CORRELATION ANALYSIS OF CYTOKINE LEVELS AND PERIPHERAL BLOOD T LYMPHOCYTE SUBSETS IN SYSTEMIC VASCULAR INFLAMMATORY DISEASE

ANALIZA POVEZANOSTI NIVOA CITOKINA I SUBPOPULACIJA T LIMFOCITA U PERIFERNOJ KRVI KOD SISTEMSKIH VASKULARNIH ZAPALJENSKIH OBOLJENJA

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Summary

Background: To measure the levels of T-cell subsets and cytokines in the peripheral blood of patients with systemic vascular inflammatory disease and to analyse the influence of different clinical characteristics of systemic vascular inflammatory disease patients on the levels of T-cell subsets and cytokines in the peripheral blood.

Methods: The case group included 80 patients with systemic vascular inflammatory disease who were diagnosed and treated at our hospital between January 2021 and December 2024. The control group consisted of 40 healthy volunteers recruited from our hospital's health assessment centre. The proportions of helper T-cell (Th)1, Th17, and regulatory T-cell (Treg) subsets in peripheral blood were analysed using flow cytometry. Serum levels of cytokine, including interleukin-2 (IL-2), interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), IL-4, IL-10, IL-17, and IL-6, were measured using enzyme-linked immunosorbent assay (ELISA).

Results: Compared with the control group, the expression levels of total T lymphoid subsets and CD8+ T and Th1 cells in patients with systemic vascular inflammatory disease were significantly higher, and there was no statistically significant difference in Th, Th17, or Treg cell expression between the groups (P>0.05). However, there were statistically significant differences between the two groups [68.75 (54.23, 80.32)] and [72.10 (62.23, 86.45)], [23.44 (12.89, 33.76)] and [31.46 (20.13, 45.26)], [10.67 (8.23, 12.35)] and [10.26, 17.96 [25.39)], Z=-3.13, -4.54, -3.97 (all P values<0.05). Compared with those in the control group, patients with sys-

Kratak sadržaj

Uvod: Ispitivan je nivo subpopulacija T ćelija i citokina u perifernoj krvi pacijenata sa sistemskim vaskularnim zapaljenskim oboljenjem, kao i analiza uticaja različitih kliničkih karakteristika ovih pacijenata na nivoe T-ćelijskih subpopulacija i citokina u perifernoj krvi.

Metode: U grupi ispitanika je bilo 80 pacijenata sa sistemskim vaskularnim zapaljenskim oboljenjem koji su dijagnostikovani i lečeni u našoj ustanovi u periodu od januara 2021. do decembra 2024. godine. Kontrolnu grupu činilo je 40 zdravih dobrovoljaca iz centra za sistematske preglede iste ustanove. Ekspresija subpopulacija T-pomoćničkih ćelija (Th1, Th17) i regulatornih T ćelija (Treg) u perifernoj cirkulaciji određivana je protočnom citometrijom. Nivoi citokina interleukina i interferona-γ (IFN-γ) u serumu mereni su metodom imunoenzimskog testa (ELISA), uključujući TNF-α, IL-4, IL-10, IL-17 i IL-6.

Rezultati: U poređenju sa kontrolnom grupom, pacijenti sa sistemskim vaskularnim zapaljenskim oboljenjem imali su značajno više nivoe ukupnih T limfocitnih subpopulacija, CD8+ T ćelija i Th1 ćelija, dok razlike u ekspresiji Th, Th17 i Treg ćelija nisu bile statistički značajne (P>0,05). Međutim, postojale su statistički značajne razlike između grupa [68,75 (54,23–80,32)] i [72,10 (62,23–86,45)], [23,44 (12,89–33,76)] i [31,46 (20,13–45,26)], [10,67 (8,23–12,35)] i [10,26–17,96 (25,39)], Z=-3,13, -4,54, -3,97 (sve P<0,05). Pacijenti su imali značajno više serumske nivoe IL-17, IL-6, TNF-α, IL-4 i IFN-γ, dok su

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temic vascular inflammatory disease had significantly higher serum levels of IL-17, IL-6, TNF-α, IL-4, and IFN-γ, whereas their IL-10 levels were significantly lower. The differences were statistically significant (Z=-4.32, -5.01, -8.18, -8.70, -3.48, and -8.30). The P values were all <0.05. Compared with that in patients without related manifestations, IL-6 expression was noticeably higher in systemic vascular inflammatory disease patients with arthritic symptoms and those with active systemic vascular inflammatory disease, and the difference was statistically significant {pg/mL: [3.23 (2.98, 5.35)] compared with [10.51 (6.72, 23.21)], [6.32 (4.79, 8.93)] compared with [9.68 (6.97, 18.73)], Z=5.47, 8.76, P values <0.05}. Compared with those in patients without relevant clinical manifestations, TNF-α levels were significantly higher in patients with arthritis, ocular, or digestive tract manifestations, and in patients with active systemic vascular inflammatory disease, whereas IFN-γ levels were substantially higher in systemic vascular inflammatory disease patients with digestive system lesions. The differences were statistically significant {pg/mL: [7.74 (6.89, 10.19)] compared with [39.84 (30.37, 50.61)], [6.12 (5.36, 9.89)] compared with [31.35 (15.71, 30.46)], [6.49 (4.78, 10.21)] compared with [19.89 (14.36, 36.21)]. [5.89 (4.61, 8.96)] than [27.91 (15.32, 37.81)], [6.89 (5.43, 14.86)] than [26.79 (15.41, 31.56)], Z= 7.70, 6.84, 6.94, 9.47, 5.70, P values <0.05}. However, there was no statistically significant difference in the expression levels of IL-4 and IL-17 between systemic vascular inflammatory disease patients with and without relevant clinical manifestations (P>0.05).

Conclusions: Patients with systemic vascular inflammatory disease exhibit an imbalance of T lymphocyte subsets and cytokines, and disease activity and clinical classification may also involve such imbalances.

