.3 and CA 549 have individually higher sensitivity in detecting metastatic breast cancer. No clinical advantage was observed for using two or more markers concurrently over CA 15.3 or CA 549 alone in the monitoring of metastatic breast cancer.

Key words: CA 15.3, CA 549, MCA, CEA, serum markers, metastatic breast cancer monitoring
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INTRODUCTION

A NUMBER of circulating tumour-associated antigens are currently extensively used in detecting and monitoring metastatic breast cancer. Carcinoembryonic antigen (CEA) was the first studied antigenic glycoprotein, and is still a reference serum marker in breast cancer. Another class of circulating tumour markers is represented by high molecular weight glycoproteins, described as mucins or mucin-like components. These can be released into the circulation because of the loss of functional polarity in tumour cells, coupled with disruption of normal tissue architecture by a developing and invasive neoplasm. Many monoclonal antibodies reactive with breast carcinoma-associated mucins have been produced. Most of these seem to define separate and distinct epitopes on mucins [1]. CA 15.3, mucin-like carcinoma-associated antigen (MCA) and CA 549 are included in this class of antigens.

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CA 15.3 is a high molecular weight glycoprotein recognised by two monoclonal antibodies; the first, 115-D8, was obtained by immunisation with human milk fat globule membranes [2]; the second, DF3, by immunisation with a membrane-enriched fraction of a human breast cancer [3]. CA 549 is a high molecular weight acid glycoprotein recognised by two different monoclonal antibodies; the first, BC4E 549, is directed against a human breast cancer line (T 417), and the second, BC4N 154, against the membranes of milk fat globules [4]. MCA is a high molecular weight acid soluble glycoprotein antigen recognised by the monoclonal antibody b-12 [5].

High serum levels of CEA were reported in up to 73% of patients with metastatic breast cancer [6], while abnormal serum levels of CA 15.3 [7], MCA [8] and CA 549 [9] occur in 70–80% of cases. Our previous observations on CA 15.3 [10] and CA 549 [11] confirmed these results.

The usefulness of simultaneous determination of two or more markers, both in recognising patients with metastatic disease and in monitoring the course of the disease, is still being debated [12], and no conclusive data are available in the literature.

The present study was aimed at comparing sensitivity of CEA, CA 15.3, MCA and CA 549 and their combinations in signalling the presence of disease in patients with documented metastatic

breast cancer. A comparison of the correlation between changes in serum levels of single tumour markers and their combinations during antitumour treatment and clinical response was also undertaken.

PATIENTS AND METHODS

Patient population

Serum samples were collected from 201 consecutive patients with documented metastatic breast cancer. The median age of the patients was 61 years (range 33–90); 172 patients (86%) were postmenopausal and 29 (14%) were premenopausal. The prevalent metastatic sites were soft tissues (ST) in 38 cases (19%), bones (O) in 58 patients (29%) and viscera (V) in 105 patients (52%). Marker values were correlated with the prevalent metastatic site and with the status of the disease at the first determination.

To evaluate the correlation between marker serum levels and the extent of the disease, the patients were divided into six subgroups, as reported previously [11] (ST1 ≤ three skin and lymph node lesions; ST2 > three lesions; O1 = prevalent bone metastases with a maximum involvement of three osseous segments; O2 = prevalent bone metastases involving more than three osseous segments; V1 = limited metastatic involvement of one viscera; V2 = diffuse metastatic involvement of one viscera or metastatic involvement of more than one viscera or simultaneous involvement of viscera and bones and/or soft tissues).

In a subset of 71 patients with progressive disease, marker levels were determined before beginning a systemic antitumour therapy, and subsequently were monitored every 4 weeks during the treatment. The behaviour of four markers and their combinations was analysed in relation to clinical response. The treatment included endocrine therapy (tamoxifen, medroxyprogesterone acetate, 4-hydroxy-androstenedione, goserelin) or chemotherapy (cyclophosphamide + fluorouracil + methotrexate = CMF regimen; or cyclophosphamide + fluorouracil + epirubicin = FEC regimen; or methotrexate plus mitomycin C plus mitoxantrone = MMM regimen).

