Cancer Management and Research

Elevate Series

Open Access Full Text Article

ORIGINAL RESEARCH

Clinical Value of Serum sTim-3, CEA, CA15-3 for Postoperative Recurrence of Breast Cancer

Ting Shen[1,*], Hongming Fang[2,*], Jialong Wu[1], Yuan Qin[1], Xiumei Zhou[1], Xueqin Zhao[1], Biao Huang[1,3], Haiyan Gao[4]

[1]College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, Zhejiang Province, People's Republic of China; [2]Affiliated Xiaoshan Hospital, Hangzhou Normal University, Hangzhou, Zhejiang Province, People's Republic of China; [3]Provincial Key Laboratory for Research and Translation on the Syndrome of Kidney Deficiency Accompanied by Blood Stasis and Turbidity, Zhejiang Sci-Tech University, Hangzhou, Zhejiang Province, People's Republic of China; [4]Department of Nuclear Medicine, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Biao Huang; Haiyan Gao, Email jswxhb@163.com; gaohaiyanhyx@med.uestc.edu.cn

Objective: To evaluate the clinical value of serum soluble T cell immunoglobulin 3 (sTim-3) on postoperative recurrence of breast cancer (BC).

Methods: A highly sensitive time-resolved fluorescence immunoassay (TRFIA) was employed to measure sTim-3. Quantification of serum sTim-3 in 172 BC patients more than one-year postoperative (96 patients with stage I + II, 76 patients with stage III + IV; 31 patients with postoperative recurrence, and 141 patients with postoperative non-recurrence) and 51 healthy controls (HC). To evaluate the difference of serum sTim-3 in different stages of BC and its clinical value for postoperative recurrence of BC.

Results: The serum sTim-3 level of BC patients with stage III + IV (21.62 (17.27, 29.78)) were significantly higher than HC (4.49 (3.30, 7.60)), patients with stage I + II (14.96 + 4.94) ($P < 0.0001$). Serum sTim-3 level of BC patients with postoperative recurrence (21.8(12.40,34.20) were significantly higher than those without recurrence (17.13 ± 6.44) ($P = 0.0130$). When the serum sTim-3 level was below 11.8 ng/mL, the negative predictive values of sTim-3, CEA and CA15-3 were 90.9%, 68.0% and 67.1%, respectively, and the negative likelihood ratios were 0.16, 0.77 and 0.81, respectively. The positive rate of combined detection of sTim-3, CEA and CA15-3 was 58.1%, higher than single detection of CEA (22.6%) and CA15-3 (19.4%).

Conclusion: Serum sTim-3 levels may assist in the staging of BC. Combined detection of sTim-3, CEA, and CA15-3 can be used to routinely monitor the progression of BC and indicate the risk of postoperative BC recurrence.

Keywords: breast cancer, biomarkers, postoperative recurrence, serum, sTim-3

Introduction

Breast Cancer (BC) is one of the most common malignant tumors in women worldwide[1] and one of the major cancers that cause death in women.[2] BC patients usually face poor prognosis,[3] high recurrence rate,[4] and short survival.[5] Prevention and early detection of postoperative recurrence are very important, and long-term monitoring of the disease is required. At present, the clinical monitoring of postoperative BC is mainly through imaging examination,[6] immunohistochemical examination,[7] tumor marker screening[8,9] and other methods. However, the imaging examination is greatly affected by the scope of examination. In the early stage of the tumor, malignant transformation of cells may have occurred, but no obvious structural changes have been formed, which makes it difficult to detect the imaging examination, so the diagnosis exists hysteresis.[10] Immunohistochemical examination is more invasive and difficult to achieve routinely monitoring.[7] CEA and CA15-3 are the most commonly used biomarkers in BC recurrence. However, the levels of CEA and CA15-3 in postoperative BC patients will fluctuate within a narrow range,[11] and their sensitivity is relatively low.[12,13] It is necessary to explore biomarkers with more detection efficacy and more suitable for routine monitoring to assist in assessing the risk of recurrence in patients with BC after surgery.

Recent studies have found that immune checkpoint T cell immunoglobulin 3 (Tim-3) plays a certain role in the auxiliary diagnosis of BC.[14] Tim-3 is an immune checkpoint molecule, available in both soluble (sTim-3) and membrane

Received: 12 December 2024
Accepted: 26 February 2025
Published: 11 March 2025

binding forms.[15] sTim-3 is produced through the cleavage of membrane-bound Tim-3 by the action of the metalloproteinases ADAM10 and ADAM17.[16] Recent studies have shown that the expression of Tim-3 can promote the progression of BC[17] and can be used as a biomarker of BC. Its expression level is significantly correlated with traditional tumor markers, which has potential application value in the diagnosis and treatment of BC.[18] These studies are based on cellular level analysis of the diagnostic value of Tim-3 in BC, and there are no reports on the indicative role of serum sTim-3 in postoperative recurrence of BC.

Therefore, in this study, we employed a high sensitivity time-resolved fluorescence immunoassay (TRFIA) to detect serum sTim-3 levels in the BC patients with postoperative and HC, and analyzed the discriminative value of sTim-3 for the postoperative recurrence of BC.

