Original Article

Significance of Serum Tumor Markers in Monitoring Advanced Breast Cancer Patients Treated with Systemic Therapy: A Prospective Study

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***Objective*:** The significance of serum tumor markers in monitoring advanced breast cancer patients is still controversial. To clarify this issue, the Tumor Marker Study Group of the Japanese Breast Cancer Society conducted a prospective study.

***Methods*:** Patients with advanced breast cancer who were treated with systemic therapy between January and December 2002 were recruited from five collaborative institutes in Japan. The patients were monitored every four weeks using three serum tumor markers, CEA, CA 15-3 and NCC-ST-439 during the therapy.

***Results*:** Findings from 108 eligible patients were analyzed. The pretreatment positivity rates were 51.9% for CEA, 50% for CA 15-3, and 34.3% for NCC-ST-439. The changes in each marker level at 8 and 12 weeks but not at 4 weeks after the start of therapy seemed to correlate with the response to therapy in pretreatment marker-positive patients but not in negative patients. The Cox proportional hazard model revealed a greater than 20% reduction in CEA, CA 15-3 or NCC-ST-439 levels at 4, 8 and/or 12 weeks after the start of therapy to be an independent predictive factor for longer time-to-progression (TTP) in pretreatment marker-positive patients.

***Conclusion*:** This prospective study supported the findings obtained from our previous retrospective study that in pretreatment marker-positive patients 1) the changes in serum tumor marker levels after the start of therapy correlate with the response to therapy; and 2) a greater than 20% reduction in the tumor marker levels was a favorable predictive factor for TTP during systemic therapy. When the pretreatment serum level of these markers is over the respective cut-off value, sequential measurement of them may be useful for evaluating the efficacy of treatment as well as monitoring the outcome of patients with advanced breast cancer.

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Key words: Breast cancer, CEA, CA 15-3, NCC-ST-439, Time-to-progression

The Clinical Guidelines of the American Society of Clinical Oncology[1] indicate that routine measurement of serum tumor markers such as CEA and CA 15-3 is not recommended to monitor the outcome of breast cancer patients because of a lack of scientific evidence showing clinical benefit. However, our previous questionnaire survey revealed that various serum tumor markers such as CEA, CA 15-3 and NCC-ST-439 are routinely measured by a majority of breast cancer experts in Japan[2]. Therefore, to clarify the significance of measuring serum tumor markers in patients with breast cancer, the Tumor Marker Study Group was organized by the Japanese Breast Cancer Society in June 2001.

This group previously conducted a large-scale retrospective study and found that changes in serum CEA and CA 15-3 levels correlated with the response to systemic therapy. A greater than 20% reduction in serum CEA or CA 15-3 levels during

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Abbreviations:
CEA, Carcinoembryonic antigen; TTP, Time-to-progression; CR, Complete response; PR, Partial response; NC, No change; PD, Progressive disease

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therapy was an independent predictive factor for longer time-to-progression (TTP) in tumor marker-positive patients with advanced breast cancer[3]. These findings suggest that the measurement of serum CEA and CA 15-3 levels is useful for monitoring patients with advanced breast cancer whose pre-treatment marker levels are over their respective cut-off values.

To clarify these results obtained from the retrospective study, the Tumor Marker Study Group conducted the present study. Patients with advanced breast cancer were recruited and monitored every four weeks using three serum tumor markers, CEA, CA 15-3 and NCC-ST-439, during various systemic therapies. Because TTP is recognized as one of most important parameters of clinical benefit obtained from systemic therapies in patients with advanced breast cancer, the primary endpoint of this study was defined as the relation between the change in tumor marker levels and TTP.

Patients and Methods

Patients

Patients were eligible for this study if they had locally advanced and/or metastatic breast cancer with at least one lesion assessable according to UICC criteria[4]. Patients were treated between January and December 2002, with systemic therapies including chemotherapy, endocrine therapy and/or trastuzumab. All patients were informed of the aims of this study and signed a consent form approved by the respective institutional review board before starting the therapies.