Marker assays

Marker serum levels were measured using commercially available kits, and the assays were performed according to manufacturers' recommendations. The 95th percentile value in 130 normal women comparable in age was chosen as the positive/negative cut-off: CEA 2.5 ng/ml, MCA 14 U/ml, CA 15.3 30 U/ml and CA 549 12 U/ml. A liquid-phase radioimmunological assay was employed to measure CEA serum levels (CEA®, Eiken Medical Systems): the CEA standard used in this kit is extracted from liver metastases of human colon cancer. Anti-CEA rabbit serum and anti-rabbit-IgG goat serum are also employed. MCA levels were determined by a solid-phase two-site immuno-enzymatic assay (MCA®, Roche); the kit employs the same monoclonal antibody to MCA (MAb b-12) in both positions of the sandwich, as this antibody recognises a repetitive epitope on the MCA molecule. To measure CA 15.3 serum levels, a solid-phase two-site immunoradiometric assay was employed (ELSA®-CA 15.3/CIS Diagnostici). To measure CA-549 serum levels, a solid-phase two-site immunoradiometric assay was used (Tandem®-R CA 549/Hybritech). The interassay coefficients of variation (at the cut-off concentration) were 6.5%, 8.6%, 8.4% and 6.6% for CEA, MCA, CA 15.3 and CA 549, respectively.

Criteria of evaluation

Sensitivity of a single marker in signalling metastatic breast cancer was calculated as the percentage of true positive values over the sum of true positive and false negative values. In calculating the sensitivity of two, three or four marker panels, at least one of the markers had to be positive.

The clinical response was evaluated according to WHO criteria [13] (including definition of complete response = CR, partial response = PR, no change = NC, progression = PD). The clinical response was assessed independently by marker levels after 1 and 3 months and subsequently at 3 month intervals. The pretreatment sample was chosen as the baseline marker value, and the determination after 3–6 months was chosen as the intratreatment value. In order to correlate marker behaviour and clinical response, the difference in marker levels has been expressed as the percentage of change between the baseline antigen level (Ag_b) and the intratreatment value (Ag_t), according to

$$\% \text{ of change Ag} = \frac{(Ag_b - Ag_t)}{Ag_b} \times 100 .$$

In the subgroup of patients with abnormal baseline marker values, according to others [9] and to the interassay coefficients of variation listed above, we considered a ± 25% baseline value change as the cut-off in order to define marker behaviour (increase ≥ 25% = PD; decrease ≤25% = PR; decrease > 25%, associated with normalisation of abnormal values = CR; neither a ≥25% increase nor a ≥25% decrease can be measured = NC). When considering a two, three or four marker panel, the patient was included in the subgroup with abnormal baseline values if at least one marker in the combination was abnormal at baseline.

As previously described by Tondini and associates [14], sensitivity and specificity of correlation with clinical response were calculated as follows:

Sensitivity of correlation in patients with PD was defined as:

$$\frac{\text{No. of pts with PD and with serial Ag levels which increase} \geq 25\%}{\text{No. of pts with PD}} \times 100 ;$$

Sensitivity of correlation in patients with NC was defined as:

$$\frac{\text{No. of pts with NC and with serial Ag level change} < 25\%}{\text{No. of pts with NC}} \times 100 ;$$

Sensitivity of correlation in patients with responsive disease was defined as:

$$\frac{\text{No. of pts with PR or CR and with serial Ag levels which decrease} \geq 25\%}{\text{No. of pts with PR or CR}} \times 100 ;$$

Specificity of correlation for patients with PD was defined as:

$$\frac{\text{No. of pts with CR or PR or NC and with serial Ag levels which do not increase} \geq 25\%}{\text{No. of pts with CR or PR or NC}} \times 100 ;$$

Specificity of correlation for patients with responsive disease was defined as:

$$\frac{\text{No. of pts with PD or NC and with serial Ag levels which do not decrease } \geq 25\%}{\text{No. of pts with PD or NC}} \times 100 .$$

Sensitivity and specificity of two, three or four marker panels were calculated considering that a true positive or negative patient was the patient in whom at least one abnormal baseline marker changed according to the 25% rule mentioned above and in whom no other marker behaved in the opposite sense. In the subgroup of patients with normal baseline marker values, only the correlation between marker behaviour and PD was evaluable. We considered positive correlation with clinical PD an increase of the marker ≥25%, associated with marker changes from within the normal range to above the assay cut-off value. A combination of two or more normal baseline markers was considered to correlate with PD when at least one of the markers in the combination met this criterion.