Keywords: systemic vascular inflammatory disease, clinical features, T lymphocyte subsets, cytokine levels

nivoi IL-10 bili značajno ni i (Z=-4,32, -5,01, -8,18, -8,70, -3,48, -8,30; svi P <0,05). Kod pacijenata sa artritičnim simptomima i aktivnim oblikom bolesti, ekspresija IL-6 bila je značajno viša nego kod pacijenata bez tih manifestacija {pg/mL: [3,23 (2,98–5,35)] prema [10,51 (6,72–23,21)], [6,32 (4,79–8,93)] prema [9,68 (6,97–18,73)], Z=5,47, 8,76, P<0,05}. Nivoi TNF-α bili su značajno viši kod pacijenata sa artritičnim, očnim i gastrointestinalnim manifestacijama, kao i kod pacijenata sa aktivnom bolešću, dok su nivoi IFN-γ bili povišeni kod pacijenata sa gastrointestinalnim lezijama (Z=7,70, 6,84, 6,94, 9,47, 5,70, svi P<0,05). Nije bilo značajnih razlika u nivoima IL-4 i IL-17 između pacijenata sa i bez odgovarajućih kliničkih manifestacija (P>0,05).

Zaključak: Pacijenti sa sistemskim vaskularnim zapaljenskim oboljenjem pokazuju disbalans subpopulacija T limfocita i citokina. Aktivnost bolesti i klinička klasifikacija mogu da budu povezane sa ovim imunološkim disbalansom.

Ključne reči: sistemsko vaskularno zapaljensko oboljenje, kliničke karakteristike, subpopulacije T limfocita, nivoi citokina

Introduction

Systemic vascular inflammatory disease is a chronic inflammatory disease with vasculitis as its pathological basis and involves multiple systems, with recurrent episodes. The damage mainly affects the oral cavity, external genitalia, and eyes and can also involve the visceral system. The different types of damage to the visceral system can be classified into vascular, neurological, gastrointestinal, etc. The aetiology and pathogenesis of this damage remain unclear. Early research revealed that a key factor in the pathophysiology of systemic vascular inflammatory disease is the helper T (Th)type 1 immunological response (1). Research has revealed that Th17/regulatory T (regulatory T) cells are important for the onset and progression of systemic vascular inflammatory disease (1–4).

Systemic vascular inflammatory disease is a complex, chronic inflammatory condition that affects multiple systems and manifests as skin lesions, eye inflammation, external genital ulcers, and mouth ulcers (5). Although its aetiology has not yet been fully clarified, many studies (6–8) have shown that immune system abnormalities, primarily imbalances

in T lymphocytes, play important roles in its pathogenesis. Research on peripheral blood cytokines and T lymphocyte subsets has steadily increased in recent years, suggesting their possible roles in the aetiology and development of Behcet's disease (9). Patients with systemic vascular inflammatory disease have aberrant peripheral blood T lymphocyte subset proportions, with Th1 and Th17 cells considerably increasing while regulatory T cells (Tregs) decline. The increase in these cytokines is closely related to disease activity. Domestic research also supports this view, showing that certain cytokines, such as IL-23 and IL-12, are significantly elevated in patients with systemic vascular inflammatory disease. In addition, studies on gene polymorphisms have shown that certain mutations in immune-regulatory genes may increase susceptibility to systemic vascular inflammatory disease (10–12). However, the specific mechanisms of action of T lymphocyte subsets and cytokines in systemic vascular inflammatory disease remain incompletely understood, and differences among studies may be due to factors such as sample size, detection techniques, and variations in patient backgrounds (13). In addition, major challenges in clinical practice remain in improving the diagnosis and treat-

ment of systemic vascular inflammatory disease by regulating these immune indicators.

This study explored the influence of various clinical characteristics in patients with systemic vascular inflammatory disease on peripheral blood T-cell subsets and cytokine levels.

Materials and Methods

Research subjects

Fifty-nine male and 21 female systemic vascular inflammatory disease patients were selected as research participants from 80 systemic vascular inflammatory disease patients hospitalised or observed in our hospital's outpatient department between January 2021 and December 2024, and whose data were complete. The average age was 32.34 ± 14.21 years, the median age of onset was 31 years, and the average disease duration was 3.76 ± 11.62 years. Twenty-eight males and twelve females, with an average age of 34.25 ± 15.32 years, were chosen as the control group from among the 40 healthy examinees whose sex and age were matched with the case group in our hospital's health examination centre during the same period. This study has been approved by the Human Medical Research Ethics Committee (No. HKYS-2025-A0158).

Test reagents and equipment

A FACSCalibur flow cytometer (systemic vascular inflammatory disease Biosciences, USA), a CO2 cell culture incubator (Thermo Fisher, USA), a centrifuge (Thermo Fisher, USA), an IFN-γ-FITC- antibody (Ebioscience, USA), an IL-17 PE, a CD3 Percp, a CD8-APC, the corresponding homolotype and corresponding membrane breakers, a PMA/TPA (PKC activator) (Biyutan, China), an ionomycin (ENZO, USA), and a Monensin (Ebioscience, USA) containing 10% fetal bovine serum 1640 medium for FCS and an enzyme-linked immunosorbent assay (ELISA) kit (Xinbosheng, Neobioscience, China) were used.

Flow cytometry detection Kit: systemic vascular inflammatory disease Biosciences, item number systemic vascular inflammatory disease 560882 (for labelling T lymphocyte subsets). Composition: Includes monoclonal antibodies such as CD3, CD4, CD8, CD25, CD45RO, etc., suitable for flow cytometry analysis. Cytokine Detection Kit: eBioscience, catalogue numbers BMS6002 (for ELISA detection of IL-6) and BMS6003 (for ELISA detection of TNF-α). This kit is suitable for quantitative analysis of IL-6 and TNF-α levels in peripheral blood. Lymphocyte isolation solution: Tsystemic vascular inflammatory disease Sciences, catalogue number: Tsystemic vascular inflammatory disease-105 (for isolation of peripheral blood mononuclear cells). This isolation solution can be used to efficiently isolate lymphocytes from peripheral blood. Flow cytometer: systemic vascular inflammatory disease Biosciences, model: systemic vascular inflammatory disease LSRFortessa, for analysing the fluorescence signals of T lymphocyte subsets. ELISA instrument: BioTek, model: BioTek ELx808, used for the detection and analysis of cytokine concentrations.

