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SERUM c-*erb*B-2 IN BREAST CANCER PATIENTS

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The c-*erb*B-2 oncogene product in serum (serum c-*erb*B-2) was measured by an enzyme-immunoassay kit. The 12 U/ml cut-off level was estimated as the mean plus two standard deviations for 250 healthy women. With this cut-off level increased serum c-*erb*B-2 was found in 12.0% of primary breast cancer cases (n = 25), in 4.9% of non-recurrent breast cancer patients (n = 82), and in 31.4% of patients with recurrent breast cancer (n = 35). In patients with primary and recurrent breast cancer, whose sera were assayed concurrently for serum c-*erb*B-2, CEA and CA15-3, the positive rates of these markers were fairly similar. However, their combined use significantly increased the sensitivity as compared to the use of any one marker alone.

More than 50 oncogenes have been identified in the many studies conducted since the late 1970s. Functional alterations of these oncogenes have been shown to be closely related to the induction of malignancy (1). The c-*erb*B-2 (HER-2/*neu*) oncogene was first identified in carcinogen-induced rat neuroblastoma (2). Gene amplification and overexpression of the c-*erb*B-2 gene product have been reported in breast, gastric and ovarian adenocarcinomas (3–5).

The c-*erb*B-2 gene-product is a protein of 185 kD with tyrosine kinase activity, and its function and structure are similar to those of the epidermal growth factor receptor (6). The c-*erb*B-2 protein is strongly stained immunohistochemically on the cell membrane of adenocarcinoma. Recent reports have shown that a soluble form of the c-*erb*B-2 protein can be observed in serum (7–9).

In the present study, c-*erb*B-2 protein in serum was measured in breast cancer patients (primary, recurrent and non-recurrent). After evaluating kit performance and determining a cut-off value based upon healthy subjects, sera of breast cancer patients were analyzed. Results were then compared to other tumor markers as CEA and CA15-3.

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Material and Methods

The control population ranged in age from 20 to 70 years and consisted of 250 healthy women (Table). The clinical cases consisted of 10 benign mastopathy patients and 142 breast cancer patients; they included 25 with primary cases, 35 with recurrence and 82 previously operated patients without recurrence. All patients were treated from August 1991 to May 1992.

Serum c-*erb*B-2 levels were measured by an enzyme-immunoassay (EIA) kit (c-*erb*B-2 Serum EIA kit, Triton Diagnostics, Alameda, CA, U.S.A.). This kit utilized two monoclonal c-*erb*B-2 antibodies which have previously been reported (8) as well as utilized in an ELISA system (9).

CEA was assayed with an automatic analyzer (AIA-1200, Tosoh Inc., Tokyo, Japan) which is applicable for EIA system and its EIA reagent (E-test 'tosoh' II CEA, Tosoh Inc., Tokyo, Japan) (10). The cut-off level of CEA was defined as 5.0 ng/ml, which corresponded to the mean ± 2 SD of 200 healthy subjects.

CA15-3 was assayed by CA15-3 RIA kit (Centocor Co., Ltd., Malvem, PA, U.S.A.). The cut-off level of CA15-3 was 30 U/ml (11).

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Table

Mean levels and ranges of serum c-erbB-2 in healthy females

Age (years)	n	Mean (U/ml)	Range: mean − 2 SD–mean + 2 SD (U/ml)
20–69	250	8	4–12
20–29	50	7	4–11
30–39	50	7	4–10
40–49	50	8	6–11
50–59	50	8	5–12
60–69	50	8	5–12

Data obtained from healthy subjects were tested with various statistical methods. Differences of mean levels in each group were analyzed by Student's t-test. McNemar's test was used to compare the clinical behavior of each tumor marker.

Results

Accuracy and reproducibility of kit. The intra-assay variability of c-*erb*B-2 serum EIA kit was 6.2% using a low control, which was adjusted to about 12 U/ml from a normal pooled serum, and 2.3% with a higher one, which was adjusted to about 182 U/ml from a breast cancer patient. Each control was assayed 10 times. The inter-assay variability of 10 assays was 3.9%, using a sample prepared to 90 U/ml from a standard. Recovery was tested to evaluate kit accuracy by means of spiking standard material of the kit to pooled serum. The rate reached about 90% as an average of the three tests.

Serum c-erbB-2 protein in healthy subjects. The distribution of serum c-*erb*B-2 in healthy subjects is shown in the Table with stratification for age (10-year groups). The mean levels and ranges from −2 SD to +2 SD were determined by Smirnov's test (12) and Grubb's test (13). The mean level (and range) was 8 U/ml (5 to 12 U/ml) in all healthy women (n = 250). No age-dependecy was observed. Fig. 1 shows the levels of serum c-*erb*B-2 in 250 healthy women. The histogram of the healthy women was observed as a log-normal distribution which skewed slightly to the right. Thus the cut-off level was set at 12 U/ml, which was a value of mean ±2 SD from its log-normal distribution as an upper limit of the normal range in a healthy female population.