A total of 133 patients with advanced breast cancer were registered for this study from five institutes (Kumamoto City Hospital, Kawasaki Medical School, Osaka City University Medical School, Hyogo Prefecture Hospital for Adult Diseases, and Osaka University School of Medicine). Of these, pretreatment serum CEA was not measured in six patients, at least one of three markers was not sequentially measured during therapy in 17 patients, and the response to therapy could not be evaluated because the follow-up time was too short in two patients. These 25 patients were excluded from this study according to the study protocol. This paper reports on 108 patients who had sequential tumor marker measurements, which were compared with their clinical outcome.

Measurement of Tumor Markers

Serum CEA levels were measured by a CEA-RIA MAb kit (Abbott Laboratories, Inc., Chicago, IL, USA) or a CEA-CLIA kit (Sysmex, Co., Ltd., Kobe, Japan). Serum CA 15-3 levels were measured by a Centocor CA 15.3 RIA kit (Fujirebio Diagnostics Inc., Malvern, PA, USA), and serum NCC-ST-439 levels were measured by a Lanazyme ST-439 plate (Nippon Kayaku Co., Tokyo, Japan). Blood samples were transferred to commercial laboratories authorized by the Ministry of Health, Labor and Welfare in Japan and assayed. Quality control for the measurement of these markers was strictly performed in each laboratory. The coefficients of intra- and interassay variations for three markers in each laboratory were less than 10%. Samples above the standard curve were retested with appropriate dilutions. According to the laboratories' recommendation, pretreatment tumor marker-positive patients were defined as patients with pretreatment CEA levels greater than 5 ng/ml, CA 15-3 levels greater than 30 units/ml or NCC-ST-439 levels greater than 7 units/ml.

Response to Therapy and Blood Sampling

Clinical assessment of therapeutic response was performed every four weeks after the start of treatment or earlier if clinically indicated and classified according to the UICC criteria into four categories: complete response (CR), partial response (PR), no change (NC), and progressive disease (PD). Tumor markers were measured using blood samples obtained within a week before the start of systemic therapies and subsequently every four weeks during therapy. To investigate changes in tumor marker levels, the percentage of marker levels when response was assessed was calculated as follows: (marker level at time of assessment/pretreatment marker level) $\times$ 100

When evaluating changes in the percentages of tumor marker levels after the start of therapy, an increase or decrease of greater than 20% was considered significant because the coefficient of assay variations for CEA, CA 15-3, and NCC-ST-439 were less than 10%.

Statistical Analysis

Numerical variables were expressed as the mean $\pm$ SD or the median and ranges. Differences among groups in continuous variables were assessed using ANOVA. Differences among groups in terms of frequency were assessed using

cross-tabulation tables, and chi-square analysis or Fisher's exact test. TTP curves were generated using the Kaplan-Meier method and analyzed by the Mantel-Cox logrank test. Two-sided p values less than 0.05 were regarded as statistically significant. All calculations were made using StatView computer software (ATMS Co., Tokyo, Japan).

To investigate the relation between changes in the marker levels and TTP, tumor marker-positive patients were divided into three groups, D, N, and U according to our previous study[3]. In groups D, N, and U, the percentages of the marker levels when responses were assessed were less than 80% (greater than 20%-reduction compared with pretreatment level), between 80-120% (a ±20%-change), and greater than 120% (greater than 20%-increase), respectively. A Cox proportional hazards model was applied at three time points, 4 weeks, 8 weeks, and 12 weeks after the start of systemic therapies. Clinical assessment of therapeutic response (presence or absence of objective response) according to UICC criteria and changes in tumor marker levels (categorized as D, N or U) were directly compared using the Cox proportional hazards model at each time point.