The positive predictive value of the tests was defined [14] as:

$$\frac{\text{sensitivity} \times C}{(\text{sensitivity} \times C) + (1 - \text{specificity}) \times (1 - C)}$$

where C represents the incidence of the clinical event (PD or regression) in our study population (42% PD and 37% PR/CR).

Statistical analysis

The data from the study were analysed by non-parametric statistics (Chi-square, Mann-Whitney rank sum test, sign test and Wilcoxon signed-rank test).

RESULTS

Sensitivity in recognising metastatic breast cancer

The data concerning sensitivity of single markers and their combinations are reported in Table 1. The overall sensitivity in detecting metastatic breast cancer independently of current status of the disease was 45, 59, 71 and 72%, for CEA, MCA, CA 15.3 and CA 549, respectively (MCA or CA 15.3 or CA 549 versus CEA: $P < 0.005$; CA 15.3 or CA 549 versus MCA: $P < 0.01$). Excluding patients with non-progressive disease at the moment of blood collection, sensitivity in 103 patients was 54, 72, 80 and 81%, respectively (MCA or CA 15.3 or CA 549 versus CEA: $P < 0.009$).

Sensitivity increased by 6% to 8% when two, three or four markers were simultaneously considered. In the PD group, the best two-marker combination was CA 15.3 plus CA 549 (87%), while the best three-marker combination was CEA plus CA 15.3 plus CA 549 (89%). The statistical comparison did not show any significant advantage when these combinations were compared to CA 15.3 or CA 549 alone. The four-marker combination did not increase sensitivity (89%).

The median marker values in relation to the prevalent metastatic site are shown in Table 2. Higher values were observed in patients with O and V metastases. The differences reached statistical significance ($P < 0.02$) when values of patients with V involvement were compared to those of patients with ST metastases, except for MCA. No statistical difference was evident between groups O and V. The analysis for extension of the disease demonstrated that in both O and V groups higher marker levels were detected in the subgroups of patients with greater tumour burden (O2 and V2). The difference was statistically significant for all four markers in comparing O1 versus O2 and for all but CEA in the comparison of V1 versus V2. No difference was evident in ST subgroups.

Table 1. Sensitivity in detecting metastatic breast cancer

	All patients (n = 201)	PD (n = 103)
CEA	90 (45%)	56 (54%)
MCA	118 (59%)	74 (72%)
CA 15.3	142 (71%)	82 (80%)
CA 549	145 (72%)	83 (81%)
CEA+MCA	139 (69%)	81 (79%)
CEA+CA 15.3	155 (77%)	89 (86%)
CEA+CA 549	154 (77%)	86 (83%)
MCA+CA 15.3	150 (75%)	88 (85%)
MCA+CA 549	149 (74%)	85 (83%)
CA 15.3+CA 549	159 (79%)	90 (87%)
CEA+CA 15.3+MCA	159 (79%)	91 (88%)
CEA+CA 15.3+CA 549	164 (82%)	92 (89%)
MCA+CA 15.3+CA 549	160 (80%)	90 (87%)
CEA+MCA+CA 549	156 (78%)	87 (84%)
CEA+MCA+CA 15.3+CA 549	165 (82%)	92 (89%)
Chi square		
CA 15.3 or CA 549 versus CEA	$P = 0.0000$	$P < 0.0001$
CA 15.3 or CA 549 versus MCA	$P < 0.01$	$P = 0.1$
MCA versus CEA	$P = 0.005$	$P = 0.009$
CA 549 versus CA 15.3+CA 549	$P = 0.1$	$P = 0.1$
CA 15.3 versus CA 15.3+CA 549	$P = 0.06$	$P = 0.1$
CA 15.3 versus CEA+CA 15.3+CA 549	$P = 0.01$	$P = 0.06$
CA 549 versus CEA+CA 15.3+CA 549	$P = 0.02$	$P = 0.08$

