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

Haiping Zhao
Xuanwu Hospital
Fangfang Li
Xuanwu Hospital
Yuyou Huang
Xuanwu Hospital
Sijia Zhang
Xuanwu Hospital
Lingzhi Li
Xuanwu Hospital
Zhenhong Yang
Xuanwu Hospital
Rongliang Wang
Xuanwu Hospital
Zhen Tao
Xuanwu Hospital
Ziping Han
Xuanwu Hospital
Junfen Fan
Xuanwu Hospital
Yangmin Zheng
Xuanwu Hospital
Qingfeng Ma
Xuanwu Hospital
Yumin Luo (yumin111@ccmu.edu.cn)
Xuanwu Hospital

Research

Keywords: Acute ischemic stroke, inflammation, sIL-2R, HGF, prognosis

Posted Date: February 6th, 2020

DOI: https://doi.org/10.21203/rs.2.21269/v2

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 204 AIS patients and 76 healthy controls, and ten cytokines (HGF, IL-1β, IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3α, 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-1β, MIP-3α, IL-2, sIL-2R, and IL-5 were significantly upregulated in AIS patients compared to controls. 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 after AIS ($p < 0.05$). 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 most common cause of disability, and there are about 25.7 million stroke survivors (71% ischemic stroke) globally [1]. There is an urgent need to accurately predict outcome after acute ischemic stroke (AIS) for physicians, patients, and their families to aid early and informed decision-making about acute therapies, palliative care, and/or rehabilitation. Blood biomarkers reflecting pathophysiological processes associated with AIS and/or other concomitant diseases might improve outcome prediction.

Inflammation is a critical factor affecting the evolution of pathology after stroke and long-term neurological outcomes [2-5]. A number of studies showed that the blood levels of cytokines such as C-reactive protein, IL-6, TNF receptor and IL-10, were associated with outcomes in AIS patients [6-8]. It was recently reported that the specific ex vivo released cytokine profile is associated with AIS outcome and improves its prediction [9]. And serum dickkopf-3, an immune modulator in peripheral CD8 T-cell tolerance [10], is associated with death and vascular events after ischemic stroke [11]. In addition to inflammatory mediators, lymphocytes produce increased amounts of acetylcholine in AIS patients, also contribute to fatal post-stroke infection and mortality [12]. Serum level of irisin, a skeletal muscle cell-derived myokine, was a powerful biological marker of risk of post-stroke depression [13]. To discover new independent blood biomarkers or to combine with promising prognostic candidate biomarkers are both important [14].

To investigate the globally alterations of plasma biomarkers in AIS patients and healthy controls, we first screened 65 cytokine/chemokine/growth factors by immune monitoring 65-Plex human panel, including APRIL, BAFF, BLC, CD30, CD40L, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, FGF-2, Fractalkine, G-CSF, GM-CSF,

GROα, HGF, IFN-α, IFN-, IL-1α, IL-1β, IL-2, IL-2R, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10, I-TAC, LIF, MCP-1, MCP-2, MCP-3, M-CSF, MDC, MIF, MIG, MIP-1α, MIP-1β, MIP-3α, MMP-1, NGF-β, SCF, SDF-1α, TNF-α, TNF-β, TNFR2, TRAIL, TSLP, TWEAK, and VEGF-A. Among them, ten cytokine/chemokine/growth factors (IL-1β, IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3α, CD40L, MMP1 and HGF) were discovered to change significantly (Supplementary Figure 1). To confirm their change and find the independent prognostic factor after AIS, we collected plasma and measured 10 cytokine/chemokine/growth factors from 204 AIS patients and 76 matched controls using a multiplex immunoassay. Multivariate logistic regression models were used to identify the significance of each factor, and NRI and IDI was calculated to explore if adding these independent biomarkers to the basic model with traditional risk factors will improve risk stratification for unfavorable outcomes.

Methods

Study Participants and outcome assessment

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. Clinical outcome was evaluated at 90 days after stroke using the mRS score, with a favorable outcome defined as mRS 0-2. The follow-up was evaluated by trained neurologists unaware of treatment assignment.

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. Stroke severity was accessed using the NIH Stroke Scale (NIHSS) by trained neurologists at admission [15]. Blood samples were drawn from each healthy control and AIS patient before any treatment into K3EDTA tubes. All plasma samples were separated and frozen at −80°C prior to the test.

Circulating biomarker measurements

The abnormally expressed ten cytokines in AIS patients were confirmed in 76 healthy controls and 204 AIS patients using ProcartaPlex (Invitrogen, PPX-10). Experienced laboratory technicians who performed

the assays were blinded to the experimental design.

