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ORIGINAL RESEARCH

Elevated Serum IL-6/IL-10 Ratio as a Promising Diagnostic and Disease Grading Biomarker for Cerebral Small Vessel Disease

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Background: Chronic inflammation and the imbalance of M1/M2 phenotype of microglia contribute to the onset and progression of cerebral small vessel disease (CSVD). Currently, no effective biomarkers for diagnosing CSVD are found. Our research aims to explore the efficacy of IL-6/IL-10 ratio in the diagnosis of CSVD and assessment of its severity, establishing IL-6/IL-10 as an independent predictive biomarker.

Patients and Methods: A total of 155 participants admitted to the First People's Hospital of Yancheng City from February 2022 to September 2024 were grouped based on MRI and CSVD imaging scoring. Clinical and laboratory data were collected and analyzed.

Results: Compared with the healthy control group, the CSVD group showed significant increases of IL-6/IL-10 ratio in peripheral blood (P=0.001); while compared with the CSVD (score 0–1) group, IL-6/IL-10 ratio was statistically significant elevation in the CSVD (score 2–4) group (P=0.002). The area under the curve (AUC) of IL-6/IL-10 ratio in the diagnosis of CSVD was 0.816, the sensitivity and specificity were 67.9% and 85.7%. Besides, the AUC of IL-6/IL-10 ratio in evaluating the severity of CSVD was 0.687.

Conclusion: IL-6/IL-10 ratio is a potential circulating biomarker for diagnosing CSVD, with good diagnostic efficacy and has the ability to distinguish the severity of the disease to a certain degree.

Keywords: cerebral small vessel disease, neuroinflammation, biomarker, microglia polarization, IL-6/IL-10

Introduction

Clinical manifestations of cerebral small vessel disease (CSVD) include stroke, progressive dementia, gait and balance problems, and emotional disorders.[1] The incidence of CSVD increases with advancing age, affecting nearly all individuals over the age of 90.[2,3] Although many studies are exploring biomarkers for acute cerebrovascular disease, however, the diagnosis and grading of CSVD currently rely heavily on the imaging findings from cranial magnetic resonance imaging (MRI), and there is a lack of reliable and effective biological markers.[4,5]

Neuroinflammation driven by microglia is integral to all pathological mechanisms, underscoring its importance in the pathogenesis of CSVD.[6–9] The activation of microglia is heterogeneous, and the interplay between the M1 phenotype, which produces cytotoxic substances such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and the M2 phenotype, which secretes neuroprotective cytokines like interleukin-4 (IL-4) and interleukin-10 (IL-10), is consistently dynamic.[10,11] IL-6 has pleiotropy, which can both induce neuronal survival after injury and cause neurodegeneration and cell death in neurodegenerative diseases.[12] L-10 is an important anti-inflammatory regulator for neuroglial activation, which can prevent inflammation mediated neuronal functional degradation under pathological conditions.[13] Changes in microglial phenotype may depend on the stage and severity of the disease, which is a complex and nuanced process.[14] Microglia can act as a double-edged sword in many diseases, while with the persistent and excessive polarization of the M1 phenotype proving detrimental to the body.[15] The inflammatory response is often accompanied by endothelial cell

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dysfunction and disruption of blood-brain barrier permeability, offering insights and opportunities for disease identification through the detection of circulating biomarkers.[3,16]

The aim of this study is to evaluate the utility of the inflammatory related biomarker IL-6/IL-10 ratio as a non-invasive indicator for the diagnosis of CSVD and the differentiation of its severity. We collected clinical data to analyze the differential expression of IL-6/IL-10 ratio in healthy subjects, patients with CSVD, and patients with varying degrees of CSVD, and explore the diagnostic efficacy, which was subsequently verified it through animal experiments.

Materials and Methods

Participants and Grouping

In this retrospective study, 112 hospitalized patients diagnosed as CSVD were enrolled from Yancheng First People's Hospital, China, from February 2022 to September 2024. Comprehensive demographic information, medical histories and laboratory data were recorded, analyzed and reviewed by two experienced neurologists to ensure the accuracy. Due to the fact that the diagnosis of 6 participants in the CSVD group was based on computed tomography (CT) of the skull, they were not included in subsequent clinical studies. The other 106 participants in the CSVD group were divided into two groups based on the CSVD imaging total burden score, namely 71 participants with a CSVD imaging total burden score of 0–1 were included in the CSVD (score 0-1) group, and 35 participants with a CSVD imaging total burden score of 2–4 were included in the CSVD (score 2-4) group.

Inclusion Criteria for Patients with CSVD

(i) High risk factors and clinical symptoms of CSVD.

(ii) At least one of the following evidence existed in cranial MRI: lacunar infarction, white matter hyperintensities (WMH), enlargement of perivascular space (PVS), cortical microinfarcts, cerebral microbleeds (CMB) and brain atrophy.

Exclusion Criteria for Patients with CSVD

(i) CSVD caused by etiologies other than vascular risk factors such as hypertension: cerebral amyloid angiopathy, genetic causes of amyloidosis, inflammatory or immune-mediated factors, causes of venous collagenization, and so on.

(ii) Diseases accompanied by other active inflammations, such as pneumonia, acute upper respiratory tract infections, cholecystitis, and so on.

Healthy Controls (HC)

43 individuals for physical examination from February 2022 to September 2024 were selected as the healthy control group. Inclusion criteria for the healthy control group: (i) No abnormalities were found on cranial MRI. (ii) The body was not in an inflammatory state.

Blood Routine Test

Electrical impedance method is performed, using Mindray hemocytometer for detection. NLR is the ratio of neutrophil count to lymphocyte count in blood routine.

Detection of High-Sensitivity C-Reactive Protein (Hs-CRP)

A high-sensitivity C-reactive protein detection kit (latex enhanced immune scattering turbidity method) was used. The reagent is mixed with the sample, and the antibody labeled latex microspheres in the reagent undergo agglutination reaction with C-reactive protein in the sample, resulting in an increase in solution turbidity. The concentration of C-reactive protein in the sample is obtained through turbidity analysis, which is further converted into the concentration of C-reactive protein in plasma or serum by the proportion of blood cell volume in the sample.