Results

Patient Characteristics

As shown in Table 1, of the 108 eligible patients, 92 (85.2%) had recurrent breast cancer and 16 had stage Ⅳ disease. The median age of the subjects was 55 years. The median disease-free interval of the patients with recurrent disease was 32 months, and the median number of previous treatments was one. Forty-eight patients (44.4%) received chemotherapy alone, 25 received chemotherapy plus trastuzumab, and 20 received endocrine therapy alone. CR was obtained in 10.2%, PR in 25%, NC in 56.5%, and PD in 8.3% of patients. The main target lesion for therapy was visceral disease in 57.4%, soft tissue disease in 24.1% and bone metastasis in 18.5% of the patients.

Pretreatment Positivity Rates

Pretreatment tumor marker positivity rates were 51.9% for CEA, 50.0% for CA 15-3, and 34.3% for NCC-ST-439. The CEA and CA 15-3 positivity rates were significantly higher than the positivity rate of NCC-ST-439 ($p = 0.001$ and $p = 0.031$, respectively).

Table 1. Patient Demographics and Disease Characteristics

No. of patients	108
Median Age (years, range)	55 (31-82)
Diseases	
Recurrent	92 (85%)
Stage Ⅳ	16 (15%)
No. of previous treatments	
Median (range)	1 (0-5)
Main target of treatment	
Visceral metastasis	62 (57%)
Soft tissue metastasis	26 (24%)
Bone metastasis	20 (19%)
Treatment	
Chemothrapy alone	48 (44%)
Chemothrapy + Trastuzumab	25 (23%)
Endocrine therapy alone	20 (19%)
Others	15 (14%)
Best clinical therapeutic response	
CR	11 (10%)
PR	27 (25%)
NC	61 (57%)
PD	9 (8%)

Correlation between Clinical Therapeutic Response and Changes in the Tumor Marker Levels

To investigate the relation between changes in the percentage of tumor marker levels and clinical therapeutic response, the patients were divided by the best therapeutic response within 12 weeks after the start of therapy. As shown in Fig 1, for pretreatment tumor marker-positive patients the percentage of each tumor marker level seemed to correlate with the clinical therapeutic response 8 and 12 weeks after the start of the therapy. In contrast, it did not correlate with the therapeutic response 4 weeks after the start of the therapy, except for pretreatment CEA-positive patients obtaining CR during treatment. However, in pretreatment tumor marker-negative patients, changes in the percentage of tumor marker levels did not correlate with clinical therapeutic response (data not shown).

Changes in Tumor Marker Levels and TTP

TTP in pretreatment CEA-positive patients was significantly shorter than in negative patients (50% TTP; 18 weeks and 25.5 weeks, respectively; $p = 0.003$). However, pretreatment tumor marker positivity for CA 15-3 and NCC-ST-439 did not significantly influence TTP.

