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SERUM TUMOR MARKERS FOR DETECTION OF BONE METASTASIS IN BREAST CANCER PATIENTS

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For the diagnosis of bone metastasis in breast cancer patients during systemic treatment serum tumor markers, including carbohydrate antigens 15-3 (CA 15-3) and 19-9 (CA 19-9), cancer antigen 125 (CA 125), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), beta-2 microglobulin (BMG), ferritin, and tissue polypeptide antigen (determined by the M3 monocolonal antibody, TPS) were measured in 22 patients with known bone metastases and in 30 patients without documented metastases. The most useful single marker was CA 15-3. By stepwise discriminant analysis, it was found that 90% of the patients could be diagnosed truly by using the markers CA 15-3, BMG and ferritin. It is concluded that monitoring with combinations of tumor markers at regular intervals increases the diagnostic efficiency.

Bone metastases are common in breast cancer and develop in approximately 70% of patients with metastatic breast cancer (1). At autopsy bone metastases are found in more than half of the patients who die of breast cancer (2–4). Radiologic examination of the bones and radionuclide bone scan have for years been the standard methods for detection of bone metastases (5–7). Some studies suggest that serum tumor markers could play some role in the diagnosis of metastases (8). Osseous metastases often cause important morbidity as fractures (9, 10). The goal of treatment for these patients is basically palliation to improve the quality of life (11). An important practical question is whether and when patients during chemotherapy should be examined by imaging methods for detection of possible non-symptomatic bone metastases. This may be especially important in postmenopausal patients who often receive adjuvant endocrine treatment for 2 to 5 years. Serum tumor markers may be useful in these circumstances. Relatively little has been published regarding the choice of investigative methods for diagnosis of osseous metastases during systemic treatment (12). The present report is a study on the efficiency of single tumor markers and combinations of such markers in the diagnosis of bone metastases in breast cancer during systemic treatment.

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Material and Methods

Included in the study were 22 breast cancer patients with known bone metastases and 30 breast cancer patients without documented metastases. The characteristics of the patients are shown in Table 1. Patients without documented metastases were eligible for inclusion in this study if they had at least 2 courses of chemotherapy after the surgery. In patients with bone metastases the samples were also taken after at least two courses of i.v. chemotherapy (13). Blood samples were taken between the third and sixth course of chemotherapy at one time only, and the sample was taken one day before the chemotherapy course. Tamoxifen (20 mg/day) was administered to the postmenopausal patients, and during the study all of the postmenopausal patients had received tamoxifen for more than 12 weeks (14).

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In all cases blood samples were separated into plain and EDTA tubes and spun at rpm for 10–15 min within one hour after removal. The plasma or serum was then immediately placed into containers and stored at −35°C and was studied within two months.

Whenever blood samples were taken, a full clinical examination was carried out and chest x-rays and abdominal ultrasonography were performed. In patients with abnormal liver function tests but with normal abdominal ultrasonograpy, CT of the abdomen was performed. Before chemotherapy, bone scanning was performed in all patients. In patients with ^{99m}Tc pyrophosphate bone scan showing more than two hot spots and without non-malignant disorders accounting for these lesions skeletal radiological surveys were performed to confirm possible bone metastases.

Complete blood count, serum calcium, total protein, albumin, alkaline phosphatase, bilirubin, gammaglutamyl transpeptidase, aspartate aminotransferase, and creatinine were determined in all patients.

Serum tumor markers including carbohydrate antigen 19-9 (CA 19-9), carbohydrate antigen 15-3 (CA 15-3), cancer antigen 125 (CA 125), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), beta-2 microglobulin (BMG), and ferritin were measured with commercially available RIA kits, and tissue polypeptide antigen was determined using the M3 monoclonal antibody (TPS) (CA 15-3 and CA 19-9: CIS Bio International, Gif-Sur-Yuette Cedex, France; CA 12-5, AFP, ferritin: Abbott Laboratories, USA; CEA: Bio Merieux, Marcy-l'Etoile, France; BMG: Behringwerke AG, Merburg, Germany; TPS: Beki Diagnostics AB, Bromma, Sweden). All measurements were performed in duplicate, and the results were expressed in arbitrary units. The cut-off values of the markers were CA 19-9: 37 U/ml; CA 15-3: 30 U/ml; CA 125: 35 U/ml; AFP: 10 IU/ml; CEA: 10 ng/ml; BMG: 2.5 mg/l; ferritin: 120 ng/ml; TPS 80 U/l.