Correlation of marker changes with clinical response during treatment

71 patients were monitored during treatment and intratreatment marker values were compared with baseline levels. In 30 patients who presented with PD the median value of each marker increased significantly: CEA from 1.3 to 3.6 ng/ml (Wilcoxon test, $P = 0.0004$); MCA from 14 to 29.5 U/ml ($P = 0.04$); CA 15.3 from 33 to 74.5 U/ml ($P = 0.0009$) and CA 549 from 16.5 to 35 U/ml ($P = 0.0008$). In 26 patients who experienced CR or PR, the median marker values decreased: CEA from 2.7 to 1.6 ng/ml ($P = 0.05$); MCA from 17 to 10 U/ml ($P = 0.0006$); CA 15.3 from 87.5 to 36 U/ml ($P = 0.0007$) and CA 549 from 26 to 12.5 U/ml ($P = 0.002$). In 15 patients with NC, the median marker values did not change significantly according to clinical response. The sensitivity and specificity of correlation between CEA, MCA, CA 15.3 and CA 549 changes, and the clinically evaluated objective responses are reported in Tables 3–5.

Patients with abnormal baseline values

In this subgroup, the sensitivity of correlation was 53, 56, 67 and 60% for CEA, MCA, CA 15.3 and CA 549, respectively (Table 3). The concordance was higher in patients with PR/CR (64–78%) and PD (47–73%) than in those with NC (14–44%). The combination of two or more markers did not increase the level of sensitivity. The specificity of marker changes (Table 4) varied from 75 (CA 549) to 85% (MCA) in patients with PD and from 67 (CEA) to 83% (CA 549) in patients with CR or PR; no statistically significant difference was observed between markers. The positive predictive values (PPV) of the tests (Table 6) ranged from 64% (CA 549) to 76% (CA 15.3) and from

*Table 2. Marker median values in relation to the extent of the disease (189 patients)**

	n	• CEA ng/ml (range)	MCA U/ml (range)	CA 15.3 U/ml (range)	CA 549 U/ml (range)
ST1	16	1.4 (0.8–3.5)	14 (3–500)	42 (14–805)	14.5 (7–95)
ST2	16	1.4 (0.7–3.1)	15.5 (2–50)	32.5 (11–100)	16.2 (8–76)
ST (Total)	32	1.4 (0.7–3.5)	15 (2–500)	39.5 (11–805)	15 (7–95)
O1	20	1.6 (0.8–11)	10 (1.5–350)	29.5 (14–440)	14.2 (6–135)
O2	36	3.9 (0.6–180)	28 (3.5–1000)	80 (12–2100)	28 (8–420)
O (Total)	56	2 (0.6–180)	19.5 (1.5–1000)	60 (12–2100)	20 (6–420)
V1	5	1.2 (0.8–3.7)	7 (4.5–23)	19 (14–140)	8.5 (7.5–30)
V2	96	3.1 (0.7–180)	20.5 (2–1500)	60 (13–4800)	29.5 (7–500)
V (Total)	101	3.1 (0.7–180)	20 (2–1500)	60 (13–4800)	29 (7–500)
Total	189	2.2 (0.6–180)	19 (1.5–1500)	50 (11–4800)	20 (6–500)

ST, soft tissue metastases; ST1, ≤ three lesions in soft tissues; ST2, > three lesions in soft tissues; O, bone metastases; O1, ≤ three osseous segments involved; O2, > three osseous segments involved; V, visceral metastases; V1, limited involvement of one viscera; V2, diffuse involvement of one viscera or metastatic involvement of more than one viscera or simultaneous involvement of viscera and bone and/or soft tissues.
*12 patients in complete remission were excluded.
Mann-Whitney test:

	ST versus O	ST versus V	O versus V	ST1 versus ST2	O1 versus O2	V1 versus V2
CEA	P = 0.004	P = 0.0000	P = n.s.	P = n.s.	P = 0.009	P = n.s.
MCA	P = n.s.	P = n.s.	P = n.s.	P = n.s.	P = 0.04	P = 0.01
CA 15.3	P = n.s.	P = 0.02	P = n.s.	P = n.s.	P = 0.02	P = 0.04
CA 549	P = n.s.	P = 0.01	P = n.s.	P = n.s.	P = 0.01	P = 0.01

Table 3. Sensitivity of changes in marker levels to monitor clinical course in patients with abnormal baseline marker value

	Clinical response			
	PD	NC	PR/CR	Total
CEA	7/11 (64%)	1/7 (14%)	9/14 (64%)	17/32 (53%)
MCA	7/15 (47%)	2/8 (25%)	14/18 (78%)	23/41 (56%)
CA 15.3	11/15 (73%)	4/9 (44%)	15/21 (71%)	30/45 (67%)
CA 549	10/17 (59%)	1/7 (14%)	16/21 (76%)	27/45 (60%)
CEA+CA 15.3	10/16 (62.5%)	2/9 (22%)	13/23 (57%)	25/48 (52%)
CEA+MCA	11/17 (65%)	1/8 (12.5%)	15/23 (65%)	27/48 (56%)
CEA+CA 549	13/18 (72%)	0/9 (−)	15/24 (62.5%)	28/51 (55%)
MCA+CA 15.3	10/16 (62.5%)	1/9 (11%)	17/22 (77%)	28/47 (60%)
MCA+CA 549	11/17 (65%)	0/9 (−)	17/22 (77%)	28/48 (58%)
CA 15.3+CA 549	13/17 (76%)	1/9 (11%)	17/24 (71%)	31/50 (62%)
CEA+MCA+CA 15.3	9/17 (53%)	1/9 (11%)	15/24 (62.5%)	25/50 (50%)
CEA+MCA+CA 549	11/18 (61%)	0/9 (−)	16/25 (64%)	27/52 (52%)
CEA+CA 15.3+CA 549	12/18 (67%)	0/9 (−)	15/26 (58%)	27/53 (51%)
MCA+CA 15.3+CA 549	10/17 (59%)	0/9 (−)	17/24 (71%)	27/50 (54%)
CEA+MCA+CA 15.3+ CA 549	10/18 (56%)	0/9 (−)	15/26 (58%)	25/53 (47%)

PD, progression; NC, no change; PR, partial remission; CR, complete remission.
Chi square
Total: CA 15.3 versus MCA, P = 0.3; CA 15.3 versus CEA, P = 0.2.
Progression: CA 15.3 versus CA 15.3 + CA 549, P = 0.9.
Partial remission: CA 15.3 or CA 549 or MCA versus MCA + CA 15.3, $P > 0.7$.

Table 4. Specificity of changes in marker levels to monitor clinical course in patients with abnormal baseline marker value

	Clinical response	
	PD	PR/CR
CEA	17/21 (81%)	12/18 (67%)
MCA	22/26 (85%)	16/23 (70%)
CA 15.3	25/30 (83%)	19/24 (79%)
CA 549	21/28 (75%)	20/24 (83%)
CEA+CA 15.3	22/32 (69%)	15/25 (60%)
CEA+MCA	22/31 (71%)	14/25 (56%)
CEA+CA 549	18/33 (54%)	17/27 (63%)
MCA+CA 15.3	25/31 (81%)	15/25 (60%)
MCA+CA 549	22/31 (71%)	17/26 (65%)
CA 15.3+CA 549	22/33 (67%)	20/26 (77%)
CEA+MCA+CA 15.3	19/33 (57%)	12/26 (46%)
CEA+MCA+CA 549	19/34 (56%)	14/27 (52%)
CEA+CA 15.3+CA 549	19/35 (54%)	16/27 (59%)
MCA+CA 15.3+CA 549	22/33 (67%)	16/26 (62%)
CEA+MCA+CA 15.3+CA 549	19/35 (54%)	13/27 (48%)

PD, progression; PR, partial remission; CR, complete remission.
Chi square
Specificity for PD: MCA versus CA 549, $P = 0.3$.
Specificity for PR/CR: CA 549 versus CEA, $P = 0.2$.

