The incremental prognostic value of sIL-2R and HGF in acute ischemic stroke

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Abstract

Background: Inflammation affect long-term neurological outcome after acute ischemic stroke (AIS). Comprehensive and insightful understanding of correlation of inflammatory mediators and stroke outcome may offer new biomarkers or therapeutic approaches for AIS.

Methods: We collected plasma from in 204 AIS patients and 76 healthy controls, and ten cytokines (HGF, IL-1 β , IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3 α , CD40L and MMP1) screened out by Immune Monitoring 65-Plex Human ProcartaPlex Panel were measured. Functional outcome 3 months after stroke was assessed using the modified Rankin Scale. To assess the prognostic ability of inflammatory mediators, we applied multivariate logistic regression and construction of multimarker score.

Results: HGF, IL-10, IL-1 β , MIP-3 α , IL-2, sIL-2R, and IL-5 were significantly upregulated in AIS patients compared to control. After multivariable adjustment, sIL-2R (OR, 1.138; 95% CI, 1.028-1.259; $P=0.012$) and HGF (OR, 1.121; 95% CI, 1.030-1.218; $P=0.008$) remained individually associated with unfavorable outcomes at 3 months ($p < 0.05$). Furthermore, adding sIL-2R and HGF to the conventional model significantly improved risk reclassification for unfavorable outcomes (continuous net reclassification improvement 32.18%, $p < 0.001$; integrated discrimination improvement 10.21%, $p < 0.001$).

Conclusions: Higher plasma sIL-2R was a new independent predictor of unfavorable outcomes in AIS, and incorporation of sIL-2R and HGF into the conventional model significantly improved risk stratification for unfavorable outcomes.

Background

Stroke is the third most common cause of disability, and there are almost 25.7 million stroke survivors (71% ischemic stroke) globally[1]. Moreover, approximately 50% of patients with stroke will suffer a recurrence within the first year after the index stroke[2]. Given the substantial costs to the community and the increasing burden of stroke, it is important to understand the mechanisms that may impact stroke outcome to achieve the best possible prognosis. However, the established risk factors cannot satisfactorily predict stroke outcome. Thus, the use of one or multiple specific biomarkers to differentiate patients who should receive aggressive treatment and intense secondary prevention seems necessary.

Convincing evidence has implicated global brain inflammation as possibly critical factors affecting the evolution of pathology after a stroke and shaping stroke patients' long-term neurological outcomes[3-6]. Exploring the inflammatory mechanisms of ischemic stroke will undoubtedly promote rehabilitation, as illustrated by the observation that pretreatment with angiotensin-converting enzyme inhibitors is a predictor of good outcomes, owing to the proinflammatory effects of angiotensin II[7]. A number of studies have investigated relationships between inflammation biomarkers, such as C-reactive protein, IL-6, TNF receptor and IL-10, and stroke outcome, and they were reported to be associated with adverse or good outcomes in AIS patients[8-10]. Additionally, our team reported a Th1 to Th2 lymphocyte shift in the acute phase of AIS patients and conspicuous Th1 to Th2-related cytokine alterations in the plasma of

middle cerebral artery occlusion mice[11]. It was recently reported that the specific ex vivo released cytokine profile is associated with ischemic stroke outcome and improves its prediction, indicating the utility of ex vivo synthesized cytokines for predicting stroke outcome[12]. Except for cytokines, lymphocytes produce increased amounts of acetylcholine in AIS patients, which might contribute to fatal post-stroke infection and mortality[13]. It was also suggested that reduced serum levels of irisin were powerful biological markers of risk of developing post-stroke depression[14], and serum dickkopf-3 is associated with death and vascular events after ischemic stroke[15]. However, to discover the role of new inflammatory factors in the prognosis of ischemic stroke, especially the combined prognostic significance of multiple inflammatory biomarkers on stroke outcome are needed [16].

We studied a broad panel of 65 circulating inflammatory biomarkers, including T and B lymphocyte-related cytokines, immune cell infiltration-related chemokines, blood brain barrier breakdown-related factors, apoptosis- and neuronal survival-related cytokines, and angiogenesis- and neurogenesis-related factors in AIS patients to enhance our understanding of the relationship between the involved inflammatory cytokines and adverse outcomes at 3 months after stroke events. We hypothesized that the simultaneous employment of multiple inflammatory cytokines may improve the prediction of unfavorable outcomes and improve risk stratification beyond pre-existing risk factors in AIS patients.

