ORIGINAL RESEARCH

Correlations Between Serum CXCL9/12 and the Severity of Acute Ischemic Stroke, a Retrospective Observational Study

Miaomiao Han[1,2], Bo Ma[3], Ruifang She[4], Yan Xing[5], Xiaohong Li[1]

[1]Department of Neurology, Jinan Central Hospital, Shandong University, Jinan City, People's Republic of China; [2]Encephalopathy Department, Zibo Hospital of Integrated Traditional Chinese and Western Medicine, Zibo City, People's Republic of China; [3]Department of Neurology, The First Affiliated Hospital of Bengbu Medical College, Bengbu City, People's Republic of China; [4]Department of Neurology, Tai' an City Central Hospital, Tai' an City, People's Republic of China; [5]Zibo Center for Disease Control and Prevention, Zibo City, People's Republic of China

Correspondence: Xiaohong Li, Department of Neurology, Jinan Central Hospital, Shandong University, No. 105, Jiefang Road, Jinan City, Shandong Province, 250013, People's Republic of China, Tel + 86 13370582096, Email lxh5231@126.com

Purpose: This retrospective observational study was conducted to determine the correlations between serum CXCL9/12 and the severity of acute ischemic stroke (AIS).

Methods: Total 138 patients with AIS were enrolled in the study. These patients underwent Brain CT on admission and blood samples were collected. Serum CXCL9 and CXCL12 were detected by ELISA assay. The correlations of serum CXCL9/12 with AIS was analyzed based on Oxfordshire Community Stroke Project (OCSP) classification, Trial of Org 10,172 in acute stroke treatment (TOAST) classification, National Institutes of Health Stroke Score (NIHSS) score, infarct volume, and modified Rankin scale (mRS) score.

Results: Compared with the controls, patients with AIS had higher levels of serum CXCL9 and CXCL12. Logistic regression analysis determined that CXCL9 and CXCL12 were independent risk factors for AIS. In addition, the increased serum CXCL9 and CXCL12 were associated with TOAST classification, NIHSS score, and infarct volume. However, serum CXCL9 and CXCL12 were not associated with functional outcomes (mRS score). CXCL9 and CXCL12 both exhibited a high diagnostic value in AIS.

Conclusion: Serum CXCL9 and CXCL12 were elevated in patients with AIS, closely correlated with the severity of AIS.

Keywords: acute ischemic stroke, CXCL9, CXCL12, stroke severity, NIHSS score

Introduction

Stroke is the second leading cause of death worldwide and the first leading cause of long-term acquired disability, resulting in a socioeconomic burden.[1] The weighted prevalence of stroke is increased annually in China, accounting from 2.28% in 2013 to 2.58% in 2019.[2] Acute ischemic stroke (AIS) manifests as sudden neurological dysfunction caused by focal cerebral ischemia accompanied by acute infarction.[3] The thrombus extracted from patients with AIS may be from large arterial atherosclerosis (LAA), cardioembolism (CE), small vessel occlusion (SVO), other strokes of established etiology, or strokes of unknown etiology.[4–6] Early recognition and urgent intervention of AIS are essential to reduce mortality and morbidity.[1]

Inflammation plays an important role in the progression of atherosclerosis, coronary artery disease, acute coronary thrombosis, and occlusion.[7] Chemokines are inflammatory mediators with a variety of biological functions. The CXC chemokine ligand-9 (CXCL9) is a member of the CXC chemokine subfamily that involved in the pathogenesis of diverse inflammatory cardiovascular diseases. A higher expression of CXCL9 has been determined in patients with coronary artery disease,[8] myocardial infarction,[9] and pulmonary arterial hypertension.[10] Similarly, CXCL12 is another important factor in regulating the inflammatory response of cardiovascular diseases. Previous studies have determined that

increased level of CXCL12 is closely associated with atherosclerosis[11,12] and coronary artery disease,[7,13] considering a potential therapeutic target. It is noteworthy that CXCL9 and CXCL12 are also up-regulated in the serum of patients with AIS.[14,15] Amin et al have reported that the elevated CXCL9 may be a predisposing factor for stroke.[14] Chen et al have found that elevated serum CXC12 is associated with adverse functional outcomes and mortality in Chinese patients with AIS at 6-month follow-up.[16] Gu et al have revealed that serum CXCL12 is a potential predictor of stroke recurrence in patients with AIS.[15] However, the detail correlations of CXCL9/12 with the clinical characteristics of AIS are not fully revealed.

