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# Assay of serum CEACAM1 as a potential biomarker for breast cancer

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ABSTRACT

*Background:* Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a widely expressed multi-functional adhesion molecule reported to serve as a serum biomarker in several types of cancer. However, the serum CEACAM1 expression in breast cancer is unclear. We investigated the serum concentrations of CEACAM1 in patients with breast cancer and determine the potential of serum CEACAM1 as a breast cancer biomarker.
*Methods:* Serum specimens were obtained from 33 patients with breast cancer, 30 patients with benign breast diseases and 34 healthy donors. The serum CEACAM1 concentrations were examined by an enzyme-linked immunosorbent assay (ELISA).
*Results:* The serum CEACAM1 concentrations in the malignant group (532 ng/ml) were significantly higher than those of the benign group (423 ng/ml) and healthy control group (386 ng/ml) (both $p < 0.001$). Based on univariable logistic regression, serum CEACAM1 concentrations significantly predicted breast cancer versus normal controls or benign breast diseases. Area under receiver operating characteristic curve (ROC) for serum CEACAM1 was 0.925(95% CI: 0.866–0.984). The optimal cut-off concentration of CEACAM1 was 475.82 ng/ml for discriminating breast cancer from normal controls.
*Conclusion:* Serum concentrations of CEACAM1 may serve as a useful indicator for the presence of breast cancer.


## 1. Introduction

Breast cancer is one of the most common malignancies and the leading cause of death in female malignant tumor diseases around the world [1]. Early detection together with curative resection is the key to improving the prognosis of breast cancer patients [2,3]. Serum biomarkers are effective and non-invasive for the early detection and prognostic evaluation of most cancer types. However, to date, a limited number of biomarkers have been verified for the clinical management of breast cancer [4]. Therefore, the identification of novel biomarkers related to breast cancer is highly valuable.

Carcinoembryonic antigen (CEA)-related cell adhesion molecule 1 (CEACAM1), a type 1 single-pass trans-membrane glycoprotein, is a member of the carcinoembryonic antigen (CEA) family, which belongs to the immunoglobulin superfamily [5]. CEACAM1 has been shown to be expressed in a large number of epithelia, endothelia as well as in the monocytes and natural killer cells [6]. CEACAM1 has a wide variety of biological functions, most of which are relevant to the hallmarks of cancer such as proliferation, apoptosis, invasion, migration, immune evasion, inflammation and angiogenesis [7]. It is well documented that CEACAM1 exists as trans-membrane form or soluble form in human body fluids, including serum, bile, urine and saliva [8,9]. Most of the previous studies were focused on the biological functions and differential expressions of trans-membrane CEACAM1 in tumor tissues, however, there were few reports regarding the soluble CEACAM1 in cancer.

Recently, some studies have shown that the serum CEACAM1 concentrations are elevated in several cancer patients, such as pancreatic adenocarcinoma [10], malignant melanoma [11], and non-small-cell lung cancer patients [12]. This increase was associated with the tumor presence, progression and survival, suggesting the potential of serum CEACAM1 as a new tumor biomarker. In breast cancer, an immunohistochemical study showed that aberrant CEACAM1 expression was associated with estrogen receptor (ER)/progesterone receptor (PR) status and the 5-year survival rate [13]. However, to date, there is little information about serum CEACAM1 expression in breast cancer. Thus, it is of interest to determine the serum CEACAM1 concentrations in patients with breast cancer and assess its potential as a novel tumor indicator.

*Abbreviations:* CEACAM1:, Carcinoembryonic antigen-related cell adhesion molecule 1.

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## 2. Materials and methods

### 2.1. Patients and specimens

All breast cancer patients enrolled were diagnosed based on histopathological evaluation for the first time at Shanghai Jiao Tong University Affiliated Sixth People's Hospital, and none received chemotherapy or radiation therapy. Patients with any other disease outside breast were excluded in this study. A total of 97 serum specimens were included in this study, including 33 samples that were collected from patients with breast cancer before surgery, 30 samples from patients with benign breast diseases and 34 samples from sex-and age-matched healthy volunteers who passed all the routine examinations without any abnormal results. The detailed clinical characteristics of patients and healthy controls were shown in Table 1. This study was approved by the Ethics Committee of Shanghai Jiao tong University in accordance with the Helsinki declaration 1975 (as revised in 2008). All individuals provided the informed consent before participation in the study.

### 2.2. Sandwich ELISA for soluble CEACAM1 in sera

The serum concentration of CEACAM1 was detected by enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech) according to the manufacturer's instructions. Firstly, a 96-well microplate was pre-coated with anti-human CEACAM1 antibodies. One hundred microliters of each standard or serum sample was added into the wells and CEACAM1 present in sample was bound to the wells by the immobilized antibodies. Then the wells were washed and added with biotinylated anti-human CEACAM1 antibodies. After washing away the unbound biotinylated antibodies, HRP-conjugated streptavidin was added to the wells. The wells were washed again, and a TMB substrate solution was added to the wells and color develops in proportion to the amount of CEACAM1 bound. Finally, the stop solution was added to each well, and the intensity of the color was measured at 450 nm.

### 2.3. Serological concentrations of CA15-3 and CEA

We also measured carcinoembryonic antigen (CEA; Architect i2000 SR, Abbott) and cancer antigen 15–3(CA15-3; cobas e601 Roche) in all the serum samples. The CEA and CA15-3 cut-off values were set up at 5.0 ng/ml and 25 U/ml, respectively. Tumor markers with serum values higher than the cut-off were defined as abnormal.