Methods

Study Participants

The data that support the findings of this study are available from the corresponding author on reasonable request. We retrospectively screened a consecutive range of patients who were diagnosed with AIS and presented within 24 hours after symptom onset at Xuanwu Hospital of Capital Medical University between November 2018 and May 2019. Our study was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University. All patients or their immediate family members provided written informed consent. Inclusion criteria included (1) presented with focal or global neurological deficits, (2) brain magnetic resonance imaging (MRI) or computed tomography (CT) confirmed the diagnosis of AIS, (3) premorbidity modified Rankin Scale (mRS) ≤ 1 , and (4) a clinical evaluation at 3 months poststroke was performed and recorded. Patients with cerebral hemorrhage within the most recent three months, cancer, rheumatic heart disease, heart failure, renal failure, liver cirrhosis, immune diseases, active infection, epilepsy and other neurological diseases were excluded from our study. Gender- and age-matched hemorrhage patients were also recruited as positive controls. These patients presented within 24 hours after symptom onset, accompanied with focal or global neurological deficits, and brain CT verified parenchymatous hematoma. The exclusion criteria were similar to those for AIS.

Clinical data and blood collection

Baseline data, including demographic characteristics, onset time, blood pressure, comorbidities, routine laboratory determinations (leukocyte counts, plasma glucose levels, blood lipids, etc.) at admission were collected from all study participants. The severity of stroke was evaluated by trained neurologists using

the NIH Stroke Scale (NIHSS) at admission[17]. Blood samples were drawn from each patient before any treatment into 4-ml K3EDTA tubes. All plasma samples were separated and frozen at -80°C prior to the test. Healthy control subjects' plasma was obtained as described above.

Circulating biomarker measurements

The abnormally expressed ten cytokines in AIS patients were confirmed in 76 healthy controls and 204 AIS patients using a customized commercially available ELISA kit (PROCARTAPLEX 10 PLEX, Invitrogen, PPX-10). Experienced laboratory technicians who performed the assays were blinded to the experimental groups, baseline characteristics and clinical outcomes of the study patients.

Outcome assessment

Outcome 90 days after stroke was represented as the mRS score. Primary outcome was an unfavorable outcome at 90 days after stroke, and mRS score ≥ 2 points indicated an unfavorable outcome. The follow-up was evaluated by trained neurologists unaware of treatment assignment.

Statistical analysis

The results of continuous data are expressed as the means $\pm$ SDs or medians (quartiles), and categorical data are expressed as percentages. Comparisons between continuous variables were carried out using Student's t test if the variables were normally distributed, or else using the Mann-Whitney U test. Comparisons between categorical variables were implemented using the χ^2 test. First, the Mann-Whitney U test was employed to identify the aberrantly expressed cytokines in AIS patients. Then, in a verified patient cohort, the correlations between those cytokines and the study outcomes were examined using univariate and multivariable regression analyses. Crude and adjusted odds ratios (ORs) of each biomarker, along with the corresponding 95% confidence intervals (CIs) were reported. There were several potential variables in the multivariable analysis, including age, admission NIHSS score, history of diabetes mellitus, coronary heart disease and atrial fibrillation, recombinant tissue-plasminogen activator (rt-PA) treatment, leukocyte count, glucose levels, total cholesterol, low-density lipoprotein, HGF, IL-1 β , IL-16, IL-2, sIL-2R and IL-5 on admission. Furthermore, receiver operating characteristic (ROC) curves were applied to calculate optimal cutoff points of HGF and sIL-2R in predicting unfavorable outcomes in AIS patients. Next, we calculated the net reclassification index (NRI) and integrated discrimination improvement (IDI)[18] to evaluate the reclassification value through adding one or more of these inflammatory biomarkers to the conventional model with existing risk factors.

All statistical analysis was conducted using SPSS 21.0 software (IBM Corp., Armonk, NY, USA) and R software (version 3.5.1). A p value ≤ 0.05 was considered significant.

