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# Correlation Between Changes in the Tumour Markers CA-M26 and CA-M29 and Standard Response Evaluation in Patients with Metastatic Breast Cancer

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**In this study we correlated response evaluated by standard WHO criteria to strict defined criteria of tumour marker response in 63 patients with metastatic breast cancer. Pretreatment sensitivity at first evaluation was 71% and 85% for CA-M26 and CA-M29, respectively. Of the 156 evaluations for CA-M26 and 178 for CA-M29 in 26 and 30 patients with evaluable lesions 72% and 67% were concordant with the results of the clinical evaluations. When the discordant evaluations due to lead time were included the concordances were 87% for CA-M26 and 83% for CA-M29. Of the 70 evaluations for CA-M26 and 92 for CA-M29 in 19 and 24 patients with non-evaluable lesions 59% and 72% were concordant with the results of the clinical evaluations. Most importantly, progressive disease according to the changes in the marker level nearly always predicted disease progression. Such knowledge obtained in a simple way may prevent continuation of ineffective treatment in patients with metastatic breast cancer.**
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## INTRODUCTION

A RELIABLE EVALUATION of response in patients with metastatic disease is important since continuation of a potentially toxic treatment in case of disease progression is not in the benefit of the patient. In patients with metastatic breast cancer the evaluation of response is frequently hampered by the lack of evaluable lesions in more than 50% of the patients. Especially in patients with bone metastases, regression or progression is very hard to ascertain even with nuclear bone scans or X-rays. Decisions to continue treatment or not are often based on subjective considerations.

Although a number of studies have investigated the role of serum tumour markers for disease monitoring, virtually none define criteria for marker response and or correlate these with response assessments according to standard WHO criteria [1–2]. In most longitudinal studies the results are often presented as case reports or as a three point analysis [3–5].

In the present study it was investigated whether two newly developed markers, CA-M26 and CA-M29, could be used for disease monitoring in patients with metastatic breast cancer. The results of response evaluations according to standard WHO criteria [6] were compared with strictly defined criteria of response evaluation by the two markers.

## MATERIALS AND METHODS

CA-M26 and CA-M29 serum levels were retrospectively analysed and subsequently compared with the results of the clinical evaluations of response in a group of 63 patients with metastatic breast cancer, who underwent either hormonal treatment or chemotherapy. In these patients blood samples for determination of CA-M26 and CA-M29 serum levels were collected at

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each visit, usually at 4–6 week intervals. The blood samples were stored at −70°C until analysis.

First-line chemotherapy in this group of patients consisted of cyclophosphamide 100 mg/m² orally days 1–14, methotrexate 40 mg/m² intravenously (i.v.) on days 1 and 8, and 5-fluorouracil 600 mg/m² i.v. on days 1 and 8 every 4 weeks for a maximum of six cycles. Second-line chemotherapy consisted of either doxorubicin 60 mg/m² i.v. every 3 weeks or mitoxantrone 10–14 mg/m² every 3 weeks. First-line hormonal treatment consisted of tamoxifen 20 mg twice a day and second-line hormonal treatment consisted of megestrolacetate 160 mg once daily or aminogluthetimide 250 mg twice a day with or without hydrocortisone.

In patients with evaluable lesions response was assessed according to standard WHO criteria. The clinical response assessment was done without knowledge of any tumour marker level. A theoretical advantage of serum tumour markers is that they may reflect the total three-dimensional body tumour load. Therefore the following response criteria for the tumour markers were used: complete response: normalisation of an elevated marker for at least 1 month; partial response: decrease of 65% or more of an elevated marker for at least 1 month; stable disease: less than 65% decrease or less than 40% increase of an elevated marker; progressive disease: more than 40% increase of an elevated marker level or a rise from below to above the cut-off level.

When both methods of evaluation yielded the same result at the same time the evaluation was called concordant.

