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ORIGINAL ARTICLE

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Evaluation of circulating tumor cells in patients with breast cancer: multi-institutional clinical trial in Japan

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Abstract
Background. With the development of the CellSearch System, it has become possible to measure circulating tumor cell (CTC) levels with high reproducibility, and the CTC test is currently being used clinically for patients with metastatic breast cancer in the United States. It is imperative that the clinical significance of the CTC test also be examined in Japan.
Methods. Using the CellSearch System, CTC levels were evaluated in 57 healthy individuals and patients with benign breast disease; 30 patients with primary breast cancer (stages 1–3); and 38 patients with metastatic breast cancer. First, the relationship between CTC levels and the presence of metastasis was examined using a cutoff score of 2 CTCs per 7.5 ml whole blood. Then, the patients with metastatic breast cancer were divided into two groups, using a cutoff score of 5 CTCs per 7.5 ml blood, and progression-free survival (PFS) and overall survival (OS) were compared in the two groups.
Results. When the clinical cutoff score was set at 2 CTCs per 7.5 ml blood, 0% of the healthy individuals and patients with benign breast disease (0/57), 3.3% of the patients with primary breast cancer (1/30), and 50% of the patients with metastatic breast cancer (19/38) were identified as as having 2 CTCs per 7.5 ml blood. Additionally, with a cutoff score of 5 CTCs, 11 patients were reported to have 5 or more CTCs and both PFS ($P = 0.0036$) and OS ($P = 0.04$) were worse for this patient population than for the population with fewer than 5 CTCs.
Conclusion. As concluded in a similar clinical trial in the United States, for patients with breast cancer, measuring CTC levels can be both an accurate indicator of metastases and an important measure of patient prognosis.

Key words Circulating tumor cells · Metastatic breast cancer · CTC in breast cancer in Japan

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Introduction

In Japan, the incidence rate of breast cancer is the highest among all cancers for women and is steadily continuing to rise. The death rate for patients with breast cancer is also increasing, and the importance of adjuvant therapy has been widely discussed. In a number of cases where patient survival is threatened by distant metastases, the disease is considered incurable and treatment is shifted to palliative care.[1] While there are several different treatment options available for such patients with metastatic disease, it is essential to consider prognosis and treatment response during clinical evaluation when determining the appropriate treatment. At present, histology, disease-free interval (DFI), performance status (PS), site of metastasis, tumor markers, degree of metastases, and speed of progression are commonly used for patient diagnosis and for determining treatment efficacy. However, it is often difficult to make conclusive clinical decisions based on this information.

Previous studies have examined the presence of circulating tumor cells (CTCs) in patients with metastatic cancers[2] and its relationship with patient prognosis.[3,4] With the development of the CellSearch System (Veridex, Warren, NJ, USA), CTCs can now be detected and captured with high reproducibility and reliability.[2,5] Using the CellSearch System, a prospective multicenter clinical trial in patients

with metastatic breast cancer was conducted in the United States. The study found that the number of CTCs was a highly sensitive independent predictor of progression-free and overall survival in patients with metastatic breast cancer.[6] Moreover, the study determined that the CTC count effectively reflected tumor burden and tumor progression. Following this study, we conducted a multicenter trial to determine the utility of the CellSearch System in patients with breast cancer in Japan.

Patients and methods

Study design

A prospective study was conducted to evaluate the clinical utility of CTC levels in patients with metastatic breast cancer who were being treated at three institutions in Japan. (a) St. Luke's International Hospital, (b) Metropolitan Komagome Hospital, and (c) National Kyushu Cancer Center Hospital. Thirty-eight patients with metastatic breast cancer were enrolled between November 2004 and March 2005 and followed to evaluate treatment response. The principle inclusion criteria were patients with progressive, metastatic breast cancer prior to the initiation of a new systemic therapy. All patients were confirmed to have distant metastasis by imaging. The patients' ages ranged from 41 to 77 years (median, 56 years). Blood samples were collected for the enumeration of baseline CTC levels. Disease status was assessed using clinical evaluation, imaging, and tumor markers in a comprehensive manner without knowledge of CTC levels, and the follow-up period ranged from 1.9 to 89.0 weeks (median, 45.5 weeks).