Laboratory testing methods

This study aims to analyse the correlation between T lymphocyte subsets and cytokines in the peripheral blood of patients with systemic vascular inflammatory disease. The experiment was conducted using flow cytometry and enzyme-linked immunosorbent assay (ELISA) for detection.

T lymphocyte subset detection

Flow cytometry uses fluorescently labelled antibodies to identify specific T cell surface markers and quantitatively analyses cell populations by laser excitation of fluorescence. Peripheral blood samples were collected from patients with systemic vascular inflammatory disease, and peripheral blood mononuclear cells were isolated using the lymphocyte isolation solution of Tsystemic vascular inflammatory disease Sciences (Catalogue number: Tsystemic vascular inflammatory disease-105). The cell suspension was mixed with the relevant fluorescently labelled antibody and incubated at room temperature in the dark for 30 minutes. Cell populations were detected by flow cytometry, and the proportion of T lymphocyte subsets was analysed.

Cytokine detection

Enzyme-linked immunosorbent assay (ELISA) is based on antigen-antibody reactions. It uses enzyme-labelled secondary antibodies for signal amplification and quantitatively determines cytokine concentrations by measuring substrate colouration. Add the peripheral plasma sample to the Wells of the ELISA plate. Add the enzyme-labelled secondary antibody, incubate at room temperature, then wash. Add the substrate solution, incubate, and then measure the absorbance using an ELISA instrument to calculate the cytokine concentration.

Detection methods

Peripheral venous blood was collected from systemic vascular inflammatory disease patients on an empty stomach in the morning (2 mL for heparin anticoagulant tubes and 2 mL for procoagulant tubes). Heparin-treated cells were subjected to flow cytometry within 4 hours, and Th1 (CD3+CD4+ IFN-γ+), Th17 (CD3+CD4+IL17+), and Treg

(CD4+CD25+Foxp3+) cells were detected within 10 hours. One of the procoagulant blood tubes was used to separate the serum, which was aliquoted and frozen at -80 °C for future use. The levels of the cytokines IFN-γ, IL-4, IL-10, IL-17, IL-6 and TNF-α were determined via ELISA. Wuhan Antujie Biotechnology Co., Ltd, carried out detection.

Statistical analysis

SPSS 21.0 software was used to perform the statistical analysis. When the distribution was nonnormal and the variance was homogeneous, the median and interquartile range (M(Q1, Q3)) were used to express the data. For group comparisons, the Wilcoxon rank-sum test was used.

Results

General clinical data of systemic vascular inflammatory disease patients

All 80 patients with systemic vascular inflammatory disease had recurrent oral ulcers (100%). Fifty-five patients had genital ulcers (68.75%). Fifty-one patients (63.75%) had skin lesions, presenting as erythema nodosum and pseudofolliculitis. 13 patients (16.25%) had positive acupuncture reactions. There were 27 cases (33.75%) of ophthalmia, all of which were uveitis. There were 33 cases (41.25%) with digestive system lesions, including multiple ulcers in the gastrointestinal tract. There were 25 cases (31.25%) of circulatory system lesions, 19 cases (23.75%) of neurological lesions, and arthritis/arthralgia in 39 cases (48.75%). Among them, 48 patients were in the active stage of the disease, and 32 patients were in the stable stage when the samples were collected.

Comparison of lymphocyte subsets between systemic vascular inflammatory disease patients and controls

The total number of T lymphoid subsets and the percentages of CD8+ T and Th1 cells in systemic vascular inflammatory disease patients were significantly greater (P<0.05), whereas there were no statistically significant differences in the numbers of Th, Th17 or Treg cells between the groups (P>0.05) (Table I, Figure 1).

Comparison of cytokine expression levels between systemic vascular inflammatory disease patients and the control group

