Original Article
Diagnostic value of breast ultrasound BI-RADS classification combined with TGF-β detection in breast cancer patients

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Abstract: To investigate the diagnostic value of breast ultrasound breast imaging reporting and data system (BI-RADS) classification combined with transforming growth factor-β (TGF-β) detection to differentiate malignant from benign tumors. This study enrolled 163 patients with breast tumor (breast cancer of 104 cases and breast benign tumor of 59 cases) who were admitted to Cangzhou Central Hospital from February 2016 to May 2017. All patients underwent breast ultrasound detection. Peripheral blood was collected before treatment and tested for mRNA expression of TGF-β by quantitative real-time PCR. Twenty age- and sex-matched healthy people were selected as controls. The area under receiver-operating characteristic curve was used to evaluate the diagnostic value of BI-RADS classification, TGF-β mRNA expression and the combination of these two. X^2 test and fisher exact test were used to evaluate the relationship between clinicopathological information and BI-RADS and TGF-β in breast cancer patients. The value of TGF-β mRNA expression were 18.29±4.66 and 2.00±0.25 in the peripheral blood for patients with breast cancer and breast benign tumors, respectively. The sensitivity of breast ultrasound BI-RADS classification was 0.731, the specificity was 0.915, the area under the curve was 0.885 (P<0.001); the diagnostic sensitivity of TGF-β mRNA expression was 0.683, the specificity was 0.814, the area under the curve was 0.770 (P<0.001); the combined sensitivity of these two was 0.817, the specificity was 0.898, and the area under the curve was 0.927 (P<0.001). In breast cancer patients, high tumor diameter and stage predicted increased BI-RADS grade. Thus, the present study suggests that the diagnostic value of breast ultrasound BI-RADS classification combined with TGF-β detection is superior to the diagnostic value of both, which may be an effective method for the diagnosis of breast cancer.

Keywords: Breast ultrasound, TGF-β, breast cancer, diagnosis

Introduction

Currently, breast cancer is the most common cancer in women of China and even the world [1, 2]. Despite the gradual decline in breast cancer mortality in recent years, the number of patients who die each year is still very large [3]. The pathological diagnosis is the gold standard for breast cancer detection. However, puncture sampling is invasive. Therefore, investigating new diagnostic methods or indicators for early diagnosis and treatment of breast cancer is of great significance.

At present, the diagnosis of breast cancer mainly includes imaging diagnosis and hematological indicators of detection. Breast ultrasound is a routine imaging for breast cancer detection, which have a certain value to distinguish benign and malignant breast tumors [4, 5]. Carbohydrate antigen 153 (CA-153) is a tumor marker for breast cancer, but the diagnostic sensitivity and specificity is poor [6, 7]. In recent years, researchers continue to explore new indicators for breast cancer diagnosis, including long-chain non-coding RNA, plasma free DNA, microRNA, vascular endothelial growth factor [8-16].

Transforming growth factor-β (TGF-β) is an immunosuppressive molecule that plays an important role in immune escape in cancer patients [17, 18]. It promotes tumor progression by inhibiting apoptosis, differentiation, promoting angiogenesis, and suppressing immune responses [19, 20]. The expression of TGF-β is negatively correlated with the prognosis of cancer patients [21]. This study aimed to detect

Table 1. Clinicopathologic characteristics of 104 breast cancer patients

Parameter	Number of patients
Median age (range)	51 (22-75)
Pathological type	
Ductal carcinoma in situ	2
Invasive ductal carcinoma	66
Invasive lobular carcinoma	6
Invasive carcinoma	30
Tumor grade	
Grade 1	5
Grade 2	55
Grade 3	23
Unknown	21
ER status	
Positive	76
Negative	28
PR status	
Positive	68
Negative	36
HER-2 status	
Positive	34
Negative	70
Tumor size	
T1	37
T2	61
T3	6
Lymph node status	
N0	59
N1	26
N2	10
N3	9
Presence of metastasis	
Yes	6
No	98
TNM stage	
Stage 1	25
Stage 2	58
Stage 3	15
Stage 4	6
BI-RADS	
3	3
4A	9
4B	15
4C	33
5	33
6	11

the expression of TGF-β mRNA in peripheral blood of patients to explore the value of differ-

Table 2. Clinicopathologic characteristics of 59 patients with benign breast tumor

Parameter	Number of patients
Median age (range)	39 (20-60)
Pathological type	
Intraductal papilloma	6
Fibroadenoma	36
Fibroadenosis	14
Other	3
BI-RADS	
3	22
4A	22
4B	10
4C	4
5	1

ential diagnosis of benign and malignant breast tumor. More importantly, we combined breast ultrasound with TGF-β to improve the differential diagnostic rate.