Materials and Methods

Instruments and Reagents

The Tim-3 antigen and two antibodies targeting different epitopes of the antigen (capture antibody and detection antibody) were purchased from Sino Biological Inc. Coating buffer, assay buffer, enhancement solution, blocking solution and washing buffer were provided by Zhejiang Bosh Biotechnology Co., Ltd.

The 96-well microtiter plates were purchased from Xiamen Yunpeng Technology Development Co., Ltd. The time-resolved fluorescence immunoassay analyzer HG-1000 was purchased from Foshan Daan Medical Equipment Co., Ltd. The washing machine DM-3 was purchased from Darui Biotechnology Co., Ltd. The micro shaker was purchased from Kangjian Medical Supplies Co., Ltd.

Serum Samples and Storage

This study was approved by the Ethics Committee of Xiaoshan Affiliated Hospital (approval No. 2021–011). The samples were collected from 172 BC patients at Xiaoshan Hospital in Zhejiang Province for more than one year from September 1, 2020 to December 31, 2022. The BC patients had symptoms such as breast lumps, sunken or red skin, nipple discharge or blood discharge, and breast shape change before surgery. All patients in this study underwent sentinel lymph node biopsy, axillary lymph node dissection and radical mastectomy. And 51 healthy subjects were collected in physical examination center of Xiaoshan Hospital of Zhejiang Province. Venous blood was collected 5mL, centrifuged at 4000rpm for 5min, serum samples were collected, and stored in the refrigerator at −80°C until use. The sample screening process is shown in Figure 1.

Inclusion criteria of healthy subjects: 1) All healthy subjects were adults; 2) The tumor markers and imaging examinations were taken in the hospital. The tumor markers were negative and the imaging examinations showed no abnormality.

Inclusion criteria for postoperative BC patients: 1) Patients diagnosed clinically and pathologically more than 1 year before blood collection underwent radical resection of tumors; 2) The stages were determined by immunohistochemical analysis more than 1 year before blood collection (stage I + II (n = 96), stage III + IV (n = 76)).

Exclusion criteria for postoperative BC patients: 1) combined with a history of other malignant tumors and other major diseases; 2) Staging was not determined by immunohistochemical analysis; 3) Patients who may interfere with the level of target biomarkers: such as patients with severe infection.

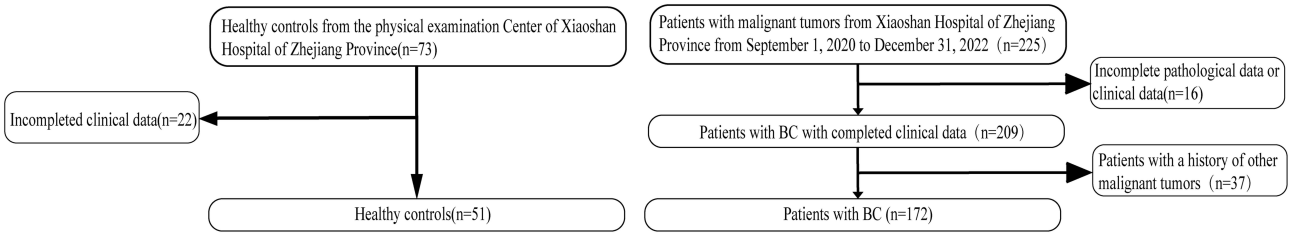

Figure 1 Flow diagram of BC patients, and healthy controls enrolled in the study.

The diagnostic criteria of postoperative recurrence and metastasis of BC are as follows:[19] 1) recurrence or metastasis confirmed by pathological biopsy; 2) Clinical diagnosis of BC recurrence through imaging, tumor marker examination, patient signs and symptoms (Postoperative recurrence of BC (n = 31), No recurrence of BC after surgery (n = 141)).

The diagnostic criteria of postoperative BC without recurrence and metastasis are as follows:[19] 1) no recurrence or metastasis confirmed by pathological biopsy; 2) Imaging, tumor marker examination, patient signs and symptoms showed no positive symptoms.

Detection Method

The TRFIA was established by Chen et al[20] was used to detect the serum sTim-3 levels in healthy subjects and BC patients. We used double antibody sandwich method for detection. The Tim-3 capture antibody was diluted to 2ug/mL with coating buffer, and 100 uL was added to each well, overnight at 4°C, and washed once with washing buffer. Add 150 uL of blocking solution and let stand for 2 h. Pour off the blocking solution and dry under vacuum drying environment for more than 2h. Add 100 uL of standard or serum, react at 37°C for 1h, wash with washing buffer twice, add 100 uL of Eu-labeled Tim-3 antibody, react at 37°C for 1h, wash the plate with washing buffer for 6 times, add 100 uL of enhancement solution and detect the level of serum sTim-3.