Serum IL-17, IL-6, TNF-α, IL-4, and IFN-γ expression levels were considerably higher in systemic vascular inflammatory disease patients than in control patients. In contrast, the amount of IL-10 was considerably reduced. Table II shows that the differences were statistically significant (P<0.05).
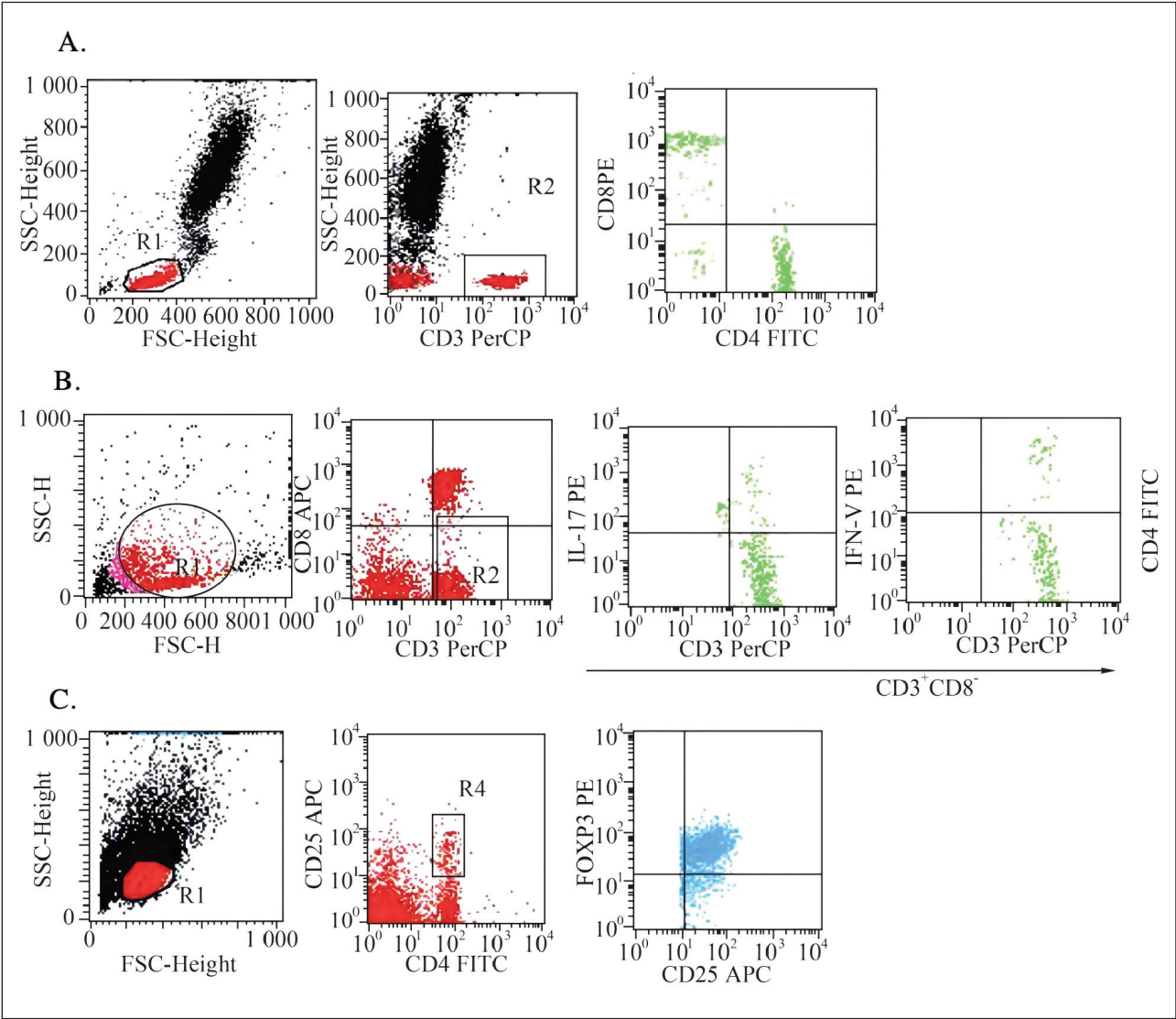
Comparison of the expression levels of IL-6 and IL-10 in systemic vascular inflammatory disease patients with different clinical manifestations

Compared with that in systemic vascular inflammatory disease patients without arthritis manifestations, the serum IL-6 level in patients with arthritis manifestations was significantly higher. Compared with that in patients with stable systemic vascular inflammatory disease, the expression level of IL-6 in patients with active systemic vascular inflammatory disease was significantly greater (P<0.05) (Table III). However, there was no statistically significant difference in IL-10 levels between patients with systemic vascular inflammatory disease without relevant clinical manifestations and those with relevant clinical manifestations (P>0.05).

Table I Comparison of lymphocyte subsets between systemic vascular inflammatory disease patients and the control group.

Project	Systemic vascular inflammatory disease group (80 cases)	Control group (40 cases)	Z value	P value
T (CD3+) (%)	72.10 (62.23, 86.45)	68.75 (54.23, 80.32)	-3.13	<0.05
Th (CD3+CD4+) (%)	35.81 (23.63, 47.82)	34.56 (20.37, 45.69)	1.54	>0.05
Ts (CD3+CD8+) (%)	31.46 (20.13, 45.26)	23.44 (12.89, 33.76)	-4.54	<0.05
Th1 (CD3+CD4+IFN-g+) (%)	17.96 (10.26, 25.39)	10.67 (8.23, 12.35)	-3.97	<0.05
Th17 (CD3+CD4+IL17+) (%)	5.88 (3.34, 12,21)	3.81 (2.83, 4.67)	-1.91	>0.05
Treg (CD4+CD25+Foxp3+) (%)	0.21 (0.04, 2.21)	0.08(0.02,1.56)	1.02	>0.05

Figure 1 Flow cytometry detection of lymphocyte subsets in BD patients.
A. Flow cytometry of T lymphocyte subsets; B. Flow cytometry of Th17 and Th1 cells; C. Flow cytometry of Treg cells.

Table II Comparison of cytokine expression levels between systemic vascular inflammatory disease patients and the control group.

Cytokine	Systemic vascular inflammatory disease group (80 cases)	Control group (40 cases)	Z value	P value
IFN-γ (pg/mL)	8.80 (4.30, 20.45)	4.78 (3.55, 7.50)	-3.48	<0.05
TNF-α (pg/mL)	12.13 (10.60, 17.24)	4.12 (1.95, 6.60)	-8.18	<0.001
IL-6 (pg/mL)	4.74 (3.59, 12.59)	3.20 (1.86, 4.41)	-5.01	<0.05
IL4 (pg/mL)	4.28 (3.50, 5.22)	1.07 (1.03, 1.12)	-8.70	<0.001
IL-10 (pg/mL)	0.55 (0.42, 0.76)	1.63 (1.33, 2.46)	-8.30	<0.001
IL-17 (pg/mL)	20.11 (10.31, 45.05)	8.59 (5.82, 13.38)	-4.32	<0.05

Table III Comparison of L-6 expression levels in systemic vascular inflammatory disease patients with different clinical manifestations.

Clinical features	Number of cases	IL6 (pg/mL)		Z value	P value
		Have relevant manifestations	No relevant manifestations		
Oral ulcer	80	4.74 (3.59, 12.59)	-	-	-
Vulvar ulcer	55	3.92 (3.13, 7.89)	5.63 (4.78, 13.63)	1.18	>0.05
Skin manifestations	51	4.21 (3.67, 9.06)	6.93 (5.47, 15.04)	1.49	>0.05
Arthritis	39	10.51 (6.72, 23.21)	3.23 (2.98, 5.35)	5.47	<0.05
Ophthalmia	27	4.32 (3.91, 9.28)	7.37 (5.52, 16.24)	1.92	>0.05
Lesions of the digestive system	33	5.69 (4.78, 10.53)	4.28 (3.68, 7.32)	1.62	>0.05
Circulatory system lesions	25	5.38 (4.69, 7.34)	4.78 (3.86, 7.39)	1.50	>0.05
Neurological disorders	19	5.24 (4.93, 7.21)	4.89 (3.95, 8.03)	0.97	>0.05
Disease activity	48	9.68 (6.97, 18.73)	6.32 (4.79, 8.93)	8.76	<0.05

Table IV TNF-α levels in systemic vascular inflammatory disease patients with different clinical manifestations.