In this study, the roles of serum CXCL9/12 relating with the clinical manifestations of AIS were explored, referring to the parameters of Oxfordshire Community Stroke Project (OCSP) classification, Trial of Org 10,172 in acute stroke treatment (TOAST) classification, National Institutes of Health Stroke Scale (NIHSS) score, infarct volume, and modified Rankin scale scores (mRS). Our study is aimed to reveal the correlations of serum CXCL9/12 with the severity of AIS, providing guidance for clinical treatments.

Materials and Methods
General Information
From December 2018 to May 2019, 152 patients with AIS were collected from our hospital. The inclusion criteria included i) > 18 years old; ii) anterior circulation infarction confirmed by brain CT/MRI; iii) admission within 24 h of stroke onset. Patients met the following criteria were excluded: i) patients with severe heart, liver, and kidney insufficiency and other serious diseases (N = 1); ii) patients with acquired or hereditary bleeding constitution; iii) brain CT was not performed within 24 h of stroke onset (N = 1); iv) patients lost follow-up (N = 7) or died (N = 4). After screened by exclusion criteria, 138 patients with AIS (89 males and 49 females; 67.15 ± 10.57 years old) were finally enrolled. Forty normal individuals (24 males and 16 females; mean 63.87 ± 9.89 years) were enrolled as the controls (Figure S1). The clinical information of AIS patients and controls are shown in Table 1, including BMI, medical history, smoking and drinking, family history, and hypertension. This study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of our hospital. Written informed consent was obtained from all patients.

Blood Samples
Venous blood samples were collected from AIS patients and controls on admission before treatment. Blood samples were centrifuged immediately at 3000 rpm for 15 min to isolate the supernatant serum. The serum concentration of CXCL9 and CXCL12 were analyzed by Enzyme-linked immunosorbent assay (ELISA) kits (Eusebio, Shanghai, China) following the instructions of the manufacturer.

Table 1 Comparisons of Clinical Information Between Control and AIS Groups

	AIS (n = 138)	Control (n = 40)	χ^2/F	P value
Sex (Female/Male)	89/49	22/18	1.191	0.275
Age (years)	67.152 ± 10.573	63.875 ± 9.8922	0.351	0.554
BMI (kg/m²)	25.454 ± 3.9328	25.466 ± 2.7559	0.745	0.389
Smoking/ drinking (%)	59 (42.75%)	11 (27.50%)	3.024	0.082
Medical history (%)	118 (85.51%)	25 (62.5%)	10.392	0.001
Family history (%)	48 (34.78%)	5 (12.50%)	7.364	0.007
Hypertension (%)	95 (68.84%)	12 (30%)	15.010	0.000

Abbreviations: AIS, acute ischemic stroke; BMI, body mass index; F, Fisher's exact test; χ^2, Chi-squared test.

284
Neuropsychiatric Disease and Treatment 2023:19

Neuroimaging Collection

The volume of cerebral infarction in all patients was based on the results of brain magnetic resonance examination within 24 h of onset. According to the calculation formula of cerebral infarction volume proposed by Pullitono: cerebral infarction volume (cm) $3=\pi/6\times$length$\times$width$\times$layer. The cerebral infarcts were graded according to the size of the cerebral infarction as follows: small infarcts (< 5 cm^3), medium infarcts (5–15 cm^3), and large infarcts (> 15 cm^3).

Ocsp

OCSP is a clinical classification based on neurological deficits.[17] There are four types of clinical OCSP classification, including total anterior circulation infarcts (TACI), posterior circulation infarcts (POCI), partial anterior circulation infarcts (PACI), and lacunar circulation infarcts (LACI).

Toast

AIS patients underwent an etiological investigation. According to TOAST classification, AIS subtypes were classified into 5 categories based on etiology, large-artery atherosclerosis (LAA), small vessel occlusion (SVO), cardioembolism (CE), stroke of other determined etiology, and stroke of undetermined etiology.

Nihss

The neurological function of AIS patients was assessed using the NIHSS score by two neurologists with more than 3 years of work experience.[18] The total score is 0–42 points, of which less than 1 means normal or normal, 1–4 means mild neurological deficit, 5–15 means moderate neurological deficit, and greater than 15 means severe neurological deficit. The average of the two was taken as the final score, and the scores were all completed within 24 h of admission.

mRS Score

mRS ranges from 0 (no neurological symptoms) to 6 (death). mRS score between 0 and 2 for patients is considered to have a good functional outcome (neurological function recovery) and an mRS greater than 2 indicates a poor functional outcome.

Statistical Analysis

Statistical data were presented as the mean ± SD. Categorical variables were compared between groups with the Chi-squared test or Fisher's exact test when appropriate, and continuous variables with the Student's t-test. The difference between two groups was determined using logistic regression for binary events. Multiple group comparisons by one-way ANOVA followed by Tukey's post-hoc test using SPSS 26.0 software. $P < 0.05$ was considered significant.