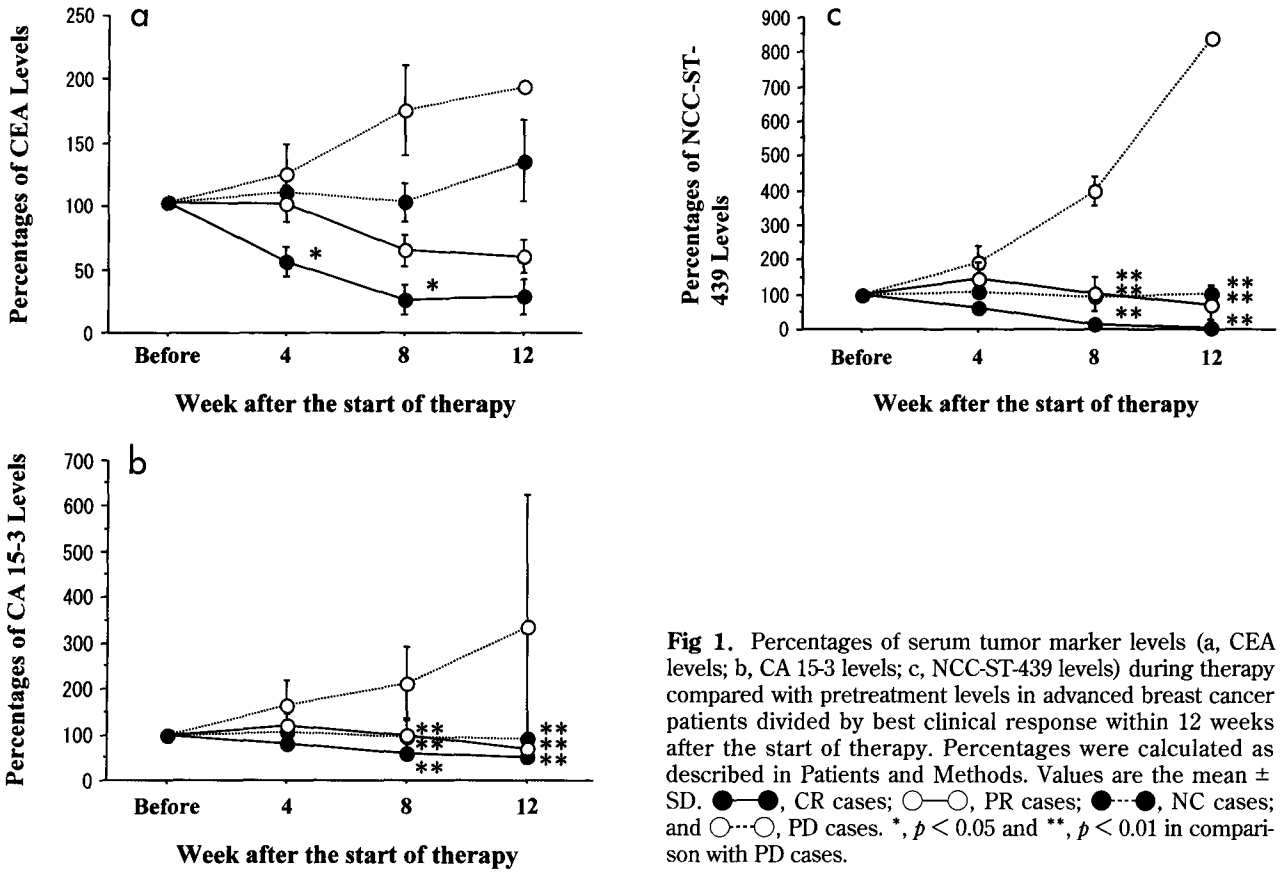

Fig 1. Percentages of serum tumor marker levels (a, CEA levels; b, CA 15-3 levels; c, NCC-ST-439 levels) during therapy compared with pretreatment levels in advanced breast cancer patients divided by best clinical response within 12 weeks after the start of therapy. Percentages were calculated as described in Patients and Methods. Values are the mean ± SD. ●—●, CR cases; ○—○, PR cases; ●···●, NC cases; and ○···○, PD cases. *, $p < 0.05$ and **, $p < 0.01$ in comparison with PD cases.

Table 2. Correlation of Changes in Tumor Marker Levels with TTP in Pretreatment Marker-Positive Patients*

	Hazard ratio	95% CI	*p* value
Pretreatment CEA-positive patients (n = 49)			
4 weeks after: No final model applicable	NA**	NA	NA
8 weeks after: Category D is an independent factor	0.274	0.100-0.749	0.012
12 weeks after: Category D is an independent factor	0.338	0.135-0.846	0.021
Pretreatment CA 15-3-positive patients (n = 49)			
4 weeks after: Category D is an independent factor	0.178	0.051-0.615	0.006
8 weeks after: Category D is an independent factor	0.250	0.107-0.585	0.001
12 weeks after: Category D is an independent factor	0.221	0.088-0.556	0.001
Pretreatment NCC-ST-439-positive patients (n = 35)			
4 weeks after: Category D is an independent factor	0.270	0.090-0.804	0.019
8 weeks after: Category D is an independent factor	0.334	0.115-0.967	0.043
12 weeks after: No final model applicable	NA	NA	NA

*Cox proportional hazards model was applied to assess the raltion between changes in tumor marker levels and TTP 4, 8 or 12 weeks after the start of therapy. Variables consited of three categories of changes in tumor marker levels (D, N and U as described in the Materials and Methods) and presence or absence of objective response.