Clinical features	Number of cases	TNF-α (pg/mL)		Z value	P value
		Have relevant manifestations	No relevant manifestations		
Oral ulcer	80	12.13 (10.60, 17.24)	-	-	-
Vulvar ulcer	55	16.63 (13.48, 20.78)	8.91 (7.63,12.39)	1.55	>0.05
Skin manifestations	51	17.68 (17.89, 23.65)	9.96 (8.89, 12.81)	1.48	>0.05
Arthritis	39	39.84 (30.37, 50.61)	7.74 (6.89, 10.19)	7.70	<0.05
Ophthalmia	27	31.35 (15.71,30.46)	6.12 (5.36, 9.89)	6.84	<0.05
Lesions of the digestive system	33	19.89 (14.36, 36.21)	6.49 (4.78, 10.21)	6.94	<0.05
Circulatory system lesions	25	16.67 (12.14, 20.21)	9.86 (7.67, 10.37)	1.76	>0.05
Neurological disorders	19	15.78 (10.63, 17.46)	10.56 (8,75, 11.34)	1.65	>0.05
Disease activity	48	27.91 (15.32, 37.81)	5.89 (4.61, 8.96)	9.47	<0.05

Comparison of TNF-α and IFN-γ expression levels in systemic vascular inflammatory disease patients with different clinical manifestations

Compared with those in patients without relevant clinical manifestations, TNF-α expression levels in systemic vascular inflammatory disease patients with arthritic, ocular, or digestive tract manifestations were significantly higher, whereas IFN-γ expression levels in systemic vascular inflammatory disease patients with digestive system lesions were substantially higher. The differences were statistically significant (*Tables IV* and *V*). However, there was no statistically significant difference in the expression levels of IL-4 and IL-17 between systemic vascular inflammatory disease patients with and without relevant clinical manifestations (P>0.05).

Table V Comparison of IFN-γ expression levels in systemic vascular inflammatory disease patients with different clinical manifestations.

Clinical features	Number of cases	IFN-γ (pg/mL)		Z value	P value
		Have relevant manifestations	No relevant manifestations		
Oral ulcer	80	8.80 (4.30, 20.45)	-	-	-
Vulvar ulcer	55	9.64 (5.43, 15.89)	8.89 (5.14, 16.68)	1.82	>0.05
Skin manifestations	51	9.23 (4.65, 20.36)	8.95 (3.98, 18.23)	1.68	>0.05
Arthritis	39	10.98 (5.67, 21.86)	7.23 (4.32, 19.73)	1.91	>0.05
Ophthalmia	27	10.35 (4.67, 17.35)	7.37 (4.32, 19.01)	1.87	>0.05
Lesions of the digestive system	33	26.79 (15.41, 31.56)	6.89 (5.43, 14.86)	5.68	<0.05
Circulatory system lesions	25	9.01 (4.89, 21.87)	7.86 (3.67, 16.92)	1.76	>0.05
Neurological disorders	19	10.86 (5.03, 23.47)	8.92 (4.01, 17.65)	1.87	>0.05

Discussion

Systemic vascular inflammatory disease is a chronic inflammatory illness that can affect multiple systems, including the gastrointestinal tract, blood vessels, and the central nervous system (14–16). The pathogenesis of systemic vascular inflammatory disease is complex, and T lymphocytes and related cytokines play important roles in its pathogenesis (17). Early studies revealed that the Th1 immune response is involved in the pathogenesis of systemic vascular inflammatory disease. Th1-related cytokines, such as IFN-γ and TNF-α, are significantly elevated in patients with systemic vascular inflammatory disease (18). Research has shown that Th17 cell levels in patients with systemic vascular inflammatory disease are elevated considerably (19). Compared with the normal control group and the stationary systemic vascular inflammatory disease group, patients with active systemic vascular inflammatory disease had higher levels of CD4+CD25+ Tregs and higher levels of Foxp3 mRNA expression (20–22). Although the Th17 and Treg cell levels in the systemic vascular inflammatory disease patient group were higher than those in the normal control group, the difference was not statistically significant. One possible explanation is that the study included fewer patients.

A study (23) on IL-17, IL-10, IL-6, and IFN-γ in patients with systemic vascular inflammatory disease revealed that IL-17 and IL-6 were expressed at high levels, whereas IL-10 was expressed at low levels. Our experimental results revealed that the levels of IL-4, IL-17, IL-6, TNF-α and IFN-γ in the case group were significantly greater than those in the normal control group, whereas the level of IL-10 decreased significantly, which was basically consistent with reports in

the abovementioned literature. IL-6 is a multifunctional proinflammatory factor that regulates immune responses and is elevated in various immune and inflammatory diseases (24). According to preliminary research, Tregs mediate their immunosuppressive activity throughout the pathophysiology of systemic vascular inflammatory disease by utilising immunosuppressive substances and by affecting the secreted products of these cells (such as Th1 cells). Th17 cells (macrophages) have both negative and positive feedback effects. IL-4 is a cytokine related to Th2 cells (25–28). Compared with the control group, IL-6 and IL-4 expression were significantly increased in patients with systemic vascular inflammatory disease, whereas IL-10 expression was significantly decreased. The expression level of IL-10 in patients with systemic vascular inflammatory disease was decreased considerably, suggesting that IL-10, a negative inflammatory factor, can inhibit the body's immune response.

