Original Article
Clinical value of serum biomarkers CA153, CEA, and white blood cells in predicting sentinel lymph node metastasis of breast cancer

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Abstract: We retrospectively analyzed preoperative serum CA153, carcinoembryonic antigen (CEA) and white blood cells (WBC) in 121 breast cancer patients who underwent breast-conserving therapy and sentinel lymph node biopsy (SLNB) between June 2017 and April 2019 in our institution. The receiver operating characteristic curve (ROC curve) was used to determine the optional cut-offs of these biomarkers for predicting SLN metastasis. The relationship between the parameters to SLN metastasis of breast cancer was assessed by univariate analysis and multivariate logistic regression models. We finally enrolled 121 breast cancer patients who all underwent SLNB, of whom 56 were confirmed as positive SLN by histopathology. ROC curve analysis calculated an ideal CA153 cutoff value of 7.85 U/ml in prediction of SLN metastasis, with a sensitivity of 73.2%, and specificity of 67.7%. The ideal cutoff value for CEA to predict SLN metastasis was 1.66 ng/ml (sensitivity 67.9%, specificity 73.8%). CA153 combined with CEA showed specificity 78.6% and specificity 76.9%. CA153 and CEA combined with WBC presented sensitivity 80.4% and specificity 78.5%. In the multivariate logistic regression analysis, CA153 (odds ratio (OR): 1.165, 95% confidence interval (CI) 1.061-1.279, P<0.001) and CEA (OR: 3.440, 95% CI: 1.859-6.366, P<0.001) were independent predictive factors of SLN metastasis in patients with breast cancer.

Keywords: Breast cancer, CA153, CEA, WBC, SLN metastasis

Introduction

Breast cancer is the most common cancer among women in the world [1]. Sentinel lymph nodes (SLN) are the first lymph nodes to be reached by primary tumor metastasis. SLN metastasis is related to the stage and prognosis of breast cancer, and is also an important factor in the selection of surgical methods [2]. Sentinel lymph node biopsy (SLNB) which has become the standard of nodal stage care in early breast cancer patients, is an important method for clinical evaluation of axillary lymph node metastasis [3]. SLNB can exempt some patients from axillary lymph node dissection and reduce the complications of axillary lymph node dissection, for example limb lymph edema and nerve injuries. However, SLNB also has some deficiencies. First, it is an invasive procedure that can lead to vital complications, such as shoulder dysfunction, nerve damage, and lymphedema, which can significantly affect the

patient's quality of life [4, 5]. Moreover, SLNB showed a false negative rate of 5.5-16.7% [6]. Third, SLNB is related to some factors, such as dye injection sites and level of experience of the operator. Therefore, it is valuable to develop a preoperative noninvasive method for predicting SLN metastasis in breast cancer.

Serum markers are used as risk factors for metastasis, prognosis, and therapeutic effectiveness in malignant tumors [6-8]. Serum markers are easier to obtain and less invasive than biopsy or surgical tissue samples. Cancer antigen 15-3 (CA153) and carcinoembryonic antigen (CEA) are both tumor-associated antigens. They are the most used and recommended tumor markers in breast cancer [9, 10]. CA153 and CEA have been evaluated in the diagnosis and prognosis of patients with breast cancer [9, 11, 12]. They are also studied as tumor markers for predicting lymph node metastasis of gastric cancer [8]. Recently, Li et al. reported

the value of CA153 and CEA in predicting the survival of different breast cancer molecular subtypes [13]. Nonetheless, little is known about the association of tumor markers (CA153 and CEA) in predicting SLN metastasis of breast cancer. Moreover, studies [14-16] have shown that the white blood cell (WBC) count is a predictor of cancer mortality and postmenopausal women with higher WBC counts have a higher risk of breast and other cancers. The relationship between WBC count and sentinel lymph nodes in breast cancer is unknown.

In this study, we aim to explore single or combinations of preoperative CA153, CEA, and WBC for the prediction of breast cancer SLN metastasis.

Methods

This is a retrospective study approved by the Institutional Ethical Board of West China Hospital of Sichuan University and the requirement for informed consent was waived.

Patients and data collection

We retrospectively reviewed consecutive 211 breast cancer patients who underwent breast-conserving therapy and SLNB between June 2017 and April 2019 in our institution. All the operations were performed by experienced surgeons and postoperative pathological results were determined by two experienced pathologists in our center. Our inclusion criteria were (1) histologically confirmed invasive breast cancer; (2) definite SLN state after operation; (3) no radiotherapy or chemotherapy before operation; (4) serum CA153, CEA and WBC count were tested within 2 weeks before surgery. Exclusion criteria included the following: (1) stage III and IV breast cancer; (2) incomplete clinicopathologic data; (3) combination with other tumors. Pathologic diagnosis is the gold standard for the diagnosis of breast cancer and SLN state. Eventually, we enrolled a total of 121 clinical stage I and II breast cancer patients in this study.

