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Original Article

Clinical significance of breast ultrasound and FOXP3 in breast cancer patients

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Abstract: To examine clinical significance of breast ultrasound together with forkhead box P3 (Foxp3) detection to istic (ROC) curve was used to assess the diagnostic value of Breast Imaging Reporting and Data System (BI-RADS) classification, Foxp3 mRNA expression and the combination of these two parameters. The value of Foxp3 mRNA expression in the peripheral blood for breast cancer patients was higher than patients with benign breast tumors significantly (P<0.001). The diagnostic sensitivity and specificity of BI-RADS classification, Foxp3 mRNA expression, and the combination of these two were 0.792 and 0.732, 0.875 and 0.625, 0.896 and 0.768, with the area under the curve (AUC) of 0.827 (P<0.001), 0.836 (P<0.001) and 0.910 (P<0.001), respectively. Therefore, this study demonstrated that breast ultrasound BI-RADS classification combined with Foxp3 detection is of higher diagnostic value than that of both, which probably be an effective approach to diagnose breast cancer.

Keywords: Breast cancer, breast ultrasound, BI-RADS, Foxp3, diagnosis

Introduction

In recent years, breast cancer has become one of the most common cancers in Chinese women and even in women around the world [1]. In spite of the gradual decrease in the mortality of breast cancer currently, the number of dead patients per year remains enormous [2, 3]. The gold standard for detecting breast cancer is pathological diagnosis [4]. However, it is invasive to puncture for sampling. Hence, it is of great significance to explore new diagnostic methods or indicators to diagnose breast cancer early and initiate treatment.

Nowadays, the main diagnostic methods of breast cancer include imaging diagnosis and detecting hematological indicators. Breast ultrasound is a regular imaging technique for screening breast cancer, which could distinguish benign and malignant breast tumors with a certain value [5, 6]. Carbohydrate antigen 153 (CA-153) is a biological marker for breast tumor, but its diagnostic sensitivity and specificity are poor [7, 8]. Currently, researchers are making great efforts to investigate new indicators to help diagnosing breast cancer, such as long-chain plasma free DNA, microRNA, non-coding RNA and vascular endothelial growth factor [9-12].

It is widely known that the transcription factor Fork head box P3 (Foxp3) plays an important role in the development of immunoregulatory T cells and achieving their function [13]. Foxp3 is considered to be a crucial factor to control oncogenic factors in epithelial cells by regulating the expression of many genes involved in cancer [14-16]. In addition, it facilitates progression and metastasis of tumor and inhibits immune responses [17-19]. The expression of Foxp3 in tumor samples is in negative correlation with the prognosis of cancer population [20-24]. We aimed to explore the expression of Foxp3 mRNA in peripheral blood and investigate the diagnostic value of Foxp3 mRNA expression, BI-RADS classification, and the combination of these two parameters for differentiating benign and malignant breast tumors.

Materials and methods

Study population

This study enrolled 96 patients with breast cancer and 56 with benign breast tumors from April

Table 1. Clinicopathologic characteristics of 96 breast cancer patients and 56 patients with benign breast tumors

Parameter	Number of patients
Breast cancer patients	
Median age (range)	54 (19-83)
Tumor size	
T1	35
T2	55
T3	6
Tumor grade	
G2	56
G3	22
Unknown	18
ER	
Positive	72
Negative	24
PR	
Positive	62
Negative	34
HER-2	
Positive	29
Negative	67
Lymph node involvement	
N0	55
N1	27
N2	6
N3	8
AJCC stage	
I	23
II	57
III	12
IV	4
BI-RADS classification	
3	2
4A	18
4B	23
4C	26
5	23
6	4
Patients with benign breast tumors	
Median age (range)	41 (21-68)
BI-RADS classification	
3	19
4A	22
4B	8
4C	6
5	1

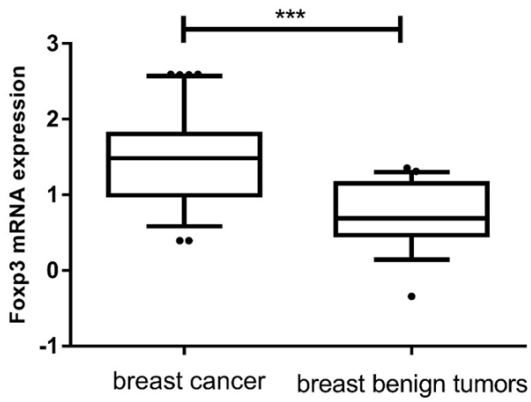

Figure 1. Foxp3 expression in patients with breast cancer and benign breast tumors

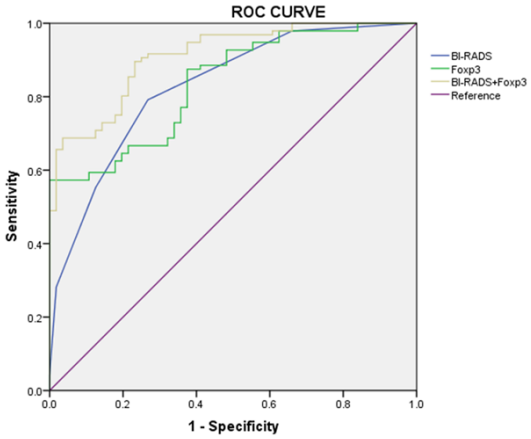

Figure 2. ROC curve for breast ultrasound and Foxp3 to distinguish breast cancer and benign breast tumors.