Sample Collection and Cytokine Detection

From 6:00 to 8:00 in the morning, collect fasting venous blood from patients. The blood sample should be ensured to be free of hemolysis, lipid blood, and blood clots, and stored at 4 °C. Then send the plasma sample to the laboratory for

centrifugation treatment, centrifuge 1000g for 30 minutes. The kit uses an 8-item cytokine detection kit. The experiment is based on the principle of immunological analysis methods, using a direct sandwich method and Raisecyte2L6C flow cytometer to detect and analyze patient samples.

Magnetic Resonance Scanning

Magnetic resonance imaging (MRI) is performed by specialized MRI technicians, and imaging sequences (T1, T2, FLAIR, SWI) are scanned. Various types of CSVD markers typically coexist, and neurological outcomes are influenced by their combined effects. The higher the score, the heavier the disease burden (Table S1).[17]

Animal Care and Grouping

C57BL/6 male mice (8–10 weeks, 23 ± 2 g) were obtained from Bengbu Yinuojia Biotechnology Co., Ltd., and housed in the Animal Center of Jiangsu Vocational College of Medicine. During the study, the mice were raised under specific conditions of a 12 / 12 h light dark cycle in an environment of 24 ± 2 °C and 50 ± 10% relative humidity. All mice had free access to water and food. Mice were randomly divided into two groups: (i) the sham-operation group, and (ii) the model group.

Establishment of the Bilateral Carotid Artery Stenosis (BCAS) Model

The mice went through fasting for 12 h before the surgery. They were anaesthetized with 0.3% pentobarbital sodium (0.1 mL/10 g) and fixed thereafter. When operated, the common carotid artery (CCA) was separated, and two 4–0 wires were respectively disposed at the distal and proximal ends of the CCA. We then gently raised the artery with silk threads and placed it between the metal micro-coil directly below the carotid bifurcation. The micro-coil was wound by rotation around the CCA. After 30 mins, another micro-coil was wound around the other CCA similarly. The skin was sutured and disinfected with 75% ethanol in the end.

Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

The cortex of the mice was dissected and preserved in −80°C refrigerator. RNA was collected by using RNA-easy isolation reagent and a tissue homogenizer. One microgram of RNA and a reverse transcription kit was prepared for cDNA synthesis according to the manufacturer's protocol. Afterwards, 2 μL of cDNA and SYBR Green were both added for qPCR. The primer sequences used in this study were listed in Table 1. GAPDH was regarded as a housekeeping gene to quantitatively analyze the target gene expression.

Statistical Analysis

GraphPad Prism 9 software and SPSS Statistics 26.0 were both used to analyze all the data. Age and laboratory results were not normally distributed and Mann–Whitney *U*-test was applied to compare nonparametric data, expressed as the median (interquartile range) [M(QR)]. For the statistical analysis of the data in the four-grid table, when the sample size $n \geq 40$ and the theoretical frequency $T \geq 5$, Pearson chi square test was used, such as the comparison of the sex of the two groups of subjects, the constituent ratio of the past history of hypertension, diabetes, hyperlipidemia and stroke. When $n \geq 40$, if $1 \leq T \leq 5$ appears in a certain grid, Yates continuity correction should be used, such as comparing the

Table 1 Primer Sequences Used in This Study

Gene	Species	Sequence
IL-6	Mouse	F: CCAAGAGGTGAGTGCTTCCC R: CTGTTGTTCAGACTCTCTCCCT
IL-10	Mouse	F: GCTCTTACTGACTGGCATGAG R: CGCAGCTCTAGGAGCATGTG
GAPDH	Mouse	F: AGGTCGGTGTGAACGGATTTG R: GGGGTCGTTGATGGCAACA

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composition ratio of coronary heart disease between two groups of medical histories. When n < 40 or T < 1 appears in any grid, Fisher's exact probability test is used, such as comparing the composition ratio of atrial fibrillation between two groups of medical histories. A two-tailed *t* test was performed to analyze differences between two independent groups like body mass index (BMI), data were presented as the mean ± standard deviation (SD). Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of each indicator. Differences for which P < 0.05 were considered statistically significant.

Results

Comparison of General Characteristics and Serum Inflammatory Markers Between the HC and CSVD Group

The overall process design of this experiment is shown in Graphical abstract. 155 participants were totally included in this study, 43 were in the HC group, and 112 were in the CSVD group. The demographic and clinical characteristics were displayed in Table 2. There were no significant differences in sex, coronary artery disease, atrial fibrillation and hyperlipidemia between the two groups ($P > 0.05$). While there were significant differences in age, BMI (Figure 1A),