We also evaluated the CTC levels of 57 healthy individuals and patients with benign breast disease, as well as the levels in 30 patients with primary breast cancer (stages I–III) as part of the study.

Isolation and enumeration of circulating tumor cells (CTCs)

Blood samples were drawn into CellSave Preservative Tubes (Immunicon, Huntingdon Valley, PA, USA); these tubes are evacuated blood drawtubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, and a cellular preservative. All samples were maintained at ambient temperature and processed within 72 h of collection. The samples were analyzed at a commercial central laboratory (SRLI. Hachioji, Tokyo, Japan), using the CellSearch System.

CTCs were enriched from 7.5 ml of blood, using ferrofluid coated with antibodies targeting the epithelial cell adhesion molecule (EpCAM). Tumor cells of epithelial origin were then magnetically isolated and fluorescently labeled with the nucleic acid dye 4′,6-diamidino-2-phenylindole (DAPI) and monoclonal antibodies specific for epithelial cells (cytokeratin 8,18,19-phycoerythrin). To distinguish leukocytes from CTCs, a pan-leukocyte monoclonal antibody (CD45-allophycocyanin) was also added. Using the CellSpotter Analyzer (Immunicon, Huntington Valley PA, USA), cell images were acquired and displayed to the user in a gallery format for final classification of the magnetically captured cells. An event was classified as a tumor cell when its morphological features were consistent with those of a cell and exhibited the correct phenotype, i.e., EpCAM+, CK+, DAPI+, and CD45–. Technical details of the CellSearch System, including analytical accuracy, reproducibility, and linearity have been detailed previously.[2]

Statistical analysis

The presence of metastases in patients with breast cancer was determined using 2 CTCs as the cutoff. A cutoff score of 5 CTCs was set as a benchmark for prognosis in the patients with metastatic breast cancer, progression-free survival (PFS) and overall survival (OS) were defined as the elapsed time between the date of the blood drawn for CTC measurement and either the time of death or last follow up. Survival curves were compared using log-rank testing. P values were two-sided. $P < 0.05$ was considered significant. The analyses of PFS and OS were performed according to the intention-to-treat principle.

Results

The demographics of the patients with metastatic breast cancer are listed in Table 1. Hormone receptor positivity was detected in 16 patients (42.1%), HER2 positivity was detected in 19 patients (50.0%). HER2 status was unknown for 5 patients. Chemotherapy including trastuzumab was administered to 35 patients. Endocrine therapy alone was administered to 3 patients.

Among healthy patients and patients with benign disease, there were no patients with 2 or more CTCs. Two patients with primary breast cancer had 1 CTC, and only one patient with primary breast cancer had 2 or more CTCs. Nineteen of the 38 patients with metastatic breast cancer had 2 or more CTCs. With the cutoff value set at 2 CTCs, sensitivity to distant metastasis was 50.0% (19/38), specificity was 96.7% (29/30), and the false-positive rate was 3.3% (1/30). CTC levels in each population are shown in Fig. 1.

With the cutoff value set at 5 CTCs, there was a significant difference in both PFS and OS between patients with fewer than 5 CTCs and those with 5 or more CTCs (P = 0.0036; Fig. 2 [PFS]; P = 0.04; Fig. 3 [OS]). The population with fewer than 5 CTCs showed significantly higher PFS and OS than the group with 5 or more CTCs. However, there was no statistically significant difference in positivity rates for the presence of visceral disease between patients with fewer than 5 CTCs and those with 5 or more CTCs, at rates of 23/27 (85.2%) and 9/11 (81.8%), respectively. There were also no significant differences in positivity rates for estrogen receptor (ER)/progesterone receptor (PR) status, at 11/27