Materials and methods

Patients

This study included 163 patients with breast tumor (breast cancer of 104 cases and breast benign tumor of 59 cases) who were admitted to our hospital from February 2016 to May 2017. All patients were diagnosed by pathology. Patients had no immune system-related diseases, severe infections, organ transplant history or other types of tumors. The clinical and pathological information of breast cancer patients was collected by using the electronic medical record management system in our hospital, including the age, pathological type, pathological grade, ER, PR, HER-2, tumor size and tumor staging. Meanwhile, 20 healthy people were included as controls. This study was approved by The Ethics Committee of Cangzhou Central Hospital. Patients and their families signed informed consent.

Breast ultrasound BI-RADS classification

According to the 5th edition BI-RADS classification: Grade 1 is negative; Grade 2 is benign; Grade 3 is considered benign and is recommended for re-examination after 6 months; grade 4A is suspected of malignancy, grade 4B is moderately suspected of malignancy, 4C level is highly suspected of malignant; all
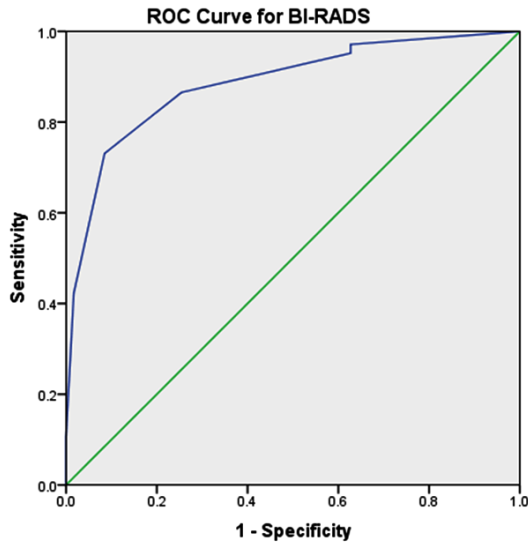
Figure 1. ROC curve for BI-RADS to distinguish breast cancer and benign tumor.

BI-RADS 4 are recommended for biopsy; BI-RADS 5 is highly suggestive of malignant, and recommended for biopsy; grade 6 is the confirmed malignant tumor.

Peripheral blood collection and qRT-PCR

Two-millimeter peripheral blood were collected from enrolled patients before surgery or other anti-tumor treatment and stored in EDTA anti-coagulant tube. We added enough red blood cell lysate to lyse red blood cells in the sample. After that, we centrifuge the blood at 2000 r/min for 5 minutes and then washed it with PBS. Finally, we took out cells and extracted RNA by using the TRizol method according to the instructions. Then, RNA was reverse transcribed to cDNA. Quantitative PCR was performed using 7500 Real-Time PCR for TGF-β detection. We used β-actin as the internal reference to calculate ΔCt and $2^{-\Delta\Delta Ct}$ method to calculate the relative quantitative value of mRNA expression. ΔΔCT was calculated by the difference between the ΔCT value of blood sample of patients and healthy controls. Primer sequences for TGF-β and β-actin were 5'CACGTGGAGCTGTACCAGA-A3' and 5'GGCGAAAGCCTTCTATTTCC3', and 5'TGACGTGGACATCCGCAAAG3' and 5'CTGGA-AGGTGGACAGCGAGG3', respectively.

Statistical analysis

The area under receiver-operating characteristic curve was used to evaluate the differential diagnostic value and calculate the sensitivity, specificity and area under the curve. The cut-off values of BI-RADS and TGF-β were according to the maximal Youden index (sensitivity+ specificity-1). The relationship between BI-RADS and TGF-β and clinicopathological information was analyzed by X^2 test or Fisher exact test. The combined predictors of BI-RADS and TGF-β were analyzed by binary logistic regression analysis. Data were analyzed using Statistical Package for Social Sciences, Version 22.0 (IBM Corporation, Armonk, NY, USA). *P*<0.05 was statistically significant.