Table 2 Demographic and Clinical Data of CSVD and Healthy Control Participants

Baseline Characteristics	**HC (n=43)**	**CSVD (n=112)**	**χ^2/Z/t**	***P* value**
Demographics				
Male (%)[a]	19 (44.2)	63 (56.3)	1.815	0.178
Age (years)[b]	45 (39; 66)	71 (67; 81)	−52.5	**<0.001**
BMI[c]	23.38±3.86	24.82±3.75	−2.116	**0.036**
Medical history				
Hypertension (%)[a]	9 (20.9)	63 (56.3)	15.583	**<0.001**
Diabetes mellitus (%)[a]	4 (9.3)	30 (26.8)	5.546	**0.019**
Coronary artery disease (%)[d]	1 (2.3)	4 (3.6)	0	1.000
Atrial fibrillation (%)[e]	0 (0)	3 (2.7)	/	0.561
Hyperlipidemia (%)[a]	17 (39.5)	42 (37.5)	0.055	0.815
Stroke history (%)[a]	2 (4.7)	38 (33.9)	13.91	**<0.001**
Laboratory characteristics				
WBC (10^9/L)[b]	5.65 (4.55; 8.00)	6.09 (5.15; 7.53)	−0.712	0.476
GRAN (10^9/L)[b]	3.33 (2.60; 5.01)	3.80 (2.82; 5.12)	−0.873	0.383
LYMPH (10^9/L)[b]	1.45 (1.21; 2.56)	1.52 (1.20; 1.91)	−1.076	0.282
PBMC (10^9/L)[b]	0.45 (0.26; 0.58)	0.48 (0.36; 0.59)	−1.116	0.264
NLR[b]	1.89 (1.50; 2.98)	2.38 (1.62; 4.42)	−1.459	0.145
LMR[b]	4.39 (3.03; 6.10)	3.29 (2.49; 4.34)	−3.109	**0.002**
Laboratory characteristics	**HC (n=43)**	**CSVD (n=112)**	**χ^2/Z/t**	***P* value**
SII[b]	401.36 (298.08; 559.61)	442.39 (255.90; 841.68)	−0.384	0.701
NMLR[b]	2.22 (1.66; 3.33)	2.62 (192; 4.82)	−1.52	0.129
PLT (10^9/L)[b]	212 (164; 239)	176 (137.75; 222)	−2.206	**0.027**
PLR[b]	111.03 (85.06; 161.15)	116.48 (81.38; 161.82)	−0.108	0.914
D-Dimer (mg/L)[b]	0.22 (0.16; 0.38)	0.51 (0.30; 0.92)	−4.724	**<0.001**
Fbg (g/L)[b]	2.55 (2.14; 3.07)	3.00 (2.43; 4.36)	−2.783	**0.005**
hs-CRP (mg/L)[b]	0.50 (0.50; 1.42)	4.18 (0.50; 16.26)	−4.187	**<0.001**
IFN-γ (pg/mL)[b]	2.64 (1.76; 5.47)	2.57 (2.07; 5.61)	−1.17	0.242
IL-12P70 (pg/mL)[b]	1.83 (1.19; 2.02)	1.50 (1.15; 1.90)	−1.609	0.108
IL-2 (pg/mL)[b]	2.07 (1.44; 2.67)	2.25 (1.67; 3.02)	−1.209	0.227

(*Continued*)

Table 2 (Continued).

IL-6 (pg/mL)[b]	2.36 (1.50; 3.62)	3.63 (2.25; 7.42)	−3.076	**0.002**
TNF-α (pg/mL)[b]	2.03 (1.94; 2.98)	2.35 (1.94; 3.20)	−1.067	0.286
IL-17 (pg/mL)[b]	2.78 (1.85; 5.30)	4.60 (2.11; 8.57)	−2.411	**0.016**
IL-4 (pg/mL)[b]	1.64 (0.94; 2.00)	1.32 (0.81; 2.00)	−1.303	0.193
IL-10 (pg/mL)[b]	2.25 (1.74; 2.94)	2.04 (1.69; 2.92)	−0.598	0.550
IL-6/IL-10[b]	1.05 (0.73; 1.43)	1.53 (0.96; 3.38)	−3.231	**0.001**
IL-6/IL-4[b]	1.33 (1.05; 2.93)	2.92 (1.32; 6.59)	−3.287	**0.001**
TNFα/IL-10[b]	0.76 (0.97; 1.38)	1.15 (0.76; 1.45)	−1.094	0.274
TNFα/IL-4[b]	1.71 (0.84; 2.49)	2.06 (1.18; 2.82)	−1.839	0.066
IL-17/IL-10[b]	1.56 (0.66; 2.52)	2.36 (1.29; 4.03)	−2.558	**0.011**
IL-17/IL-4[b]	1.86 (1.32; 3.51)	3.49 (1.93; 6.34)	−3.061	**0.002**

Notes: The bold numbers in the table indicate that the data are statistically significant. Statistical method: [a]chi square test; [b]Mann Whitney *U*-test; [c]T-test; [d]Yates continuity correction; e: Fisher's exact probability test.

hypertension, diabetes mellitus and stroke history ($P < 0.05$). Between the two groups, serum inflammatory and related indicators were compared (Table 2). The results showed that compared with the HC, there was a statistically significant increase in serum lymphocyte-to-monocyte ratio (LMR), platelet (PLT), D-Dimer, Fbg, hypersensitive C-reactive protein (hs-CRP), IL-6, interleukin 17 (IL-17), IL-6/IL-10, IL-6/IL-4, IL-17/IL-10, and IL-17/IL-4 ($P < 0.05$, Figure 1B–L). No statistically significant difference was found in serum white blood cell (WBC), neutrophilicgranulocyte (GRAN), lymphocyte (LYMPH), peripheral blood mononuclear cell (PBMC), neutrophil-to-lymphocyte ratio (NLR), systemic immune inflammation index (SII), (neutrophil + monocyte)-to-lymphocyte ratio (NMLR), platelet-to-lymphocyte ratio (PLR), interferon gamma (IFN-γ), interleukin 12P70 (IL-12P70), interleukin 2 (IL-2), TNF-α, IL-4, IL-10, TNFα/IL-10, and TNFα/IL-4 ($P > 0.05$).