Statistical analysis

Data were analyzed with SPSS 21.0 software (IBM Corp., Armonk, NY, USA) and R software (version 3.5.1). Statistical significance was set at $p<0.05$. Continuous variables of a normal distribution are expressed as the means ± SDs and analyzed using Student's t test. Non-normally distributed variables are expressed as medians with inter-quartile ranges (IQR) and analyzed using the Mann-Whitney U test. Meanwhile, the Chi-Squared test was used to compare frequency and percentage in categorical variables. The relationship between plasma cytokines and 3-months functional outcomes of AIS patients were analyzed by univariate and multivariable logistic regression analysis. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of each biomarker were calculated.

Potential covariates 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-1β, IL-16, IL-2, sIL-2R and IL-5 on admission. Receiver operating characteristic (ROC) curve was applied to determine the values of HGF and sIL-2R to predict prognosis in AIS patients. We calculated the net reclassification index (NRI) and integrated discrimination improvement (IDI) [16] to evaluate the reclassification value through adding one or more of these inflammatory biomarkers to the conventional model with established risk factors.

Results

Differentially expressed plasma cytokines between AIS patients and controls

Ten cytokine/chemokine/growth factors (HGF, IL-1β, IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3α, CD40L and MMP1) were discovered to be differentially expressed between AIS patients (n=29) and healthy controls (n=18) by immune monitoring 65-Plex human procartaPlex panel (Supplementary Figure 1). Further verification was conducted in 204 AIS patients and 76 healthy controls, and we found that levels of HGF, IL-10, IL-1β, MIP-3α, IL-2, sIL-2R, and IL-5 were markedly upregulated in AIS patients (Table 1). And ROC curves demonstrated that all of them (AUC>0.5) has the discriminatory power in the diagnosis of AIS (Figure 1).

Comparisons of baseline characteristics and cytokine levels between AIS patients with good and poor outcome

Baseline characteristics of the 204 AIS patients are shown in table 2, stratified by functional outcome (mRS score) at 3 months. Older age, higher NIHSS scores, odds of atrial fibrillation history, white blood cell counts, glucose levels, and lower triglyceride levels and total cholesterol levels predicts unfavorable outcomes. 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-1β (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 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-1β were individually associated with unfavorable outcomes at 3 months after AIS ($p < 0.05$, Table 3). After adjusting for gender, age, admission NIHSS score, onset time, systolic BP, history of hypertension, diabetes mellitus, coronary heart disease, and other variables in binominal multivariate analysis (model 2), only HGF and sIL-2R remained significant for the prediction of unfavorable outcome ($p < 0.05$ for both). The multivariable adjusted OR (95% CIs) for HGF (each 10 pg/ml increase) was 1.121 (1.030-1.218), and sIL-2R (each 100 pg/ml increase) was 1.138 (1.028-1.259) (Table 3).

Receiver-operating characteristic curve demonstrated the greatest discriminatory accuracies for HGF (AUC 0.786; cut-off value 117.915 pg/ml; 95% CI 0.719–0.854; sensitivity 65.8%; specificity 83.2%) and sIL-2R (AUC 0.768; cut-off value 971.44 pg/ml; 95% CI 0.702–0.835; sensitivity 75.3%; specificity 67.2%). HGF levels ≥ 117.915 pg/ml and sIL-2R ≥ 971.44 pg/ml were both associated with an increased risk of the primary outcome at 3 months after AIS (Table 3).

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

We further construct a continuous multimarker score, based on the estimates of beta coefficients of these 2 biomarkers obtained from logistic regression model to additionally assess the effect of multiple biomarkers (Table 4). Individually, adding each of them to the conventional model significantly improved the risk reclassification for unfavorable outcomes ($p<0.05$ for both NRI and IDI). Furthermore, simultaneously adding HGF and sIL-2R to the conventional model substantially improved the risk stratification for primary outcome, shown by an increase in category-free NRI (NRI 32.18%, $p < 0.001$; IDI 10.21%, $p < 0.001$).

Discussion

In the present study, we investigated diagnostic and prognositic value of a panel of 10 circulating biomarkers representing various pathophysiologic pathways in AIS patients. Individually, we observed that elevated plasma HGF and sIL-2R were associated with increased risk of unfavorable outcomes at 3 months. More importantly, simultaneously adding HGF and sIL-2R to basic model with established risk factors substantially improved the risk stratification for primary outcomes. Data confirm an important conclusion that sIL-2R is a new independent prognostic marker of functional outcome in Chinese patients with AIS and adds significant additional predictive information to the clinical score of the NIHSS.