Results

Differentially expressed inflammatory cytokines in AIS patients

By immune monitoring 65-Plex human procartaPlex panel, ten significantly changed cytokines (HGF, IL-1 β , IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3 α , CD40L and MMP1) were screened out (Supplementary Figure 1). Then we collected plasma from in 204 acute ischemic stroke (AIS) patients and 76 healthy controls, and the above cytokines were measured. We found that HGF, IL-1 β , IL-2, sIL-2R, IL-5, IL-10, and MIP-3 α were significantly upregulated in AIS patients (Table 1). We further calculated AUCs using ROC curves, and the seven plasma cytokines demonstrated superior discrimination capacities between AIS patients and healthy controls (Figure 1).

Comparisons of baseline characteristics and cytokine levels between the group with good and poor outcome

The patient's characteristics and the ten aberrantly expressed cytokines, stratified by functional outcome according to the mRS score at 3 months (Table 2). Patients with unfavorable outcomes tended to be older, had higher baseline NIHSS scores, less likelihood of receiving rt-PA treatment, higher odds of atrial fibrillation history, higher baseline white blood cell counts and baseline glucose levels, and lower triglyceride levels and total cholesterol levels. Notably, we observed significant increases in HGF (138.64 versus 89.09, $p < 0.000$), IL-16 (87.03 versus 56.09, $p < 0.000$) and sIL-2R levels (1346.94 versus 812.92, $p < 0.000$), and significant reductions in IL-1 β (4.83 versus 6.34, $p = 0.024$) and IL-2 levels (23.15 versus 29.74, $p = 0.013$) in patients with unfavorable outcomes compared with patients with favorable outcomes (Table 2, Figure 2).

Higher plasma sIL-2R and HGF are independent predictors of unfavorable outcomes in AIS

In the univariate analysis, HGF, sIL-2R, IL-16, IL-2, and IL-1 β were all associated with unfavorable outcomes at 3 months after AIS ($p < 0.05$ for all, Table 3). After adjusting for gender, age, baseline NIHSS score and other clinical variables possibly associated with unfavorable outcomes in binominal multivariate analysis (model 2), only plasma HGF and sIL-2R remained significant for prediction of an unfavorable outcome 3 months after AIS ($p < 0.05$ for both). The multivariable adjusted OR (95% CIs) of each 10 pg/ml higher of HGF was 1.121 (1.030-1.218), and the multivariable adjusted OR (95% CIs) of each 100 pg/ml higher of IL-2R was 1.138 (1.028-1.259) (Table 3).

Next, we conducted ROC curve analysis to obtain the optimal cut-off points for HGF and sIL-2R. The optimal cut-off value of HGF was 117.915 pg/ml, with a sensitivity of 65.8% and a specificity of 83.2%, and the AUC was 0.786 (95% CI 0.719–0.854). The optimal cut-off value of sIL-2R was 971.44 pg/ml, with a sensitivity of 75.3% and a specificity of 67.2%, and the AUC was 0.768 (95% CI 0.702–0.835). We also found that HGF levels ≥ 117.915 pg/ml and sIL-2R ≥ 971.44 pg/ml were both associated with an increased risk of the primary outcome 90 days after AIS (Table 3).

Incremental predictive value of plasma sIL-2R and HGF for the prognosis of AIS

To construct multimarker score, we used two cytokines that remained significantly associated with poor outcome in the final backward elimination model: HGF and sIL-2R. We examined whether adding plasma

HGF or sIL-2R to a conventional model could improve the predictive value for the prognosis of AIS. Individually, adding each of them to the conventional model consisting of risk factors in model 2 significantly improved the risk reclassification for unfavorable outcomes ($p < 0.05$ for both NRI and IDI) (Table 4). Furthermore, adding plasma HGF and sIL-2R at the same time to the conventional model offered the greatest incremental predictive capacity for the primary outcome (continuous NRI 32.18%, $p < 0.001$; IDI 10.21%, $p < 0.001$).

Discussion

In the present study, we first investigated 65 circulating inflammatory biomarkers simultaneously in AIS patients and their relationship with long-term neurological outcomes. We found that plasma HGF and sIL-2R were each independently associated with increased risk of unfavorable outcomes at 3 months after AIS in our study. Furthermore, adding plasma HGF or sIL-2R to traditional risk factors could improve the risk stratification for unfavorable outcomes. And adding HGF and sIL-2R simultaneously to the basic model offered the greatest incremental predictive capacity for the primary outcome. These findings indicated that increased plasma HGF and sIL-2R were associated with unfavorable prognosis of AIS and might be potential prognostic biomarkers for AIS.