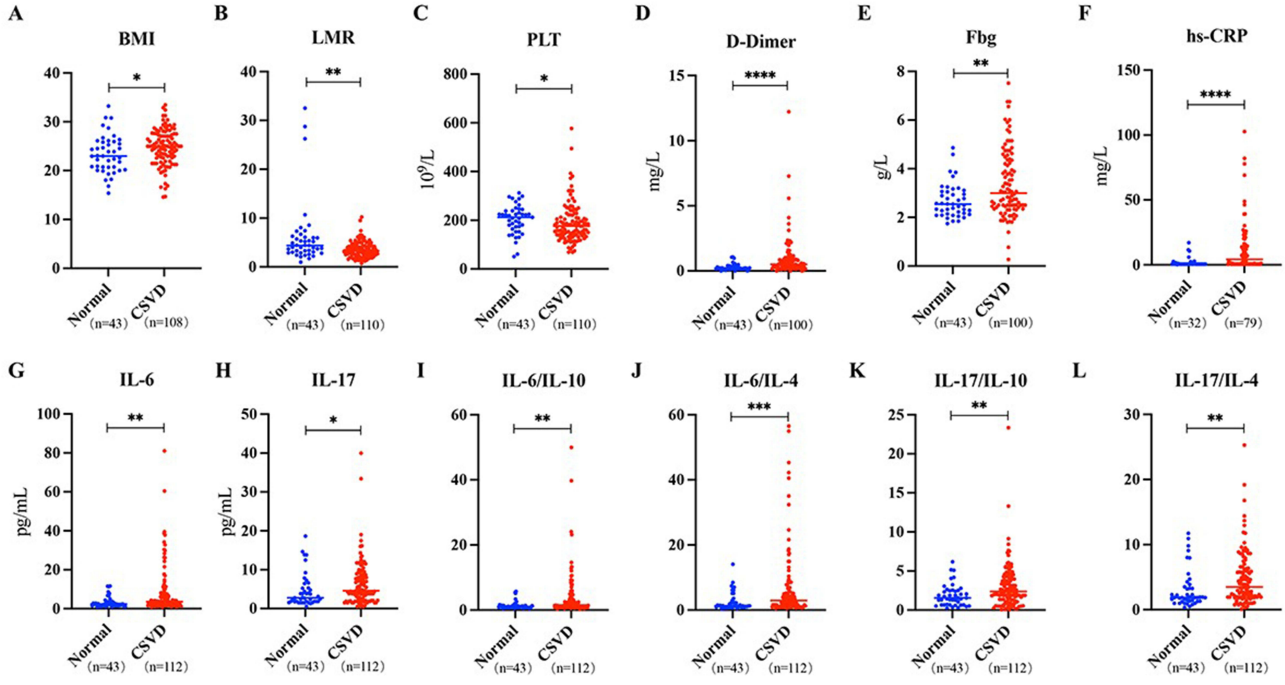

Figure 1 Serum levels of inflammatory indicators, inflammatory related indicators and BMI tested in normal and CSVD subjects. (**A**) BMI was compared between two groups. (**G** and **H**) Serum levels of hs-CRP, IL-6, IL-17 and IL-10 were detected by flow cytometry between two groups. (**I–L**). Inflammatory factor ratios of IL-6/IL-10, IL-6/IL-4, IL-17/IL-10, IL-17/IL-4 were compared between two groups. Statistical method used in (**A**): *T*-test; (**B–L**): Mann–Whitney *U*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, Normal vs CSVD.

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Comparison of General Characteristics and Serum Inflammatory Markers Between the CSVD (Score 0-1) and CSVD (Score 2-4) Group

The CSVD group was divided into two subgroups based on imaging scores, and baseline data and serum inflammatory markers were compared between the two groups (Table 3). The results indicated that there was a statistically significant difference in age, GRAN, LYMPH, NLR, LMR, SII, NMLR, PLR, IL-2, IL-6, TNF-α, IL-6/IL-10, and IL-6/IL-4 ($P < 0.05$, Figure 2A–L). There was no statistically significant difference in sex, BMI, hypertension, diabetes mellitus, coronary artery disease, atrial fibrillation, hyperlipidemia, stroke history, WBC, PBMC, PLT, hs-CRP, IFN-γ, IL-12P70, IL-17, IL-4, IL-10, TNFα/IL-10, TNFα/IL-4, IL-17/IL-10, and IL-17/IL-4 ($P > 0.05$).

Table 3 Demographic and Clinical Data of CSVD (Score 0–1) and CSVD (Score 2–4)

Baseline Characteristics	**CSVD (Score 0–1) (n=71)**	**CSVD (Score 2–4) (n=35)**	**χ^2/Z/t**	***P* value**
Demographics				
Male (%)[a]	39 (54.9)	20 (57.1)	0.047	0.829
Age (years)[b]	71 (58; 80)	75 (69; 80)	−2.161	**0.031**
BMI[c]	25.06±3.49	24.80±4.32	1.192	0.236
Medical history				
Hypertension (%)[a]	38 (53.5)	21 (60.0)	0.399	0.528
Diabetes mellitus (%)[a]	19 (26.8)	11 (31.4)	0.252	0.616
Coronary artery disease (%)[d]	3 (4.2)	1 (2.9)	0	1.000
Atrial fibrillation (%)[e]	2 (2.8)	1 (2.9)	/	1.000
Hyperlipidemia (%)[a]	31 (43.7)	11 (31.4)	1.467	0.226
Stroke history (%)[a]	21 (29.6)	14 (40.0)	1.151	0.283
Laboratory characteristics				
WBC (10^9/L)[b]	5.84 (5.07; 7.34)	6.62 (5.39; 8.02)	−1.507	0.132
GRAN (10^9/L)[b]	3.34 (2.74; 4.84)	4.60 (3.48; 6.04)	−2.491	**0.013**
LYMPH (10^9/L)[b]	1.58 (1.19; 2.09)	1.35 (1.00; 1.67)	−1.961	**0.050**
PBMC (10^9/L)[b]	0.44 (0.34; 0.59)	0.51 (0.36; 0.58)	−0.693	0.488
NLR[b]	2.01 (1.46; 3.51)	3.23 (2.05; 5.84)	−2.938	**0.003**
LMR[b]	3.76 (2.66; 4.72)	3.09 (1.94; 3.91)	−2.100	**0.036**
Laboratory characteristics	**CSVD (score 0–1) (n=71)**	**CSVD (score 2–4) (n=35)**	**χ^2/Z/t**	***P* value**
SII[b]	339.71 (228.29; 711.59)	562.28 (380.26; 1051.81)	−2.709	**0.007**
NMLR[b]	2.31 (1.67; 3.88)	3.46 (2.35; 6.48)	−2.966	**0.003**
PLT (10^9/L)[b]	176 (136; 210.5)	161.5 (139.5; 246.25)	−0.281	0.779
PLR[b]	109.86 (73.73; 147.77)	133.61 (99.41; 188.50)	−2.190	**0.029**
hs-CRP (mg/L)[b]	2.05 (0.50; 14.07)	6.68 (0.51; 15.36)	−1.068	0.286
IFN-γ (pg/mL)[b]	2.57 (2.06; 5.64)	2.57 (2.08; 5.75)	−0.266	0.791
IL-12P70 (pg/mL)[b]	1.50 (1.17; 1.97)	1.55 (1.14; 1.76)	−0.034	0.973
IL-2 (pg/mL)[b]	2.05 (1.52; 2.84)	2.61 (1.90; 3.30)	−2.237	**0.025**
IL-6 (pg/mL)[b]	3.09 (1.71; 5.33)	6.15 (3.24; 24.39)	−3.648	**<0.001**
TNF-α (pg/mL)[b]	2.11 (1.94; 3.04)	2.41 (2.03; 4.03)	−1.965	**0.049**
IL-17 (pg/mL)[b]	4.27 (1.93; 8.01)	5.70 (2.90; 8.92)	−1.059	0.290
IL-4 (pg/mL)[b]	1.32 (0.81; 2.00)	1.61 (0.81; 2.00)	−0.222	0.824
IL-10 (pg/mL)[b]	2.04 (1.66; 2.88)	2.10 (1.73; 2.91)	−0.625	0.532
IL-6/IL-10[b]	1.25 (0.81; 2.49)	2.45 (1.24; 7.02)	−3.127	**0.002**
IL-6/IL-4[b]	2.51 (1.15; 4.41)	4.18 (2.30; 13.11)	−3.057	**0.002**

