Evaluation of Serum KL-6, a Mucin-like Glycoprotein, as a Tumor Marker for Breast Cancer

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ABSTRACT
The utility of serum KL-6 as a tumor marker for breast cancer was evaluated in this study. The sera from 146 patients with breast cancer, 13 with benign breast disease, and 108 healthy individuals were measured for KL-6 titer using a sandwich enzyme immunoassay method. Carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA15-3) titers were also tested in the same sera from the patients. The mean KL-6 titer of patients with primary breast cancer was 673 units/ml, which was significantly higher than that of benign and healthy individuals (P = 0.037 and P < 0.0001, respectively). The titer of patients with relapsed breast cancer was 1964 units/ml, which was also higher than that of primary cancer (P = 0.013). KL-6 titer was related to tumor stage, distant metastasis, and relapse site (P = 0.0053, P < 0.0001, and P = 0.0251, respectively). Using the cutoff value of 467 units/ml, the sensitivity of KL-6 was 31% for primary breast cancer (16% for stage I and 29% for stage II) and 73% for relapsed breast cancer (50% for local relapse and 89% for distant relapse). The specificity was 92%. The sensitivity of KL-6 was higher than that of CA15-3 and CEA. Combination of the three markers, followed by KL-6 and CEA, raised the sensitivity for primary breast cancer. Single use of KL-6 demonstrated a higher sensitivity than in each combination for relapsed breast cancer. In conclusion, serum KL-6 may be helpful for clinical use as a tumor marker for breast cancer, and it may play an important role, especially in the surveillance of disease relapse.

INTRODUCTION
In various markers, the best single and established marker for breast cancer is CA15-3, followed by CEA (1). Nevertheless, the American Society of Clinical Oncology (2) has stated in the Clinical Practice Guidelines for the Use of Tumor Markers that neither CA15-3 nor CEA is recommended for routine use for diagnosing breast cancer, and new powerful markers for breast cancer are needed.

KL-6 is a high molecular weight, mucin-like glycoprotein that was originally discovered as a circulating pulmonary adenocarcinoma-associated antigen (3, 4). Expression of KL-6 has been observed in both various carcinomas and normal cells (3), and it is thought to be released into the serum in cases of cell damage (5). Biochemical properties of KL-6 are similar to those of other MUC-1 mucins (6). KL-6 does not directly reflect events in the process of tumorigenesis. The clinical value of serum KL-6 has been recognized as a marker for the disease activity of interstitial pneumonitis (5, 7). Kohno et al. (3) has reported that serum KL-6 levels were also elevated in breast and pancreatic cancers.

In this study, we analyzed the serum KL-6 titer in breast cancer and evaluated the clinical value of KL-6 as a tumor marker for breast cancer.

MATERIALS AND METHODS
Clinical Materials. Sera from 146 female patients with breast cancer treated at the First Department of Surgery, Osaka City University Hospital, were analyzed. Sera were obtained before therapy. Among the patients, 131 had primary invasive breast cancer, and 15 had relapsed cancer. The mean age of the patients was 54 years (range, 28–81 years). None of the patients had other malignancies or active pulmonary disease. The characteristics of the patients are summarized in Table 1. Clinicopathological factors were abstracted from the initial surgical pathology reports. Staging was determined according to the TNM Classification of Malignant Tumors set by the Union International Contre Cancer (8). Histological grade was analyzed according to the Scarff-Bloom-Richardson histological grading system (9). The survival period was defined as the interval between the time point at which the serum sample was obtained and the date of first relapse or death.

Serum samples from 108 healthy females, who had voluntarily cooperated in a blood donation campaign in Osaka, with a mean age of 31 years (range, 18–63 years) served as healthy controls. Sera from 13 patients with benign breast disease (2 phylloides tumors, 4 intraductal papillomas, 4 mastopathies, 1 fibroadenoma, 1 adenoma, and 1 mastitis) treated in our hospital

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2 The abbreviations used are: CA15-3, carbohydrate antigen 15-3; CEA, carcinoembryonic antigen.
were also analyzed for KL-6. Additionally, the titers of CEA and CA15-3 were measured in the same sera from patients.

**Assay for Serum KL-6, CEA, and CA15-3.** The serum samples were stored at −40°C until use. The samples were assayed for KL-6 with a sandwich-type enzyme-linked immunoassay using the Eitest KL-6 kit (Sanko Jyunyaku Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. The kit was composed of mouse monoclonal antibody against KL-6 antigen. The coefficient of variation of the kit was <10% in each of four examinations using the 2.5 and 10 units/ml KL-6 control samples. In brief, 20 μl of serum samples diluted to 1:201 using Tris-buffer with bovine albumin or 20 μl of known concentration KL-6-controls, with an addition to 100 μl of Tris buffer with normal rabbit serum, were pipetted into the wells of the microplate, which had been precoated with a mouse monoclonal antibody for KL-6. After mixing, the plate was covered with plate sealer and incubated at room temperature for 2 h. Each well was washed thoroughly three times with 0.85% NaCl. One hundred μl of appropriately diluted antibody to KL-6 conjugated to horseradish peroxidase was pipetted into each well. After incubation at room temperature for 1 h, wells were washed, and 100 μl of substrate with 2,2'-azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid were added for the color development. After incubation at room temperature for 30 min, the color reaction was terminated by the addition of 100 μl of stop solution. The absorbance of each well was determined by a MTP-120 micro plate reader (Corona Electric Co., Ibaragi, Japan) set to 405 nm. The concentration of each serum sample was determined from the standard curve using the KL-6-controls.

