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Original Article

Expression and significance of T lymphocyte subsets, RANTES and inflammatory factors levels in serum of patients with abdominal aortic aneurysm

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Abstract: Objective: To investigate the expression levels and significance of T lymphocyte subsets, RANTES (regulated on activation, normal T cell expressed and secreted), and inflammatory factors in serum of patients with abdominal aortic aneurysm (AAA). Methods: 32 patients each with large (large AAA) and small (small AAA) groups were selected, and 32 normal subjects were selected as control group. Serum C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), RANTES and CD4+ T cells, CD8+ T cells and CD4+/CD8+ expressions in peripheral blood were compared among the three groups. Results: Compared with control group, CRP, TNF-α, IL-6, RANTES and CD8+ T cells levels were higher in large and small AAA groups, while CD4+ T cells and CD4+/CD8+ levels were lower (P<0.05). Compared with small AAA group, CRP, TNF-α, IL-6, RANTES and CD8+ T cells levels in large AAA group were higher, while CD4+ T cells and CD4+/CD8+ levels were lower (P<0.05). The abdominal aorta diameter was positively correlated with CRP, TNF-α, IL-6, RANTES and CD8+ T cells levels, while negatively correlated with CD4+ T cells and CD4+/CD8+ levels (P<0.001). Receiver operating characteristic curve results showed that the areas under the curve of CRP, TNF-α, IL-6 and RANTES in the diagnosis of AAA were all more than 0.800 (P<0.001). Conclusion: The serum of patients with AAA was in a state of inflammatory activation, and the expression of T lymphocytes was abnormal. The levels of T lymphocyte subsets, RANTES and inflammatory factors were closely related to abdominal aorta diameter. CRP, TNF-α, IL-6 and RANTES levels could be used as auxiliary indicators for the diagnosis of AAA.

Keywords: Abdominal aortic aneurysm, T lymphocyte subsets, RANTES, inflammatory factors

Introduction

Abdominal aortic aneurysm (AAA) is a type of immune-related chronic inflammatory reaction, and its main clinical manifestation is localized and permanent expansion of abdominal aortic wall [1-4]. The incidence of AAA ranks first in the aortic aneurysm, and with the development of society, its incidence is increasing. AAA's case fatality rate is as high as 50%-70%, which can promote the rupture of aortic aneurysm when the disease progresses rapidly. When the case fatality rate of ruptured AAA is as high as 80%-95%, it will seriously threaten the life and health of patients [5-7]. At present, aortic aneurysm diameter exceeding 5 cm is usually indicated clinically for surgical treatment, but it is often difficult to accurately predict the risk of AAA rupture based on tumor diameter alone [8]. Therefore, it is of great clinical significance to explore the mechanism of disease occurrence and progression in the prevention and treatment of AAA.

It is now clinically believed that immune response and chronic inflammation are the key factors leading to the occurrence and development of AAA [9]. Human regulated on activation, normal T cell expressed and secreted (RANTES) can promote the release of inflammatory factors, which in turn mediates the inflammatory response and the occurrence and development of tumors [10]. Kuivaniemi et al. confirmed that the level of CD4+CD28- T cells in patients with AAA continued to increase, which in turn promoted the expression of inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), there-

by participating in the occurrence and development of AAA, leading to local chronic inflammatory migration of AAA [11]. In addition, the larger the surface area of the AAA, the higher the serum IL-6 level of patients [12]. However, previous studies have focused on the difference in the expression of inflammatory factors and T lymphocytes in patients with AAA and normal people, and there are few reports about the above indicators in the auxiliary diagnosis of AAA [11, 12]. Based on this, this study investigated the expression and significance of T lymphocyte subsets, RANTES and inflammatory factors levels in serum of patients with AAA, aiming to provide relevant evidence for clinical treatment of AAA.

Materials and methods

General information

From January 2017 to January 2020, 32 patients with large AAA (large AAA group, abdominal aortic diameter ≥5 cm) and 32 patients with small AAA (small AAA group, 3 cm ≤ abdominal aortic diameter <5 cm) admitted to our hospital were selected, and 32 subjects with healthy physical examinations were selected as control group. This study was approved by the Ethics Committee of our hospital.

Inclusion and exclusion criteria

Inclusion criteria for AAA group: (1) patients those met the relevant diagnostic criteria for AAA in the *Guidelines for Diagnosis and Treatment of Abdominal Aortic Aneurysm* compiled by Guo in 2008 [13]; (2) patients aged ≥18 years old; (3) patients without previous history of aortic aneurysm; (4) patients without history of cardiovascular and cerebrovascular diseases; (5) patients without history of autoimmune diseases; (6) patient and his/her family members were informed and consent.