(*Continued*)

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Table 3 (Continued).

TNFα/IL-10[b]	1.15 (0.74; 1.38)	1.29 (0.91; 1.69)	−1.567	0.117
TNFα/IL-4[b]	2.00±1.04	2.34±1.37	−1.427	0.156
IL-17/IL-10[b]	2.33 (1.05; 4.26)	2.47 (1.72; 4.04)	−0.410	0.682
IL-17/IL-4[b]	2.80 (1.93; 6.26)	3.90 (1.86; 7.00)	−0.554	0.579

Notes: The bold numbers in the table indicate that the data are statistically significant. Statistical method: [a]chi square test; [b]Mann Whitney *U*-test; [c]T-test; [d]Yates continuity correction; e: Fisher's exact probability test.

The Diagnostic Value of IL-6/IL-10 Ratio Depicted by Receiver Operating Characteristic (ROC) Curve for CSVD

In order to clarify the diagnostic value of various indicators for CSVD, the optimal cut-off values, area under the curve (AUC), 95% confidence interval (CI), sensitivity, and specificity of LMR, Fbg, IL-6, IL-6/IL-10, IL-6/IL-4 were calculated in Table 4. ROC curve analysis showed that the diagnostic performance of IL-6/IL-10 ratio was significantly better than other indicators, the AUC of IL-6/IL-10 ratio was 0.816, while the sensitivity was 74.1% and the specificity was 79.1% (Figure 3 and Table 4).

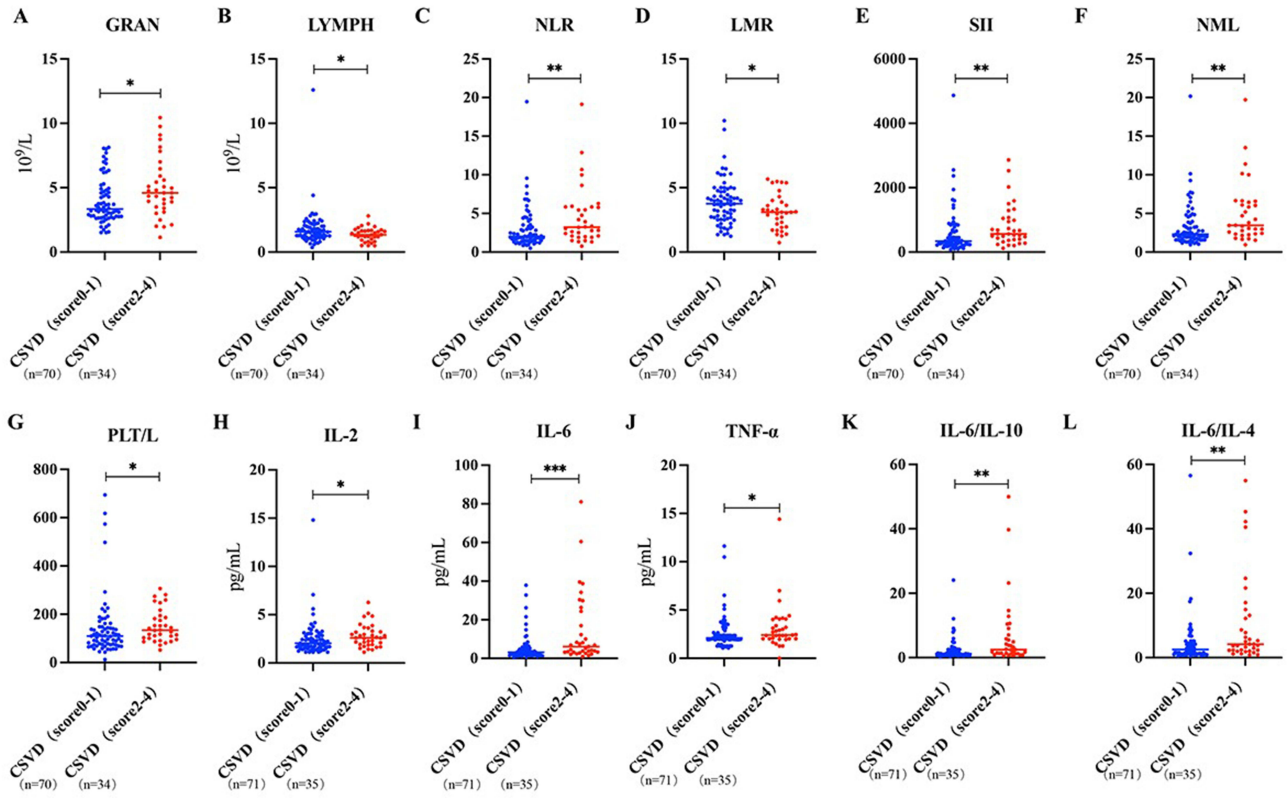

Figure 2 Serum levels of inflammatory indicators and inflammatory related indicators in CSVD (score 0–1) vs CSVD (score 2–4) subjects. (**A**–**G**) Serum levels of GRAN, LYMPH, NLR, LMR, SII, NML and PLT/L were between two groups. (**H**–**J**) Serum levels of IL-2, IL-6 and TNF-α were compared between two groups. (**K**–**L**) Inflammatory factor ratios of IL-6/IL-10 and IL-6/IL-4 were compared between two groups. Statistical method used in (**A**–**L**): Mann–Whitney *U*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, CSVD (score 0–1) vs CSVD (score 2–4).