Results

Comparisons of General Data Between AIS and Control

There were no significant differences in gender, age, BMI, and history of smoking and drinking. These data showed that patients with the medical history, family history, and hypertension were more likely to develop AIS (Table 1).

Comparisons of Blood Index Test results Between AIS and Control

Compared with control, patients with AIS had higher levels of serum CXCL9 and CXCL12 on admission (Table 2). AIS was positively correlated with the serum content of CXCL9, CXCL12, white blood cells (WBC), red blood cells (RBC), high hemoglobin (Hb), glucose (Glu), Creatinine (Cr), blood urea nitrogen (BUN), and D-dimer ($P < 0.05$). There was no significant difference in serum concentration of platelets (PLT), triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), apolipoprotein B (ApoA), apolipoprotein B (ApoB), lipoprotein a, glycosylated hemoglobin (GHb), uric acid (UA), homocysteine (HCY), and fibrinogen (FIB) (Table 2).

Table 2 Comparisons of Blood Parameters Between AIS and Control Groups

	AIS (n = 138)	Control (n = 40)	t/F/Z	*P* value
WBC (10⁹/L)	7.17 ± 1.96	5.59 ± 1.20	39.302	0.000
RBC (10¹²/L)	4.57 ± 0.55	4.30 ± 0.48	2.833[a]	0.005
Hb (g/L)	141.44 ± 16.60	134.38 ± 14.09	2.448[a]	0.015
PLT (10⁹/L)	225.28 ± 61.35	213.28 ± 45.67	1.318	0.252
TG (mmol/L)	1.52 ± 1.12	1.43 ± 0.86	1.191	0.662
TC (mmol/L)	4.41 ± 1.17	4.35 ± 1.02	0.079	0.778
LDL (mmol/L)	2.72 ± 0.89	2.54 ± 0.80	1.150[a]	0.252
HDL (mmol/L)	1.21 ± 0.34	1.22 ± 0.35	0.013	0.908
ApoA (g/L)	1.34 ± 0.30	1.35 ± 0.33	−0.157[a]	0.876
ApoB (g/L)	1.15 ± 0.30	1.07 ± 0.27	1.456[a]	0.147
Lipoprotein a (mmol/L)	161.90 (66.43–287.35)	131.90 (75.13–325.40)	−0.080[b]	0.936
Glu (mmol/L)	7.17 ± 2.48	5.92 ± 1.52	15.299	0.000
GHb (%)	6.44 ± 1.57	6.21 ± 1.27	0.722	0.397
UA (μmol/L)	297.98 ± 72.43	316.23 ± 97.76	1.665	0.199
HCY (μmol/L)	16.01 ± 10.68	14.15 ± 6.67	1.090	0.298
Cr (μmol/L)	64.16 ± 18.19	57.58 ± 12.17	7.114	0.009
BUN (mmol/L)	5.66 ± 1.77	4.93 ± 1.13	9.782	0.002
FIB (g/L)	3.14 ± 1.98	2.98 ± 1.05	0.247	0.620
D-dimer (mg/L)	0.39 (0.22–0.70)	0.23 (0.17–0.37)	−2.736[b]	0.006
CXCL9 (pg/mL)	679.3 ± 199.77	354.26 ± 276.14	68.313	0.000
CXCL12 (pg/mL)	6.96 ± 2.52	4.19 ± 1.53	73.180	0.000

Notes: [a]Student's *t*-test (t); [b]Z test; Others are Fisher's exact test (F).
Abbreviations: WBC, white blood cells; RBC, red blood cells; Hb, high hemoglobin; PLT, Platelets; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoA, apolipoprotein B; ApoB, apolipoprotein B; Glu, glucose; GHb, glycosylated hemoglobin; UA, uric acid; HCY, homocysteine; Cr, Creatinine; BUN, blood urea nitrogen; FIB, fibrinogen.

Binary Logistic Regression Analysis of Independent Influencing Factors of AIS

Taking the occurrence of AIS as the dependent variable, the variables with statistical difference between the two groups (previous medical history, family history, hypertension, WBC, RBC, Hb, Glu, Cr, BUN, D-dimer, CXCL9, and CXCL12 were subjected to binary logistic regression analysis. The results showed that medical history, hypertension, CXCL9, and CXCL12 were independent risk factors for AIS (Table 3).