Table 5. Sensitivity and specificity of changes in marker levels to monitor clinical course in patients with normal baseline marker value (only progression is evaluable in this group)

	Sensitivity	Specificity
CEA	8/19 (42%)	14/20 (70%)
MCA	8/15 (53%)	14/15 (93%)
CA 15.3	13/15 (87%)	7/7 (100%)
CA 549	9/13 (69%)	9/13 (69%)
CEA+CA 15.3	12/14 (86%)	3/9 (33%)
CEA+MCA	9/13 (69%)	6/10 (60%)
CEA+CA 549	8/12 (67%)	5/8 (62.5%)
MCA+CA 15.3	13/14 (93%)	6/10 (60%)
MCA+CA 549	10/13 (77%)	7/10 (70%)
CA 15.3+CA 549	12/13 (92%)	4/8 (50%)
CEA+MCA+CA 15.3	12/13 (92%)	2/8 (25%)
CEA+MCA+CA 549	9/12 (75%)	3/7 (43%)
CEA+CA 15.3+CA 549	11/12 (92%)	1/6 (17%)
MCA+CA 15.3+CA 549	12/13 (92%)	4/8 (50%)
CEA+MCA+CA 15.3+CA 549	11/12 (92%)	1/6 (17%)

Chi square
Sensitivity
CA 15.3 versus MCA, $P = 0.04$; CA 15.3 versus CEA, $P = 0.008$; CA 15.3 versus CA 549, $P = 0.25$.
CA 549 versus MCA, $P = 0.2$; CA 549 versus CEA, $P = 0.1$.
CA 15.3 versus MCA + CA 15.3, $P = 0.5$.
Specificity
CA 15.3 versus CA 549, $P > 0.09$.

Table 6. Positive predictive values of marker changes to monitor clinical course in patients with metastatic breast cancer

	Clinical response		
	Patients with abnormal baseline marker value		Patients with normal baseline marker value
	PD	PR/CR	PD
CEA	71%	53%	50%
MCA	69%	60%	85%
CA 15.3	76%	66.5%	100%
CA 549	64%	72%	62%
CEA+CA 15.3	59%	45.5%	48%
CEA+MCA	62%	46%	55.5%
CEA+CA 549	53%	50%	56%
MCA+CA 15.3	70%	53%	63%
MCA+CA 549	62%	56%	65%
CA 15.3+CA 549	70%	64%	59%
CEA+MCA+CA 15.3	48%	40%	47%
CEA+MCA+CA 549	50%	44%	49%
CEA+CA 15.3+CA 549	51%	45%	44.5%
MCA+CA 15.3+CA 549	56%	52%	57%
CEA+MCA+CA 15.3+CA 549	47%	39.5%	44.5%

PD, progression; PR, partial remission; CR, complete remission.

53% (CEA) to 72% (CA 549) in patients with PD or with objective remission, respectively. The specificity and the PPV progressively decreased when two or more markers were considered simultaneously.

Patients with normal baseline values

Data on sensitivity and specificity are reported in Table 5. In this subgroup, the sensitivity of correlation with PD was 42, 53, 87 and 69% for CEA, MCA, CA 15.3 and CA 549, respectively. CA 15.3 was significantly better than CEA ($P = 0.008$) or MCA ($P = 0.04$). Once again, the combination of two or more markers in this subgroup did not significantly increase the correlation between marker behaviour and clinical response. The specificity of marker serum level changes in relation to PD was 70, 93, 100 and 69% for CEA, MCA, CA 15.3 and CA 549, respectively (not significantly different). The PPVs (Table 6) were 50% (CEA), 84% (MCA), 100% (CA 15.3) and 62% (CA 549). Also in this subgroup of patients, the specificity and the PPVs were reduced by the combinations of two or more markers.