Serum c-erbB-2 in breast disease. Serum c-*erb*B-2 in breast diseases is illustrated by a dotted pattern in Fig. 2. In breast cancer patients, the mode was at 7 U/ml and 18 positive cases (> 12 U/ml) were found among the 142 patients analyzed. Eleven of these 18 cases were found in recurrent breast cancer patients. There were no positive cases, and the mean ±SD was 7.3 ± 2.5 U/ml in 10 benign mastopathies. However, there were 3 positive cases (12%)

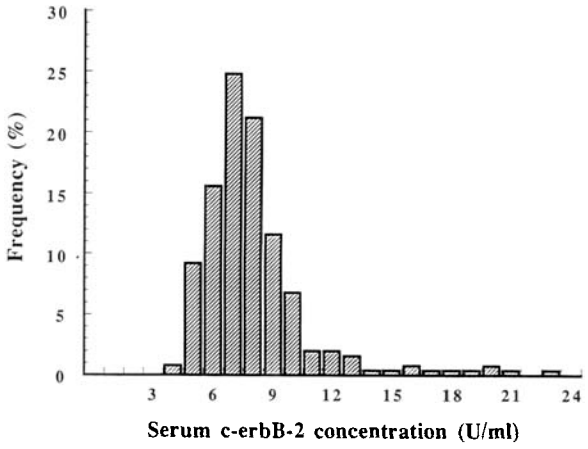

Fig. 1. Histogram of serum c-*erb*B-2 levels in healthy women. It shows a log-normal distribution with a skewness of 0.164 ($p < 0.01$) and a kurtosis was 3.100 ($p < 0.01$). The population consisted of 250 women of whom 9 with a serum c-*erb*B-2 value 16 U/ml were excluded from the histogram.

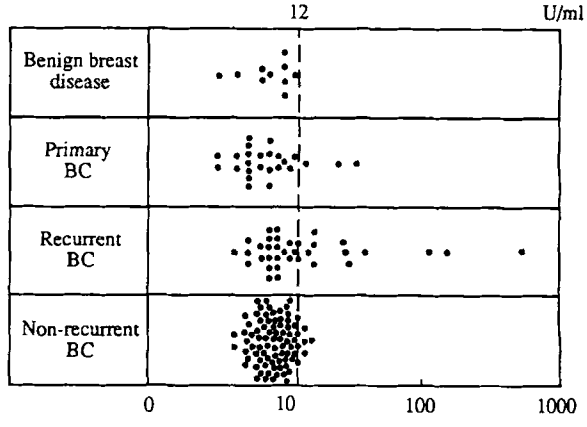

Fig. 2. Distribution of serum c-*erb*B-2 levels in breast diseases. BC: Breast cancer.

and a mean of 8.0 ± 6.0 U/ml among 25 primary breast cancer patients, 4 positive cases (4.9%) and a mean of 8.2 ± 2.3 U/ml among 82 patients without recurrence, and 11 positive cases (31.4%) and a mean of 67.9 ± 304.0 U/ml in 35 patients with recurrence, which was significantly higher ($p < 0.01$) than the other groups described above. The 3 positive primary cases were classified as stage 0, IIA and IIB, according to UICC. Three of 11 recurrent cases with elevated serum c-*erb*B-2 had values greater than 100 U/ml.

Comparison between serum c-erbB-2, CEA and CA15-3. In order to compare serum c-*erb*B-2 with other tumor markers, 127 breast cancer patients were examined and their sera assayed concurrently for serum c-*erb*B-2, CEA and CA15-3. The 127 cases included 16 primary breast cancer patients, 31 recurrent and 80 non-recurrent breast cancer patients. Here non-recurrence means clinically free of relapse when the assays were performed. From the