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Table 4 Diagnostic Value for CSVD Using LMR, Fbg, Hs-CRP, IL-6, IL-6/IL-10, and IL-6/IL-4

Item	Cut-Off value	AUC	95% CI	*P*	Sensitivity (%)	Specificity (%)
LMR	—	0.338	0.239–0.437	0.002	—	—
Fbg	3.305 g/L	0.649	0.558–0.740	0.005	42.0	88.4
IL-6	2.905 pg/mL	0.660	0.568–0.752	0.002	65.2	65.1
IL-6/IL-10	1.474	0.816	0.746–0.885	<0.001	74.1	79.1
IL-6/IL-4	1.513	0.505	0.407–0.604	0.920	50.9	60.5

The Ability of IL-6/IL-10 Ratio to Distinguish the Severity of CSVD Evaluated by ROC Curve

The optimal cut-off values, AUC, 95% CI, sensitivity, and specificity of LMR, Fbg, IL-6, IL-6/IL-10, IL-6/IL-4 were also calculated in Table 5 to explore the diagnostic efficacy of disease grading. The diagnostic performance of IL-6/IL-10 ratio was slightly inferior to IL-6, but superior to other indicators, and the AUC of IL-6/IL-10 ratio was 0.687, while the sensitivity was 45.7% and the specificity was 85.9% (Figure 4 and Table 5).

Changes in IL-6/IL-10 Ratio Expression in the Cortex of a Mouse Model of CSVD

To further investigate the expression changes of IL-6/IL-10 ratio in CSVD, a mouse model of bilateral common carotid artery stenosis (BCAS) was constructed to simulate the disease. Compared with the sham-operation group, the mRNA level of IL-6 and IL-6/IL-10 ratio was significantly increased in the model group (Figure 5A and C), while there was no difference in the the mRNA level of IL-10 between the two groups (Figure 5B).

Discussion

The persistent and excessive polarization of the M1 phenotype in microglia is a critical factor contributing to the onset and progression of CSVD, and the dynamic balance of M1/M2 is disrupted in this disease. Our findings indicated there were significant differences in the expression levels of IL-6/IL-10 ratio between healthy controls, patients diagnosed with

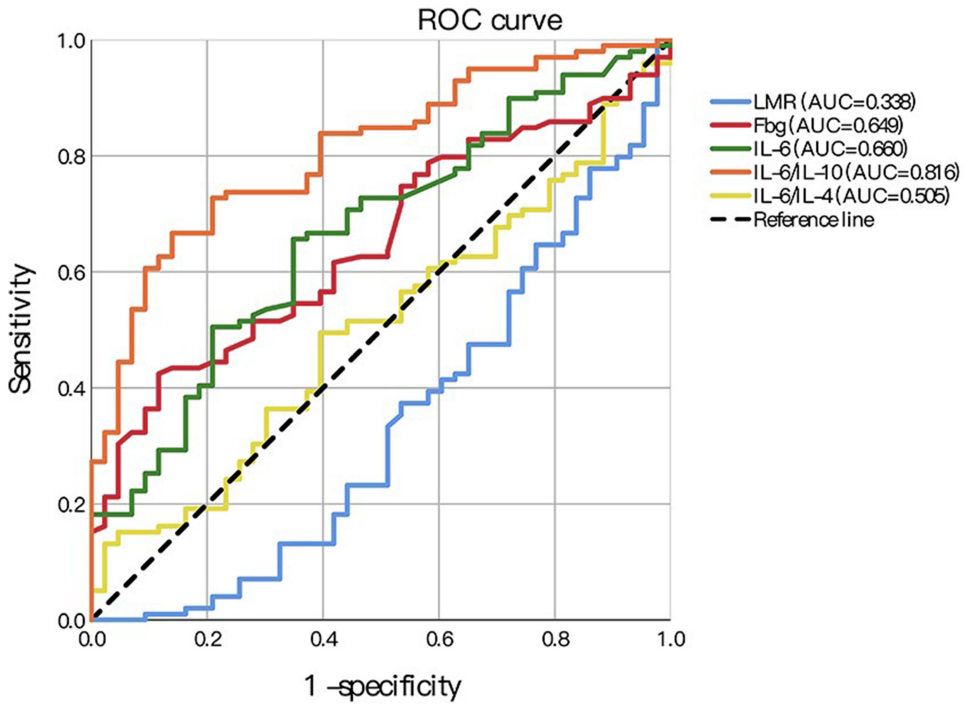

Figure 3 ROC curve analysis of LMR, Fbg, IL-6, IL-6/IL-10 and IL-6/IL-4 as diagnostic biomarkers for CSVD.

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Table 5 Diagnostic Value for Severity of CSVD Using LMR, Fbg, Hs-CRP, IL-6, IL-6/IL-10, and IL-6/IL-4

Item	Cut-off value	AUC	95% CI	*P*	Sensitivity (%)	Specificity (%)
LMR	—	0.373	0.258–0.487	0.036	—	—
Fbg	3.85 g/L	0.561	0.438–0.685	0.328	42.4	75.4
IL-6	3.21 pg/mL	0.719	0.615–0.822	<0.001	80.0	57.7
IL-6/IL-10	3.256	0.687	0.577–0.797	0.002	45.7	85.9
IL-6/IL-4	1.839	0.683	0.576–0.790	0.002	85.7	47.9

CSVD, and patients exhibiting varying degrees of CSVD. Additionally, results from animal models also corroborate this observation. We report for the first time, evidence suggesting that serum IL-6/IL-10 ratio may serve as a potential biomarker for the diagnosis of CSVD, and possess the capacity to differentiate the severity of the disease to a certain extent.