**Not assessable

In pretreatment CEA-positive patients (n = 49), a greater than 20% reduction in the CEA level was a significantly better predictive factor for TTP than the presence or absence of an objective response 8 and 12 weeks after the start of therapy (hazard ratio [HR], 0.274; $p = 0.012$ 8 weeks after and HR, 0.338; $p = 0.021$ 12 weeks after; Table 2). In pretreatment CA 15-3-positive patients (n = 49), a

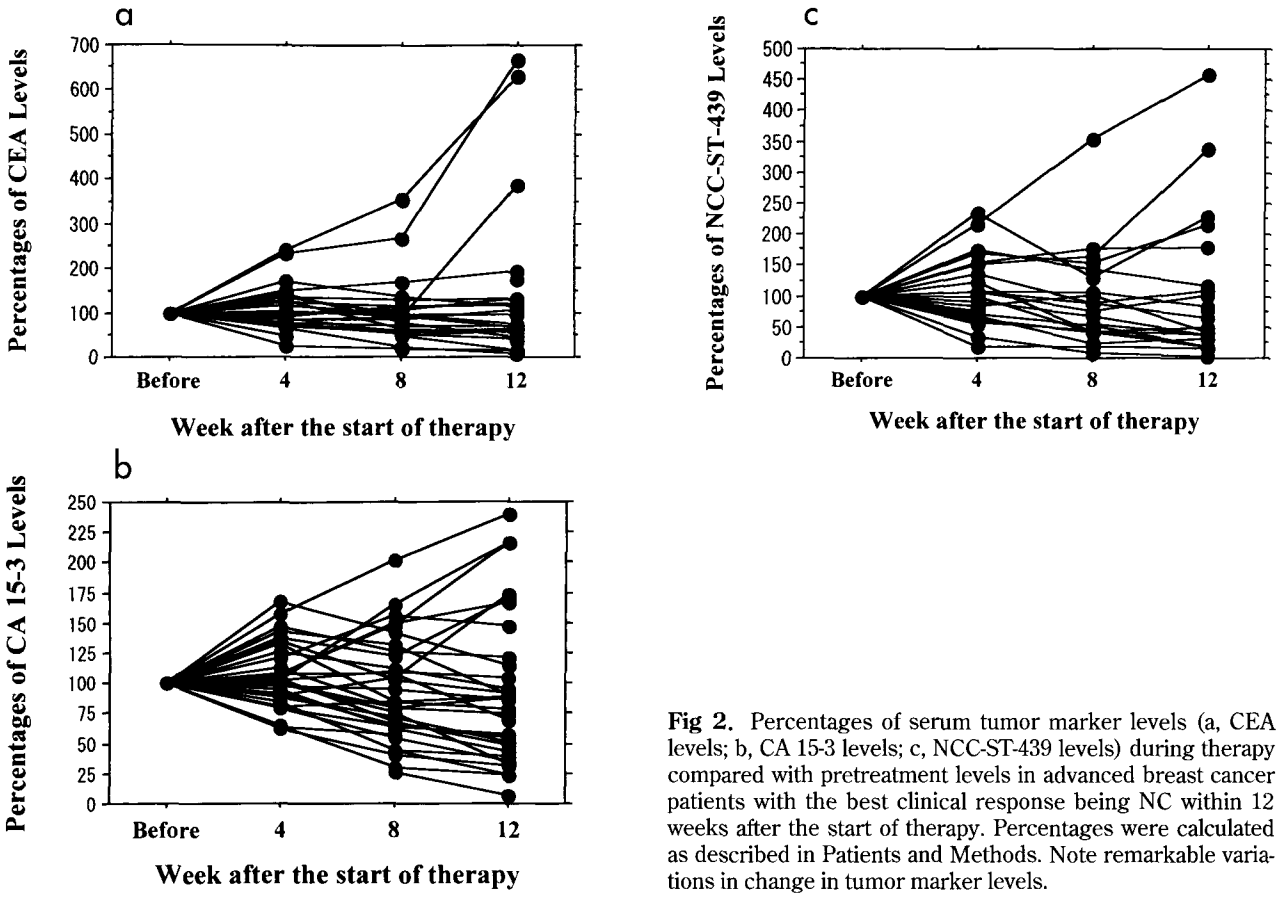

Fig 2. Percentages of serum tumor marker levels (a, CEA levels; b, CA 15-3 levels; c, NCC-ST-439 levels) during therapy compared with pretreatment levels in advanced breast cancer patients with the best clinical response being NC within 12 weeks after the start of therapy. Percentages were calculated as described in Patients and Methods. Note remarkable variations in change in tumor marker levels.

greater than 20% reduction in the CA 15-3 level was a significantly better predictive factor for TTP than the presence or absence of objective response 4, 8, and 12 weeks after the start of therapy (HR, 0.178; $p = 0.006$, HR, 0.250; $p = 0.001$ and HR, 0.221; $p = 0.001$, respectively; Table 2). In pretreatment NCC-ST-439-positive patients (n = 35), a greater than 20% reduction in the NCC-ST-439 level was a significantly better predictive factor for TTP than the presence or absence of objective response 4 and 8 weeks after the start of therapy (HR, 0.270; $p = 0.019$ for 4 weeks after and HR, 0.334; $p = 0.043$ for 8 weeks after; Table 2).