Currently, relatively few reports on lymphocyte subsets and cytokines in patients with systemic vascular inflammatory disease exist, and the results are not entirely consistent. The reason may be differences in the pathogenesis of systemic vascular inflammatory disease and the complex, diverse clinical phenotypes (29). Our research revealed that serum IL-6 levels were significantly elevated in patients with systemic vascular inflammatory disease in the active stage and with arthritis manifestations. Tocilizumab (TCZ), a humanised IL-6 inhibitor, has achieved specific therapeutic effects. Studies (30–32) have shown that the serum level of IL-10 in patients with systemic vascular inflammatory disease is positively correlated with ocular manifestations, whereas the serum level of IFN-γ is negatively correlated with ocular manifestations (33). The immune response in these patients is believed to

be mainly mediated by Th1 cells rather than Th17 cells. The pathogenesis of gastrointestinal systemic vascular inflammatory disease may differ from that of other systemic vascular inflammatory diseases (34–36). Another study (37) revealed that levels of IL-17 and IL-23, which are associated with Th17 cells, and of IL-35 and IL-10 were significantly increased in the intestinal systemic vascular inflammatory disease group. This study revealed that, compared with those in patients without digestive tract manifestations, the IFN-γ and IL-17 levels in systemic vascular inflammatory disease patients with digestive tract manifestations were slightly greater. The heterogeneity of the above studies may be due to differences in study populations, specimen collection methods (local tissue fluid vs. tissue or serum), and other factors.

Conclusion

The clinical manifestations of systemic vascular inflammatory disease vary, and its pathogenesis is complex. Further in-depth research with larger sample sizes is still needed. Given the limited sample size in this study, it is necessary to increase the sample size further and improve monitoring methods in the future to provide a basis for understanding the pathogenesis and treatment of systemic vascular inflammatory disease.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

References

1. Lavalle S, Caruso S, Foti R, Gagliano C, Cocuzza S, La Via L, Parisi FM, Calvo-Henriquez C, Maniaci A. Behcet's Disease, Pathogenesis, Clinical Features, and Treatment Approaches: A Comprehensive Review. Medicina (Kaunas) 2024 Mar 29; 60(4): 562. doi: 10.3390/medicina60040562. PMID: 38674208; PMCID: PMC11051811.

2. Zhong Z, Su G, Yang P. Risk factors, clinical features and treatment of Behcet's disease uveitis. Prog Retin Eye Res 2023 Nov; 97: 101216. doi: 10.1016/j.preteyeres.2023.101216. Epub 2023 Sep 19. PMID: 37734442.

3. Alharthy F, Almaqati AS, Alsulami S, Ahmad A. Behcet's Disease in Saudi Arabia: Clinical and Demographic Characteristics. Cureus 2023 Feb 11; 15(2): e34867. doi: 10.7759/cureus.34867. PMID: 36923194; PMCID: PMC10010633.

4. Bergamo S. The diagnosis of Adamantiades-Behçet disease: Clinical features and diagnostic/classification criteria. Front Med (Lausanne) 2022 Dec 9; 9: 1098351. doi: 10.3389/fmed.2022.1098351. PMID: 36569165; PMCID: PMC9780472.

5. Bergamo S. The diagnosis of Adamantiades-Behçet disease: Clinical features and diagnostic/classification criteria. Front Med (Lausanne) 2022 Dec 9; 9: 1098351. doi: 10.3389/fmed.2022.1098351. PMID: 36569165; PMCID: PMC9780472.

6. Ding Y, Hu W, Li L, Guan W, Guan K, He Y, Liu S, Li TF. Clinical features and independent predictors of Behcet's disease associated with myelodysplastic syndrome. Clin Exp Rheumatol 2023 Sep; 41(9): 1823–30. doi: 10.55563/clinexprheumatol/04us5e. Epub 2023 Feb 7. PMID: 36762734.

7. Kidd DP. Neurological involvement by Behcet's syndrome: clinical features, diagnosis, treatment and outcome. Pract Neurol 2023 Oct; 23(5): 386–400. doi: 10.1136/pn-2023-003875. Epub 2023 Sep 29. PMID: 37775123.

8. Zhan H, Cheng L, Li Y. Neuro-Behçet's disease: an update of clinical diagnosis, biomarkers, and immunopathogenesis. Clin Exp Immunol 2025 Jan 21; 219(1): uxae123. doi: 10.1093/cei/uxae123. PMID: 39774671; PMCID: PMC11755846.

9. Tono T, Kikuchi H, Sawada T, Takeno M, Nagafuchi H, Kirino Y, Tanaka Y, Yamaoka K, Hirohata S. Clinical features of Behcet's disease patients with joint symptoms in Japan: A national multicenter study. Mod Rheumatol 2022 Oct 15; 32(6): 1146–52. doi: 10.1093/mr/roab092. PMID: 34850090.

10. Çakan ZK, Ya ız B, Pehlivan Y. Clinical features of Behcet's disease and prediction of the use of biologics in 488 cases: a single tertiary center study. Rheumatol Int 2023 Nov; 43(11): 2089–97. doi: 10.1007/s00296-023-05416-0. Epub 2023 Aug 22. PMID: 37604938.

11. Konak HE, Erden A, Arma an B, Güven SC, Apaydın H, Da lı PA, Uzun Y, Kaygısız M, Küçük ahin O, Omma A. Effects of breast milk on Behcet's disease clinical features. Turk J Med Sci 2023 Feb; 53(1): 121–9. doi: 10.55730/1300-0144.5565. Epub 2023 Feb 22. PMID: 36945965; PMCID: PMC10387996.

12. Wu L, Zheng Y, Liu J, Luo R, Wu D, Xu P, Wu D, Li X. Comprehensive evaluation of the efficacy and safety of LPV/r drugs in the treatment of SARS and MERS to provide potential treatment options for COVID-19. Aging (Albany NY) 2021 Apr 20; 13(8): 10833–52. doi: 10.18632/aging.202860. Epub 2021 Apr 20. PMID: 33879634; PMCID: PMC8109137.