Inflammation is involved in the pathological process of ischemic stroke. Few studies have globally investigated the alterations of inflammatory cytokines in AIS patients and healthy controls. We first screened 65 inflammatory cytokines in our patient cohort by Immune Monitoring 65-Plex Human ProcartaPlex Panel. Ten cytokines (HGF, IL-1 β , IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3 α , CD40L and MMP1) were screened to change significantly (Supplementary Figure 1). Then we collected plasma from in 204 acute ischemic stroke (AIS) patients and 76 healthy controls, and found that seven cytokines including HGF, IL-1 β , IL-2, sIL-2R, IL-5, IL-10, and MIP-3 α indeed upregulated and had diagnostic capacity in AIS patients. Therefore, our first contribution was to identify two new biomarkers sIL-2R and MIP-3 α , which can provide references to other studies and help target particular pathways to prevent the progression of ischemic stroke.

Then, in the univariate analyses, higher plasma HGF, sIL-2R and IL-16 levels were found to be associated with increased risk of unfavorable outcomes, while higher IL-2, and IL-1 β levels were associated with decreased risk of unfavorable outcomes. Next, after adjusting for potential risk factors in binominal multivariate analysis, HGF and sIL-2R remained associated with increased risk of unfavorable outcomes, suggesting that plasma HGF and sIL-2R at baseline may be potential predictive biomarkers for prognosis of AIS. Furthermore, the addition of plasma HGF, sIL-2R or both to conventional risk factors was shown to improve risk predictions for the primary outcome. Therefore, this is the first research showed that plasma sIL-2R might be useful in risk stratification in AIS prognosis and could be beneficial for the selection of high-risk patients who should receive aggressive monitoring and therapeutic interventions in future clinical practice.

In recent years, serum HGF has emerged as a novel biomarker for cardiovascular and cerebrovascular diseases. Bielinski et al[19] reported that HGF is a biomarker of atherosclerotic disease and is associated with subclinical and incident coronary heart disease. Moreover, Susen et al[20] found that high serum HGF is an independent predictor of a composite of death and myocardial infarction. In terms of stroke, HGF was proved to be positively associated with the incidence of stroke[21, 22], and Zhu et al[23] reported that higher HGF was associated with mortality but not disability at 3 months after ischemic stroke onset. However, Zhu et al included patients within 48 hours of symptom onset and excluded patients treated with rt-PA. By comparison, patients' blood in our study was collected within 24 hours before they received any treatment. Thus, the level of HGF in our study could reflect more about the authentic pathological change of AIS without the influence of other factors. Next, we included patients who received rt-PA therapy. rt-PA therapy is a widely accepted treatment strategy for AIS patients within 4.5 hours after symptom onset and can reduce patients' disability to a large extent[24]. Hence, most patients will accept rt-PA treatment if their onset time is less than 4.5 hours, and including patients with rt-PA therapy could better fit real clinical settings. Moreover, Zhu's study only ruled out AIS patients in deep coma, without considering patients whose premorbid mRS ≥ 2 . However, for patients with a severe disability, they had a higher probability of having a poor prognosis, which may result in statistical bias. Therefore, we only included patients whose premorbid mRS ≤ 1 minimize this bias. The mechanism of HGF was clarified in the basic research. HGF is a pleiotropic cytokine that can regulate different cellular functions in developmental and pathological situations. According to prior studies, HGF can enhance the proliferation of neural precursor cells and increase neuronal differentiation, thus protecting against ischemic stroke[25]. The other underlying mechanisms might include promoting the migration of immune cells and secretion of pro-inflammatory chemokines[26] and accelerating the progression of atherosclerotic lesions[27], thus increasing the risk of unfavorable prognosis.