Data Analysis

IBM SPSS Statistics version 26 was used for data analysis. Binary logistic regression was used to fit the combined variables, and the receiver operating characteristic (ROC) curve of the combined detection was constructed using the fitted probability variables. Statistic data are expressed as quartiles Q2 (Q1, Q3). Analysis of variance (ANOVA) was used to analyze the differences between groups. Statistical analysis was performed using GraphPad Prism 10.1 (GraphPad Software Company), and the best sensitivity and specificity of the combined diagnosis was determined by Jorden index.

Results

Comparison of Indicators (sTim-3, CEA, CA15-3) Between Healthy Individuals and Patients

Serum sTim-3 levels in BC patients (17.40 (12.95, 21.71)) were significantly higher than HC (4.49 (3.30, 7.60)) (Figure 2A; $P < 0.0001$); CEA levels (1.49 (1.00, 2.43)) were slightly higher than HC (0.79 (0.53, 1.58)) (Figure 2B; $P = 0.1092$), but not statistically significant; CA15-3 levels (7.50 (5.60, 12.70)) were slightly higher than HC (6.00 (5.10, 9.10)) (Figure 2C; $P = 0.1302$), but not statistically significant (Table 1).

Comparison of Indicators (sTim-3, CEA, CA15-3) Between Healthy Individuals and Patients with BC by Clinical Stage

The analysis of BC patients who were grouped by immunohistochemical analysis found that (Figure 2D–F. Table 2), serum sTim-3 level increased with the increase of stage (HC (4.49(3.30,7.60)); Stage I + II (14.60 (11.90, 18.94)); Stage III + IV (21.62(17.27,29.78))). However, CEA and CA15-3 levels did not change significantly.

Comparison of Indicators (sTim-3, CEA, CA15-3) Between Postoperative Recurrence and Non-Recurrence of BC Patients

There were 31 patients with BC recurrence through follow-up, and the serum sTim-3 level was higher in BC patients with recurrence (21.8(12.40,34.20) than non-recurrence (17.27 (12.99, 20.85)) (Figure 2G, $P = 0.0130$). Although there were differences in the levels of CEA (Figure 2H, $P < 0.0001$) and CA15-3 (Figure 2I, $P = 0.0320$) between patients with recurrence and non-recurrence, their levels were mostly lower than cut-off value, and the difference was not significant compared with HC (Table 3).