Statistical analysis. Before statistical analysis logarithmic transformation was performed for all tumor marker values to normalize the frequency distribution.

For comparison of mean values Student's t-test, for median values Mann-Whitney U test, and for ratios Yates' χ^2-test were used. Although discriminant analysis is most often employed to explain or describe factors that discriminate among groups of interest, the procedure can also be used to classify future subjects (15, 16). Classification involves the determination of a separate prediction equation corresponding to each group that gives the probability of belonging to that group. For classification of a future subject, a prediction is calculated for each group and the individual is classified as belonging to the group he or she most closely resembles (15, 16). To detect the combination of markers that could best differentiate the patients with and without bone metastases discriminant analysis was used. Due to the limited sample of patients we did not use the discriminant analysis in pre- and postmenopausal patients separately. Stepwise discriminant analysis was performed by using SPSS/PC+ package program.

Results

The results are shown in Tables 1–5 and Figs. 1 and 2. As renal function tests were normal in all patients (creatinine < 12 mg/l), corrections for serum BMG level were not performed (17). Except one case with liver metastasis, alkaline phosphatase and liver enzyme levels were within the normal ranges in all patients. In one patient with liver metastasis gammaglutamyl transpeptidase level was elevated. There was no significant differences between the metastatic and non-metastatic cases as regards blood counts, liver function tests, renal function tests, total protein, albumin and calcium levels ($p > 0.05$).

Table 1

Patients' characteristics

Variable	Bone metastases	
	Present	Absent
Number of patients	22	30
Age		
Mean ± SD	46 ± 11	52 ± 11
Premenopausal/ postmenopausal	9/13	17/13
Previous treatment		
Surgery (breast)	22	0
RT (breast)	21	0
Systemic treatment		
Chemotherapy	18	0
Endocrine treatment (tamoxifen)	13	0
Therapy during the study		
Surgery (breast)	0	30
Surgery (skeleton)	0	0
RT (breast)	0	10
RT (skeleton)	13	0
Systemic treatment		
Chemotherapy	21	23
Endocrine treatment (tamoxifen)	13	13
Time to beginning of chemotherapy (mean weeks ± SD)	14.6 ± 3.3	14.8 ± 3.7
Time to beginning of endocrine treatment (median weeks)	48	18
Sites of metastatic involvement		
Lung	6	0
Lung and brain	1	0
Brain	1	0
Liver and brain	1	0

Table 2

Analysis of tumor marker levels with respect to bone metastases [a]

Markers	Non-metastatic patients	Metastatic patients	p-value	Specificity %	Sensitivity %	AE [b] %
	Median (n)	Median (n)				
CA 15-3 U/ml	22.6 (30)	43.5 (22)	0.001	73	73	73
BMG mg/l	2.75 (30)	3.95 (22)	0.002	43	86	69
Ferritin ng/ml	34 (30)	115 (22)	0.001	87	50	71
TPS U/l	69 (30)	110 (20)	0.02	43	75	56

[a] The levels are median values. The analysis was performed with the Mann-Whitney U-test. The non-significant markers are not written.
[b] Actual efficiency.

Table 3

Relation between tumor marker levels and occurrence of bone metastases analyses by stepwise discriminant analysis (n = 52)

Markers	Wilks' lambda	Significance p-value	Specificity %	Sensitivity %	AE %
CA 15-3	0.63	< 0.001			
BMG	0.46	< 0.001			
Ferritin	0.42	< 0.001			
			97	82	90

The non-significant markers are not included.

Table 4

Fisher's linear discriminant functions

Markers	Non-metastatic A	Metastatic B
M1: CA 15-3	a1: 14.7	b1: 20.4
M2: BMG	a2: 29.5	b2: 40.1
M3: Ferritin	a3: 7.7	b3: 9.9
(constant)	C1: −22.9	C2: −41.4

Function A = (a1 × log M1) + (a2 × log M2) + (a3 × log M3) + (C1)
Function B = (b1 × log M1) + (b2 × log M2) + (b3 × log M3) + (C2)

An example of a case: The real group is the 'non-metastatic group'.

Marker	Level	LogM	Function A	Function B
M_1: CA 15-3	13.7 U/ml	1.14		
M_2: BMG	2.9 mg/l	0.46		
M_3: Ferritin	34.2 ng/ml	1.53		
			19.2	15.5
			Function A >	Function B

As function A is larger than function B, this case is in group A (non-metastatic group).