Table 3 Binary Logistic Regression Analysis of Independent Influencing Factors of AIS

	B	*SE*	Wald Value	OR	*P* value	95% CI
Medical history	1.86	0.865	4.63	6.425	0.031	1.18–34.974
Family history	1.093	0.756	2.088	2.982	0.148	0.677–13.125
Hypertension	1.584	0.709	4.995	4.876	0.025	1.215–19.568
WBC	0.192	0.21	0.831	1.211	0.362	0.802–1.829
RBC	1.178	1.287	0.838	3.249	0.36	0.261–40.463
Hb	−0.021	0.042	0.246	0.98	0.62	0.903–1.063
Glu	0.074	0.188	0.155	1.077	0.694	0.745–1.557
Cr	0.047	0.029	2.744	1.048	0.098	0.991–1.109
BUN	0.425	0.272	2.446	1.529	0.118	0.898–2.605
D-dimer	0.63	0.475	1.754	1.877	0.185	0.739–4.766
CXCL9	0.006	0.002	11.232	1.006	0.001	1.002–1.009
CXCL12	0.694	0.24	8.358	2.002	0.004	1.25–3.204

Abbreviations: WBC, white blood cells; RBC, red blood cells; Hb, high hemoglobin; Glu, glucose; UA, uric acid; Cr, Creatinine; BUN, blood urea nitrogen; B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

Relationships Between Serum CXCL9/12 and OCSP Classification

We further compared the serum levels of CXCL9 and CXCL12 in AIS patients with different OCSP subtypes. Patients were clinically classified as TACI (11 patients), POCI (47 patients), PACI (73 patients), and LACI (7 patients). There was no significant difference in serum CXCL9 (F = 0.181) and CXCL12 (F = 0.041) between different subtypes of OCSP ($P > 0.05$) (Table 4).

Relationships Between Serum CXCL9/12 and TOAST Classification

As shown in Table 5, the etiological TOAST classification for 138 patients was as follows: LAA (63 patients), SVO (68 patients), and CE (7 patients). The serum CXCL9 in LAA subtype patients was significantly higher than that in SAO subtype patients ($P < 0.05$). Serum CXCL9 was statistically different between TOAST subtypes (F = 6.071, P = 0.003). In addition, Serum CXCL12 in LAA subtype patients was markedly higher compared with SAO patients ($P < 0.05$). There was a significant difference in serum levels of CXCL12 between different TOAST subtypes (F = 4.642, P = 0.011).

Relationships Between Serum CXCL9/12 and NIHSS Score

There were 85 patients presented with mild AIS, 47 patients presented with moderate, and 6 patients in the severe group. The difference of serum CXCL9 (F = 6.839, P = 0.014) and CXCL12 (F = 8.383, P = 0.000) between different NIHSS score groups was statistically significant. Serum CXCL9 and CXCL12 in the severe group were significantly higher compared with the mild and moderate groups ($P < 0.05$, Table 6).

Table 4 Relationships Between Serum CXCL9/12 and OCSP Classification (n=138)

	TACI	**POCI**	**PACI**	**LACI**	**F**	**P**
n	11	47	73	7		
CXCL9	686.65 ± 159.73	693.99 ± 236.75	671.83 ± 186.98	647.05 ± 125.69	0.181	0.909
CXCL12	6.99 ± 2.26	6.85 ± 2.20	7.01 ± 2.81	7.01 ± 2.24	0.041	0.989

Abbreviations: TACI, total anterior circulation infarcts; POCI, posterior circulation infarcts; PACI, partial anterior circulation infarcts; LACI, lacunar circulation infarcts; F, one-way ANOVA.

Table 5 Relationships Between Serum CXCL9/12 and TOAST Classification (n=138)

	LAA	**SVO**	**CE**	**F**	**P**
n	63	68	7		
CXCL9	723.08 ± 121.73	648.11 ± 152.22*	774.04 ± 167.93	6.071	0.003
CXCL12	7.71 ± 1.83	6.89 ± 1.34*	7.89 ± 2.13	4.642	0.011

Notes: *P < 0.05 vs LAA (Tukey's post-hoc test).
Abbreviations: LAA, large-artery atherosclerosis; SVO, small vessel occlusion; CE, cardioembolism; F, one-way ANOVA.

Table 6 Relationships Between Serum CXCL9/12 and NIHSS Score

	Mild (≤ 4)	**Moderate (5–14)**	**Severe (≥ 15)**	**F**	**P**
n	85	47	6		
CXCL9	643.94 ± 117.42*	677.20 ± 191.78*	1196.78 ± 425.31	6.839	0.014
CXCL12	6.81 ± 2.47*	6.72 ± 2.00*	10.87 ± 3.96	8.383	0.000

Notes: *P < 0.05 vs Severe (Tukey's post-hoc test).
Abbreviation: F, one-way ANOVA.