Changes in Tumor Markers Levels in Patients for Whom the Best Clinical Response was NC

As shown in Fig 2a, 2b and 2C, all tumor marker levels gradually increased or fell in a majority of the NC patients. Therefore, to clarify the hypothesis that a change in the tumor marker level is a reliable predictor for TTP, changes in tumor marker levels were divided into U, N and D groups and TTP was compared among the groups in patients for whom the best clinical response within 12 weeks after the start of systemic therapy was NC. In pretreatment CA 15-3-positive patients (n = 32), TTP was significantly longer in the D group than the U group at 8 and 12 weeks after the start of therapy (median TTP, 37 weeks for D group and 18 weeks for U group at 8 weeks after; $P = 0.021$; 35 weeks for D group and 18 weeks for U group at 12 weeks after; $P = 0.003$; Fig 3a and 3b). In pretreatment CEA-positive patients (n = 25), TTP was significantly longer in the D group than the U group at 12 weeks after the start of therapy (median TTP, 28 weeks for the D group and 16 weeks for the U group; $P = 0.046$; Fig 3c). No such significant difference in TTP was observed in pretreatment NCC-ST-439-positive patients (n = 22).

Discussion

Our previous study[3] which analyzed over 300 patients with advanced breast cancer showed the following: 1) the pretreatment positivity rate of CA 15-3 was higher than that of CEA, 2) changes in CEA and CA 15-3 levels correlated well with clinical therapeutic response only in pretreatment