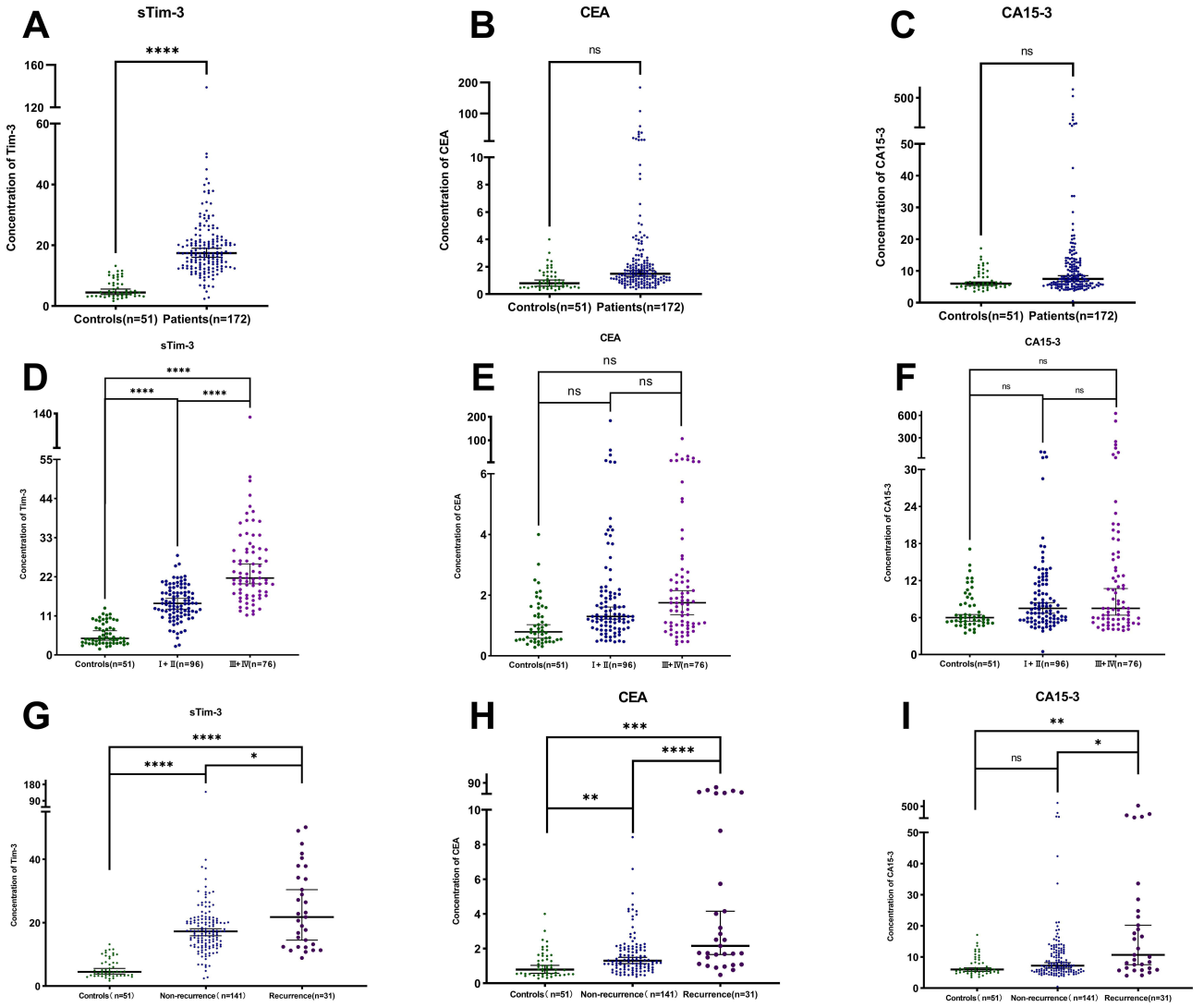

Figure 2 (**A**) sTim-3 levels in controls and BC patients; (**B**) CEA levels in controls and BC patients; (**C**) CA15-3 levels in controls and BC patients; (**D**) sTim-3 levels in controls, stagel+II, stage III+IV BC patients; (**E**) CEA levels in controls, stagel+II, stage III+IV BC patients; (**F**) CA15-3 levels in controls, stagel+II, stage III+IV BC patients; (**G**) sTim-3 levels in controls, non-recurrence and recurrence; (**H**) CEA levels in controls, non-recurrence and recurrence; (**I**) CA15-3 levels in controls, non-recurrence and recurrence.

Notes: *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001.

Evaluation Value of sTim-3, CEA and CA15-3 in Postoperative Recurrence of BC

The cut-off values of CEA and CA15-3 were provided by the clinic, and CEA was 10 ng/mL and CA15-3 was 31.3 U/mL. The cut-off value of sTim-3 of postoperative BC patients was determined to be 11.8 ng/mL by means of mean+2*SD of HC. ROC curves for patients with and without recurrence were constructed (Figure 3). According to the Youden index, the cut-off value of serum sTim-3 level in BC patients with postoperative recurrence was 21.7 ng/mL (Table 4). Through the analysis of HC and recurrence patients, it was found (Table 5) that the positive predictive values of CEA and CA15-3 were 100%, indicating the recurrence of BC. CEA negative patients accounted for 96.5% and CA15-3 negative patients accounted for 95.7% in the BC patients with postoperative non-recurrence, while CEA negative patients accounted for 77.4% and CA15-3 negative patients accounted for 80.6% in the BC patients with postoperative recurrence. In other words, CEA and CA15-3 levels were mostly negative in the HC, the BC patients with postoperative non-recurrence, and the BC patients with postoperative recurrence. However, when the cut-off value of serum sTim-3 was 11.8 ng/mL, the negative predictive value of serum sTim-3 was significantly higher than that of CEA and C15-3, and the negative likelihood ratio was significantly lower than that of CEA and C15-3. According to the analysis of BC patients with and without recurrence (Table 6), when the cut-off value of serum

 https://doi.org/10.2147/CMAR.S508321

Table 1 Serum Indices in the Healthy Control Group and Patients With BC

Index	Controls (n = 51)	BC Patients (n = 172)	P value
sTim-3 (ng/mL)	4.49 (3.30, 7.60)	17.40 (12.95, 21.71)	<0.0001
CEA (ng/mL)	0.79 (0.53, 1.58)	1.49 (1.00, 2.43)	0.1092
CA15-3 (U/mL)	6.00 (5.10, 9.10)	7.50 (5.60, 12.70)	0.1320
ER+	/	(73/172)	/
PR+	/	(71/172)	/

Notes: Statistic data are expressed as Q2 (Q1, Q3). ER+ and PR+ information is expressed as (number of positives/total number of groups). And the difference was analyzed by ANOVA followed by Tukey's multiple comparison test. P value means the difference between the group of controls and the group of BC patients.
Abbreviations: ER+, Estrogen receptor-positive; PR+, Progesterone receptor positive.

Table 2 Serum Indices of Stage I + II BC, and Stage III +IV BC Groups

Index	Stage I + II (n = 96)	Stage III +IV (n = 76)	P value
sTim-3 (ng/mL)	14.60 (11.90, 18.94)	21.62 (17.27, 29.78)	<0.0001
CEA (ng/mL)	1.30 (0.93, 2.09)	1.75 (1.01, 2.76)	0.2677
CA15-3 (U/mL)	7.50 (5.60, 11.80)	7.50 (5.70, 15.80)	0.0121

Notes: Statistic data are expressed as quartile Q2 (Q1, Q3). And the difference was analyzed by ANOVA followed by Tukey's multiple comparison test. P value means the difference between the group of the Stage I + II patients and the group of Stage III + IV patients.