Associations Between Infarct Volume and Serum CXCL9/12

There were 111 patients in the small infarction group, 21 patients in the moderate infarction group, and 6 patients in the large infarction group (Table 7). The difference in serum CXCL9 between different MRI infarct volume groups was statistically significant (F = 7.833, P = 0.003). The serum CXCL9 and CXCL12 in the large infarction group was significantly increased compared with the small infarction and moderate infarction groups ($P < 0.05$). Serum CXCL12 was significantly different between MRI infarcts of different volumes (F = 11.081, P = 0.002). Results showed that infarct volume was associated with increasing CXCL9 and CXCL12 levels.

Associations Between Serum CXCL9/12 and mRS Score

After 3 months, 83 patients showed a good functional outcome (mRS score ≤ 2) and 48 patients had a poor functional outcome (mRS score > 2). There no significant differences in serum CXCL9 (t = 0.133, P = 0.894) and CXCL12 (t = −0.061, P = 0.952) were revealed between patients with good and poor functional outcomes. Differently, the serum IL-6 (t = −2.458, P = 0.015) and TNF-α (t = −2.77, P = 0.006) were significantly higher in patients with poor functional outcome than those with good functional outcome (Table 8).

ROC Curve Analysis of CXCL9/12

The ROC curve based on CXCL9 and CXCL12 is shown in Figure 1 and Table 9. For CXCL9, the optimal cutoff value was 470.335 ng/mL, which had a sensitivity of 0.949 and specificity of 0.850. The AUC (area under the curve) for CXCL9 was 87.1%. For CXCL12, ROC curve analysis showed that the area under the AUC curve of CXCL9 was 89.3%, and the optimal cutoff value was 4.5775 ng/mL, which had a sensitivity of 0.899 and specificity of 0.800. The results suggested that CXCL9 and CXCL12 have a high diagnostic value in AIS.

Discussion

A stroke is a neurological emergency that causes damage to the brain's blood supply mechanisms, further leading to ischemia and death of brain cells.[19] Studies have reported that patients with AIS exhibit several severe deficits, such as impairments in memory, thinking, language, and movement.[14] In China, the prognosis of stroke patients is improved in

Table 7 Relationships Between Serum CXCL9/12 and Infarct Volume (n = 138)

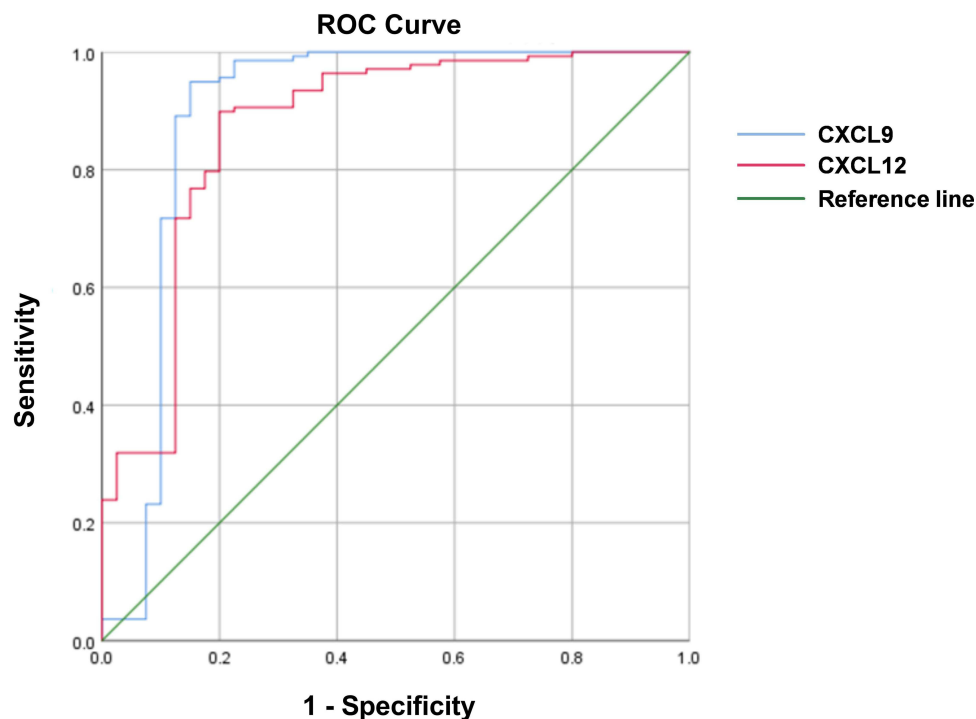|	Small Infarction (<5)	Moderate Infarction (5–15)	Large Infarction (> 15)	F	P
n CXCL9 CXCL12	111 634.49 ± 118.45* 6.38 ± 1.57*	21 749.74 ± 201.75* 8.16 ± 3.17*	6 1261.83 ± 386.51 13.37 ± 4.19	 7.833 11.081	 0.003 0.002

Notes: *$P < 0.05$ vs large infarction (Tukey's post-hoc test).
Abbreviation: F, one-way ANOVA.

