EXPRESSION OF SAA1 IN PERIPHERAL BLOOD AND TISSUES OF BREAST CANCER PATIENTS AND ITS VALUE IN EARLY WARNING OF BREAST CANCER

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ABSTRACT

Objective: *To explore the expression of SAA1 in the peripheral blood and tissues of patients with breast cancer and understand its value in the early detection of breast cancer.*

Methods: *From June 2017 to May 2018, 80 patients with breast cancer who underwent surgical resection in our hospital were collected as the observation group, and samples of breast cancer tissue and normal paracancerous tissue were collected during those procedures. Another 50 adults who had a physical examination in our hospital at the same time were collected as the control group. Enzyme-linked immunosorbent assay (ELISA) and fluorescence quantitative PCR were used to detect the levels of serum amyloid A 1 (SAA1) and mRNA expression in all tissues of all participants, and the value of SAA1 in the early detection of breast cancer was analysed by drawing the ROC curve.*

Results: *The level of SAA1 in the observation group was significantly higher than that in the control group (P<0.05). The positive expression rate of SAA1 in breast cancer was 41.25% (33 / 80), which was significantly higher than levels in normal breast tissue. The expression of SAA1 in breast cancer was correlated with tumour size, lymph node metastasis and histological grade (P<0.05), but not with age and clinical stage (P>0.05). Lymph node metastasis and SAA1 expression are independent risk factors for the prognosis of patients with breast cancer. The area under the curve of SAA1 mRNA in plasma and tissue was 0.718 and 0.902, respectively.*

Conclusion: *The changes in the SAA1 level in the course of breast cancer occurrence and development can provide a reference for early detection and the evaluation of clinical treatment and SAA1 can also be an independent risk factor affecting the prognosis of patients with breast cancer, which is of great significance to evaluate the prognosis of patients.*

Keywords: *Breast cancer, SAA1, peripheral blood, prediction, detection, prognosis.*

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Introduction

At present, breast cancer has become the most common malignant tumour in the world. According to the latest data, there are about 1.2 million patients diagnosed with breast cancer every year around the world, and about 500,000 patients die of breast cancer every year. The incidence of breast cancer in developed countries is relatively high[1-2]. Affected by economic development, living habits and changes in diet structure, the incidence and mortality of patients with breast cancer in China are increasing year by year, and the trend for diagnosis is trending younger. According to the latest data in China, the incidence of female malignant tumours is the highest in China, and the number of patients continues to increase[3-4]. The onset and development of breast cancer are closely related to genetic factors, endocrine disorders, dietary structure and other factors[5]. Surgery, radiotherapy, chemotherapy and targeted therapy are commonly used to treat breast cancer in the clinic, of which

surgical resection is the most important treatment, but the prognosis of patients is poor[6]. If patients with breast cancer relapse, it can cause a significant decline in the survival rate, the five-year survival rate is less than 20%[7].

Therefore, to explore a specific biomarker that can effectively evaluate patients with early breast cancer is of great significance for early clinical diagnosis and treatment. Serum amyloid A1 (SAA1) is an acute reactive protein, which is mainly synthesised by the liver[8]. Clinical research shows that it plays an important role in the onset and development of many tumours such as gastric cancer, sarcoma, nasopharyngeal carcinoma, and renal cell carcinoma[9].

Therefore, the purpose of this study is to explore the value of SAA1 in the early detection of breast cancer by identifying the expression level of SAA1 in peripheral blood and tissues of patients with breast cancer.

Materials and methods

General information

From June 2017 to May 2018, 80 patients with breast cancer, who were operated upon in our hospital's breast surgery clinic, were collected as the observation group, and samples of breast cancer tissues and normal paracancerous tissues from these patients were collected at the same time.

Another 50 adults who had a physical examination in our hospital at the same time were collected as the control group. The average age of the observation group was (42.34±6.32) years, and that of the control group was (42.51±6.48) years.

Inclusion criteria

• All patients were diagnosed as having breast cancer;

• All patients were receiving treatment for the first time;

• Age > 18;

• Clinical data files were complete;

• All patients signed a written informed consent;

• The protocol was approved by the ethics committee of our hospital.

Exclusion criteria

• Exclusion of patients previously treated by radiotherapy, chemotherapy, targeted therapy, etc.;

• Exclusion of patients treated by biological immunotherapy for nearly half a year;

• Exclusion of patients who need to be hospitalised for serious infection;

• Exclusion of patients with serious open wound and blood disease;

• Exclusion of patients with a malignant tumour.

There was no significant difference in general data between the two groups (P>0.05).

Methods

• The SAA1 level was detected by an enzyme-linked immunosorbent assay in strict accordance with the instructions of the enzyme-linked immunosorbent kit. 0.01 mol/L PBS was used to dilute the serum according to 1:4. The absorbance of the sample was measured at 450 nm and the curve was drawn, and the expression level of SAA1 was calculated.