We collected patients' clinical and histopathologic data which included age, tumor size, CA-153 level, CEA level, WBC count, estrogen receptor [ER], progesterone receptor [PR], human epidermal growth factor receptor-2 [HER2], proliferation marker Ki-67 and SLD statue (SLN

with macrometastasis or micrometastasis was considered positive).

Statistical analysis

Characteristics of the patients were expressed with frequency or descriptive analysis. Continuous variables were presented as means ± standard deviation and were analyzed using the Student t test. The receiver operating characteristic (ROC) curves were plotted and the areas under the curve (AUC) were calculated to determine the suitable cut-off value for predicting the SLN metastatic. We calculated the sensitivity and specificity of CA153, CEA and WBC according to the cut-off value. Univariate and multivariate logistic regression were performed to identify independent factors for SLN metastasis. The P value <0.05 was deemed significant for all analyses. The statistical analysis was performed using the SPSS version 25.0 (IBM, Armonk, NY, USA).

Results

Patients' characteristics

We enrolled a total of 121 female patients with breast cancer, including 56 SLN positive and 65 SLN negative. These patients all underwent breast-conserving therapy and SLNB. The average age at surgery was 47.9 years old (range: 23-91 years old), of whom, 70 (57.9%) were >45 years old. According to the 2013 St. Gallen guidelines [17], of these patients, twenty-two (18.2%) patients were pathologically diagnosed with luminal A, forty-nine (40.5%) were luminal B, thirty-three (27.3%) were HER2-enriched, and seventeen (14.0%) were basal-like breast cancer. Clinicopathological characteristics of the 121 patients are shown (**Table 1**). No significant differences were found in age, proliferation index (Ki-67) and molecular subtypes between the two groups.

ROC analysis

ROC analysis predicted SLN metastasis of breast cancer (**Figure 1** and **Table 2**). We calculated AUC and cut-off value. The AUC (**Table 2**) of CA153; CEA; WB; CA153 and CEA; Combination of WBC, CA153 and CEA was 0.725 (CI 0.634-0.816, P<0.001), 0.741 (CI 0.652-0.831, P<0.001), 0.53 (CI 0.505-0.711, P=0.042), 0.789 (CI 0.704-0.874, P<0.001),

Table 1. Patient characteristics

Characteristic	Patients (n=121)
Age[a] (years)	47.9±12.8 (23-91)
Tumor size[a] (cm)	2.1±1.1 (0.5-5.0)
Molecular subtype	
Luminal A	22
Luminal B	49
HER2 positive	33
Triple negative	17
Maximum tumor diameter (cm)	
≤2	78
>2 but ≤5	42
>5	1
CA153[a] (ng/ml)	9.92±5.29 (3.33-26.29)
CEA[a] (U/ml)	1.78±1.55 (0.35-10.33)
WBC[a] (10⁹/L)	5.89±1.65 (3.07-11.65)

CA153 cancer antigen 15-3, CEA carcinoembryonic antigen, WBC white blood cell. [a]Parameters are presented as median, Standard Deviation, and range.
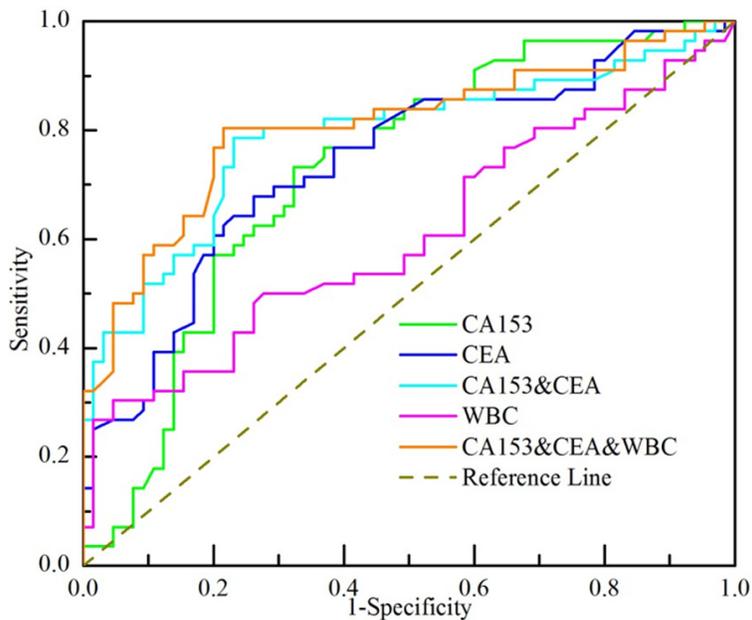
Figure 1. Receiver operating characteristic (ROC) curve analysis of different markers to predict the SLN metastatic status.