Inclusion criteria for control group: (1) those who were healthy during physical examination within the past one month; (2) those aged ≥18 years old; (3) the subjects and their family members were informed and consent.

Exclusion criteria for AAA group: (1) those who complicated with neurological or psychiatric diseases; (2) those with severe infection or inflammatory reaction; (3) those with malignant tumors; (4) those during pregnancy or lactation; (5) those who took immunosuppressant and other drugs that might affect the experimental results in the past one month; (6) those with poor compliance; (7) those who participated in other research projects.

Exclusion criteria for control group: (1) those during pregnancy or lactation; (2) those with poor compliance.

Methods

In control group, 5 mL×2 tubes of venous blood were extracted from all people on an empty stomach in the morning of the next day after enrolling the group. In AAA group, 5 mL×2 tubes of venous blood were extracted from all patients on an empty stomach before treatment. For one tube, CD4+ T cells (BD Bioscience Company, USA), CD8+ T cells (BD Bioscience Company, USA), and CD4+/CD8+ levels were measured using flow cytometry (BD FACSCalibur flow cytometry, BD Bioscience Company, USA); the other tube was centrifuged at 3000 r/min for 5 min, the serum was separated, and the ELISA method (Hamilton microlab star multi-function microplate reader, Switzerland) was used to detect C-reactive protein (CRP, Abcam Company, USA), TNF-α (Abcam Company, USA), IL-6 (Abcam Company, USA) and RANTES (Abcam Company, USA) levels.

Outcome measures

Primary outcome measures: the three groups' CRP, TNF-α, IL-6, RANTES, CD4+ T cells, CD8+ T cells and CD4+/CD8+ levels; correlation between CRP, TNF-α, IL-6, RANTES, CD4+ T cells, CD8+ T cells, CD4+/CD8+ levels and abdominal aorta diameter.

Secondary outcome measures: comparison of general information of the three groups; the clinical value of CRP, TNF-α, IL-6 and RANTES levels in the diagnosis of AAA.

Statistical analysis

Statistical analysis was conducted by SPSS22.0 software. The count data were expressed as (n, %), and the comparison was performed by χ^2 test. The measurement data were expressed by mean ± standard deviation ($\overline{x}\pm$sd). The measurement data among multiple groups was compared by one-way ANOVA test, and pairwise comparison between groups was performed

Table 1. Comparison of general information (n, %, $\bar{x}\pm sd$)

Indicator	Large AAA group (n=32)	Small AAA group (n=32)	Control group (n=32)	Statistic	P
Age (year)	61.6±5.5	62.1±5.9	61.9±5.8	0.062	0.941
Gender				0.638	0.727
Male	21	19	22		
Female	11	13	10		
Hypertension history				0.251	0.882
Yes	14	16	15		
No	18	16	17		
History of diabetes				0.706	0.703
Yes	9	11	8		
No	23	21	24		
Total cholesterol (mmol/L)	4.54±0.93	4.48±1.01	4.51±0.97	0.031	0.970
Triglyceride (mmol/L)	1.31±0.62	1.38±0.64	1.33±0.71	0.096	0.908
Low density lipoprotein (mmol/L)	2.81±0.84	2.73±0.75	2.44±0.59	2.251	0.111

Note: AAA: abdominal aortic aneurysm.

Table 2. Comparison of T lymphocyte levels (n, %, $\bar{x}\pm sd$)

Group	CD4+ T cells	CD8+ T cells	CD4+/CD8+
Large AAA group (n=32)	3.08±0.35[a,b]	3.75±0.79[a,b]	0.94±0.34[a,b]
Small AAA group (n=32)	3.51±0.44[a]	3.56±0.90[a]	1.20±0.31[a]
Control group (n=32)	4.66±0.46	3.02±0.47	1.83±0.41
F	121.228	17.422	51.700
P	<0.001	<0.001	<0.001

Note: Compared with control group, [a]P<0.05; compared with small AAA group, [b]P<0.05. AAA: abdominal aortic aneurysm.

using LSD-t test. The correlation between two continuous variables was analyzed by Pearson. CRP, TNF-α, IL-6, RANTES, CD4+ T cells, CD8+ T cells and CD4+/CD8+ levels were independent variables, and abdominal aorta diameter was dependent variable. The clinical value of CRP, TNF-α, IL-6 and RANTES levels in the diagnosis of AAA was analyzed using receiver operating characteristic (ROC) curve. P<0.05 was considered statistically significant.