*CA-M26 and CA-M29 assay*

Serum CA-M26 and CA-M29 concentrations were determined using a immunoassay kit (Genetic Systems Corp., Seattle, Washington, U.S.A.), which are based on the method described by Linsey *et al.* [7]. In short, on microtitre plates the microwells are coated with a monoclonal antibody to the cancer associated mucin epitope (M26 and M29). In the assay, after incubation with the sample, an enzyme-labelled second monoclonal antibody (M38 in both test systems) is used as a tracer. The antigen concentration is approximated with the help of two standard points. Cut off levels used in this study are 77 U/ml for CA-M26 and 15 U/ml for CA-M29 [8]. The coefficients for intra-assay variation were in the range of 3–10% and 7–11% for the inter-assay variation, except for the lowest CA-M26 concentration tested [9].

## RESULTS

*Sensitivity*

Additional clinical characteristics are listed in Table 1. The number of patients with an elevated CA-M26 and/or CA-M29 at first evaluation of the markers is listed in Table 2. The overall sensitivity for both markers was 89%. In 7 patients neither one of the markers was elevated. 4 of these patients had a limited tumour load such as only local regional disease or a limited number of skin metastases. Only 3 patients with extensive metastases had normal values for both markers. Median CA-M26 was 110 U/ml (range 4.3–76.121) and median CA-M29 was 28 U/ml (range 8–1287) for all patients. A significant correlation was observed between the two studied markers ($r = 0.56$, $P < 0.001$). A scatter diagram is depicted in Fig. 1.

*Table 1. Patients' characteristics*

| | |
|---|---|
| No. patients | 63 |
| Median age (years) | 55 |
| Range | 27–82 |
| Evaluable patients | 39 |
| Non-evaluable patients | 24 |
| Sites of metastases | |
| Bone | 30 |
| Liver | 5 |
| Lung and pleura | 11 |
| Loco-regional disease | 24 |
| Other | 16 |

*Table 2. Sensitivity of CA-M26 and CA-M29 in patients with metastatic breast cancer*

| | CA-M26 positive | CA-M26 negative |
|---|---|---|
| CA-M29 positive | 43 (68%) | 11 (17%) |
| CA-M29 negative | 2 (3%) | 7 (11%) |

*CA-M26 during follow-up in evaluable patients*

In 26 patients with an elevated CA-M26 and evaluable lesions 156 evaluations for response were performed. The concordance between the results of the clinical evaluations according to WHO criteria and the changes in tumour marker according to the above mentioned criteria was 72%.

Seventeen of the 44 discordant evaluations could be explained by positive lead time which means that the change in the tumour marker preceded the results obtained by the clinical evaluation by 1 or 2 months. A negative lead time, which means that the change in the tumour marker followed the results obtained by the clinical evaluation by 1 or 2 months, was observed on six occasions. In all these 6 cases the clinical evaluation was partial response while the tumour marker met the criteria for partial response 4–8 weeks later.

Four evaluations yielded a partial response by the marker and stable disease by the clinical evaluation.

In 1 patient with clinical stable disease the marker indicated progression (three evaluations).

With 14 evaluations the clinical response was progressive disease and the marker response stable disease. In seven of these

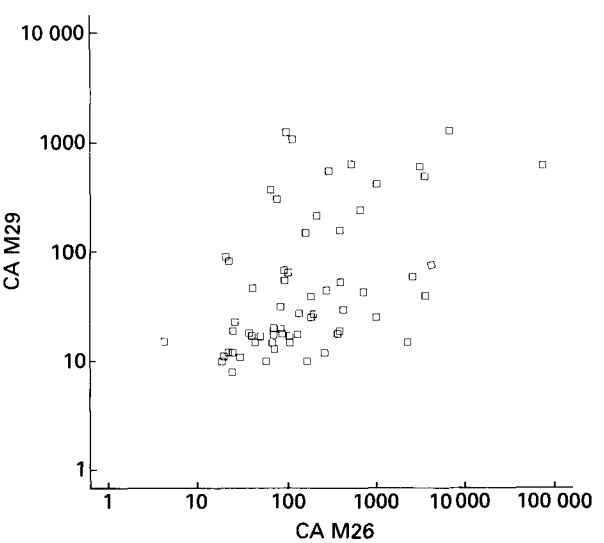


**Fig. 1. Scatterdiagram of CA-M26 vs. CA-M29.**

evaluations the clinical evaluation was based on progression of a local regional recurrence or progression of skin metastases mostly in patients with only slightly elevated marker levels. In 2 patients with clinical progressive disease (two evaluations) there was an increase in the marker level but not sufficient to meet the criteria set for marker progression. With five evaluations the clinical progression was based on the appearance of new lesions such as the development of pleural effusion or a new bone lesion. It seems obvious that in a patient with extensive metastatic disease the appearance of a small new lesion does not represent a substantial increase in total tumour load.