0.806 (CI 0.725-0.888, P<0.001) respectively. The cut-off value of CA153 was 7.85 U/ml which generated a sensitivity of 73.2%, specificity of 67.7%. The CEA cut-off value of 1.66 ng/ml generated a sensitivity of 67.9%, specificity of 73.8% while WBC was 7.51 10⁹/L showed a sensitivity of 30.4%, a specificity of 95.4%. The combination of CEA and CA153 resulted in a sensitivity of 78.6% and specificity of 76.9%. The combination of all three markers resulted in a sensitivity of 80.4% and specificity of 78.5% for prediction of SLN metastasis

Univariate and multivariate analysis the correlation of preoperative factors with SLN metastasis

Univariate and multivariate analysis for SLN metastasis prediction in breast cancer patients was presented (**Table 3**). In the univariate analysis, CA153, CEA, WBC, tumor size and hormone receptor state were significant predictors for SLN metastasis. In the multivariate logistic regression analysis, CA153 (OR: 1.165, 95% CI: 1.061-1.279, P<0.001), CEA (OR: 3.440, 95% CI: 1.859-6.366, P<0.001) and WBC (OR: 1.475, 95% CI: 1.077-2.022, P<0.001), these three factors remained independent predictors.

Discussion

Breast cancer is the most common malignant tumor and the second leading cause of cancer-related mortality in women [1, 18]. Early breast cancer is considered a curable disease while metastatic breast cancer is incurable with currently practicable therapeutic regimens. Currently, SLNB has been used for some breast cancer patients as a routine medical service. It protects patients from many of the side effects of axillary lymph node dissection. However, SLNB still can cause lymphedema, upper limb pain, numbness and dye allergy in a small number of patients [20]. Whether SLNB can be avoided or replaced by other more suitable methods is a great challenge. In this study, we systematically assessed the ability of the role of CA153, CEA, and WBC alone and in combination for predicting SLN metastasis in breast cancer.

Table 2. Area under the curve

Preoperative Factor	Area	Std. Error[a]	P	95% CI	
				Lower Bound	Lower Bound
CA153	0.725	0.046	<0.001	0.634	0.816
CEA	0.741	0.046	<0.001	0.652	0.831
CA153 and CEA	0.789	0.043	<0.001	0.704	0.874
WBC	0.608	0.053	0.042	0.505	0.711
CA153 and CEA and WBC	0.806	0.041	<0.001	0.725	0.888

[a]Under the nonparametric assumption.

Table 3. Univariate and multivariate analysis of SLN multivariate patients

Preoperative Factors	Univariate	Multivariate	P	OR	95% CI for OR	
	p	Wald			Lower	Upper
Age	0.188	NA	NA	NA	NA	NA
CA153	0.000	10.289	0.001	1.165	1.061	1.279
CEA	0.000	15.482	<0.001	3.440	1.859	6.366
Tumor size	0.030	0.533	0.465	1.181	0.756	2.022
WBC	0.009	5.855	0.016	1.475	1.077	2.022
HER2	0.221	NA	NA	NA	NA	NA
ER	0.032	NA	NA	NA	NA	NA
PR	0.010	NA	NA	NA	NA	NA
Ki-67	0.442	0.001	0.977	0.957	0.046	19.771
Molecular subtypes	0.768	0.087	0.768	1.083	0.637	1.842

NA, not available.

The collection of serum specimens is relatively noninvasive, easily-accessible and cost-effective in patient care. Serum biomarkers play an important role in many malignances. CA153, a specific solute transmembrane glycoprotein [21], has been used as serum biomarker for breast cancer since 1980s. CEA, a tumor-associated antigen, is widely used as a serum tumor marker for breast cancer and other malignancy. Both of them are FDA-approved for monitoring breast cancer [22]. Previously many studies [23-25] suggested CA153 and CEA can be applied for early detection of breast cancer metastasis. Moreover, CEA is inferior to CA153. Our study showed CA153 and CEA for the prediction of SLN metastasis were similar and the sensitivity and specificity of CA153 were 73.2% and 67.7%, respectively, and CEA had 67.9% and 73.8%, respectively. When the two tumor markers were combined, the sensitivity and specificity were slightly improved. Wang et al. [25] found that the sensitivity of CA153 in the diagnosis of metastatic breast cancer was 44.5%, and that of CEA was 56.7%. Petra et al. [23] showed that the sensitivity of CA153 and CEA alone in the detection of metastatic breast cancer was 55.6% and 40.6% respectively, and the sensitivity could be increased to 66.3% when the two were combined. This is consistent with our findings.

Previous studies [14-16] have linked higher WBC count to an increased risk of breast cancer and other cancers in postmenopausal women. However, little is known about the value of WBC in predicting SLN metastasis. Routine blood sampling is the most common test for admission, so we also include WBC count. Our study suggested that WBC has statistical significance and it has a surprising sensitivity of up to 95.4%, but the specificity is low. The reason for this result needs further exploration.

There are some limitations to our study. This was a single-center retrospective study. Moreover, we collected onlydata from patients who underwent SLNB in our center; small sample size may cause selection bias which can influence the representativeness. Therefore, further prospective multi-center studies with larger sample sizes are warranted to better understand the relationship between multiple clinical

factors and sentinel lymph node metastasis in breast cancer.

In conclusion, our study determined that pre-operative serum CA153, CEA, and WBC can be a favorable, cost-effective method for predicting SLN metastasis in breast cancer.

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Disclosure of conflict of interest

None.

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