Results

Comparison of general information

There was no significant difference in general information such as age and gender among the three groups (P>0.05). See **Table 1**.

Comparison of T lymphocyte levels

Compared with control group, CD4+ T cells and CD4+/CD8+ levels were lower in small and large AAA groups, while CD8+ T cells levels were higher (P<0.05). Compared with small AAA group, CD4+ T cells and CD4+/CD8+ levels were lower in large AAA group, while CD8+ T cells level was higher (P<0.05). See **Table 2**.

Comparison of CRP, TNF-α, IL-6 and RANTES levels

Compared with control group, CRP, TNF-α, IL-6 and RANTES levels were higher in small and large AAA groups (P<0.05). Compared with small AAA group, CRP, TNF-α, IL-6 and RANTES levels were higher in large AAA group (P<0.05). See **Table 3**.

Correlation analysis

The results of correlation method displayed that abdominal aorta diameter was positively correlated with CRP, TNF-α, IL-6, RANTES and CD8+ T cell levels, while negatively correlated with CD4+ T cells and CD4+/CD8+ levels (P< 0.001). See **Table 4**.

ROC curve

The results showed that when CRP's cut-off value was 6.001 mg/L, the area under the curve (AUC) for the diagnosis of AAA was 0.887; when TNF-α's cut-off value was 24.050 pg/mL, the AUC for the diagnosis of AAA was 0.827; when IL-6's cut-off value was 72.149 pg/mL, the AUC for the diagnosis of AAA was 0.878; when RANTES's cut-off value was 42.555

Table 3. Comparison of CRP, TNF-α, IL-6 and RANTES levels (n, %, $\bar{x}\pm sd$)

Group	CRP (mg/L)	TNF-α (pg/mL)	IL-6 (pg/mL)	RANTES (μg/L)
Large AAA group (n=32)	9.64±2.44[a,b]	40.69±7.33[a,b]	119.27±27.66[a,b]	59.71±14.36[a,b]
Small AAA group (n=32)	6.80±1.58[a]	32.17±4.34[a]	93.37±21.13[a]	47.66±8.21[a]
Control group (n=32)	3.60±2.61	18.47±7.52	49.59±37.95	33.34±12.57
F	57.545	93.368	44.905	38.771
P	<0.001	<0.001	<0.001	<0.001

Note: Compared with control group, [a]P<0.05; compared with small AAA group, [b]P<0.05. AAA: abdominal aortic aneurysm; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; RANTES: regulated on activation, normal T cell expressed and secreted.

Table 4. Correlation analysis results

Statistic	CRP (mg/L)	TNF-α (pg/mL)	IL-6 (pg/mL)	RANTES (μg/L)	CD4+ T cells	CD8+ T cells	CD4+/CD8+
r	0.753	0.639	0.687	0.658	-0.861	0.592	-0.756
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; RANTES: regulated on activation, normal T cell expressed and secreted.

Table 5. ROC curve results

Indicator	Cut-off value	AUC	P	95% CI		Sensitivity	Specificity
				Lower limit	Upper limit		
CRP (mg/L)	6.001	0.887	<0.001	0.799	0.975	0.891	0.906
TNF-α (pg/mL)	24.050	0.827	<0.001	0.732	0.921	0.906	0.688
IL-6 (pg/mL)	72.149	0.878	<0.001	0.797	0.959	0.906	0.719
RANTES (μg/L)	42.555	0.876	<0.001	0.793	0.959	0.813	0.875

Note: ROC: receiver operating characteristic; AUC: area under the curve; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; RANTES: regulated on activation, normal T cell expressed and secreted.

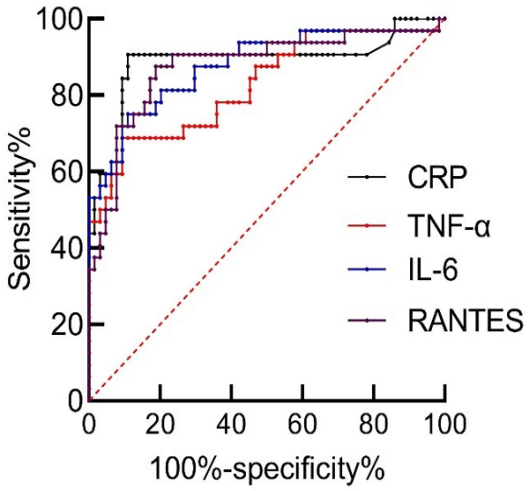

Figure 1. ROC curve results. ROC, receiver operating characteristic; CRP, C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; RANTES: regulated on activation, normal T cell expressed and secreted.