*CA-M29 during follow-up in evaluable patients*

In 30 patients with an elevated CA-M29 and evaluable lesions 178 evaluations for antitumour reponse were performed. The concordance between the results of the clinical evaluations according to WHO criteria and the changes in tumour marker according to the earlier mentioned criteria was 67%.

Fourteen of the discordant 59 evaluations could be explained by a positive lead time. A negative lead time was observed on 16 occasions most often in patients with a clinical partial response and stable marker levels. Twelve evaluations yielded a partial response by the marker and stable disease by the clinical evaluation.

On one occasion in a patient with a recent change of therapy and clinical stable disease during the first month after start of treatment an increase in the marker level occurred sufficient enough to meet the criteria for progression after which the marker again returned to the previous level (surge phenomenon). Interestingly, the CA-M26 level in this patient remained stable.

In 1 patient the marker indicated progression and the clinical evaluation remained as stable disease.

With 14 evaluations the clinical response was progressive disease and the marker response stable disease. Seven of these 14 evaluations concerned patients with a limited tumour load such as a local regional recurrence or skin metastases. Of the seven remaining discordant evaluations on four occasions the marker level increased when the clinical progression was documented but not sufficient enough to meet the criteria for marker progression.

In 1 patient with clinical progressive disease the CA-M29 evaluation revealed a partial response, while at the same time the evaluation of CA-M26 indicated progression. Probably the tumour of this patient consisted of at least two subpopulations of which the CA-M29-positive population responded to treatment and the CA-M26-positive population progressed.

*CA-M26 during follow-up in non-evaluable patients*

In 19 patients with an evaluated CA-M26 and non-evaluable lesions 76 evaluations for anti-tumour response were performed. Since these patients had non-evaluable lesions clinical response was mostly recorded as either stable disease or progression. Clinical progression was based on an increase of hotspots on bone scans, an increase of effusions, appearance of new lesions or an increase of pain.

A concordance between the results of the clinical evaluations and the changes in the tumour marker was found in 55% of the evaluations. On seven occasions the discordance could be explained by a positive or negative lead time of the marker. With 21 evaluations the marker response was stable disease while clinically disease progression was assumed. Clinical progression was based on an increase of bone pain with seven evaluations, progression of hot spots on the bone scan with eight evaluations and a progression of a pleural effusion with one evaluation.

Progression of the marker with clinically stable disease was observed three times and a partial response of the marker with clinically stable disease also on three occasions.

*CA-M29 during follow-up in non-evaluable patients*

In 23 patients with an elevated CA-M29 and non-evaluable lesions, 98 evaluations for anti-tumour response were performed. A concordance between the results of the clinical evaluations and the changes in the tumour marker was found in 72% of the evaluations. Eight of the 27 discordant evaluations could be explained by a positive or negative lead time of the marker. With 13 evaluations the marker response was stable disease while clinically disease progression was assumed. Clinical progression was based on an increase of bone pain with four evaluations, a progression of a pleural effusion with two evaluations and progression of a local-regional recurrence with also one evaluation. A clinical response was recorded with five evaluations based on regression or diminishing of effusions while the marker response remained stable. These patients had also bone metastases which probably represented the bulk of the tumour load. A partial response of the marker and clinical progressive disease was observed on one occasion. This patient had received radiotherapy for painful bone metastases which might be an explanation for the decrease in the marker level.