Table 3 Serum Indices of Recurrence and Non-Recurrence

Index	Recurrence (n=31)	Non-Recurrence (n=141)	P value
sTim-3 (ng/mL)	21.8 (12.40, 34.20)	17.27 (12.99, 20.85)	0.0130
CEA (ng/mL)	2.16 (1.12, 8.79)	1.36 (0.93, 2.15)	<0.0001
CA15-3 (U/mL)	10.70 (6.40, 24.80)	7.20 (5.40, 11.85)	0.0320

Notes: Statistic data are expressed as quartile Q2 (Q1, Q3). And the difference was analyzed by ANOVA followed by Tukey's multiple comparison test. P value means the difference between the group of the BC recurrence patients and the group of BC non-recurrence patients.

sTim-3 was 21.7 ng/mL, the combined detection of sTim-3, CEA and CA15-3 showed that, the positive detection rate of postoperative recurrence group (58.1%) was significantly higher than that of CEA (22.6%) and CA15-3 (19.4%) (Table 6).

Discussion

Postoperative recurrence of BC is a complex problem, with initial tumor diameter, tumor stage and lymphatic infiltration having different effects on recurrence.[21] Postoperative recurrence has always been a constant concern for patients with BC, and timely postoperative follow-up is a key measure to prevent BC recurrence. Patients are typically assessed for disease progression by monitoring tumor marker levels and by imaging for potential BC recurrence.[22] Both CEA and CA15-3 are markers associated with BC, but the rate of positive detection for CEA in BC patients with recurrent is relatively low.[23,24] CA15−3[25] as an indicator for adjuvant diagnosis of BC, postoperative follow-up and metastasis recurrence, exhibits limited sensitivity to BC[26,27] and poor evaluation effect on tumor recurrence. Although imaging detection is also used for routine radiologic control of BC, because the tumor cells have not undergone morphological changes in the early stage, and exited hysteresis in diagnosis, and the recurrence of BC cannot be predicted in time.[28] Combined detection of multiple indicators can provide a more reliable monitoring approach for patients at high risk of postoperative recurrence of BC.

https://doi.org/10.2147/CMAR.S508321

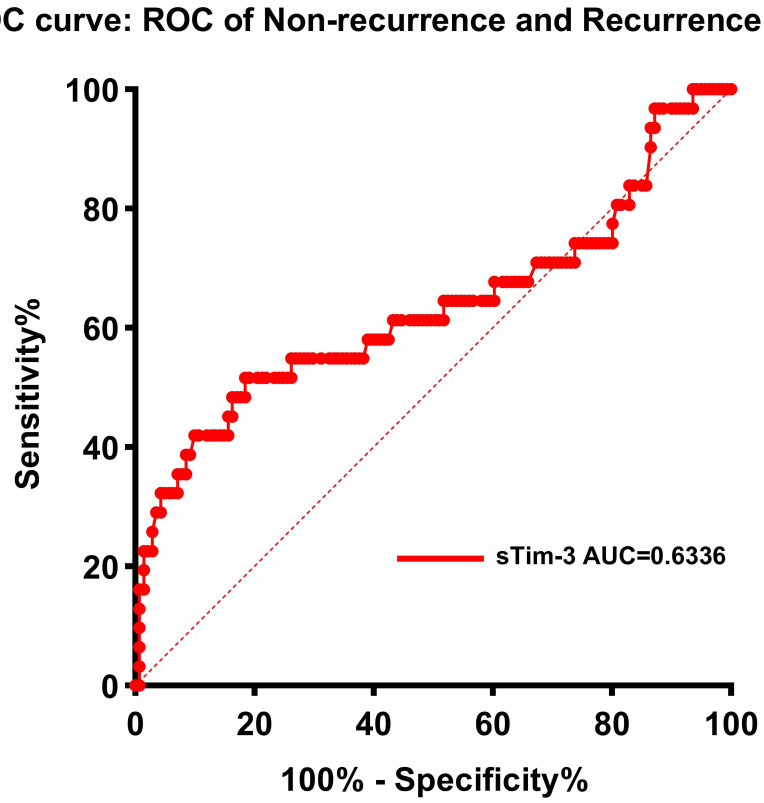

Figure 3 ROC curve of recurrence and non-recurrence of sTim-3.

Tim-3 is expressed on a variety of immune cells, including CD8+ T cells, Th1 cells, and Th17 cells that produce IFN-γ. In the development of BC, Tim-3 is upregulated on tumor-infiltrating lymphocytes (TILs), which promotes immune evasion by the tumor and is correlated with poor prognosis in BC patients.[29] As mentioned above, membrane-bound Tim-3 is cleaved to form sTim-3 by metalloproteinases ADAM10 and ADAM17. In this study, it is found that the level of sTim-3 in postoperative BC patient group is significantly higher than that in HC group, while CEA and CA15-3 shows no difference between HC group and postoperative BC patient group. This may be because all or part of the tumor cells have been removed after BC surgery, and the level of tumor markers is reduced,[30,31] and the sensitivity is not high in patients with BC recurrence, so the difference is not significant compared with HC. However, Tim-3 is expressed on T cells, which depletes after continuous stimulation by tumor antigens. Even if tumor cells are removed, T cells within the tumor immune microenvironment persist in expressing Tim-3 due to prior tumor stimulation, thereby maintaining high levels post-surgery,[32] which is significantly different from that of HC. Therefore, the serum sTim-3 level may serve as an indicator for routine monitoring of postoperative BC patients, aiding in monitoring the progression of BC.