CSVD has emerged as a common factor in age-related diseases such as stroke and dementia.[18] Research indicated that an increase in BMI was associated with impaired white matter microstructural integrity and an increased risk of CSVD and other neurodegenerative diseases.[19,20] Elevated systolic blood pressure in patients with CSVD was significantly correlated with their total imaging burden.[21] Other notable, diabetes and previous stroke history were related to CSVD as well.[22] The analysis of data from our clinical retrospective studies showed that the risk of CSVD increased with age when compared with the healthy controls. Furthermore, this risk was associated with BMI, hypertension, diabetes and a history of strokes. Additionally, age also correlated with the severity of classification based on CSVD imaging scores.

Blood-based biomarkers have demonstrated significant diagnostic value in various neurological diseases.[23–26] In patients with non-small cell lung cancer, LMR levels were found to be negatively correlated with the possibility of brain metastasis.[27] Forty-eight hours LMR can serve as an independent predictor of favorable discharge outcomes in patients who have undergone intravenous thrombolysis.[28] The persistent elevation of Fbg levels is closely associated with the abnormal activation of microglia and chronic inflammation, playing a role in the pathogenesis of multiple neurological

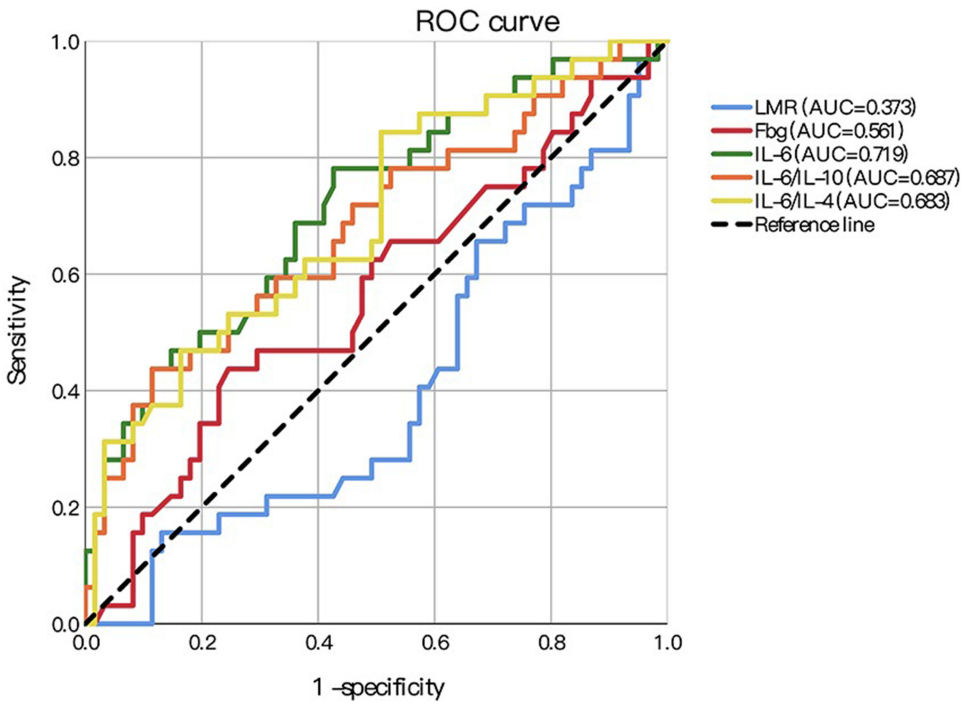

Figure 4 Receiver operating characteristics (ROC) curve analysis of LMR, Fbg, IL-6, IL-6/IL-10 and IL-6/IL-4 as diagnostic biomarkers for severity of CSVD.

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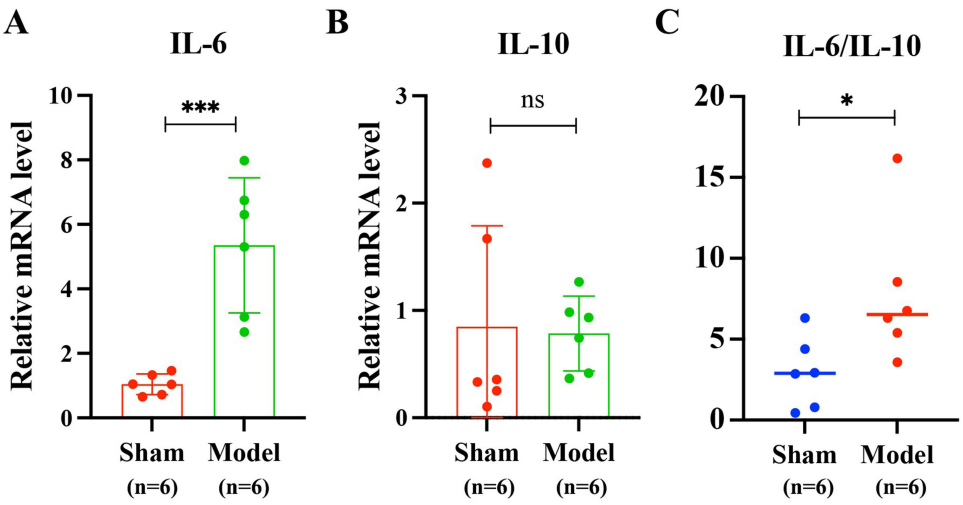