2015 to August 2017. Pathological diagnosis was accomplished for all the patients. Inclusion criteria included patients without other types of tumors, severe infections, immune system-related diseases or organ transplant history. The electronic medical record in our hospital system was used to collect clinical and pathological information of breast cancer patients, including age, pathological type and grade, ER, PR, HER-2, size and staging of tumor. This research was approved by The Ethics Committee of The First Affiliated Hospital of Hainan Medical University. Informed consent was acquired from the patients.

BI-RADS classification

In accordance with the 5th edition BI-RADS classification: 1-negative radiologic findings; 2-benign findings; 3-probably benign findings,

Table 2. Diagnostic sensitivity and specificity of BI-RADS and Foxp3 in differentiating breast cancerand benign breast tumors

Parameters	Sensitivity	Specificity	Youden Index
BI-RADS	0.792	0.732	0.524
Foxp3	0.875	0.625	0.500
BI-RADS+Foxp3	0.896	0.768	0.664

Table 3. AUC according to BI-RADS classification and Foxp3

Parameters	Cut-off	AUC	95% CI	P
BI-RADS	4B	0.827	0.760-0.894	<0.001
Foxp3	7.4	0.836	0.774-0.898	<0.001
BI-RADS+Foxp3	-	0.910	0.866-0.954	<0.001

with recommendation for reassessment at 6 months; 4-suspicious findings; and 5-highly suspicious findings, with recommendation for histological correlation.

Blood sample collection and qPCR

Peripheral blood of two-milliliter was collected from included patients before any anti-tumor treatment including surgery and stored in EDTA anticoagulant tubes. Enough red blood cell lysate was added to lyse red blood cells in the sample. Then, the blood sample was centrifuged for 5 minutes at 2000 r/min and was washed with PBS after centrifugation. In the next, the cells were taken out and RNA was extracted with the TRizol method in line with the instructions. At last, we reverse transcribed the RNA to cDNA and performed quantitative PCR with 7500 Real-Time PCR for detecting Foxp3. *β-actin was* used as the internal reference to calculate ΔCt and $2^{-\Delta\Delta Ct}$ method was applied to calculate the relative quantitative value of mRNA expression. The difference between the ΔCT value of patient's blood sample and that of healthy controls was calculated asΔΔCT. The foxp3 primer sequences are 5'CCACTTGCAGACACCATTTG3' and 5'CATGATCAGCCTCACACCAC3'.

Statistical analysis

The area under receiver-operating characteristic (ROC) curve was used to assess the diagnostic value of different methods and calculate the sensitivity, specificity and area under the curve (AUC). The best cut-off values of BI-RADS and Foxp3 were set based on the maximal Youden index (sensitivity + specificity -1). The χ^2 test or Fisher exact test was applied to test the relationship between clinicopathologic parameters and BI-RADS and Foxp3. Binary logistic regression analysis was used to analyze the combination diagnostic value of BI-RADS and Foxp3. All the data were analyzed with Statistical Package for Social Sciences, Version 20 (IBM Corporation, Armonk, NY, USA). $P<0.05$ was statistically significant.

Results

Clinicopathologic characteristics

Clinicopathologic characteristics of 96 breast cancer patients were shown in **Table 1**. The median age of patients with breast cancer was 54 (19-83) years old. The patients number of tumor grade 2, grade 3 and unknown was 56, 22 and 18 respectively; 72 patients were ER positive and the rest were ER negative; PR positive was seen in 62 patients, negative in 34 patients; 29 patients of HER-2 positive, 67 patients of negative; tumor size of 35 patients was less than 2 cm, 61 patients had tumor size ≥2 cm; In accordance with the breast cancer staging criteria in the 7th edition of the American Cancer Joint Committee, 23 patients had tumor in stage I, 57 in stage II, 12 in stage III and 4 in stage. Patients with benign breast tumors had the median age of 41 (21-68) years old.

Diagnostic performance of BI-RADS and Foxp3

In this research, the number of patients in BI-RADS grade 3, grade 4A, grade 4B, grade 4C, grade 5 and grade 6 were 2, 18, 23, 26, 23, and 4, respectively; in patients with benign tumors, the tumors of 19 were in grade 3, 22 in grade 4A, 8 in grade 4B, 6 in grade 4C, 1 case in grade 5 (**Table 1**). Higher expression of Foxp3 mRNA was detected in patients with breast cancer compared with patients with benign tumors (**Figure 1**).

The best cut-off value to differentiate benign and malignant tumors was BI-RADS grade 4B with a maximal Youden index (0.524) demonstrated in ROC. The sensitivity and specificity of diagnosis were 0.792 and 0.732, respectively. The AUC was 0.827 (0.760-0.894) (**Figure 2**, **Tables 2**, **3**). It was shown that the Foxp3 mRNA expression had a cut-off value of 7.4 with a maximal Youden index (0.500), and the sensitivity and specificity for diagnosing were 0.875 and 0.625, respectively, with the AUC of Foxp3 was 0.836 (0.774-0.898) (**Figure 2**, **Tables 2**, **3**).