Analysis of the tumor marker levels with respect to bone metastases is shown in Table 2. Concerning CEA, CA 125, CA 19-9 and AFP there were no statistically significant differences between the metastatic and non-metastatic cases. CEA levels were elevated in 10 patients (46%) with metastases and in 11 patients (37%) without metastases. The median in the former group was 8.95 ng/ml and the latter 6.8 ng/ml ($p > 0.21$). CA 125 was elevated in 6 patients (27%) with metastases and in one of the patients (0.03%) without metastases. The median in the former group was 16.55 U/ml and in the latter group 12.4 U/ml ($p > 0.26$). CA 19-9 levels were elevated in 10 patients (46%) with metastases and in 8 patients (27%) without metastases. The median of the former was 35.4 U/ml and of the latter 23.5 U/ml ($p > 0.13$). AFP levels were elevated in 2 patients (9%) with metastases and in none of the patients without metastases. The median of the former was 4.15 IU/ml and of the latter 2.3 IU/ml ($p > 0.32$) (Figs. 1 and 2).

Table 5

The diagnostic value of tumor markers alone or in combination in relation to occurrence of bone metastases

CA 15-3 >30 U/ml	BMG >2.5 mg/l	Ferritin >120 ng/ml	Specificity	%	Sensitivity	%	AE
+	−	−	22/30	73.3	16/22	72.7	73
−	+	−	14/30	46.7	19/22	86.4	63
−	−	+	26/30	86.7	11/22	50.0	71
+	+	−	26/30	86.7	13/22	59.0	75
−	+	+	27/30	90.0	11/22	50.0	73
+	−	+	27/30	90.0	7/22	31.8	65
+	+	+	2/30	6.7	7/22	31.8	17

AE: Actual efficiency; + and −: indicate whether the mode of investigation in question was taken into account in calculating the predictive values.