Figure 5 mRNA levels of IL-6 and IL-10 evaluated by qRT-PCR between the sham-operation group and the model group. (**A**) The comparison of inflammatory cytokine of IL-6 between two groups. (**B**) The comparison of inflammatory cytokine of IL-10 between two groups. (**C**) The comparison of inflammatory cytokine ratio of IL-6/IL-10 between two groups. N = 7 mice in sham-operation group, n = 8 mice in model group. Statistical method used in (**A** and **C**): *T*-test, statistical method used in (**B**): Mann–Whitney *U*-test, **P* < 0.05, ****P* < 0.001.
Abbreviation: ns, no statistical significance.

diseases.[29] The elevation of baseline Fbg levels in patients with ischemic stroke was closely related to secondary brain atrophy and white matter lesions, indicating its potentiality as a predictive indicator.[30] Previous literature has reported a strong association between LMR, Fbg and central nervous system inflammation respectively. Our statistical analysis also revealed significant differences in the expression levels of LMR and Fbg between different patient groups, suggesting their potential utility as biomarkers for identifying and grading CSVD. However, the results of the ROC curve analysis were not ideal. Future investigations are needed to assess the diagnostic efficacy of these two biomarkers through more refined subgroup analyses, such as categorization based on clinical symptoms or grouping based on imaging characteristics.

Neuroinflammation is considered to be a major pathological marker in neurodegenerative diseases characterized by the proliferation and activation of microglia, which are associated with the release of cytokines and the generation of chemokines.[31] The activation of microglia exhibits heterogeneity, and the relationship between the cytotoxic M1 phenotype and the neuroprotective M2 phenotype is constantly evolving. The inclination towards M1/M2 balance may depend on the stage and severity of the disease, representing a complex and intricate process.[14] In the context of CSVD, the disruption of the blood-brain barrier may lead to elevated serum levels of inflammatory factors, serving as peripheral indicators of an intracranial inflammatory response. Several studies have indicated the relationship between serum inflammatory factors levels and various clinical symptoms or underlying mechanisms.[32,33] Notably, circulating IL-6 levels have been linked to the pathological aging process, particularly in conditions associated with inflammation and fibrosis, making its monitoring crucial for the diagnosis and prognosis of diseases.[34] Moreover, IL-6 had a predictive effect on the recurrence of acute stroke patients.[35] The high expression of IL-6 was associated with poor prognosis in patients with low-grade gliomas and a prognostic indicator with predictive value for the survival status of such patients.[36] Our study found that the serum levels of IL-6 in the CSVD group was significantly higher than that in the healthy control group. Furthermore, a high imaging score in patients with CSVD indicated a relatively severe condition, whose IL-6 level were also higher, and the efficacy of IL-6 in assessing the severity of CSVD was obviously superior to other indicators. IL-6, as a non-specific systemic inflammatory response marker and a product of M1 microglia activation, further demonstrated the important role of inflammatory mechanisms in the occurrence and development of CSVD. IL-6 may play an important predictive role in the severity, outcome, and prognosis of the disease, which requires further research.

What's more, the disruption of the balance and transformation of the relationship between the M1 and M2 microglial phenotypes may exert an important role in regulating the onset and progression of central nervous system diseases.[37] The

balance between TNF-α/IL-10 in maternal prenatal serum was gender dependent and correlated with depression.[38] The ratio of pro-inflammatory to anti-inflammatory factors can serve as an indicator for diagnosing diseases, assessing disease severity, and predicting prognosis and treatment efficacy. Higher levels of IL-6/IL-10 ratio were associated with cognitive decline, impaired immediate memory, and visual spatial performance in elderly individuals.[39] Exploring the immune mechanisms underlying antibodies against glutamic acid decarboxylase (GADA) related autoimmune epilepsy, it was found that an increase in circulating IL-6 concentrations was correlated with an increase in GADA titers. However, there was no significant difference in IL-6/IL-10 levels among the three groups.[40] Our research findings indicated that the IL-6/IL-10 ratio demonstrated good diagnostic efficacy in CSVD. However, it had certain ability in judging the severity of the disease but was not as effective as IL-6. To some extent, the IL-6/IL-10 ratio reflects the imbalance between pro-inflammatory and anti-inflammatory responses in the pathogenesis of CSVD. The expression of immune regulatory molecules may result in persistent inflammation and disrupt the anti-inflammatory balance, potentially leading to adverse outcomes.

Conclusion

The equilibrium between M1/M2 phenotype is broken down in CSVD. Notably, the IL-6/IL-10 ratio has demonstrated its ability in diagnosing CSVD and may serve as useful prognostic indicators for CSVD. Further research is necessary to ascertain the applicability of this indicator for subsequent prognostic assessments and treatment monitoring.

Abbreviations

AUC, area under the curve; BMI, body mass index; CCA, common carotid artery; CI, confidence interval; CSVD, cerebral small vessel disease; CT, computed tomography; Fbg, fibrinogen; GADA, antibodies against glutamic acid decarboxylase; GRAN, neutrophilicgranulocyte; HC, healthy control; hs-CRP, hypersensitive C-reactive protein; IFN-γ, interferon gamma; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; IL-12P70, interleukin 12P70; IL-17, interleukin 17; LMR, lymphocyte-to-monocyte ratio; LYMPH, lymphocyte; MRI, magnetic resonance imaging; NLR, neutrophil-to-lymphocyte ratio; NMLR, (neutrophil + monocyte)-to-lymphocyte ratio; PBMC, peripheral blood mononuclear cell; PLR, platelet-to-lymphocyte ratio; PLT, platelet; qRT-PCR, Quantitative reverse-transcription polymerase chain reaction; ROC, receiver operating characteristics; SII, systemic immune inflammation index; TNF-α, tumor necrosis factor-α; WBC, white blood cell.

Ethics Approval and Consent to Publication

The studies involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration. This clinical work was approved by the Ethics Committee of Yancheng First People's Hospital (approval No: 2024-K(YJ)-043). Animal care and surgical procedures were executed stick to the Guide for the Care and Use of Laboratory Animals promulgated in 1996. Experimental schemes were approved by the Animal Experiment Ethics Committee of Jiangsu Vocational College of Medicine (approval No. XMLL-2024-911). Informed consent has been obtained from the research participants.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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