Further analysis reveals that there is no significant distinction in CEA levels between BC patients with stages I + II and III + IV; however, CA15-3 levels do differ between patients with stages I + II and III + IV. Moreover, serum sTim-3 levels are significantly higher in BC patients with stage III + IV compared to those with stage I + II and HC. The serum

Table 4 The Diagnostic Value of Serum Markers in Recurrence and Non-Recurrence Patients

	Cut-Off	Sensitivity	Specificity	AUC	95% CI	P-value
sTim-3 (ng/mL)	21.77	51.61%	81.56%	0.6336	0.5056 to 0.7616	0.0200

Notes: The diagnostic value of variables was analyzed by ROC analysis. Youden index was used to determine the optimal cut-off value (cut-off). $P < 0.05$ was considered statistically significant.
Abbreviations: AUC, area under the curve; CI, confidence interval.

Table 5 Comparison of Positive Predictive Value, Negative Predictive Value and Negative Likelihood Ratio Between HC and Postoperative Recurrence Group

Index	PPV	NPV	-LR
sTim-3 < 11.8 ng/mL	96.3%	90.9%	0.16
CEA < 10 0.0 ng/mL	100%	68.0%	0.77
CA15-3 < 31.3 U/mL	100%	67.1%	0.81

Notes: The cut-off value of CEA was 10 ng/mL, and the cut-off value of CA15-3 was 31.3 U/mL, which was provided by the clinic. The cut-off value of HC and postoperative BC patients was 11.8 ng/mL by means of mean+2SD.

Abbreviations: PPV, Positive Predictive Value; NPV, Negative Predictive Value; -LR, the negative likelihood ratio.

Table 6 Comparison of Various Data of HC, BC Patients with Postoperative Recurrence and Non-Recurrence Group

Index	Controls (n = 51)	Recurrence Group (n = 31)	Non-Recurrence Group (n = 141)
sTim-3 > 11.8 ng/mL	1 (2.0%)	26 (83.9%)	98 (69.5%)
sTim-3 > 21.7 ng/mL	0 (0%)	16 (51.6%)	19 (13.5%)
CEA > 10 0.0 ng/mL	0 (0%)	7 (22.6%)	5 (3.5%)
CA15-3 > 31.3 U/mL	0 (0%)	6 (19.4%)	6 (4.3%)
CEA > 10 0.0 ng/mL + CA15-3 > 31.3 U/mL + sTim-3 > 21.7 ng/mL	0 (0%)	18 (58.1%)	19 (13.5%)

Notes: The cut-off value of CEA was 10ng/mL, and the cut-off value of CA15-3 was 31.3U/mL, which was provided by the clinic. The cut-off value of HC and postoperative BC patients was 11.8 ng/mL by means of mean+2SD. The cut-off value of patients with and without recurrence of BC was determined by Youden index, which was 21.7 ng/mL.

sTim-3 level appears to increase with the advancement of BC stage, which may be associated with the biological functions of sTim-3 in promoting the proliferation, invasion, and migration of BC cells,[16] so we hypothesize that the serum sTim-3 level could assist in the clinical determination of BC stage.

In addition, studies have found that the serum sTim-3 level appears to increase with the advancement of BC stage, which may be associated with the biological functions of sTim-3 in promoting the proliferation, invasion, and migration of BC cells.[16] We find that no difference in CEA between stage I + II and stage III + IV BC patients through studies. Although CA15-3 was different in BC patients with stage I + II and stage III + IV, the difference of sTim-3 level was more significant in BC patients with stage I+ II and stage III + IV. Therefore, we speculate that serum sTim-3 level can assist clinical determination of BC stage.

In addition, BC has a poor prognosis and is prone to recurrence. In the analysis of various indicators in the group of BC patients with recurrence, we find that the positive prediction rates of CEA and CA15-3 were 100%, that is, when the level of CEA and CA15-3 is higher than the cut-off value, it can indicate BC recurrence. However, we find that 77.4% of BC patients with postoperative recurrence have negative CEA levels and 80.6% have negative CA15-3 levels. There is no significant difference between these patients and the HC group and the group with postoperative non-recurrence. Therefore, negative CEA and CA15-3 levels cannot indicate that there is no recurrence risk after BC surgery, nor does it mean that the postoperative prognosis of BC is good. This is because CEA and CA15-3 are less sensitive in patients with recurrence. The levels of CEA and CA15-3 were still negative.[11] Our analysis of HC group, BC patients with postoperative recurrence and non-recurrence find that there are significant differences in serum sTim-3 levels among HC group, BC patients with postoperative recurrence group and with non-recurrence group. When the serum sTim-3 level was lower than 11.8 ng/mL, the negative prediction rate of serum sTim-3 in the HC group and the postoperative

recurrence group is 90.9%, and the negative likelihood ratio is 0.16, indicating that serum sTim-3 has high specificity and is more reliable in excluding disease. This is due to the enhanced immunosuppression in the tumor microenvironment after BC recurrence, which promotes T cell depletion, increases serum sTim-3 expression, and significant changes in serum sTim-3 levels in BC patients with post-operative recurrence[33]. Therefore, when the serum sTim-3 level is below 11.8 ng/mL, it can indicate a low risk of BC recurrence.