Results

Clinicopathologic characteristics

Table 1 shows the clinicopathologic characteristics of 104 breast cancer patients. The median age of breast cancer patients was 51 (22-75) years old. There were 2 cases of ductal carcinoma in situ, 66 cases of invasive ductal carcinoma, 6 cases of invasive lobular carcinoma, 30 cases of invasive carcinoma; 5 cases of tumor grade 1, 55 cases of grade 2, 23 cases of grade 3, 21 cases of grade unknown; 76 cases of ER positive, 28 cases of negative; 68 cases of PR positive, 36 cases of negative; 34 cases of HER-2 positive, 70 cases of negative; tumor size <2 cm in 37 cases, ≥2 cm in 67 cases; According to the 7th edition of the American Cancer Joint Committee breast cancer staging criteria, the number of patients with lum stage were 25, 58, 15, 6, respectively. **Table 2** shows the clinicopathologic characteristics of 59 patients with benign breast tumors. The median age of patients with benign tumors was 39 (20-60) years, including intraductal papilloma in 6 cases, 36 cases of fibroadenoma, fibroadenosis in 14 cases, the other 3 cases (**Table 2**).

Diagnostic performance of BI-RADS and TGF-β

In this study, there were 3 breast cancer patients with BI-RADS grade 3, 9 patients with grade 4A, 15 with grade 4B, 33 with grade 4C, 33 with grade 5, and 11 with grade 6; in patients with benign tumors, there were 22 cases with grade 3, 22 cases with grade 4A, 10 cases with grade 4B, 4 cases with grade 4C, 1 case with grade 5. TGF-β mRNA expression for breast cancer patients was 18.29±4.66; the
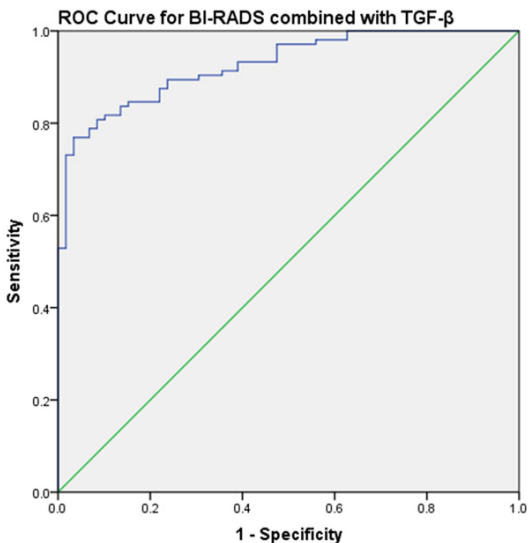
Table 3. Diagnostic performance of parameters by ROC curve and AUC analyses

Parameters	Cut-off	AUC (95% CI)	P	Sensitivity	Specificity	Youden Index
BI-RADS	4C	0.885 (0.834-0.937)	<0.001	0.731	0.915	0.646
TGF-β	3.15	0.770 (0.699-0.841)	<0.001	0.683	0.814	0.497
BI-RADS+TGF-β	/	0.927 (0.890-0.964)	<0.001	0.817	0.898	0.715
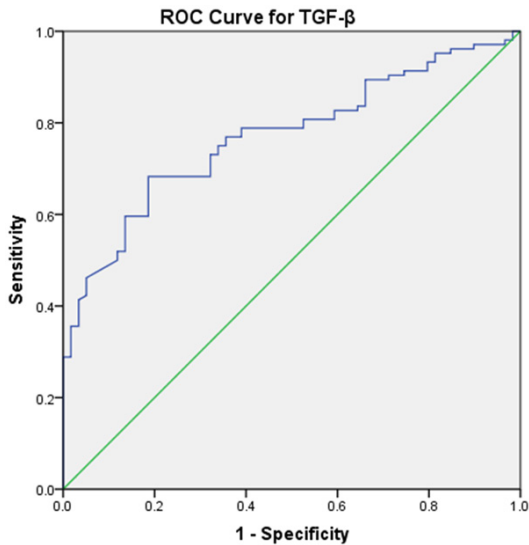
Figure 2. ROC curve for TGF-β expression to distinguish breast cancer and benign tumor.

Figure 3. ROC curve for BI-RADS in combination with TGF-β to distinguish breast cancer and benign tumor.

malignant tumors with a maximum Youden index (0.646, P<0.001). The diagnostic sensitivity and specificity were 0.731 and 0.915, respectively. The area under curve was 0.885 (0.834-0.937) (**Figure 1**; **Table 3**). The cut-off value of TGF-β mRNA expression was 3.15 with a maximum Youden index (0.497, P<0.001). The diagnostic sensitivity and specificity for TGF-β were 0.683 and 0.814, respectively. The area under curve of TGF-β was 0.770 (0.699-0.841) (**Figure 2**; **Table 3**).

The combined predictors of BI-RADS and TGF-β were calculated by using binary logistic regression analysis and ROC curve analysis. The sensitivity and specificity for the combination were 0.817 and 0.898, respectively. The area under curve was increased to 0.927 (0.890-0.964), which was larger than BI-RADS and TGF-β alone (**Figure 3**; **Table 3**).