Moreover, we first reported that sIL-2R was independently associated with increased risk of unfavorable outcomes in AIS. It can be seen in our data that sIL-2R is a high abundance protein in plasma. Therefore, it is easy to detect and suitable as a molecular marker. sIL-2R, a membrane receptor for IL-2, is expressed on the surface of activated T-cells and is shed into the circulation in a soluble form as sIL-2R. Previous research indicated that elevated serum levels of sIL-2R were associated with a poor prognosis in autoimmune diseases, such as multiple sclerosis and follicular lymphoma[28]. Peter et al reported that sIL-2R was positively associated with internal carotid wall thickness, cardiovascular disease mortality, incident cardiovascular disease and stroke[29]. In addition, sIL-2R was significantly higher in ischemic left ventricular dysfunction patients[30] and was associated with a worse prognosis for dilated cardiomyopathy patients[31]. Similarly, we first found that sIL-2R was positively associated with poor functional outcomes in AIS patients. We speculate that abnormal expression of sIL-2R is associated with the aberrant activation of T cells and promotion of neuroinflammation after ischemic stroke[32]. Importantly, according to our supplementary data (Tables 1 and 2), the number of neutrophils in the HGF high expression group was higher than that in the low HGF expression group, while the number of lymphocytes in the high sIL-2R expression group was lower than that in the low sIL-2R expression group. And previous studies suggested that high levels of neutrophils and low levels of lymphocytes were both

associated with poor functional outcomes after AIS[33]. Above all, it is of interest to further elucidate the precise mechanisms between increased sIL-2R levels and unfavorable prognosis of AIS.

However, there are some limitations in our study. First, our study lacked data on infarct volume in MRI or CT scan. Patients who met the criterion of intravenous therapy should receive CT scans to exclude cerebral hemorrhage and should be infused with thrombolysis drugs as early as possible[34]. So nearly half of the patients in our study did not have a premorbid MRI scan. Besides, infarcts are not obvious and stable on early CT scans, and the severity of neurological dysfunction is not always proportional to the size of infarct volume; hence, it was reasonable that we did not include infarct volume in our study. Second, our study was performed mainly in Chinese individuals, and the patient sample was somewhat small, limiting the generalizability of the results to other ethnicities. Further studies with larger sample sizes are needed to verify our findings.

Conclusions

In conclusion, our study is the first to comprehensively study inflammatory cytokines in acute ischemic stroke and found that higher plasma sIL-2R was a new independent predictor of unfavorable outcomes in AIS. Additionally, adding plasma sIL-2R and HGF to conventional risk factors significantly improved risk stratification for poor outcomes in AIS patients, indicating that plasma sIL-2R and HGF may be potential prognostic markers for AIS.

Abbreviations

HGF, hepatocyte growth factor; sIL-2R: soluble interleukin-2 receptors; MIP-3 α : macrophage inflammatory protein-3 alpha; CD40L: CD40 Ligand; MMP1: matrix metalloproteinase-1; mRS: Modified Rankin Scale; NIHSS: National Institute of Health Stroke Scale; AUC: Under receiver operator curves; IDI: Integrated discrimination improvement; NRI: Net reclassification improvement.

Declarations

Acknowledgements

Not applicable

Authors' contributions

HPZ and FFL prepared the study protocol; collected, analyzed, and interpreted the data; and prepared the manuscript. YYH, SJZ, LZL, ZHY, RLW, ZT, ZPH and JFF, YMZ collected the data. HPZ and FFL performed the cytometric assay and analyzed the data. QFM and YML prepared the study protocol; analyzed and interpreted the data; supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

Written informed consent was obtained from each patient included in the study. The study protocol was approved by the Xuanwu Hospital of Capital Medical University.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Comparison of cytokines levels between acute ischemic stroke and control

Characteristic	Control (n=76)	AIS (n=204)	p value
Age, yr; mean $\pm$ SD	62.63 $\pm$ 11.38	64.16 $\pm$ 13.27	0.168
Male, n (%)	51 (67.11)	150 (73.53)	0.288
Inflammatory cytokines, median (IQR), pg/ml			
HGF	70.94 (57.76-104.25)	101.11 (77.01-132.21)	0.000
IL-10	2.24 (1.44-3.04)	3.07 (1.89-4.75)	0.000
IL-1 β	4.35 (2.53-6.14)	5.78 (3.49-9.61)	0.001
MIP-3 α	13.09 (9.49-17.29)	15.67 (11.38-20.81)	0.002
IL-2	21.20 (12.94-32.77)	28.08 (17.28-41.02)	0.002
IL-2R	812.80 (612.91-1170.57)	947.70 (689.61-1382.07)	0.033
IL-5	53.72 (30.47-81.11)	63.18 (37.27-103.52)	0.015
IL-16	58.63 (40.76-82.24)	67.50 (47.22-108.95)	0.129
MMP-1	5.23 (4.24-7.10)	5.67 (4.46-7.21)	0.270
CD40L	8.10 (5.68-15.15)	7.99 (5.37-13.40)	0.293