Through the combined detection of serum sTim-3, CEA and CA15-3, we find that the positive rate of the combined detection is higher than that of the single detection of CEA and CA15-3 when analyzing the BC patients with postoperative recurrence and non-recurrence. That is, when sTim-3 is higher than 21.7 ng/mL, CEA is higher than 10 ng/mL, and CA15-3 is higher than 31.3 U/mL, it can indicate a high risk of BC recurrence. Therefore, serum sTim-3 can be applied with CEA and CA15-3 in the routine monitoring of BC as a judgement indicator for assessing BC recurrence.

In addition, this study is a cross-sectional study, so it cannot accurately determine the causal relationship between serum sTim-3 level and postoperative progression of BC. Future prospective studies are needed to explore the changes in serum sTim-3 level during the development of BC. Secondly, the study lacks long-term follow-up data. Follow-up studies can focus on establishing a follow-up information management data platform from the beginning of BC diagnosis, and accumulating follow-up data until the patients are cured or died, so as to deeply explore the clinical application value of serum sTim-3 level in BC prognosis.

In summary, this study quantitatively measured serum sTim-3 levels in postoperative BC patients. The study found that the combined detection of serum sTim-3, CEA, CA15-3 levels is helpful to determine the clinical staging of BC. When the serum sTim-3 level is less than 11.8 ng/mL, it can be used to clinically indicate a lower risk of BC recurrence. Serum sTim-3 level can be used for routine monitoring of BC progression, and can be used as an auxiliary indicator of BC recurrence risk.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Xiaoshan Affiliated Hospital (approval No. 2021-011).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Funding was provided by the Sichuan Science and Technology Program (No. 2024NSFSC0667), the "Pioneer" and "Leading Goose" R&D Program of Zhejiang (No.2023C03075, No.2022C03118), the Chinese National Natural Science Foundation (No.82172336), the Natural Science Foundation of Zhejiang Province (LQ23H050005), Scientific research project of Zhejiang Provincial Education Department (Y202250731), the Medical and Health Science and Technology Project of Zhejiang Province (2022KY265, 2020KY753).