DISCUSSION

Many recent contributions are available in the literature on the sensitivity of serum tumour markers in metastatic breast cancer, and good reviews are also available [15]. Sensitivity of mucin-like glycoproteins, such as CA 15.3, MCA and CA 549, is reported to be in the order of 70–90%. The sensitivity and specificity of CA 15.3 were found to be superior to those of CEA [7, 10, 16]. Similarly, MCA [8, 17] and CA 549 [9, 18] were also reported superior to CEA in signalling patients with progression of breast cancer. Although similar levels of sensitivity were reported for CA 15.3 and MCA [19, 20] and for CA 15.3, MCA and CA 549 [21], in a relative operating-characteristics analysis examining the clinical applicability of CA 15.3, MCA

and CEA in metastatic breast cancer, the discrimination power of CA 15.3 was statistically superior to those of MCA and CEA [22].

The addition of CEA to CA 15.3 slightly increases (by approximately 7%) the sensitivity of the latter, and even if of statistically significant power [16], it is of doubtful clinical significance [23]. The utility of combinations of two or more mucin-like glycoproteins in the detection of metastatic breast cancer is uncertain. On the one hand, a study revealed that the combination of CA 15.3 and MCA seems to improve diagnostic sensitivity [24]; on the other, no advantage was obtained with the same combination [25] or in combining CA 15.3, MCA and CA 549 in another study [21]. No contribution on the simultaneous use of CEA, CA 15.3, MCA and CA 549 was found in the literature.

In the present study, two major issues concerning the utility of the use of a four serum marker panel in metastatic breast cancer were investigated: (i) the sensitivity of expression in metastatic disease; and (ii) the correlation of their changes with clinical response in patients monitored during antitumour treatment.

In the first part of the study, the four marker panel was examined in a series of 201 consecutive patients with clinically documented metastatic breast cancer regardless of whether they were on treatment, but knowing the status of the disease at that time. Our results confirm that the mucin-like glycoproteins have individually higher sensitivity than CEA in signalling metastatic breast cancer, with CA 549 and CA 15.3 having the highest sensitivity (81 and 80%, respectively). When the whole series of patients was considered, including patients with PD and also those who, at the time of examination, were on treatment with the disease not progressing, MCA had a lower sensitivity than CA 15.3 and CA 549. However, the difference was not statistically significant if only patients with PD were considered. The combination of two, three or all four markers presented a moderately higher level of sensitivity. In patients with PD, this increase in sensitivity compared to either CA 15.3 or CA 549 alone was 8% with the CEA + CA 15.3 + CA 549 panel, but was not statistically significant. In contrast, the panel CEA + CA 15.3 + CA 549 in the whole series improved the sensitivities of CA 15.3 and CA 549 alone by 11% ($P = 0.01$) and 10% ($P = 0.02$), respectively. We think that the results observed in patients with PD have more clinical relevance because one of the principal aims in the use of the markers is the early detection of recurrent disease in patients considered to lack evidence of disease.

Patients with ST lesions had mean marker levels lower than patients with O or V lesions. However, the differences were statistically significant for CEA (ST versus O and ST versus V) and for CA 15.3 and CA 549 (ST versus V), while the differences were not significant for MCA. The mean marker levels also statistically correlated with tumour burden in O (all four markers) and V (all but CEA) groups but never in the ST group. The arbitrary criteria of subdivision in the groups of prevalent metastatic disease in order to express different tumour burden proved to be reliable, at least in O and V groups. These findings are in accordance with those of other authors [16], who found disease extension the only parameter of significant correlation with CA 15.3 serum levels.

Currently, variations of tumour markers during treatment are not considered useable in the standard measurements of response to antitumour treatments. This is the consequence of non-optimal sensitivity and specificity in detecting metastatic breast cancer and the existence of paradoxical changes, particularly in the early phase of treatment [26, 27]. However, a correlation between response and marker changes has been reported for principal tumour markers. In particular, CA 15.3 was reported to be significantly correlated with disease response to treatment more than CEA [10, 14, 28]. A high level of concordance with clinical response was reported with MCA [17]. CA 549 substantiated clinical information and provided a potentially useful approach to monitoring metastatic breast cancer [29]. Very few data exist on the simultaneous use of two or more markers in monitoring clinical response.