To date, limited studies have investigated the global alterations, diagnostic, prognostic value of cytokines in AIS patients [14, 16, 17], and the prognostic significance of multiple novel biomarkers after ischemic stroke is not fully understood. Previously, a prospective cohort study investigated the predictive value of

18 blood markers in 270 patients with acute ischemic cerebrovascular events [16]. Recently, Lin and and colleagues evaluated the prognostic potential of 35 serum cytokines, chemokines, and growth factors in patients with acute ischemic stroke within 72 hours after stroke onset [17]. The present study is the first report to demonstrate the simultaneous measurement of 65 plasma cytokines in AIS patient within 24 hours after stroke onset by multiplexing method. Ten cytokines (HGF, IL-1β, IL-2, sIL-2R, IL-5, IL-10, IL-16, MIP-3α, CD40L and MMP1) were screened to change significantly (Supplementary Fig 1) between 29 AIS patients and 18 controls. Upon further examination in a larger sample size, we found that seven cytokines including HGF, IL-1β, IL-2, sIL-2R, IL-5, IL-10, and MIP-3α were definitely upregulated in 204 AIS patients compared to 76 healthy controls and had diagnostic value. Since HGF, IL-1β, IL-2, IL-5, and IL-10 have been reported previously [17-19], our findings confirmed and extended prior studies on the relations between various biomarkers and diagnosis after AIS. Our first contribution was to identify two totally new biomarkers sIL-2R and MIP-3α for AIS patients, which can provide references to other studies and help target particular pathways to prevent the progression of ischemic stroke.

We further examine the predictive value of ten cytokines for the risk of poor outcomes after AIS, and found that higher plasma levels of HGF, sIL-2R and IL-16 were associated with increased risk of unfavorable outcomes in the univariate analyses, while higher plasma levels of IL-2 and IL-1β were associated with decreased risk of unfavorable outcomes. However, 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 may be independent predictive biomarkers for prognosis of AIS. Interestingly, the addition of plasma HGF, sIL-2R or both to conventional risk factors was shown to improve risk predictions for the primary outcome. Therefore, our second contribution was to show that plasma HGF and sIL-2R might be useful in AIS risk stratification and could be beneficial for the selection of high-risk patients who should receive aggressive monitoring and therapeutic interventions in future clinical practice.

HGF is a pleiotropic cytokine that can regulate different cellular functions in developmental and pathological situations. According to previous experimental studies, HGF can enhance the proliferation of neural precursor cells and increase neuronal differentiation, thus protecting against ischemic stroke [20]. The other underlying mechanisms might include promoting the migration of immune cells and secretion of pro-inflammatory chemokines [21] and accelerating the progression of atherosclerotic lesions [22], thus increasing the risk of unfavorable prognosis. Clinically, HGF is a biomarker of atherosclerotic disease [23] and positively associated with the incidence of stroke [24, 25]. Zhu et al reported that HGF was associated with mortality but not disability at 3 months after ischemic stroke onset [18]. However, they included patients within 48 hours of symptom onset and excluded patients treated with rt-PA [18]. By comparison, patients' blood in our study was collected within 24 hours before they received any treatment. Thus, the level of HGF could reflect more about the authentic pathological change of AIS without the influence of other factors. In addition, we included patients who received rt-PA therapy for AIS patients within 4.5 hours after symptom onset [26]. Moreover, Zhu's study only ruled out AIS patients in deep coma, without considering patients whose premorbid mRS $\geq$ 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. In general, although the inclusion criteria of AIS patients are partly different, our results are consistent with previous clinical studies that HGF is an independent risk factor for the prognosis of AIS.

More importantly, we first reported that sIL-2R was independently associated with unfavorable outcomes in AIS. It can be seen from 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 [27]. Peter et al reported that sIL-2R was positively associated with internal carotid wall thickness, cardiovascular disease mortality, incident cardiovascular disease and stroke [28]. In addition, sIL-2R was significantly higher in ischemic left ventricular dysfunction patients [29] and was associated with a worse prognosis for dilated cardiomyopathy patients [30]. 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 [31]. 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 [32]. Since ischemic stroke is a heterogeneous disease and different inflammatory processes may jointly lead to adverse outcomes, it is particularly important to incorporate multiple biomarkers covering distinct pathways to improve the risk stratification in AIS patients. 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 CT or MRI 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 [33]. So nearly half of the patients in our study did not have a premorbid MRI scan. Besides, infarcts are not obvious 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 size 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