Table 2. Baseline characteristics of AIS patients with favorable or unfavorable outcomes

Baseline characteristics	All (204)	Favorable outcome (131)	Unfavorable outcome (73)	p value
Age, y	64.16±13.27	61.85±12.28	68.29±14.06	0.001
Male, n (%)	150 (73.53)	99 (77.57)	51 (69.86)	0.376
Time from onset, h	3.00 (1.50-5.10)	2.90 (1.30-4.50)	3.20 (1.80-6.60)	0.098
Baseline systolic BP, mmHg	150 (140-170)	152 (140-170)	148 (139-169)	0.306
Baseline diastolic BP, mmHg	87.5 (78.0-93.0)	85.0 (78.0-94.0)	90.0 (79.0-92.0)	0.816
Baseline NIHSS	5.0 (3.0-11.0)	4.0 (2.0-6.0)	13.0 (8.5-17.0)	0.000
Rt-PA administration, n (%)	92 (45.10)	68 (51.91)	24 (32.88)	0.009
Risk factors, n (%)				
Hypertension	134 (65.69)	83 (63.36)	51 (69.86)	0.348
Diabetes mellitus	83 (40.69)	47 (35.88)	36 (49.32)	0.061
Coronary heart disease	46 (22.55)	24 (18.32)	22 (30.14)	0.053
Atrial fibrillation	32 (15.69)	11 (8.40)	21 (28.77)	0.000
Stroke etiology, n (%)				
Thrombotic	150 (73.53)	95 (72.52)	55 (75.34)	-
Embolic	15 (7.35)	10 (7.63)	5 (6.85)	-
Lacunar	39 (19.18)	26 (19.85)	13 (17.81)	-
Clinical parameters, median (IQR)				
White blood cells, ×10 ⁹ /L	7.42 (6.18-8.83)	7.18 (5.95-8.47)	8.23 (6.80-9.41)	0.004
Neutrophils, ×10 ⁹ /L	5.07 (3.96-6.45)	4.58 (3.72-5.77)	6.07 (4.30-7.69)	0.000
Lymphocytes, ×10 ⁹ /L	1.60 (1.16-2.18)	1.80 (1.31-2.24)	1.31 (0.83-1.91)	0.000
Neutrophils to lymphocytes ratio	2.89 (2.01-5.18)	2.55 (1.78-4.06)	4.53 (2.35-8.92)	0.000
Baseline glucose, mmol/L	6.72 (5.71-8.96)	6.32 (5.57-8.03)	8.30 (6.39-10.97)	0.000
Triglyceride, mmol/L	1.47 (0.95-2.48)	1.69 (1.04-2.69)	1.26 (0.77-1.78)	0.006
Total cholesterol, mmol/L	4.54 (3.82-5.44)	4.73 (3.94-5.59)	4.27 (3.60-5.11)	0.012
High-density lipoprotein, mmol/L	1.18 (1.00-1.39)	1.17 (0.97-1.39)	1.23 (1.05-1.39)	0.413
Low-density lipoprotein, mmol/L	2.72 (2.04-3.42)	2.79 (2.05-3.62)	2.50 (1.99-3.14)	0.095
Biomarkers (pg/ml), median (IQR)				
HGF	101.11 (77.01-132.21)	89.09 (67.61-108.22)	138.64 (99.82-200.48)	0.000
IL-2R	947.70 (689.61-1382.07)	812.92 (630.21-1158.13)	1346.94 (959.59-1777.25)	0.000
IL-16	67.50 (47.22-108.95)	56.09 (43.96-85.28)	87.03 (61.63-146.47)	0.000
IL-2	28.08 (17.28-41.02)	29.74 (18.77-46.44)	23.15 (15.49-36.41)	0.013
IL-1β	5.78 (3.49-9.61)	6.34 (3.70-10.95)	4.83 (3.03-8.70)	0.024
IL-5	63.18 (37.27-103.52)	65.79 (44.57-110.26)	57.92 (33.86-96.16)	0.116
CD40L	7.99 (5.37-13.40)	7.61 (4.90-12.37)	9.07 (5.59-14.21)	0.127
MIP-3α	15.67 (11.38-20.81)	15.67 (11.38-21.19)	15.77 (10.48-20.38)	0.615
IL-10	3.07 (1.89-4.75)	3.07 (1.89-4.79)	3.32 (1.89-4.70)	0.887
MMP-1	5.67 (4.46-7.21)	5.63 (4.46-7.17)	5.83 (4.34-7.51)	0.732