In our study, 71 patients underwent a regular marker monitoring once a month during antitumour treatment. Comparing pretreatment values and intratreatment values at the time of first clinical evaluation (median at 3 months of treatment) the correlation between clinical response and marker changes was studied. We choose to consider as an "on treatment" value the marker serum level measured at the moment of clinical evaluation: under this operative condition, it is possible, in our opinion, to avoid the problem of possible paradoxical marker behaviour in the initial phases of antitumour therapy. The mean changes of all markers were in accordance with clinical response: a statistically significant decrease or increase occurred in the group of patients who experienced PD or CR/PR; at the same time no significant changes occurred in the group of patients who were classified NC.

In order to evaluate sensitivity and specificity of the correlation between marker changes and clinical response, patients were subdivided into two groups: those with abnormal and those with normal baseline values. In the latter group, only correlation with PD was considered evaluable. In patients with an abnormal baseline level, overall sensitivity of marker changes was between 53% (CEA) and 67% (CA 15.3). Compared to patients with NC (14% CEA, 14% CA 549, 44% CA 15.3) sensitivity of correlation was higher in patients with an objective response (64% CEA–78% MCA), and for patients with PD (47% MCA–73% CA 15.3). Any difference in sensitivity between the four markers was statistically significant. The low level of overall sensitivity of concordance with clinical response was at least partially due to the lack of precision in the clinical definition of NC. Using simultaneously two, three or four markers, the overall sensitivity of correlation with response progressively decreased. Specificity of correlation with PD in patients with elevated baseline values was quite similar for the four markers (75–85%), while in responsive patients, it was between 67% (CEA) and 83% (CA 549) without significant differences. Specificity progressively decreased if two or more markers were considered. The analysis of the positive predictive value of the tests demonstrated that marker changes correctly indicated disease progression in 64% (CA 549) to 76% (CA 15.3) of cases, and disease regression in 53% (CEA) to 72% (CA 549) of cases. The combination of two or more markers reduced the PPV of the tests.

When sensitivity of correlation with PD was calculated in patients with normal baseline values, CA 15.3 (87%) was significantly better than CEA (42%) and MCA (53%). Accordingly, the changes in CA 15.3 correctly indicated disease progression (PPV: 100%), better than the other markers. Using two, three or four markers simultaneously, the sensitivity of correlation with PD did not change, while the PPVs decreased. Specificity of correlation with PD was higher for CA 15.3 (100%), but no difference between markers was statistically significant. Specificity decreased if two or more markers were considered simultaneously.

In summary, this study confirms that the mucin-like glycoproteins CA 15.3 and CA 549 are useful in staging metastatic breast cancer with a sensitivity to detect the disease at approximately 80%, with a good correlation with the extent of the disease and prevalent metastatic site. Currently, the non-optimal level of sensitivity (60–65%) and specificity (75–80%) of the correlation with clinical response limits the use of these serum markers as criteria for clinical response evaluation, as confirmed by the analysis of PPVs. Specifically oriented studies should better explore this problem and verify, for example, whether subsets of metastatic disease exist for which tumour marker variations indicate their changes under treatment. A positive experience with CA 549 [29] and with a multiple marker panel, including CEA and CA 15.3 [30], was recently reported. In spite of the limitations observed in the monitoring of the disease, CA 15.3 demonstrated good performance in signalling disease progression in patients with normal basal values.

The most important finding of this study is the demonstration of a lack of clinical utility in the use of multiple marker panels including CEA, CA 15.3, MCA and CA 549 in the routine monitoring of metastatic breast cancer when compared to CA 15.3 or CA 549 alone. Considering the high cost of these tests, an implication of this study is that only a mucin-like marker (in our opinion CA 15.3 or as an alternative CA 549) should be used in standard monitoring of metastatic breast cancer.

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