μg/L, the AUC for the diagnosis of AAA was 0.876 (all P<0.001). See **Table 5** and **Figure 1**.

Discussion

This study investigated the expression levels and significance of T lymphocyte subsets, RANTES and inflammatory factors in serum of AAA patients. The results displayed that the serum of patients with AAA was in a state of inflammatory activation, and the expression of T lymphocytes was abnormal. The levels of T lymphocyte subsets, RANTES and inflammatory factors were closely related to abdominal aorta diameter. CRP, TNF-α, IL-6 and RANTES levels could be used as auxiliary indicators for the diagnosis of AAA.

The main pathological features of aortic aneurysm are infiltration of quantities of local inflammatory cells and activation of immune cells, so

abnormal immune function plays a vital role in aortic aneurysm's occurrence and development [14]. T lymphocyte levels are strongly associated with autoimmune disease. T lymphocytes can be divided into CD4+ T cells and CD8+ T cells subsets. Among them, CD4+ CD25+ Tr in CD4+ T cells represent regulatory cell immunity and mainly play the role of benign regulation [15]. On the contrary, CD8+ T cells maintain the stability of immune response by cooperating with CD4+ T cells [16]. Under normal physiological conditions, the ratio of CD4+ T cells to CD8+ T cells is in dynamic balance, while under external stimulation, the balance of CD4+/CD8+ is broken, leading to the disorder of the body's immune internal environment [15]. The results of this study showed that the proportion of CD4+ T cells and the ratio of CD4+/CD8+ decreased in patients with AAA, while the proportion of CD8+ T cells increased; moreover, the proportion of CD4+ T cells and the ratio of CD4+/CD8+ decreased more significantly in patients with large AAA, while the proportion of CD8+ T cells increased significantly, indicating that the ratio of CD4+ T cells to CD8+ T cells is unbalanced in patients with AAA [14]. During the period of small AAA, the expression of CD8+ T cells is promoted and the secretion of CD4+ T cells is inhibited under the stimulation of certain antigens in the body, which leads to the imbalance of CD4+/CD8+ and accelerates the inflammatory response [16]. However, during the period of large AAA, with the expansion of AAA and the persistence of inflammatory response, the imbalance is aggravated [16]. The results of correlation analysis showed that abdominal aorta diameter was positively correlated with the level of CD8+ T cells, while negatively correlated with levels of CD4+ T cells and CD4+/CD8+, which was also consistent with the above conclusions. However, the exact mechanism of the correlation remains to be further explored.

The imbalance of T lymphocytes further promotes inflammatory response, which plays a key role in the occurrence and development of AAA [9, 10]. As inflammatory factors, TNF-α, CRP and IL-6 have been widely used in inflammatory or infectious diseases [17-21]. The results of this study showed that TNF-α, CRP and IL-6 levels increased in AAA patients, and were positively correlated with tumor diameter, suggesting that inflammatory factors may be involved in AAA's occurrence and development. Besides, ROC curve results indicated that CRP, IL-6 and TNF-α level had certain clinical value in the diagnosis of AAA, which also provided a new idea for the auxiliary diagnosis of AAA. RANTES is a member of the CC family of chemokines, which can regulate the inflammatory response of the body, promote the activation of lymphocytes, and regulate the growth and proliferation of cells [22]. RANTES has a pro-inflammatory effect and can participate in inflammatory response by promoting the expression of inflammatory factors such as IL-6 [23]. In this study, RANTES also increased in patients with AAA, and was positively correlated with aortic aneurysm diameter. However, when the cut-off value of RANTES was 42.555 μg/L, the AUC for the diagnosis of AAA was 0.876, and the sensitivity and specificity were 0.813 and 0.875, respectively, suggesting a high diagnostic value.

However, this study also has some limitations, such as a small sample size and limitations of the indicators involved in the study, so further confirmation is needed in subsequent studies.

In conclusion, the expression levels of T lymphocyte and inflammatory factors were abnormal in patients with AAA, the levels of T lymphocyte subsets, RANTES and inflammatory factors were closely correlated with abdominal aorta diameter, and the levels of CRP, TNF-α, IL-6 and RANTES could be used as auxiliary indicators for the diagnosis of AAA.

Disclosure of conflict of interest

None.

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