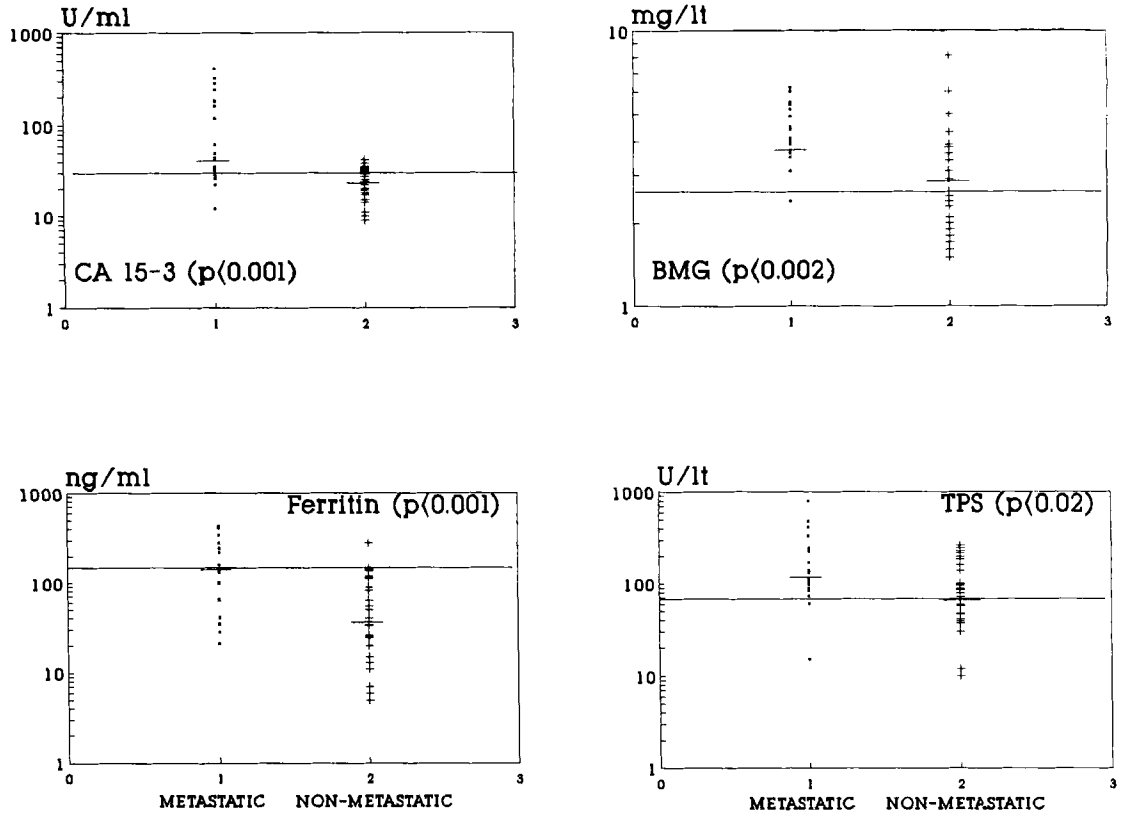

Fig. 1. Cut-off levels (long horizontal lines), medians (short horizontal lines) and serum levels of the markers CA 15-3, BMG, ferritin and TPS (statistical analysis was performed with Mann-Whitney U test).

When stepwise discriminant analysis was used for detection of the most significant tumor markers for diagnosis of bone metastases, CA 15-3, BMG and ferritin were found to be the most useful markers when they were combined ($p < 0.001$). When three markers were used together, sensitivity, specificity and actual efficiency (AE: overall percentages of cases that were correctly classified) were 82%, 97% and 90% respectively (Table 3 and 4).

The diagnostic value of CA 15-3, BMG and ferritin alone or in combination with respect to their cut-off values, in relation to occurrence of bone metastases is shown in Table 5. In 22 patients (100%) with bone metastases and in 18 (60%) of the patients without metastases one or both of the CA 15-3 and BMG levels were elevated (AE: 65%). In 17 patients (77%) with bone metastases and in 6 patients (20%) without metastases, any two of these three marker levels were higher than the cut-off values (AE: 79%). In 22 patients (100%) with bone metastases and in 21 patients (70%) without metastases one, two or three of these marker levels were elevated (AE: 60%).

When the patients were grouped as premenopausal ($n = 26$) and postmenopausal ($n = 26$) and analyzed by the

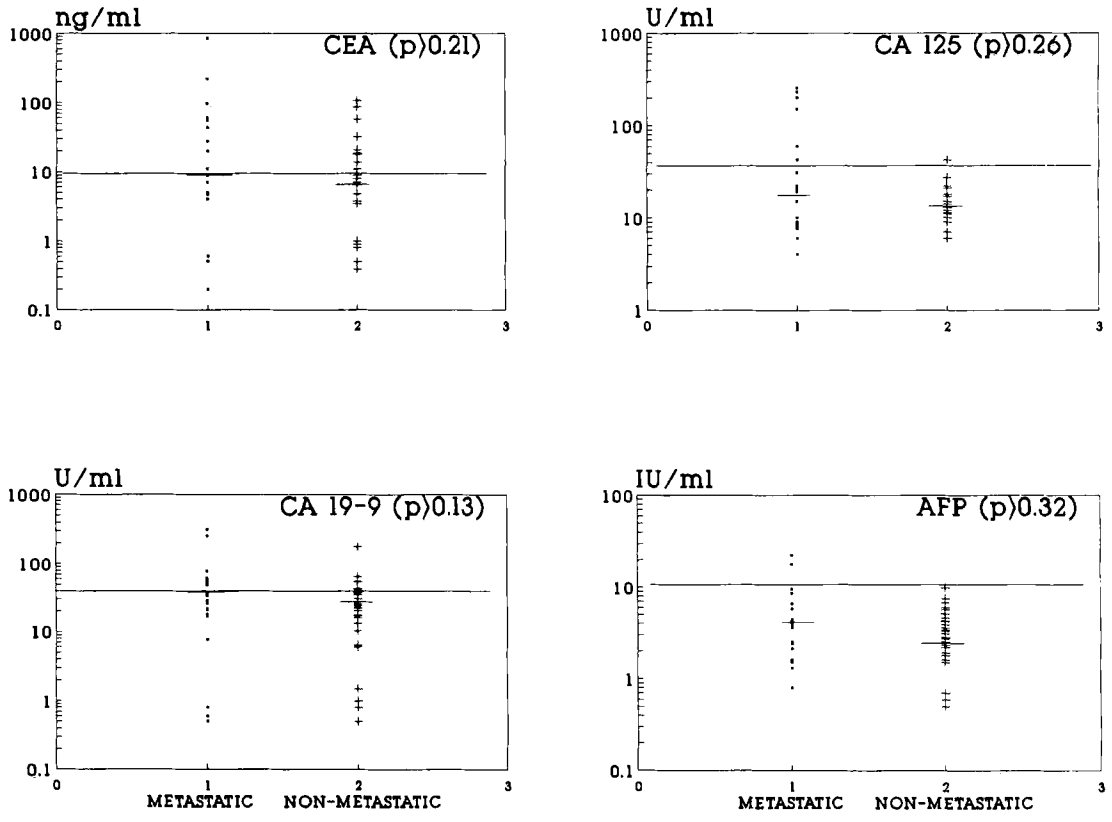

Fig. 2. Cut-off levels (long horizontal lines), medians (short horizontal lines) and serum levels of the markers CEA, CA 125, CA 19-9 and AFP (statistical analysis was performed with Mann-Whitney U test).