13. Romão VC, Khmelinskii N. Clinical and ultrasonographic features of erythema nodosum in Behcet's disease. Rheumatology (Oxford) 2022 Aug 3; 61(8): e254. doi: 10.1093/rheumatology/keac035. PMID: 35048944.

14. Al-Omoush O, AlBarakat MM, Alasmar D, Al-Khalaileh A, Alzoubi A, Tarakhan H, Shakhatreh Z, Hatamleh Z, Abu-Saleh M, Saleh O, Altiti A, A Hazaymeh W, Alshwayyat S, Hanifa H, Hazaimeh E. Clinical and imaging features of parenchymal and nonparenchymal neuro-

Behçet's disease: a systematic review of case reports and series. BMC Neurol 2025 May 26; 25(1): 225. doi: 10.1186/s12883-025-04250-0. PMID: 40419971; PMCID: PMC12107814.

15. Wu L, Zhong Y, Wu D, Xu P, Ruan X, Yan J, Liu J, Li X. Immunomodulatory Factor TIM3 of Cytolytic Active Genes Affected the Survival and Prognosis of Lung Adenocarcinoma Patients by Multi-Omics Analysis. Biomedicines 2022 Sep 10; 10(9): 2248. doi: 10.3390/biomedicines10092248. PMID: 36140350; PMCID: PMC9496572.

16. Lee S, Kang S, Eun Y, Kim H, Lee J, Koh EM, Kim DK, Cha HS. Clinical characteristics and radiographic outcomes of vascular Behcet's disease involving the aorta or its major branches. Clin Rheumatol 2022 Jun; 41(6): 1769–77. doi: 10.1007/s10067-021-06031-9. Epub 2022 Feb 7. PMID: 35128590.

17. Triggianese P, Vitale A, Lopalco G, Mayrink Giardini HA, Ciccia F, Al-Maghlouth I, Ruscitti P, Sfikakis PP, Iannone F, de Brito Antonelli IP, Patrone M, Asfina KN, Di Cola I, Laskari K, Gaggiano C, Tufan A, Sfriso P, Dagna L, Giacomelli R, Hinojosa-Azaola A, Ragab G, Fotis L, Direskeneli H, Spedicato V, Dagostin MA, Iacono D, Ali HH, Cipriani P, Sota J, Kardasini RC, Bindoli S, Campochiaro C, Navarini L, Gentileschi S, Martín-Nares E, Torres-Ruiz J, Saad MA, Kourtesi K, Alibaz-Oner F, Sevik G, Iagnocco A, Makowska J, Govoni M, Monti S, Maggario MC, La Torre F, Del Giudice E, Hernández-Intern Emerg Med 2023 Nov; 18(8): 2231–43. doi: 10.1007/s11739-023-03408-3. Epub 2023 Oct 12. Erratum in: Intern Emerg Med 2024 Jan; 19(1): 255–257. doi: 10.1007/s11739-023-03511-5. PMID: 37828268; PMCID: PMC10635948.

18. Wu L, Liu Q, Ruan X, Luan X, Zhong Y, Liu J, Yan J, Li X. Multiple Omics Analysis of the Role of RBM10 Gene Instability in Immune Regulation and Drug Sensitivity in Patients with Lung Adenocarcinoma (LUAD). Biomedicines 2023 Jun 29; 11(7): 1861. doi: 10.3390/biomedicines11071861. PMID: 37509501; PMCID: PMC10377220.

19. Casares-Marfil D, Esencan D, Alibaz-Oner F, Çefle A, Yazıcı A, Duzgun N, Aşık MA, Özbek S, Cinar M, Alpsoy E, Bilge SY, Kasifoglu T, Saruhan-Direskeneli G, Direskeneli H, Sawalha AH. Clinical trait-specific genetic analysis in Behcet's disease identifies novel loci associated with ocular and neurological involvement. Clin Immunol 2023 Aug; 253: 109657. doi: 10.1016/j.clim.2023.109657. Epub 2023 Jun 2. PMID: 37271218; PMCID: PMC10529450.

20. Zhang B, Yang Y, Kuang T, Xu Z, Gong J. Analysis of clinical and imaging features of Behcet's disease-related pulmonary lesions: A series of eight cases. Chin Med J (Engl). 2022 Aug 20; 135(16): 2011–3. doi: 10.1097/CM9.0000000000002338. PMID: 36378138; PMCID: PMC9746756.

21. Wu L, Zheng Y, Ruan X, Wu D, Xu P, Liu J, Wu D, Li X. Long-chain noncoding ribonucleic acids affect the survival and prognosis of patients with esophageal adenocarcinoma through the autophagy pathway: Construction of a prognostic model. Anticancer Drugs 2022 Jan 1; 33(1): e590–e603. doi: 10.1097/CAD.0000000000001189. PMID: 34338240; PMCID: PMC8670349.

22. Muñoz SA, Kostianovsky A, Allievi A, Orden AO. Behcet disease in Latin American countries: A systematic literature review of demographic and clinical features, and HLA-B*51 allele frequency. Reumatol Clin (Engl Ed) 2023 Aug-Sep; 19(7): 386–91. doi: 10.1016/j.reumae.2022.12.005. PMID: 37661116.

23. Wu YC, Fu YJ, Xia HJ, Zhu J, Huang Y, Jiang ZN. Ileocecal involvement in intestinal Behcet's disease and Crohn's disease: Comparison of clinicopathological and immunophenotypic features. J Dig Dis 2023 Nov; 24(11): 594–602. doi: 10.1111/1751-2980.13236. Epub 2023 Nov 21. PMID: 37864553.

24. Ding X, Yang J, Han X, Shen YF, Yang K, Shangguan Y, Dong Y, Ye X. Clinical features and novel pathogenic variants of patients with Behcet's disease like trisomy 8. Orphanet J Rare Dis 2025 Jul 4; 20(1): 340. doi: 10.1186/s13023-025-03878-y. PMID: 40616106; PMCID: PMC12228353.