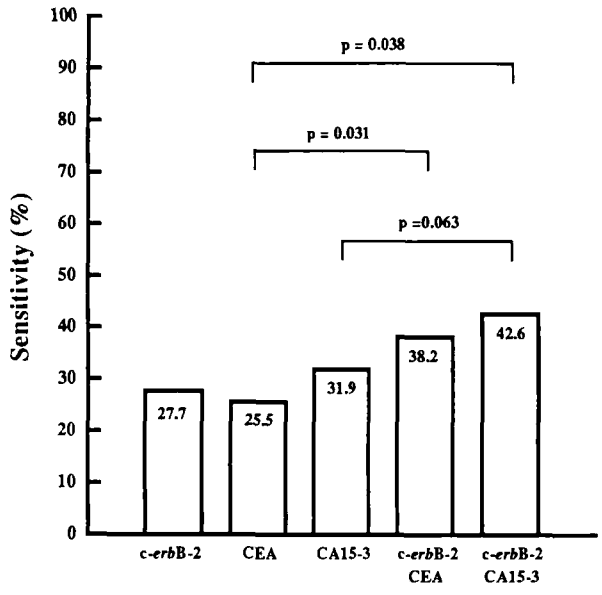

Fig. 3. Sensitivities of three tumor markers when used alone or paired with serum c-*erb*B-2. Statistic analysis was performed with the McNemar's test.

evaluation of 80 non-recurrent breast cancer patients, false-positive rates were determined to be 5.0%, 1.2% and 0.0% for c-*erb*B-2, CEA and CA15-3 respectively. Based on the evaluation of 47 patients with breast cancer (including 16 primary and 31 recurrent cases), the true positive rate (sensitivites of serum c-*erb*B-2, CEA and CA15-3 was 27.7%, 25.5% and 31.9% respectively (Fig. 3). Therefore accuracy of the above markers was estimated as 70.1%, 71.1% and 74.8% respectively. No statistically significant differences were found between these three markers concerning sensitivity, specificity and accuracy. When the above 47 paired data were tested to evaluate the relationship between c-*erb*B-2 and CEA or between c-*erb*B-2 and CA15-3, the correlation coefficient (r) was 0.075 (NS) and 0.462 ($p < 0.005$) respectively. Combining c-*erb*B-2 with CEA or with CA15-3 significantly increased the sensitivity from 25.5% for CEA alone to 38.2% ($p = 0.031$) and from 31.9% for CA15-3 alone to 42.6% ($p = 0.063$) respectively (Fig. 3).

Discussion

Many reports have indicated that amplification of c-*erb*B-2 oncogene is related to a poor prognosis of breast cancer (4, 5, 14–20). Although the product of c-*erb*B-2 oncogene can be observed in sera of cancer patients, there have been only a few reports in which the serum c-*erb*B-2 level has been evaluated in healthy subjects and patients with benign and malignant breast diseases (7–9).

In the present study, we described our evaluation of a commercially available EIA kit. Our findings suggested that this kit had sufficient sensitivity and reliability for clinical analysis of sera from breast cancer patients. The concentration of c-*erb*B-2 protein in normal sera was shown to be log-normal distributed. The cut-off level could be set at 12 U/ml from detailed analysis of many healthy subjects.

It may be suggested that the serum c-*erb*B-2 level is closely related to tumor mass, since the serum c-*erb*B-2 level in recurrent disease were found to be significantly higher than in non-recurrent disease. Therefore, the measurement of serum c-*erb*B-2 may be useful for monitoring recurrence after operation. Conversely, due to the observed lower sensitivity in primary breast cancer patients, this marker may not have any utility for screening. As the three positive cases we found among the primary breast cancer patients were classified as stage 0, IIA and IIB, according to UICC it seems to be no obvious correlation between the elevation of c-*erb*B-2 and the clinical stage.

In this study, serum c-*erb*B-2 was compared with two well-known markers for breast cancer, CEA and CA15-3. There was no difference between serum c-*erb*B-2 and CA15-3 or CEA in terms of sensitivity, specificity or accuracy. Since there was no strong correlation between these three markers, serum c-*erb*B-2 can be expected to detect some breast cancer patients that are missed by CA15-3 or CEA. In fact, in this study the combination of c-*erb*B-2 with CA15-3 or CEA in primary and recurrent breast cancer patients resulted in a significantly higher sensitivity than for any individual marker alone.

A panel of several tumor markers is often used for monitoring patients postoperatively and this can compensate for the low sensitivity or specificity of the individual markers. Serum c-*erb*B-2 is a unique and new tumor marker, that, when used in combination with other markers, such as CA15-3 or CEA, may be of some use for detecting recurrence of breast cancer patients.

Newly developed anti-c-*erb*B-2 monoclonal antibodies that bind with high affinity to the extracellular domain of c-*erb*B-2 protein were recently found to have some growth inhibitory effects on tumor cells overexpressing c-*erb*B-2. One of these monoclonal antibodies is currently undergoing evaluation in the treatment of patients with breast and ovarian cancers found to overexpress c-*erb*B-2 (21). The measurement of serum c-*erb*B-2 may play an important role in the evaluation of its therapeutic effects.

Since the physiologic function of soluble c-*erb*B-2 is not known, continued study of breast cancer patients with elevated serum c-*erb*B-2 is of considerable interest.

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