Table 1. Patient demographics

Demographic	All patients (n = 38)	
	No.	Percentage
Age (years)		
Mean	57.0	
Standard deviation	8.0	
Median	56	
ER/PR status		
ER- or PR-positive	16	42
ER- and PR-negative	17	45
Unknown	5	13
HER2 status		
HER2-negative, 0, 1+, 2+ (FISH–)	14	37
HER2-positive, 2+ (FISH+), 3+	19	50
Unknown	5	13
Previous adjuvant chemotherapy		
Yes	28	74
No	9	24
Unknown	1	3
Type of therapy for metastatic disease		
Hormone alone	3	8
Hormone + trastuzumab	0	0
Chemo alone	20	53
Chemo + trastuzumab	8	21
Chemo + hormone + trastuzumab	1	3
Trastuzumab alone	5	13
Unknown	1	3
Site of metastasis		
Visceral	32	84
Nonvisceral	6	16
Survival status		
Alive	23	61
Dead	15	39
Average time to first follow-up (weeks; n = 38)[a]		
Mean	4.3	
Standard deviation	1.6	
Median	6.9	

ER, Estrogen receptor; PR, progesterone receptor; Chemo, chemotherapy
[a] From date of baseline blood withdrawal

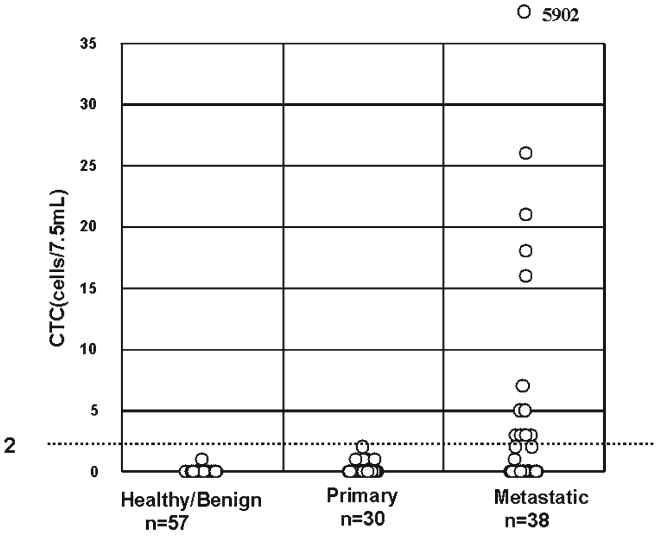

Fig. 1. The subjects involved in the study were classified into three categories, healthy individuals and patients with benign breast disease (*healthy/benign*); patients with primary breast cancer (*primary*), and patients with metastatic breast cancer (*metastatic*). The cutoff value of 2 circulating tumor cells (*CTCs*; per 7.5 ml blood) is indicated by the *dotted line*

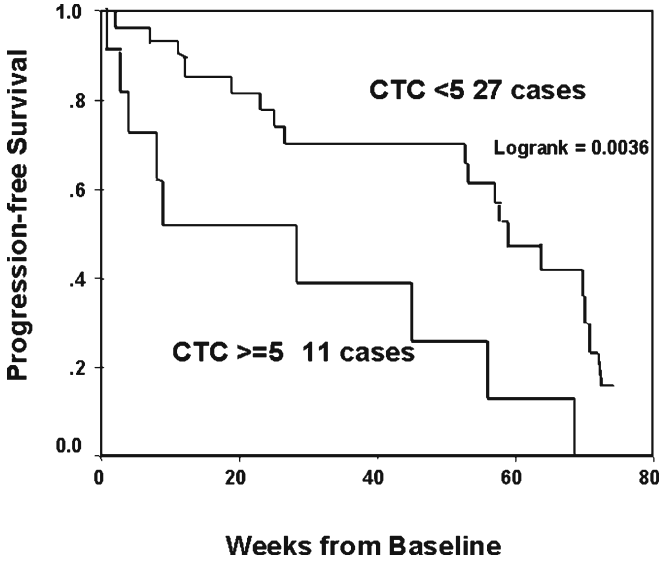

Fig. 2. Kaplan-Meier plots of progression-free survival (PFS) in patients with metastatic breast cancer with fewer than 5 circulating tumor cells (*top line*) and 5 or more circulating tumor cells (*bottom line*) at baseline. PFS was calculated from the date of the baseline blood withdrawal taken before the initiation of a new line of therapy. *Logrank* indicates the *P* value