Table 8 Associations Between Serum CXCL9/CXCL12/IL-6/TNF-α and mRS Score (n = 138)

	Good Functional Outcome (≤ 2)	Poor Functional Outcome (> 2)	t	P
n CXCL9 CXCL12 IL-6 TNF-α	83 681.16 ± 204.63 6.95 ± 2.55 17.93 ± 5.98 29.53 ± 8.07	55 676.50 ± 194.03 6.97 ± 2.50 20.58 ± 6.52 33.10± 6.19	 0.133 −0.061 −2.458 −2.77	 0.894 0.952 0.015 0.006

Abbreviation: t, Student's t-test.

Figure 1 ROC curve analysis of CXCL9/12 for AIS.

recent years, presenting an in-hospital death rate of 1.9% for inpatients, a 12-month fatality rate of 8.6% for discharged patients, and a 12-month disability rate of 16.6% and a recurrence rate of 5.7% for survivors.[20] However, the burden of stroke continues to grow in China with the deepening of aging and increased risk factors. It is known that AIS is associated with a variety of risk factors, including age, heart disease, vascular disease, and coagulation disorders.[7,21] Therefore, it is crucial to develop effective diagnostic and prognostic methods to improve AIS. In this study, our findings suggested that CXCL9 and CXCL12 levels were significantly increased following AIS, which were independent risk factors for AIS and also closely related to the severity of AIS.

We found serum levels of CXCL9 and CXCL12 were up-regulated in AIS patients compared with control. In addition, past medical history, hypertension, CXCL9, and CXCL12 were independent risk factors for AIS. Inflammation is a key progression that begins after ischemia and stroke, and chemokines function as important cytokines in inflammation.[22] Studies have reported that high levels of chemokines are associated with stroke severity and prognosis.[23] Chemokines and their receptors ensure normal brain function by driving crosstalk between neurons, glial cells, and peripheral immune cells.[24] At present, some studies have focused on the correlation of CXCL9 and CXCL12 with cerebrovascular diseases, such as stroke. For example, several studies have shown that a lack of CX3CL1/CX3CR1 reduces pro-inflammatory cytokines release, neuronal apoptosis, and infarct size after cerebral ischemia in mice with ischemic stroke.[25,26]

Chronic subdural hematoma patients have a significantly higher concentration in hematoma fluid for CXCL9 and CXCL10 compared to venous blood, which is associated with inflammatory response and risk of recurrence.[27] CXCL9-CXCR3 is

Table 9 ROC Curve Analysis of Serum CXCL9/12 for AIS

	Optimal Cutoff Value	Sensitivity	Specificity	Area Under the Curve (95% CI)	P
CXCL9	470.335	0.949	0.850	89.3% (0.808–0.979)	< 0.0010
CXCL12	4.5775	0.899	0.800	87.1% (0.798–0.944)	< 0.0010

associated with B-cell trafficking in giant cell arteritis and polymyalgia rheumatica.[28] An elevated level of serum CXCL9 is associated with unstable asymptomatic carotid plaques, which may contribute to clinically determining which patients are more likely to develop cerebrovascular events.[29] Moreover, a study has reported that CXCL9 mRNA levels are elevated at 7 days after ischemic injury in mice with permanent middle cerebral artery occlusion.[30] In this study, we found that higher CXCL9 and CXCL12 levels were correlated with NIHSS score and infarct volume. Analogously, the CXCL12 pathway excessive activation after stroke contributes to depression of neurologic function, while inhibition of CXCL12 attenuates the immune response after stroke, thereby improving functional recovery after stroke.[31] Studies have reported that elevated serum CXCL12 levels on admission are strongly associated with future recurrence of ischemic stroke of AIS patients in China.[15] Serum CXCL12 is positively correlated with stroke severity and infarct volume.[32] Duan et al have indicated that serum CXCL12 level is up-regulated at admission, and ROC curve showed the optimal cutoff value of serum CXCL12 levels as an indicator for auxiliary diagnosis of AIS was projected to be 3.5 ng/mL, which yielded a specificity of 73.5% and a sensitivity of 88.1%, with the area under the curve at 0.907.[5] Consistent with previous findings, we found that CXCL12 and CXCL9 levels may be used as the indicators for auxiliary diagnosis with the area under the curve at 87.1% (95% CI 0.798–0.944) and 89.3% (95% CI 0.808–0.979), respectively. In addition, severity and infarct volume were positively correlated with serum CXCL12. Severe AIS patients have higher serum CXCL9 and CXCL12. Taken together, these results suggested that CXCL9 and CXCL12 may be independent diagnostic and prognostic markers in patients with AIS.