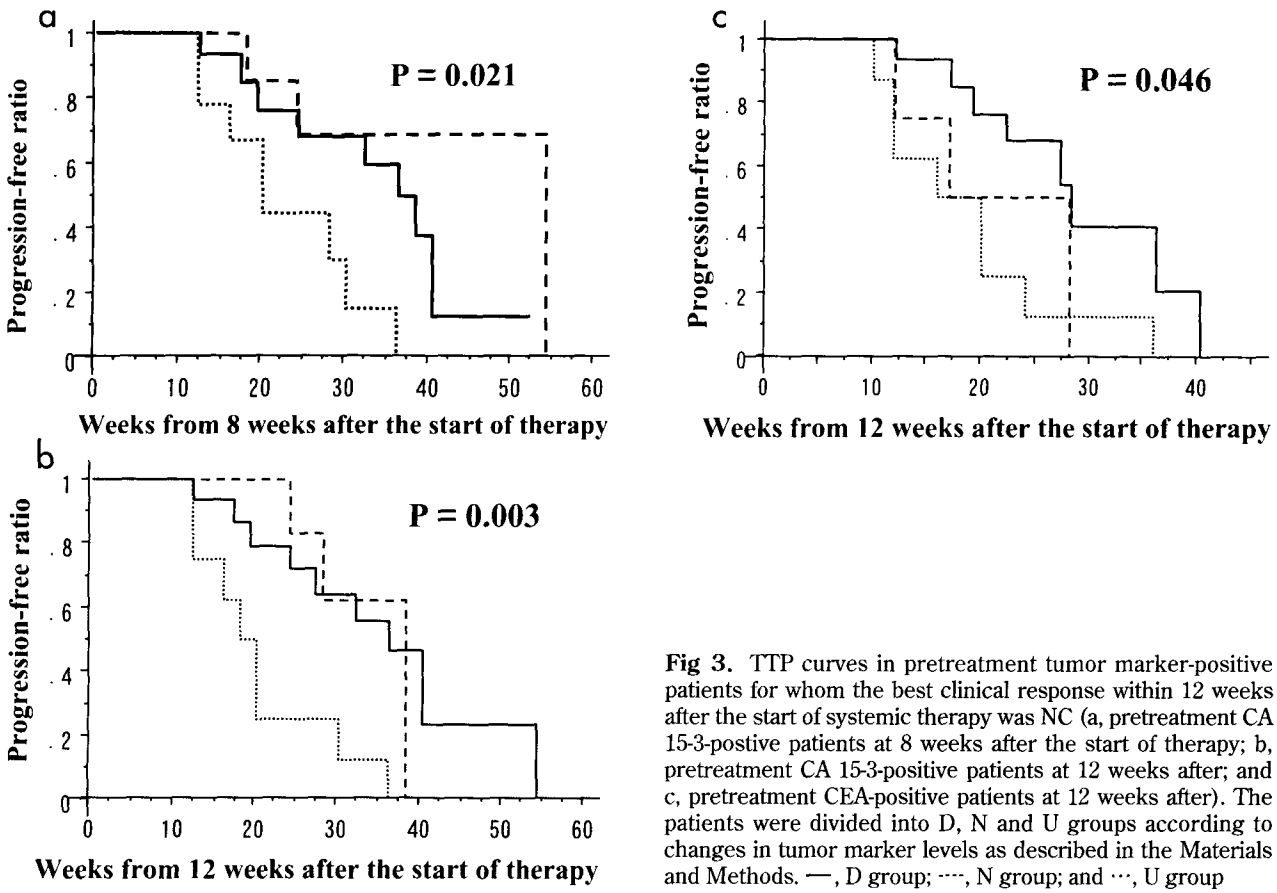

Fig 3. TTP curves in pretreatment tumor marker-positive patients for whom the best clinical response within 12 weeks after the start of systemic therapy was NC (a, pretreatment CA 15-3-postive patients at 8 weeks after the start of therapy; b, pretreatment CA 15-3-positive patients at 12 weeks after; and c, pretreatment CEA-positive patients at 12 weeks after). The patients were divided into D, N and U groups according to changes in tumor marker levels as described in the Materials and Methods. —, D group; ····, N group; and ···, U group

marker-positive patients, 3) a high pretreatment level of CA 15-3 was an independent risk factor for shorter TTP, and 4) a greater than 20%-reduction in the serum CEA or CA 15-3 levels during therapy was an independent favorable factor for longer TTP in pretreatment marker-positive patients. However, we concluded that prospective studies are needed to make definitive findings on the clinical utility of tumor markers for monitoring patients with advanced breast cancer. Therefore, the Tumor Marker Study Group subsequently conducted the present study.