## DISCUSSION

A number of serum tumour markers have been developed in recent years for patients with breast cancer [10]. Most of these markers are concerned with glycoproteins which can be found in the serum of patients with advanced adenocarcinomas of various origins. Due to a lack of specificity and/or sensitivity most of these markers are not useful for screening, for diagnosis, for establishing prognosis or for early detection of a relapse [1].

Only a limited number of studies have investigated the role of serum tumour markers for monitoring therapeutic response. If a serum tumour marker is suitable for monitoring therapeutic response the level of the marker should be a reflection of the total body tumour load, all marker-producing tumour cells should produce the marker at a constant rate and the amount of the markers produced should be reliably reflected in the serum marker level.

Several problems can be easily recognised when marker changes are correlated to a WHO response assessment. First of all the assessment of response according to WHO criteria is done by a bidimensional measurement of one or more marker lesions whereas the serum marker level is supposed to represent three-dimensionally the total tumour load. Therefore,we adapted the criteria of marker response in such a way that we were able to compare the changes in marker levels with the response evaluation by bidimensional measurements. It is, however, obvious that a conventional bidimensional measurement according to standard WHO criteria is also only a crude estimation of the total tumour load since the margins of a tumour lesion are usually not defined by the product of the largest perpendicular diameters. Other pitfalls are that changes of an evaluable marker lesion when the bulk of the disease is represented by non-evaluable lesions may not give a good reflection of the changes of the total tumour load. Finally, the appearance of a new lesion, which by definition is disease progression according to the WHO criteria, does most often not imply a 25% increase of the total tumour load.

In this study we observed a concordance between the response evaluated by standard WHO criteria and response evaluation by

*Table 3. Discordant evaluations of CA-M26 in 26 patients with evaluable lesions*

| | |
|---|---|
| Total number of evaluations | 156 |
| Number of discordant evaluations | 44 |
| Positive lead time | 17 |
| Negative lead time | 6 |
| Partial response marker/stable disease according WHO | 4 |
| Progressive disease marker/stable disease according WHO | 3 |
| Stable disease marker/progressive disease according WHO | 14 |
| Only loco-regional disease | 7 |
| Insufficient rise of the marker level to meet the criteria set for disease progression | 2 |
| Appearance of new lesions | 5 |

*Table 4. Discordant evaluations of CA-M29 in 30 patients with evaluable lesions*

| | |
|---|---|
| Total number of evaluations | 176 |
| Number of discordant evaluations | 59 |
| Positive lead time | 14 |
| Negative lead time | 16 |
| Partial response marker/stable disease according WHO | 12 |
| Progressive disease marker/stable disease according WHO | 1 |
| Partial response marker/progressive disease according WHO | 1 |
| Marker surge | 1 |
| Stable disease marker/progressive disease according WHO | 14 |
| Only loco-regional disease | 7 |
| Insufficient rise of the marker level to meet the criteria set for disease progression | 4 |

changes in the levels of CA-M26 and CA-M29 of 72% and 67%, respectively, in patients with evaluable disease. When the number of discordant evaluations due to lead time are regarded as concordant then these percentages are 87% for CA-M26 and 83% for CA-M29. Possibly if other tumour marker response criteria had been used a different percentage of concordance would have been found. Further studies are certainly necessary to define the most optimal criteria which give the best correlation between the standard WHO criteria and the changes in the tumour marker. Besides lead time for most discordant observations plausible explanations could be found such as listed in Tables 3 and 4. Interestingly, a marker surge was only observed on one occasion. The incidence of a marker surge is probably higher when more frequent determinations are done in the first days or weeks after start of a new treatment [11].

The percentage of concordant evaluations between response evaluation by CA-M26 and CA-M29 and clinical response evaluation in patients with non-evaluable lesions was more or less the same as for patients with evaluable lesions. Most importantly, progressive disease according to the changes in tumour marker level almost always predicted disease progression. Such an evaluation obtained in a simple way, which does not burden the patient, may prevent continuation of ineffective treatment. Further studies are warranted to investigate whether monitoring disease activity by one or a panel of selected tumour markers in combination with patient history and physical examination can safely replace response evaluation by conventional time-consuming and burdening methods.

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