However, this study still has some limitations. First, the dynamic changes of CXCL9/12 over the course of follow-up are not detected. Second, whether the effectual treatment can reduce serum CXCL9/12 is still questionable. Third, the retrospective nature and relatively small number of participants are also great limitations. Prospective researches based on a large population are still needed in future.

Conclusions

In this study, we enrolled 138 patients admitted within 24 h after the onset of stroke and collected their blood samples immediately after admission. We found high levels of serum CXCL9 and CXCL12 in patients with AIS. In addition, CXCL9 and CXCL12 levels were positively correlated with TOAST classification, NIHSS score, and infarction volume. Our findings indicate that serum CXCL9 and CXCL12 are positively associated with the severity of AIS, which may serve as promising biomarkers. The early prediction of AIS severity is of great significance in guiding active treatments, achieving better therapeutic outcomes.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study adhered to the tenets of the Declaration of Helsinki and was approved by the ethics committee of Jinan Central Hospital, Shandong University.(No. JNHB-097F) Written informed consent was obtained from all patients.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Herpich F, Rincon F. Management of acute ischemic stroke. *Crit Care Med*. 2020;48:1654–1663. doi:10.1097/CCM.0000000000004597
2. Tu W-J, Hua Y, Yan F, et al. Prevalence of stroke in China, 2013–2019: a population-based study. *Lancet Reg Health West Pac*. 2022;28:100550. doi:10.1016/j.lanwpc.2022.100550
3. Mendelson SJ, Prabhakaran S. Diagnosis and management of transient ischemic attack and acute ischemic stroke: a review. *JAMA*. 2021;325:1088–1098. doi:10.1001/jama.2020.26867
4. Jolugbo P, Ariëns RAS. Thrombus composition and efficacy of thrombolysis and thrombectomy in acute ischemic stroke. *Stroke*. 2021;52:1131–1142. doi:10.1161/STROKEAHA.120.032810
5. Duan XX, Zhang GP, Wang XB, et al. The diagnostic and prognostic value of serum CXCL12 levels in patients with ischemic stroke. *Neurol Sci*. 2015;36:2227–2234. doi:10.1007/s10072-015-2341-8
6. Chung JW, Park SH, Kim N, et al. Trial of ORG 10172 in acute stroke treatment (TOAST) classification and vascular territory of ischemic stroke lesions diagnosed by diffusion-weighted imaging. *J Am Heart Assoc*. 2014;3. doi:10.1161/JAHA.114.001119
7. Tavakolian Ferdousie V, Mohammadi M, Hassanshahi G, et al. Serum CXCL10 and CXCL12 chemokine levels are associated with the severity of coronary artery disease and coronary artery occlusion. *Int J Cardiol*. 2017;233:23–28. doi:10.1016/j.ijcard.2017.02.011
8. de Oliveira RT, Mamoni RL, Souza JR, et al. Differential expression of cytokines, chemokines and chemokine receptors in patients with coronary artery disease. *Int J Cardiol*. 2009;136:17–26. doi:10.1016/j.ijcard.2008.04.009
9. Lin CF, Su CJ, Liu JH, et al. Potential effects of CXCL9 and CCL20 on cardiac fibrosis in patients with myocardial infarction and isoproterenol-treated rats. *J Clin Med*. 2019;8. doi:10.3390/jcm8050659
10. Dong H, Li X, Cai M, et al. Integrated bioinformatic analysis reveals the underlying molecular mechanism of and potential drugs for pulmonary arterial hypertension. *Aging*. 2021;13:14234–14257. doi:10.18632/aging.203040
11. Murad HAS, Rafeeq MM, Alqurashi TMA. Role and implications of the CXCL12/CXCR4/CXCR7 axis in atherosclerosis: still a debate. *Ann Med*. 2021;53:1598–1612. doi:10.1080/07853890.2021.1974084
12. Gao JH, Yu XH, Tang CK. CXC chemokine ligand 12 (CXCL12) in atherosclerosis: an underlying therapeutic target. *Clin Chim Acta*. 2019;495:538–544. doi:10.1016/j.cca.2019.05.022
13. Farouk SS, Rader DJ, Reilly MP, et al. CXCL12: a new player in coronary disease identified through human genetics. *Trends Cardiovasc Med*. 2010;20:204–209. doi:10.1016/j.tcm.2011.08.002