Table 4. Correlation between BI-RADS/Foxp3 expression and clinicopathologic parameters inbreast cancer patients

Parameter	Foxp3			BI-RADS		
	<7.4	≥7.4	P	3-4A	4B-6	P
Tumor size						
T1	5	30	0.753	9	26	0.437
T2-3	7	54		11	50	
Tumor grade						
Grade 2	11	45	0.162	12	44	0.901
Grade 3	1	21		5	17	
Lymph node involvement						
N0	9	46	0.225	13	42	0.461
N1-3	3	38		7	34	
AJCC stage						
I	5	18	0.152	7	16	0.255
II-IV	7	66		14	59	
ER						
Positive	10	62	0.724	15	57	1.000
Negative	2	22		5	19	
PR						
Positive	7	55	0.749	12	50	0.630
Negative	5	29		8	26	
HER-2						
Positive	5	24	0.502	5	24	0.569
Negative	7	60		15	52	

Binary logistic regression analysis and ROC curve were used to calculate the predictive value of the combination of BI-RADS and Foxp3. The sensitivity and specificity for these two combined predictors were 0.896 and 0.768, respectively, with a larger AUC of 0.910 (0.866-0.954) compared with BI-RADS and Foxp3 separately (**Figure 2**, **Tables 2**, **3**).

The correlation between BI-RADS/Foxp3 expression and clinicopathologc characteristics in breast cancer

Table 4 shows the association between clinicopathologic characteristics and BI-RADS and Foxp3. It reveals that Foxp3 was not significantly correlated with clinicopathologic parameters. Nevertheless, a trend between increased Foxp3 expression and higher tumor grade and AJCC stage was detected in this study. In addition, there was no correlation between clinicopathologic parameters and BI-RADS score in patients with breast cancer.

Discussion

In this research, we figured out that BI-RADS score of breast ultrasound and the expression of Foxp3 mRNA alone were helpful to distinguish malignant and benign breast tumors with an acceptably high sensitivity (0.792 and 0.875) and specificity (0.732 and 0.625). Besides, it is of great clinical importance that the combined predictor of the abovementioned two parameters could increase the sensitivity (0.896) and specificity (0.768) significantly.

As a molecule of suppressing immune responses, Foxp3 was highly expressed in many different tumor samples of patients and was in negative correlation with patients' prognosis [15, 16, 25, 26]. Researchers found that the lacking expression of FOXP3 in node-positive patients with breast tumor was significantly associated with improved prognosis in comparison with FOXP3-positive patients (probability of 10-year survival, 89% vs 59%) [27]. The expression of Foxp3 mRNA in patients' peripheral blood was analyzed in this study and its relation with clinical pathology and diagnostic value were explored. Previous research had demonstrated that the expression of Foxp3 was related to tumor grade and AJCC stage [20]. Our results indicated that Foxp3 expression was not correlated with patients' clinicopathological information, which may be attributed to small number of samples. As far as we know, there are few studies focused on exploring Foxp3 and its diagnostic value in patients with breast cancer. It is shown in our study that Foxp3 has a best cut-off value of 7.4 for differentiating malignant and benign breast tumors, with the sensitivity and specificity of 0.875 and 0.625 and the AUC of 0.836 (0.774-0.898).

It is a routine protocol and of great value to use breast ultrasound to distinguish malignant and benign breast tumors. Kennedy et al. [28] reported that BI-RADS 3 had a positive predictive value of 9.1%, 5.9% in terms of BI-RADS 4, and 66.7% referring to BI-RADS 5. BI-RADS classification was used by Jeffers et al. [29] to predict the risk of breast cancer with an AUC of 0.68. Besides, the prognostic value of BI-RADS classification was also assessed by some researchers and was proved to be a negative indicator for prognosis [28, 30]. Considering the enrolled patients' ethnic variations and the ultrasonic differences, receiver operating curve

was applied to investigate the cut-off value of Foxp3 mRNA expression and ultrasonic BI-RADS score. As a consequence, the diagnostic sensitivity and specificity of BI-RADS 4 were 0.792 and 0.732 with the AUC of 0.827 (0.760-0.894). These results are in consistency with previous findings.

With regard to the combination of Foxp3 mRNA expression and ultrasound BI-RADS score, the sensitivity (0.896) and specificity (0.768) were increased statistically ($P<0.05$) and its AUC was 0.910 (0.866-0.954). Our study demonstrated a clear advantage and important clinical application in comparison with other studies focusing on joint diagnosis [9, 31-33].

Two main limitations exist concerning this study. It was conducted only in one center. Besides, the diagnostic value could possibly be affected by individual variations in mammographic BI-RADS score. Despite of the above-mentioned limitations, this study has revealed the significant diagnostic value of the combination of Foxp3 with BI-RADS classification for detecting for breast cancer.

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Disclosure of conflict of interest

None

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