### 2.4. Statistical analysis

Most of the data were not normally distributed. Thus, they were presented as a median or a range. Univariable and multivariable logistic regression analyses were used to assess the significance of each biomarker in predicting breast cancer. Nonparametric received operating characteristic (ROC) curves were generated to assess the diagnostic efficiency. The nonparametric Mann–Whitney test was used to determine the significance of two independent groups. All the analyses were performed with SPSS19.0 for Windows (SPSS Inc.). Statistical significance in this study was set at $p < 0.05$, and all reported $p$ values were 2 sides.

**Table 1**
Clinical characteristics of study subjects.

| Clinical characteristics | Malignant group (n = 33) | Benign group (n = 30) | Healthy controls (n = 34) |
|---|---|---|---|
| Age (median/range) | 59 (36–83) | 52 (25–63) | 63 (31–82) |
| Histological type | Invasive ductal (26)<br>Medullary (4)<br>Mucinous (2)<br>Tubular(1) | Fibroadenoma (20)<br>Fibocystic lesions (4)<br>Adenosis (2)<br>Hyperplasia (2)<br>Papilloma (2) | |

## 3. Results

### 3.1. Serum CEACAM1 concentrations

The serum concentration of soluble CEACAM1 was examined in 33 patients with breast cancer, 30 patients with benign breast disease and 34 healthy donors. The breast cancer group consists of 26 invasive ductal cancers, 4 medullary cancers, 2 mucinous cancers and 1 tubular cancer. The median serum CEACAM1 concentration was statistically significantly higher in breast cancer group than that in healthy donor group or benign breast disease group (both $p < 0.001$). Moreover, we found that the serum CEACAM1 concentration was remarkably higher in benign disease group as compared to healthy control group ($p < 0.01$) (Fig. 1A). For patients with breast cancer, the median serum CEACAM1 concentration was 532 ng/ml (range 393–795 ng/ml); for patients with benign breast diseases, the median was 423 ng/ml (range 286–554 ng/ml); and for healthy donors, the median was 330 ng/ml (range 227–490 ng/ml).

### 3.2. Diagnostic value of serum CEACAM1

Univariable logistic regression analysis showed that higher serum CEACAM1 concentrations significantly predicted breast cancer versus normal controls (OR: 1.032; 95% CI: 1.016–1.049; $p < 0.001$) or benign breast diseases (OR: 1.017; 95% CI: 1.008–1.026; $p < 0.001$). The ability of serum CEACAM1 concentrations to predict the presence of breast cancer was further evaluated by nonparametric ROC analysis. When used to discriminate breast cancer from normal controls, the AUC (area under the curve) for serum CEACAM1 was 0.925(95% CI: 0.866–0.984)(Fig. 1B). Using the cut-off concentration of 475.82 ng/ml (according to Youden index), serum CEACAM1 produced a sensitivity of 76%, a specificity of 97%, a positive predictive value (PPV) of 96% and a negative predictive value (NPV) of 81%. ROC curves were also generated to compare the utility of CEACAM1 in differentiating serum samples from breast cancer versus benign diseases, and the results showed that the AUC for CEACAM1 was 0.815 (Fig. 1C). Taken together, these data strongly suggest the promising potential of CEACAM1 as a novel biomarker for breast cancer.

### 3.3. Comparison of the diagnostic values of serum CEACAM1, CEA and CA15-3

For the comparison purpose, we also determined concentrations of CEA and CA15-3, 2 widely used tumor biomarkers to breast cancer, in the same serum samples. ROC analysis showed that the AUC of CEACAM1 was 0.925, higher than those of CEA (0.696) and CA15-3 (0.853) (Fig. 1B). The AUC for the combination of CEACAM1 and CA15-3 was 0.940 (Fig. 1D), better than either biomarker alone, whereas the AUC for CEACAM1 in conjunction with CEA was 0.924, less than that of CEACAM1 alone. In addition, when these three biomarkers were used together for ROC analysis, the AUC was 0.941, similar to the combination of CEACAM1 and CA15-3. Furthermore, univariable analyses indicated that CEACAM1 and CA15-3 but not CEA significantly predicted breast cancer versus normal controls. The odds ratios (OR) for CEACAM1 and CA15-3 were 1.032 (95% CI: 1.016–1.049; $p < 0.001$) and 1.258 (95% CI: 1.099–1.440; $p < 0.001$), respectively. Based on multivariable logistic regression analysis, only CEACAM1 significantly predicted breast cancer versus normal controls, whereas CA15-3 and CEA did not. The adjusted odds ratio (OR) for CEACAM1 was 1.030 (95% CI: 1.014–1.047; $p < 0.001$). Moreover, only CEACAM1 was significant in predicting breast cancer versus benign diseases (OR: 1.018; 95% CI: 1.007–1.029; $p < 0.001$). Sensitivity, specificity, PPV, NPV, and accuracy were also measured for all three biomarkers. As shown in Table 2, when used in discriminating breast cancer from normal controls, CEACAM1 had a significantly higher sensitivity (76%) than both CEA (21%) and CA15-3(27%). Although the specificity of CEACAM1 (97%) was similar to those of CEA (91%) and CA15-3(94%),