14. Amin M, Vakilian A, Mahmoodi MH, et al. Circulatory levels of C-X-C motif chemokine ligands 1, 9, and 10 are elevated in patients with ischemic stroke. *Eurasian J Med*. 2017;49:92–96. doi:10.5152/eurasianjmed.2017.17022
15. Gu XL, Liu L, Lu XD, et al. Serum CXCL12 levels as a novel predictor of future stroke recurrence in patients with acute ischemic stroke. *Mol Neurobiol*. 2016;53:2807–2814. doi:10.1007/s12035-015-9151-0
16. Cheng X, Lian YJ, Ma YQ, et al. Elevated serum levels of CXC chemokine ligand-12 are associated with unfavorable functional outcome and mortality at 6-month follow-up in Chinese patients with acute ischemic stroke. *Mol Neurobiol*. 2017;54:895–903. doi:10.1007/s12035-015-9645-9
17. de Andrade JBC, Mohr JP, Timbó FB, et al. Oxfordshire community stroke project classification: a proposed automated algorithm. *Eur Stroke J*. 2021;6:160–167. doi:10.1177/23969873211012136
18. Wang Y, Pan Y, Li H, et al. Efficacy and safety of ticagrelor and aspirin in patients with moderate ischemic stroke: an exploratory analysis of the THALES randomized clinical trial. *JAMA Neurol*. 2021;78:1091–1098. doi:10.1001/jamaneurol.2021.2440
19. Huang JB, Chen NC, Chen CL, et al. Serum levels of soluble triggering receptor expressed on myeloid cells-1 associated with the severity and outcome of acute ischemic stroke. *J Clin Med*. 2020;10:61. doi:10.3390/jcm10010061
20. Tu WJ, Chao BH, Ma L, et al. Case-fatality, disability and recurrence rates after first-ever stroke: a study from bigdata observatory platform for stroke of China. *Brain Res Bull*. 2021;175:130–135. doi:10.1016/j.brainresbull.2021.07.020
21. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. *J Transl Med*. 2009;7:97. doi:10.1186/1479-5876-7-97
22. Chen C, Chu SF, Liu DD, et al. Chemokines play complex roles in cerebral ischemia. *Neurochem Int*. 2018;112:146–158. doi:10.1016/j.neuint.2017.06.008
23. Li X, Lin S, Chen X, et al. The prognostic value of serum cytokines in patients with acute ischemic stroke. *Aging Dis*. 2019;10:544–556. doi:10.14336/AD.2018.0820
24. Le Thuc O, Blondeau N, Nahon JL, et al. The complex contribution of chemokines to neuroinflammation: switching from beneficial to detrimental effects. *Ann N Y Acad Sci*. 2015;1351:127–140. doi:10.1111/nyas.12855
25. Cipriani R, Villa P, Chece G, et al. CX3CL1 is neuroprotective in permanent focal cerebral ischemia in rodents. *J Neurosci*. 2011;31:16327–16335. doi:10.1523/JNEUROSCI.3611-11.2011
26. Tang Z, Gan Y, Liu Q, et al. CX3CR1 deficiency suppresses activation and neurotoxicity of microglia/macrophage in experimental ischemic stroke. *J Neuroinflammation*. 2014;11:26. doi:10.1186/1742-2094-11-26
27. Pripp AH, Stanišić M. The correlation between pro- and anti-inflammatory cytokines in chronic subdural hematoma patients assessed with factor analysis. *PLoS One*. 2014;9:e90149. doi:10.1371/journal.pone.0090149
28. Graver JC, Abdulahad W, van der Geest KSM, et al. Association of the CXCL9-CXCR3 and CXCL13-CXCR5 axes with B-cell trafficking in giant cell arteritis and polymyalgia rheumatica. *J Autoimmun*. 2021;123:102684. doi:10.1016/j.jaut.2021.102684
29. Schiro A, Wilkinson FL, Weston R, et al. Elevated levels of endothelial-derived microparticles, and serum CXCL9 and SCGF-β are associated with unstable asymptomatic carotid plaques. *Sci Rep*. 2015;5:16658. doi:10.1038/srep16658
30. Perego C, Fumagalli S, Miteva K, et al. Combined genetic deletion of IL (Interleukin)-4, IL-5, IL-9, and IL-13 does not affect ischemic brain injury in mice. *Stroke*. 2019;50:2207–2215. doi:10.1161/STROKEAHA.119.025196
31. Ruscher K, Kuric E, Liu Y, et al. Inhibition of CXCL12 signaling attenuates the postischemic immune response and improves functional recovery after stroke. *J Cereb Blood Flow Metab*. 2013;33:1225–1234. doi:10.1038/jcbfm.2013.71
32. Liu P, Xiang JW, Jin SX. Serum CXCL12 levels are associated with stroke severity and lesion volumes in stroke patients. *Neurol Res*. 2015;37:853–858. doi:10.1179/1743132815Y.0000000063