Association between BI-RADS/TGF-β expression and clinicopathologic characteristics in breast cancer

Table 4 summarizes the relationship between clinicopathologic characteristics and BI-RADS and TGF-β. Increased BI-RADS score was correlated with the positivity of her-2 status (P<0.001). Tumor size was positively associated with BI-RADS score (P<0.001). Compared to clinical stage 1, patients with stage 2-4 showed higher BI-RADS score (P=0.018).

In addition, we also investigated the relationship between TGF-β mRNA expression and clinicopathologic parameters of breast cancer patients. The results showed that there was no significant correlation between them (**Table 4**).

Discussion

In this study, we found that breast ultrasound BI-RADS score and TGF-β mRNA expression alone were useful to differentiate benign and malignant breast tumors with sensitivity (0.731 and 0.683) and specificity (0.915 and 0.814). More importantly, the combination of these

value for patients with benign tumors was 2.00±0.25.

ROC analysis showed that BI-RADS grade 4C was the cut-off value to distinguish benign and

Table 4. Association between BI-RADS and TGF-β expression and clinicopathologic characteristics in breast cancer patients

Parameter	BI-RADS			TGF-β		
	3-4B	4C-6	P	3.15	≥3.15	P
Tumor grade						
Grade 1-2	19	41	0.620	22	38	0.873
Grade 3	6	17		8	15	
ER status						
Positive	21	55	0.522	21	55	0.139
Negative	6	22		12	16	
PR status						
Positive	17	51	0.759	18	50	0.113
Negative	10	26		15	21	
HER-2 status						
Positive	0	34	<0.001	8	26	0.210
Negative	27	43		25	45	
Tumor size						
T1	19	18	<0.001	12	25	0.909
T2-3	8	59		21	46	
Lymph node status						
N0	17	42	0.447	22	37	0.163
N1-3	10	35		11	34	
TNM stage						
Stage 1	11	14	0.018	9	16	0.599
Stage 2-4	16	63		24	55	

the cut-off value of BI-RADS 4C, the sensitivity and specificity were 0.731 and 0.915; the area under the curve was 0.885 (0.834-0.937). The results are consistent with previous findings.

TGF-β, as an immunosuppressive molecule, was highly expressed in peripheral blood of patients with various tumors and was negatively correlated with the prognosis of patients [17, 21, 25-30]. We analyzed the expression of TGF-β mRNA in peripheral blood of patients and its relationship with clinical pathology, and to explore its diagnostic value. A previous study showed the correlation between TGF-β expression and tumor size and grade [31]. Our results showed that there was no significant correlation between TGF-β expression and clinicopathological information, which may account for limited samples. To our best knowledge, it is very rare to investigate the diagnostic value of TGF-β in breast cancer patients. With the cut-off value of 3.15, we found that the sensitivity and specificity of TGF-β to differentiate breast cancer and benign tumor were 0.683 and 0.814; the area under the curve was 0.770 (0.699-0.841).

two parameters could significantly improve the sensitivity (0.817) and specificity (0.898), which was of clinical significance. In addition, the results of this study suggest that BI-RADS score is higher in patients with larger tumor size and tumor stage.

Breast ultrasound is routinely used to differentiate breast cancer and benign tumor with a great value. Evans et al. [4] found that the sensitivity of the ultrasound BI-RADS score to identify benign and malignant breast tumors was 0.95 and the specificity was 0.69. Jeffers et al. [22] used BI-RADS classifications to predict breast cancer risk with an area under curve of 0.68. In addition, researchers also evaluated the prognostic value of BI-RADS classifications and found that it's a negative prognostic indicator [23, 24]. In view of the differences in the ethnicity of the patients enrolled in the study and the differences in the ultrasonography itself, we used the ROC curve to determine the cut-off value of ultrasound BI-RADS score and TGF-β mRNA expression. Consequently, with

The sensitivity (0.817) and specificity (0.898) were significantly improved by the combination of ultrasound BI-RADS score and TGF-β mRNA expression (P<0.05). Meanwhile, the area under the curve was 0.927 (0.890-0.964). Compared with other studies of the joint diagnosis, our results showed a certain advantage and great clinical use [11, 13, 32].

There are several limitations for the present study. First, there are individual differences in the BI-RADS score of the mammography, which may affect the diagnostic value. Second, this is a single center study. In spite of these limitations, our study suggests that breast ultrasound BI-RADS classification combined with TGF-β detection for breast cancer diagnosis is of great value.

Disclosure of conflict of interest

None.

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