• The 50 mg samples were crushed in liquid nitrogen, and total RNA was extracted by the Trizol method and phenol-chloroform method.

• The expression of SAA1 mRNA in breast cancer and normal control tissues was detected by fluorescence quantitative PCR. The tissue cRNA was synthesised and amplified by PCR, then the PCR products were identified by electrophoresis. mRNA expression level = the starting amount of the target gene/the starting amount of the internal reference gene.

• The expression of SAA1 protein in breast cancer and normal paracancerous tissues was detected by immunohistochemistry. The staining results were judged by the staining intensity and the percentage of positive cells. If the score was three or more, it was a high expression, and if the score was less than three, it was low expression.

• Draw the ROC curve to analyse the value of SAA1 in the early detection of breast cancer.

Statistical method

SPSS 23.0 software was used to input and analyse the data.

The T-test and one-way ANOVA were used to measure the data, and (x±s) was used to represent the level of SAA1 and mRNA expression in the observation group and the control group. χ^2 was used to test the relationship between SAA1 expression and clinicopathological parameters of patients with breast cancer.

A COX proportional risk regression model was used to analyse the independent risk factors influencing the prognosis of patients with breast cancer. At the same time, using the software to draw the ROC curve and calculate the area under the curve, analyse the role of SAA1 in early detection of breast cancer. P<0.05 means the difference was statistically significant.

Results

Comparison of SAA1 level in plasma and mRNA expression in the tissue from two groups of patients with breast cancer

The level of SAA1 in the observation group was significantly higher than that in the control group (P<0.05). The positive expression rate of SAA1 in breast cancer was 41.25% (33/80), which was significantly higher than that in normal breast tissue. See Tables 1, 2, Figure 1.

Group	Plasma SAA1 (μg/mL)
Control group	2.42±0.95
Observation group	3.66±2.98
t	3.979
P	<0.001

Table 1: Comparison of SAA1 levels between two groups of patients with breast cancer ($\bar{x}\pm s$).

Group	mRNA expression level
Paracancerous tissue	0.77±0.65
Breast cancer tissue	1.62±1.42
t	0.849
P	<0.001

Table 2: Comparison of mRNA expression levels in breast cancer tissues between the two groups.

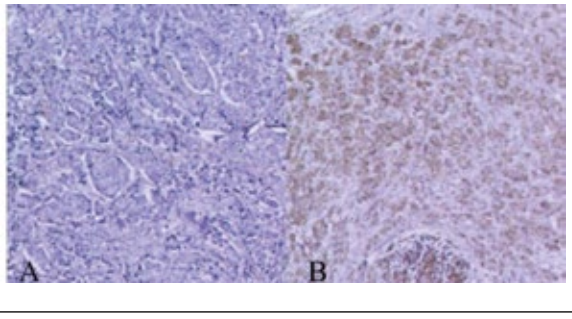

Figure 1: Positive expression of SAA1 in normal paracancerous and breast cancer tissues.
Figure A: protein negative expression of SAA1 in normal paracancerous tissues; Figure B: protein positive expression of SAA1 in breast cancer tissues.

Correlation between the expression of SAA1 in breast cancer and clinicopathological characteristics of patients with breast cancer

The expression of SAA1 in breast cancer was correlated with tumour size, lymph node metastasis and histological grade (P<0.05), but not with age and clinical stage (P>0.05). See Table 3.

Clinicopathological parameters	SAA1 expression			P
	n	Low expression	High expression	
Age				0.585
≤45 years	53	30	23	
>45 years	27	17	10	
Tumour size				0.034
≤2cm	28	12	16	
>2cm	52	35	17	
Clinical stages				0.256
Phase II	30	21	19	
Phase III	50	26	14	
Lymph node metastasis				<0.001
Yes	45	17	28	
No	35	30	5	
Histological classification				0.013
I	24	14	10	
II	49	29	20	
III	7	4	3	

Table 3: Correlation between the expression level of SAA1 in breast cancer and clinicopathological characteristics of patients with breast cancer.

Analysis of COX proportional risk regression model in patients with breast cancer

Lymph node metastasis and SAA1 expression are independent risk factors for prognosis of patients with breast cancer. See Table 4.

Clinicopathological parameters	95%CI	P
Age	0.695~1.957	0.602
Tumour size	0.544~1.444	0.603
Clinical stages	1.026~1.958	0.072
Lymph node metastasis	2.474~5.116	0.039
Histological classification	0.923~2.306	0.119
SAA1 expression	1.513~4.343	<0.001

Table 4: COX proportional risk regression model analysis of patients with breast cancer.

ROC curve analysis of SAA1 level and mRNA expression level in two groups

The ROC curve was established. The results showed that the area under the SAA1 curve was 0.718 in plasma and 0.902 in tissue.

The sensitivity and specificity of the ROC curve were high, which suggested that it had a strong diagnostic ability in the early detection of breast cancer and possessed a certain value.