Mann-Whitney U test, only ferritin levels were found to be significantly higher in postmenopausal patients ($p < 0.02$). In postmenopausal patients only CA 15-3 showed higher levels in patients with bone metastases than in non-metastatic patients ($p < 0.001$). With respect to the cut-off values specificity, sensitivity and AE were 69%, 85% and 77% respectively. In premenopausal patients the serum levels of ferritin, BMG, TPS and CA 15-3 levels were higher in patients with bone metastases ($p < 0.001$, $p < 0.004$, $p < 0.015$, $p < 0.021$; AE: 85%, 69%, 69% and 69% respectively).

Discussion

Detection of metastatic disease in patients with breast cancer might be important for choice of adequate therapy and simplified detection of metastases could facilitate the care of the patients. Serum tumor markers, including CEA, AFP, CA 15-3, CA 19-9, CA 125 and TPA have been established and used for the detection of carcinomas (18–20). CA 15-3 which is considered as a specific marker of breast cancer has been considered as particularly valuable for detection of bone metastases. It has been suggested that the CA 15-3 levels are related to bulk of the disease (21). BMG may reflect the tumor burden and during follow-up, occult tumor cells may activate the immune system (22, 23). Ferritin is an acute phase protein rising in patients with advanced disease and ferritin concentrations have been found to be significantly higher in patients with advanced breast cancer than in tumor-free control patients (24). TPS seems to indicate the rate of proliferation rather than the tumor burden (25, 26). In the present study all these four markers were found to be valuable for the diagnosis of bone metastases.

Each tumor marker may play an independent biological role (27). Changes in tumor marker levels are a reflection of cytolysis, altered production, secretion and clearance factors and all these factors may be altered by treatment. Different tumor cells have different properties to home to specific sites (28). The sites of the metastases may thus influence the alterations of tumor marker levels. It has previously been reported that the sensitivity of CA 15-3 is greater than that of CEA in patients with only local metastases and in patients with only bone metastasis (21). In the present study, 41% of the patients with bone metastases also had metastases in other organs, which possibly contributed to the tumor marker results.

The results suggest that CA 15-3, BMG and ferritin are markers of tumor burden and that a combination of these three markers may be valuable in the diagnosis of bone metastases during therapy. If only one marker had been used, the highest AE would be 73%, and if the efficiencies

of combinations of these markers with respect to their cut-off values were calculated, the highest AE would be 79%. This was the value obtained when any two or three markers were higher than their cut-off levels. By stepwise discriminant analysis it was shown that 90% of the patients who had bone metastases could be identified by using these three markers. As this AE was not dependent on the cut-off values of the markers, it suggests that determining the serum tumor markers at regular intervals is especially efficient for the diagnosis of bone metastases. It also suggests that tumor marker values lower than the cut-off levels can be useful for the detection of metastases (Table 4).

When the patients are analyzed with respect to the menopausal status CA 15-3 was the only single marker that showed statistically significant differences between the metastatic and non-metastatic cases. In the present study, one of the main differences between premenopausal and postmenopausal patients was in the systemic treatment, since tamoxifen had been administered to all of the postmenopausal patients. Effects of the tamoxifen on the metastatic cells could be the reason for the different tumor marker levels in the two groups. This subject should be studied further.

These results suggest that multiple factors influence the serum marker levels. As each tumor marker probably has an independent biologic significance, combination of all the tumor markers at regular intervals will increase the diagnostic efficiency. A useful mathematical formulation for the diagnosis of metastases might be constructed by using stepwise discriminant analysis (Tables 3 and 4). In order to establish a general formulation, the normal ranges of tumor marker measurements must be the same in all laboratories. However, the present as well as previous ones shows that there is a wide overlap of the values between the study groups. This causes problems in the use of tumor markers at an individual level. Tumor markers will certainly not replace the imaging methods but might, by using some formulation, be useful as guidance for recommending imaging methods during the follow-up.

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