25. Wu L, Zhong Y, Yu X, Wu D, Xu P, Lv L, Ruan X, Liu Q, Feng Y, Liu J, Li X. Selective poly adenylation predicts the efficacy of immunotherapy in patients with lung adenocarcinoma by multiple omics research. Anticancer Drugs 2022 Oct 1; 33(9): 943–59. doi: 10.1097/CAD.0000000000001319. Epub 2022 Aug 9. PMID: 35946526; PMCID: PMC9481295.

26. Liu Z, Yang C, Bai X, Shen K, Qiao L, Wang Q, Yang H, Qian J. Clinical features and prognosis of patients with gastrointestinal Behcet's disease-like syndrome and myelodysplastic syndrome with and without trisomy 8. Semin Arthritis Rheum 2022 Aug; 55: 152039. doi: 10.1016/j.semarthrit.2022.152039. Epub 2022 Jun 3. PMID: 35689913.

27. Gürel Bedir A, Kilic SS. Clinical characteristics and quality of life in children with PFAPA syndrome and Behcet's disease. Rheumatol Adv Pract 2025 Feb 11; 9(1): rkaf015. doi: 10.1093/rap/rkaf015. PMID: 39959133; PMCID: PMC11829805.

28. Ruscitti P, Natoli V, Consolaro A, Caorsi R, Rosina S, Giancane G, Naddei R, Di Cola I, Di Muzio C, Berardicurti O, Iacono D, Pantano I, Rozza G, Rossi S, De Stefano L, Balduzzi S, Vitale A, Caso F, Costa L, Prete M, Navarini L, Iagnocco A, Atzeni F, Guggino G, Perosa F, Cantarini L, Frediani B, Montecucco C, Ciccia F, Cipriani P, Gattorno M, Giacomelli R, Ravelli A. Disparities in the prevalence of clinical features between systemic juvenile idiopathic arthritis and adult-onset Still's disease. Rheumatology (Oxford) 2022 Oct 6; 61(10): 4124–9. doi: 10.1093/rheumatology/keac027. PMID: 35078234; PMCID: PMC9536787.

29. Yoo JW, Jo SI, Shin DW, Park JW, Kim SE, Lim H, Kang HS, Moon SH, Kim MK, Kim SY, Hwang SW, Soh JS. Clinical Usefulness of Immune Profiling for Differential Diagnosis between Crohn's Disease, Intestinal Tuberculosis, and Behcet's Disease. Diagnostics (Basel) 2023 Sep 11; 13(18): 2904. doi: 10.3390/diagnostics13182904. PMID: 37761270; PMCID: PMC10529363.

30. Murakami K, Arai J, Ihara S, Tsuchida Y, Shoda H, Tsuboi M, Kurokawa K, Shiomi C, Suzuki N, Hayakawa Y, Fujio K, Fujishiro M. Upper gastrointestinal involvement of Behcet's disease in Japan: Endoscopic findings and clin-

ical features. J Gastroenterol Hepatol 2024 Apr; 39(4): 708–15. doi: 10.1111/jgh.16479. Epub 2024 Jan 7. PMID: 38185774.

31. Makkawi S, Aljafari D, Alsharif R, Maaddawi H, Alwagdani A, Aljumah T, Alghweinem Z, Alshehri S, Khojah O, Halawani A, Ahmed Adas R, Abulaban A, Al Malik Y. The clinical and radiological features and prevalence of Neuro-Behçet's Disease: A retrospective cohort multicenter study in Saudi Arabia. Mult Scler Relat Disord 2024 May; 85: 105558. doi: 10.1016/j.msard.2024.105558. Epub 2024 Mar 20. PMID: 38569381.

32. Zhou J, Zhang D, Wang Z, Hou Y, Liu J, Zeng X, Wang Y, Zheng W. The clinical features, image findings and risk factors for vena cava syndrome in Behcet's syndrome. Clin Exp Rheumatol 2022 Sep; 40(8): 1526–34. doi: 10.55563/clinexprheumatol/r6her6. Epub 2022 Jul 11. PMID: 35819814.

33. Li Y, Xiong Z, Jiang Y, Shen Y, Hu X, Hu D, Li Z. Enhancing the Differentiation between Intestinal Behcet's Disease and Crohn's Disease through Quantitative Computed Tomography Analysis. Bioengineering (Basel) 2023 Oct 17; 10(10): 1211. doi: 10.3390/bioengineering10101211. PMID: 37892941; PMCID: PMC10604024.

34. Prtenjača L, Mayer M, Karanović B, Rukavina K, Bakula M, Padjen I, Šćepović-Ljucović M, Tomičević LS, Anić B, Barešić M. Demographics and clinical manifestations in patients with Behcet's disease: A retrospective observational study from a nonendemic country. Rheumatol Int 2025 Apr 15; 45(5): 102. doi: 10.1007/s00296-025-05855-x. PMID: 40232346.

35. Kaymaz S, Aydın D, U ur K, Çobankara V, Tan S. Expression Levels and Clinical Values of miR-195, miR-424, miR-10b, miR-103a-3p, and miR-542-3p in Vasculo-Behçet's Disease. Mediterr J Rheumatol 2023 Sep 7; 35(2): 255–62. doi: 10.31138/mjr.030623.elc. PMID: 39211027; PMCID: PMC11350416.

36. Elidrissi EH, Elaissat EM, Assoufi N, Tbouda M. Association of Sweet's Syndrome and Behcet's Disease: Is It Possible? A Case Report. Eur J Case Rep Intern Med 2023 Sep 19; 10(10): 004073. doi: 10.12890/2023_004073. PMID: 37789984; PMCID: PMC10545150.

37. Park Y, Ahn JB, Kim DH, Park IS, Son M, Kim JH, Ma HW, Kim SW, Cheon JH. Integrated Analysis of Microbiome and Metabolome Reveals Disease-Specific Profiles in Inflammatory Bowel Diseases and Intestinal Behcet's Disease. Int J Mol Sci 2024 Jun 18; 25(12): 6697. doi: 10.3390/ijms25126697. PMID: 38928402; PMCID: PMC11203907.