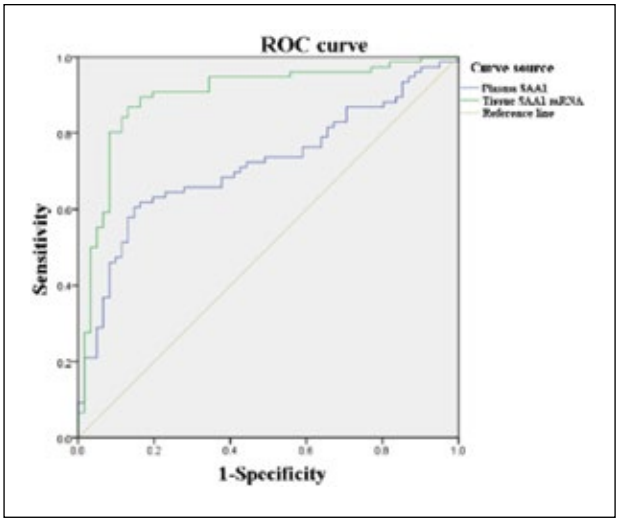

Figure 2: ROC curve analysis of the prediction of breast cancer by the expression level of SAA1 mRNA in plasma and tissue.

Discussion

At present, the comprehensive treatment of breast cancer has achieved remarkable results, but the effect on some different types of breast cancer is not satisfactory. Therefore, early diagnosis and treatment are the key points to improve the clinical efficacy and prolong the survival period of patients with breast cancer. Clinically, breast cancer screening is primarily through X-ray and CT histopathological diagnosis. However, to a certain extent, these methods can cause damage to the patient's body, or have low sensitivity and specificity[10-11]. Therefore, it is critical to identify an effective, simple and less invasive method for the early detection of breast cancer. To some extent, tumour markers have the ability to diagnose breast cancer, but no high sensitivity and specificity markers have been found. Therefore, we selected the expression of SAA1 in the plasma and tissues of patients with breast cancer as a viable option to explore for its value in the early detection of breast cancer.

SAA1 is a highly homologous acute apolipoprotein reactant. When the body has an inflammatory reaction, the level of SAA1 in the peripheral blood will rise. Therefore, when the body has trauma or infection, the level of SAA1 will rise to limit the external stimulation of the body[12]. In recent years, there has been more attention paid to the role of SAA1 in tumourigenesis and development. Many reports found that SAA1 plays an important role in the progression and prognosis of many malignant tumours and many cell activities. When cells migrate, SAA1 can enhance cell adhesion and promote the infiltration of inflammatory mediators[13]. SAA1 can not only enhance the activity of matrix metalloproteinase but also activate the expression of interleukin-6 cyclooxygenase-2 and other factors[14].

Chronic inflammation can cause a significant increase in SAA1 level, and this increase in the SAA1 level can cause tumour spread and metastasis, ultimately leading to poor prognosis and a low five-year survival rate[15-16]. It has been reported that the increase of SAA1 level in plasma can lead to metastasis of cervical cancer and other tumours[17].

Chao et al.[18] detected SAA1 level in patients with gastric cancer undergoing a radical gastrectomy. The results showed that the risk of death in patients with a high expression of SAA1 was significantly increased, and SAA1 was of great significance in predicting the survival period of patients with gastric cancer. In addition, the SAA1 level of patients with lung cancer was significantly higher than that of the healthy control group[19].

The study of tumour proteomics found that the increase of SAA1 level in peripheral blood can increase the incidence of lung cancer[20].

The results showed that the level of SAA1 in the observation group was significantly higher than that in the control group ($P<0.05$), and the expression of SAA1 mRNA in breast cancer tissue was significantly higher than that in the adjacent tissue ($P<0.05$). The expression level of SAA1 was correlated with tumour size, lymph node metastasis and histological grade of patients with breast cancer ($P<0.05$), but not with age and clinical stage of patients with breast cancer ($P>0.05$). These results suggest that SAA1 may be involved in the process of breast cancer onset and progression.

At the same time, we established a Cox model and found that lymph node metastasis and SAA1 expression are independent risk factors for the prognosis of patients with breast cancer, indicating that the high expression of SAA1 may indicate the poor prognosis of patients with breast cancer.

The area under the SAA1 curve was 0.718, which suggested that SAA1 was of high value in early detection of breast cancer. The area of SAA1 mRNA under the curve was 0.902, and the sensitivity and specificity were high, which suggested that SAA1 had a strong diagnostic ability in allowing for an early warning of breast cancer and it possessed a certain value.

In conclusion, SAA1 level changes abnormally during the occurrence and development of breast cancer, which can provide a reference for early de-

tection and on-going evaluation of the clinical treatment effect of breast cancer, and SAA1 can also be an independent risk factor affecting the prognosis of patients with breast cancer, which is of great significance for the evaluation of patients' prognosis.

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