With regard to pretreatment positivity rates of tumor markers, CA 15-3 and CEA were significantly more sensitive than NCC-ST-439 in this study population. Although CA 15-3 was significantly more sensitive than CEA in our previous study[3] and others[5-8], no difference was found in the positivity rates between CA 15-3 and CEA in this study. Pretreatment positivity rates of tumor markers depend on the tumor burden and distribution of main metastatic lesions in study subjects. Our retrospective and prospective studies suggest that changes in tumor marker levels correlate well with the clinical therapeutic response only in pretreatment marker-positive patients. Therefore, the pretreatment positivity rates of tumor markers are important for choosing appropriate monitoring markers in patients with advanced breast cancer. It may be beneficial if several tumor markers are simultaneously measured before the start of treatment and only positive markers are selected and used for monitoring patients.

To determine the most useful timing of tumor marker sampling for monitoring patients with advanced breast cancer during systemic therapy, three tumor markers, CA 15-3, CEA, and NCC-ST-439, were measured 4, 8, and 12 weeks after the start of therapy and clinical assessment of therapeutic response was obtained at the same time. Only in pretreatment marker-positive patients did changes in all tumor marker levels 8 or 12 weeks after the start of therapy correlate well with the best clinical response within 12 weeks but not 4 weeks after the start of therapy. Tumor marker levels rapidly elevate after the start of therapy in some patients who show an objective response to therapy[9, 10]. This "spike phenomenon" probably caused the discrepancy between changes in tumor marker levels 4 weeks after the start of therapy

and the best clinical response. An over 20% increase in tumor marker levels was observed in 33%, 21%, and 27% of patients who showed an objective response for CEA, CA 15-3 and NCC-ST-439, respectively, in this study. These findings suggest that tumor marker levels 4 weeks after the start of therapy should be carefully evaluated by taking into consideration a possible "spike phenomenon".

For most patients with advanced breast cancer, in particular those with distant metastasis, the main purpose of therapy is palliation of their symptoms and extending their lives. Curing these patients by systemic therapy is almost hopeless. Therefore, TTP is one of the most important parameters of clinical benefit obtained from systemic therapies. When a certain therapy provides a longer objective response or stable disease to a patient with metastatic disease, more efficient palliation and longer life-extension is expected. These findings directed us to analyze the predictive power of changes in serum tumor marker levels for TTP in this study.

The most important finding in this study is that an over 20% decrease in tumor marker levels can predict a longer TTP as opposed to the presence or absence of a clinical objective response in pretreatment marker-positive patients with advanced breast cancer (Table 2). In addition, changes in tumor marker levels are predictive for TTP in patients for whom the best clinical response was NC (Fig 3a, 3b and 3c). Although the clinical evaluation of therapeutic response depends on information obtained from imaging the disease, metastatic diseases such as bone metastases and diffuse micrometastases are sometimes difficult to image and therefore it is also difficult to evaluate the efficacy of therapy. These findings suggest that changes in tumor marker levels might be more predictive for disease progression than the clinical evaluation of therapeutic response in patients with advanced breast cancer and elevated tumor marker levels.

It is frequently hard to obtain imaging examinations such as computed tomography for liver metastases and bone scintigraphy for multiple bone metastases on breast cancer patients with multiple distant metastases. These imaging modalities are costly and time-consuming for such patients. In contrast, measuring serum tumor markers monthly is cheaper and easier. Robertson *et al.*[11] suggested that assessments of equivalent quality can be obtained at lower cost by using serum markers in advanced breast cancer patients. However, some advanced breast cancer patients have no elevated serum tumor markers before the start of therapy. Our retrospective and prospective studies suggested that changes in serum tumor marker levels do not correlate with a response to therapy in patients with marker levels within normal limits. Therefore, more sensitive tumor markers for breast cancer should be developed.

Acknowledgements

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