Our findings have important clinical implications. We first comprehensively investigated inflammatory cytokines in AIS patients and found that higher plasma sIL-2R was a new independent predictor of unfavorable outcomes in AIS patients. Additionally, adding plasma sIL-2R and HGF to established risk

factors substantially improved risk stratification for poor outcomes in AIS patients, indicating that plasma sIL-2R and HGF may be potential prognostic markers for AIS outcome. A combination of biomarkers in conjunction with clinical and imaging examination will yield the greatest accuracy for predicting poor stroke outcome.

Abbreviations

HGF, hepatocyte growth factor; sIL-2R: soluble interleukin-2 receptors; MIP-3α: 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.

Funding

This project was supported by the National Natural Science Foundation of China (81771413, 81771412, and 81971222) and Beijing Natural Science Foundation Program and the Scientific Research Key Program of the Beijing Municipal Commission of Education (KZ201810025041).

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.

Author details

[1]Cerebrovascular Diseases Research Institute and Department of Neurology, Xuanwu Hospital of Capital Medical University, 45 Changchun Street, Beijing, China. [2]Beijing Institute for Brain Disorders, Beijing, China. [3]National Clinical Research Center for Geriatric Disorders, Beijing, China.

References

1. Feigin VL, Norrving B, Mensah GA: **Global Burden of Stroke.** *Circ Res* 2017, **120:**439-448.
2. Shi K, Tian DC, Li ZG, Ducruet AF, Lawton MT, Shi FD: **Global brain inflammation in stroke.** *Lancet Neurol* 2019.
3. Salmeron KE, Maniskas ME, Edwards DN, Wong R, Rajkovic I, Trout A, Rahman AA, Hamilton S, Fraser JF, Pinteaux E, Bix GJ: **Interleukin 1 alpha administration is neuroprotective and neuro-restorative following experimental ischemic stroke.** *J Neuroinflammation* 2019, **16:**222.
4. Jin R, Zhong W, Liu S, Li G: **CD147 as a key mediator of the spleen inflammatory response in mice after focal cerebral ischemia.** *J Neuroinflammation* 2019, **16:**198.
5. Dabrowska S, Andrzejewska A, Lukomska B, Janowski M: **Neuroinflammation as a target for treatment of stroke using mesenchymal stem cells and extracellular vesicles.** *J Neuroinflammation* 2019, **16:**178.
6. Li J, Zhao X, Meng X, Lin J, Liu L, Wang C, Wang A, Wang Y, Wang Y, Investigators C: **High-Sensitive C-Reactive Protein Predicts Recurrent Stroke and Poor Functional Outcome: Subanalysis of the Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events Trial.** *Stroke* 2016, **47:**2025-2030.
7. Boehme AK, McClure LA, Zhang Y, Luna JM, Del Brutto OH, Benavente OR, Elkind MS: **Inflammatory Markers and Outcomes After Lacunar Stroke: Levels of Inflammatory Markers in Treatment of Stroke Study.** *Stroke* 2016, **47:**659-667.
8. Zhu Y, Yang H, Diao Z, Li Y, Yan C: **Reduced Serum Level of Interleukin-10 is Associated with Cerebral Infarction: A Case-Control and Meta-Analysis Study.** *Mol Neurobiol* 2016, **53:**2698-2704.
9. Klimiec-Moskal E, Piechota M, Pera J, Weglarczyk K, Slowik A, Siedlar M, Dziedzic T: **The specific ex vivo released cytokine profile is associated with ischemic stroke outcome and improves its prediction.** *J Neuroinflammation* 2020, **17:**7.
10. Papatriantafyllou M, Moldenhauer G, Ludwig J, Tafuri A, Garbi N, Hollmann G, Kublbeck G, Klevenz A, Schmitt S, Pougialis G, et al: **Dickkopf-3, an immune modulator in peripheral CD8 T-cell tolerance.** *Proc Natl Acad Sci U S A* 2012, **109:**1631-1636.