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Akram M, Iqbal M, Daniyal M, et al. Awareness and current knowledge of breast cancer. *Biol Res*. 2017;50(1):33. doi:10.1186/s40659-017-0140-9
2. Nagarajan D, McArdle SEB. Immune Landscape of Breast Cancers. *Biomedicines*. 2018;6(1):20. doi:10.3390/biomedicines6010020
3. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121(7):2750–2767. doi:10.1172/JCI45014
4. McBain CA, Young EA, Swindell R, et al. Local recurrence of breast cancer following surgery and radiotherapy: incidence and outcome. *Clin Oncol*. 2003;15(1):25–31. doi:10.1053/clon.2002.0165
5. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol*. 2023;41(10):1809–1815. doi:10.1200/JCO.22.02572
6. Lowry KP, Callaway KA, Lee JM, et al. Trends in annual surveillance mammography participation among breast cancer survivors from 2004 to 2016. *J Natl Compr Canc Netw*. 2022;20(4):379–386.e9. doi:10.6004/jnccn.2021.7081
7. Bonacho T, Rodrigues F, Liberal J. Immunohistochemistry for diagnosis and prognosis of breast cancer: a review. *Biotech Histochem*. 2020;95(2):71–91. doi:10.1080/10520295.2019.1651901
8. Tarighati E, Keivan H, Mahani H. A review of prognostic and predictive biomarkers in breast cancer. *Clin Exp Med*. 2023;23(1):1–16. doi:10.1007/s10238-021-00781-1
9. A MB, Stankowski R, Engel J, et al. Breast cancer biomarkers: risk assessment, diagnosis, prognosis, prediction of treatment efficacy and toxicity, and recurrence. *Curr Pharm Des*. 2014;20(30):4879–4898. doi:10.2174/1381612819666131125145517
10. Jafari SH, Saadatpour Z, Salmaninejad A, et al. Breast cancer diagnosis: imaging techniques and biochemical markers. *J Cell Physiol*. 2018;233(7):5200–5213. doi:10.1002/jcp.26379
11. Pedersen AC, Sørensen PD, Jacobsen EH, et al. Sensitivity of CA 15-3, CEA and serum HER2 in the early detection of recurrence of breast cancer. *Clin Chem Lab Med*. 2013;51(7):1511–1519. doi:10.1515/cclm-2012-0488
12. Iwase H, Kobayashi S, Itoh Y, et al. Evaluation of serum tumor markers in patients with advanced or recurrent breast cancer. *Breast Cancer Res Treat*. 1995;33(1):83–88. doi:10.1007/BF00666074
13. De La Lande B, Hacene K, Floiras J-L, et al. Prognostic value of CA 15.3 kinetics for metastatic breast cancer. *Int J Biol Markers*. 2002;17(4):231–238. doi:10.1177/172460080201700403
14. Virassamy B, Caramia F, Savas P, et al. Intratumoral CD8(+) T cells with a tissue-resident memory phenotype mediate local immunity and immune checkpoint responses in breast cancer. *Cancer Cell*. 2023;41(3):585–601.e8. doi:10.1016/j.ccell.2023.01.004
15. Marcq E, Siozopoulou V, De Waele J, et al. Prognostic and predictive aspects of the tumor immune microenvironment and immune checkpoints in malignant pleural mesothelioma. *Oncoimmunology*. 2017;6(1):e1261241. doi:10.1080/2162402X.2016.1261241
16. Geng H, Zhang G-M, Li D, et al. Soluble form of T cell Ig mucin 3 is an inhibitory molecule in T cell-mediated immune response. *J Immunol*. 2006;176(3):1411–1420. doi:10.4049/jimmunol.176.3.1411
17. Cheng S, Han F, Xu Y, et al. Expression of Tim-3 in breast cancer tissue promotes tumor progression. *Int J Clin Exp Pathol*. 2018;11(3):1157–1166.
18. de Mingo Pulido Á, Gardner A, Hiebler S, et al. TIM-3 regulates CD103(+) dendritic cell function and response to chemotherapy in breast cancer. *Cancer Cell*. 2018;33(1):60–74.e6. doi:10.1016/j.ccell.2017.11.019
19. Chen M, Wang L, Wang Y, et al. Soluble Tim3 detection by time-resolved fluorescence immunoassay and its application in membranous nephropathy. *J Clin Lab Anal*. 2020;34(6):e23248. doi:10.1002/jcla.23248
20. Okamoto T, Obara T, Izuo M, et al. Decision making using postoperative CEA and CA 15-3 for detection of breast cancer recurrence. *Breast Cancer*. 1995;2(2):127–131. doi:10.1007/BF02966951
21. Zhang X, Liu Y, Mu D. Influence of prognostic nutritional index on the surveillance after surgery-based systematic therapy for breast cancer. *Am Surg*. 2023;89(12):6157–6171. doi:10.1177/00031348231191200
22. Tang S, Zhou F, Sun Y, et al. CEA in breast ductal secretions as a promising biomarker for the diagnosis of breast cancer: a systematic review and meta-analysis. *Breast Cancer*. 2016;23(6):813–819. doi:10.1007/s12282-016-0680-9
23. Hall C, Clarke L, Pal A, et al. A review of the role of carcinoembryonic antigen in clinical practice. *Ann Coloproctol*. 2019;35(6):294–305. doi:10.3393/ac.2019.11.13
24. Seale KN, Tkaczuk KHR. Circulating Biomarkers in Breast Cancer. *Clin Breast Cancer*. 2022;22(3):e319–e331. doi:10.1016/j.clbc.2021.09.006
25. Tomlinson IP, Whyman A, Barrett JA, et al. Tumour marker CA15-3: possible uses in the routine management of breast cancer. *Eur J Cancer*. 1995;31a(6):899–902. doi:10.1016/0959-8049(94)00447-1
26. Given M, Scott M, Mc Grath JP, et al. The predictive of tumour markers CA 15-3, TPS and CEA in breast cancer recurrence. *Breast*. 2000;9(5):277–280. doi:10.1054/brst.1999.0154
27. Sakuishi K, Apetoh L, Sullivan JM, et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med*. 2010;207(10):2187–2194. doi:10.1084/jem.20100643
28. Zhang SJ, Hu Y, Qian H-L, et al. Expression and significance of ER, PR, VEGF, CA15-3, CA125 and CEA in judging the prognosis of breast cancer. *Asian Pac J Cancer Prev*. 2013;14(6):3937–3940. doi:10.7314/APJCP.2013.14.6.3937
29. Cheung KL, Graves CR, Robertson JF. Tumour marker measurements in the diagnosis and monitoring of breast cancer. *Cancer Treat Rev*. 2000;26(2):91–102. doi:10.1053/ctrv.1999.0151
30. Burugu S, Gao D, Leung S, et al. TIM-3 expression in breast cancer. *Oncoimmunology*. 2018;7(11):e1502128. doi:10.1080/2162402X.2018.1502128
31. Chen C, Zhao F, Peng J, et al. Soluble Tim-3 serves as a tumor prognostic marker and therapeutic target for CD8(+) T cell exhaustion and anti-PD-1 resistance. *Cell Rep Med*. 2024;5(8):101686. doi:10.1016/j.xcrm.2024.101686
32. Kumagai S, Itahashi K, Nishikawa H. Regulatory T cell-mediated immunosuppression orchestrated by cancer: towards an immuno-genomic paradigm for precision medicine. *Nat Rev Clin Oncol*. 2024;21(5):337–353. doi:10.1038/s41571-024-00870-6