BP: Blood pressure; NIHSS: NIH Stroke Scale; Rt-PA: recombinant tissue-plasminogen activator; IQR: interquartile range.

Table 3. Biomarkers and risk of the primary outcome after AIS

	Model 1		Model 2	
	OR (95% CI)	p value	OR (95% CI)	p value
Biomarkers (as continuous variables)				
HGF, 10 pg/ml per increase	1.222 (1.135-1.315)	0.000	1.121 (1.030-1.218)	0.008
sIL-2R, 100 pg/ml per increase	1.236 (1.144-1.336)	0.000	1.138 (1.028-1.259)	0.012
IL-16, 10 pg/ml per increase	1.109 (1.054-1.167)	0.000	-	-
IL-2, 1 pg/ml per increase	0.975 (0.956-0.993)	0.008	-	-
IL-1 β , 1 pg/ml per increase	0.924 (0.868-0.984)	0.014	-	-
IL-5, 10 pg/ml per increase	0.942 (0.881-1.007)	0.081	-	-
CD40L, 1 pg/ml per increase	1.004 (0.998-1.010)	0.208	-	-
MMP-1	1.028 (0.948-1.116)	0.501	-	-
MIP-3 α , 1 pg/ml per increase	0.992 (0.955-1.030)	0.661	-	-
IL-10, 1 pg/ml per increase	0.999 (0.914-1.092)	0.982	-	-
Biomarkers (as categorical variables)				
HGF, ≥ 117.915 pg/ml	9.513 (4.887-18.516)	0.000	6.178 (2.452-15.562)	0.000
sIL-2R, ≥ 971.44 pg/ml	6.253 (3.280-11.921)	0.000	3.401 (1.366-8.466)	0.009

Model 1 was an unadjusted logistic regression model. Model 2 was adjusted for gender, age, admission NIHSS score, onset time, systolic BP, history of hypertension, diabetes mellitus, coronary heart disease, and atrial fibrillation, rt-PA treatment, baseline white blood cell counts, glucose levels, triglyceride levels, total cholesterol levels, high-density lipoprotein levels and low-density lipoprotein levels.

Table 4. Reclassification of the primary outcome by plasma cytokines among AIS patients

Models	Continuous NRI		IDI	
	Estimate (95% CI), %	p value	Estimate (95% CI), %	p value
Conventional model	Reference	-	Reference	-
Conventional model + HGF	28.67(17.65-40.58)	< 0.001	7.65 (3.05-12.26)	0.001
Conventional model + sIL-2R	24.70 (11.28-37.78)	< 0.001	7.43 (2.88-11.97)	0.001
Conventional model + HGF + sIL-2R	30.96 (17.22-45.14)	< 0.001	10.71 (5.49-15.93)	< 0.001

Abbreviations: CI = confidence interval; IDI = integrated discrimination index; NRI = net reclassification improvement. The conventional model included gender, age, admission NIHSS score, onset time, systolic BP, history of hypertension, diabetes mellitus, coronary heart disease, and atrial fibrillation, rt-PA treatment, baseline white blood cell counts, glucose levels, triglyceride levels, total cholesterol levels, high-density lipoprotein levels and low-density lipoprotein levels.

Figures

Figure 1

Forest plot showing the discrimination capacities of these inflammatory cytokines in dividing AIS patients from healthy controls.

Figure 2

Comparison of inflammatory cytokines in favorable (mRS=0-2) and unfavorable outcomes (mRS=3-6). N=131 in the favorable outcome group, n=73 in the unfavorable outcome group. ****p < 0.0001, *p < 0.05.

Supplementary Files

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- [SupplementFig1.jpg](#)