CEA was measured by a counting immunoassay using a commercially available kit (Runreem CEA; TOA Medical Electronics, Kobe, Japan) in conjunction with an automated PAMTA-100 analyzer (TOA Medical Electronics). CA15-3 was measured by a two-step sandwich immunoradiometric assay using a commercially available kit [CA15-3 RIA kit (TFB), Fujirebio Diagnostics, Inc., Malvern, PA).

**Statistical Analysis.** The assays were read without knowledge of case and control status. The correlation coefficient was assessed by simple linear regression analysis. The Mann-Whitney U test and Kruskal Wallis one-way analysis were used for the comparison. Survival analysis was estimated by the Kaplan-Meier method and examined by the log-rank test. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

**Analyses of KL-6 Titers.** The linear regression analysis of all sera from the cases and controls did not show a correlation between KL-6 titer and age \(( r = 0.142; \ P = 0.24)\), although there was a sizeable difference between the ages of the cases and controls. The mean ± SD of serum KL-6 titer in healthy controls was 267 ± 200 units/ml (range, 0–1291 units/ml), and that of patients with benign disease was 297 ± 58 units/ml (range, 200–395 units/ml). The titer in patients with breast cancer ranged from 10 to 10297 units/ml. It was 673 ± 1310 units/ml in primary cancer and 1964 ± 2915 units/ml in relapsed cancer (Fig. 1). The titers in patients with primary cancer were significantly higher than those of healthy controls and patients with benign disease \(( P < 0.0001 \) and \( P = 0.037 \), respectively), and the difference in KL-6 titers between patients with primary and relapsed cancer was significant \(( P = 0.013)\).

The correlation of serum KL-6 titer to clinicopathological factors in primary breast cancer and relapse sites in relapsed cancer is shown in Table 1. The titer of KL-6 increased according to tumor size \(( P = 0.0053)\). The titers were significantly higher in patients with distant metastasis or stage IV disease \(( P < 0.0001, \) both), and the titers in patients relapsed at distant...
sites were significantly higher than those in patients who had relapsed at loco-regional sites ($P < 0.0251$).

One hundred thirteen curatively operated patients were divided into high and low KL-6 groups using a serum value of 673 units/ml, which was the mean titer in patients with primary breast cancer. There was no difference of disease-free and overall survival rates between the two groups in the median follow-up term of 74 months ($P = 0.70$ and $P = 0.45$, respectively).

Comparison of Serum KL-6 with CEA and CA15-3.

The cutoff value of KL-6 was determined as 467 units/ml, which was the mean of healthy individuals plus the SD. The cutoff values of CEA and CA15-3 recommended by the manufacturers are 6.5 ng/ml and 30 units/ml, respectively. Cases and controls were divided into positive and negative groups using these cutoff values.

Ten of 108 healthy controls had positive KL-6 levels. Among the 13 patients with benign disease, none had a positive KL-6 level, whereas one had both positive CEA and CA15-3. The specificity of KL-6 for breast cancer was 92%. Table 2 shows the sensitivity of each marker. For primary breast cancer, the sensitivity of KL-6 was 31%. It was higher than that of CEA and CA15-3. In staging analysis, the sensitivity of KL-6 was next to that of CEA in stage I, and it was higher than those of CEA and CA15-3 in other stages. The combination of three markers had the highest sensitivity in each stage, and it was followed by the combination of KL-6 and CEA. For relapsed breast cancer, the sensitivity of KL-6 was 73%. It was 50% in patients who had relapsed at the loco-regional site and 89% in patients who had relapsed at distant sites. None of the combinations was more sensitive than KL-6 alone.

**DISCUSSION**

Our results revealed that serum KL-6 titer was elevated in patients with breast cancer. These results support the report by Kohno et al. (3). The difference between the age of the cases and controls should not affect the elevation of KL-6 titer in breast cancer, because our results revealed that age did not correlate with the titer. KL-6 titer was elevated according to tumor growth, distant metastasis, and staging, and the titer was markedly elevated in patients with relapsed disease, especially in patients who had relapsed at distant sites. These results suggest that an increase in the bulk of a tumor might raise the serum KL-6 level.

Tumor markers are expected to play a role in the differential diagnoses, early detection of cancer, prognostic predictions, monitoring of treatment efficacy, and surveillance of disease relapse (1). For differential diagnosis, the sensitivity and the specificity of KL-6, which were 31 and 92%, may not be
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The role of prognostic prediction may be limited to such factors instead of tumor markers. The issue of monitoring treatment efficacy could not be addressed in this study. It must be examined in future studies. As indicators of disease relapse, the sensitivities of CEA and CA15-3 in other reports were summarized as 50 and 67%, respectively (2). Low sensitivities of CEA and CA15-3 in our results may not reveal their actual potential because the number of relapsed patients was small in our study. However, the sensitivity of KL-6 was higher than those of CEA and CA15-3, and KL-6 can detect the relapsed cases with negative CEA and CA15-3 in our study. The sensitivity of KL-6 was up to 89% in patients who had relapsed at distant sites. Compared with cases of relapse at loco-regional sites, those who had relapsed at visceral organs are difficult to screen and diagnosis. Therefore, KL-6 could play an important role for surveillance of disease relapse in these cases.

Among the five roles expected for tumor markers, monitoring treatment efficacy and surveillance of disease relapse may be most important for breast cancer. From our results, serum KL-6 might be appropriate for clinical usage as a marker for breast cancer.

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