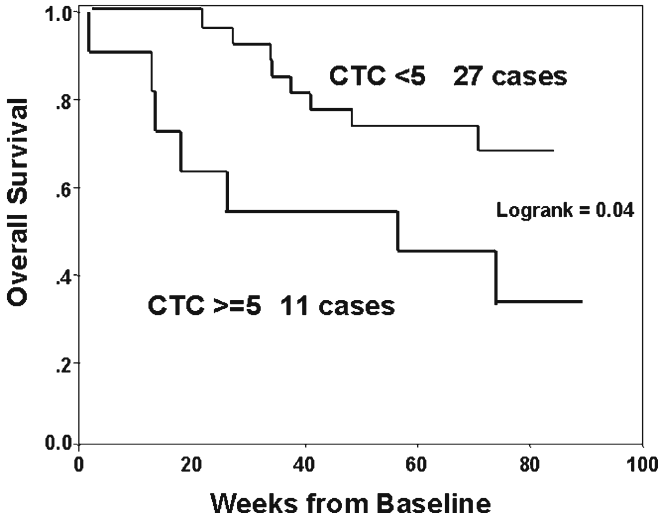

Fig. 3. Kaplan-Meier plots of overall survival (OS) in patients with metastatic breast cancer with fewer than 5 circulating tumor cells (*top line*) and five or more circulating tumor cells (*bottom line*) at baseline. OS was calculated from the date of the baseline blood withdrawal taken before initiation of a new line of therapy. *Logrank* indicates the *P* value

(40.7%) and 5/11 (45.5%), respectively, or for HER2 status, at 13/27 (48.1%) and 5/11 (45.5%), respectively.

Discussion

In their multicenter trial in the United States, Cristofanilli et al.[6] concluded that the number of circulating tumor cells (CTCs) was a strong predictor of progression-free survival (PFS) and overall survival (OS) for patients with metastatic breast cancer. Patients with 5 or more CTCs per 7.5 ml of whole blood had a poorer prognosis than patients with fewer than 5 CTCs. Moreover, at the first follow-up visit after the initiation of therapy (4–5 weeks), this difference in PFS and OS persisted. This outcome implied that measuring CTCs may provide significant value for clinical evaluation and for determining treatment options for patients. A subsequent retrospective analysis of this clinical data set found that CTC levels detected disease progression more accurately than imaging.[7]

The outcome of our trial had results comparable to the data from the United States study. With a cutoff score of 5 per 7.5 ml whole blood, CTCs can be considered an effective marker for prognosis, and the CellSearch test can be considered to have great value in Japan. Currently, an additional clinical trial is being conducted in Japan. In this trial, evaluation of the health-economic benefits of using the CellSearch test to determine CTC levels, early evaluation of treatment efficacy based on CTC levels, and the phenotypic properties of HER2 and the efficacy of treatment with HER antagonists are being investigated. One important case that is currently being examined is that of a patient who was HER-2 negative at the primary stage, but who was positive for CTCs (i.e., had more than 5 CTCs per 7.5 ml whole blood) and has responded to trastuzumab.[8]

As discussed previously, current practice incorporates collective information drawn from tumor markers, imaging, and clinical evaluation for use as prognostic data points. However, this can present challenges. In patients with bone metastasis and lymphangitis, clinical evaluation based on imaging is difficult, while in other patients it is difficult to distinguish between tumor progression and inflammation. The CellSearch System can be used not only prior to treatment of the disease, but also during treatment. By measuring CTC levels during treatment, clinicians may effectively determine the efficacy of the treatment currently provided to the patient. Additionally, while false-positives are often problematic with imaging or the use of tumor markers, the data from our study showed that in healthy subjects and patients with benign disease, there were no individuals with more than 2 CTCs. Moreover, of the patients with primary breast cancer, only one had 2 CTCs. By setting the CTC cutoff level at 2, there were virtually no false-positives, and therefore there is the potential that the CTC test can be considered to be extremely valuable in measuring recurrent disease.

Conclusion

The CTC test is simple to perform and places a limited physical burden on the patient, while being a highly sensitive measure of treatment efficacy. Treatment should be continued for patients in whom the treatment has been determined to be effective based on CTC levels. For patients whose treatment has been determined to be ineffective, treatment should be discontinued or changed, or shifted to palliative care. This allows the adverse effects caused by treatment to be minimized, reduces unneeded treatment, and further, has a health-economic benefit by reducing costs to the overall healthcare system.

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