11. Zhu Z, Guo D, Zhong C, Wang A, Xu T, Peng Y, Peng H, Li Q, Ju Z, Geng D, et al: **Serum dickkopf-3 is associated with death and vascular events after ischemic stroke: an observational study from CATIS.** *J Neuroinflammation* 2020, **17:**12.
12. Yuan M, Han B, Xia Y, Liu Y, Wang C, Zhang C: **Augmentation of peripheral lymphocyte-derived cholinergic activity in patients with acute ischemic stroke.** *BMC Neurol* 2019, **19:**236.
13. Tu WJ, Qiu HC, Liu Q, Li X, Zhao JZ, Zeng X: **Decreased level of irisin, a skeletal muscle cell-derived myokine, is associated with post-stroke depression in the ischemic stroke population.** *J Neuroinflammation* 2018, **15:**133.
14. Zhong C, Zhu Z, Wang A, Xu T, Bu X, Peng H, Yang J, Han L, Chen J, Xu T, et al: **Multiple biomarkers covering distinct pathways for predicting outcomes after ischemic stroke.** *Neurology* 2019, **92:**e295-e304.
15. Guo D, Zhu Z, Zhong C, Peng H, Wang A, Xu T, Peng Y, Xu T, Chen CS, Li Q, et al: **Increased Serum Netrin-1 Is Associated With Improved Prognosis of Ischemic Stroke.** *Stroke* 2019, **50:**845-852.
16. Whiteley W, Wardlaw J, Dennis M, Lowe G, Rumley A, Sattar N, Welsh P, Green A, Andrews M, Sandercock P: **The use of blood biomarkers to predict poor outcome after acute transient ischemic attack or ischemic stroke.** *Stroke* 2012, **43:**86-91.
17. Li X, Lin S, Chen X, Huang W, Li Q, Zhang H, Chen X, Yang S, Jin K, Shao B: **The Prognostic Value of Serum Cytokines in Patients with Acute Ischemic Stroke.** *Aging Dis* 2019, **10:**544-556.
18. Zhu Z, Xu T, Guo D, Huangfu X, Zhong C, Yang J, Wang A, Chen CS, Peng Y, Xu T, et al: **Serum Hepatocyte Growth Factor Is Probably Associated With 3-Month Prognosis of Acute Ischemic Stroke.** *Stroke* 2018, **49:**377-383.
19. Nayak AR, Kashyap RS, Purohit HJ, Kabra D, Taori GM, Daginawala HF: **Evaluation of the inflammatory response in sera from acute ischemic stroke patients by measurement of IL-2 and IL-10.** *Inflamm Res* 2009, **58:**687-691.
20. Doeppner TR, Kaltwasser B, ElAli A, Zechariah A, Hermann DM, Bahr M: **Acute hepatocyte growth factor treatment induces long-term neuroprotection and stroke recovery via mechanisms involving neural precursor cell proliferation and differentiation.** *J Cereb Blood Flow Metab* 2011, **31:**1251-1262.
21. Komarowska I, Coe D, Wang G, Haas R, Mauro C, Kishore M, Cooper D, Nadkarni S, Fu H, Steinbruchel DA, et al: **Hepatocyte Growth Factor Receptor c-Met Instructs T Cell Cardiotropism and Promotes T Cell Migration to the Heart via Autocrine Chemokine Release.** *Immunity* 2015, **42:**1087-1099.
22. Kawamoto R, Oka Y, Yoshida O, Takagi Y: **Significance of serum circulating hepatocyte growth factor in the development of carotid atherosclerosis.** *J Atheroscler Thromb* 2003, **10:**154-159.
23. Bielinski SJ, Berardi C, Decker PA, Larson NB, Bell EJ, Pankow JS, Sale MM, Tang W, Hanson NQ, Wassel CL, et al: **Hepatocyte growth factor demonstrates racial heterogeneity as a biomarker for coronary heart disease.** *Heart* 2017, **103:**1185-1193.
24. Bell EJ, Larson NB, Decker PA, Pankow JS, Tsai MY, Hanson NQ, Wassel CL, Longstreth WT, Jr., Bielinski SJ: **Hepatocyte Growth Factor Is Positively Associated With Risk of Stroke: The MESA (Multi-Ethnic Study of Atherosclerosis).** *Stroke* 2016, **47:**2689-2694.

25. Rajpathak SN, Wang T, Wassertheil-Smoller S, Strickler HD, Kaplan RC, McGinn AP, Wildman RP, Rosenbaum D, Rohan TE, Scherer PE, et al: **Hepatocyte growth factor and the risk of ischemic stroke developing among postmenopausal women: results from the Women's Health Initiative.** *Stroke* 2010, **41:**857-862.
26. Berekashvili K, Zha AM, Abdel-Al M, Zhang X, Soomro JH, Prater SJ, Grotta JC: **Emergency Medicine Physicians Accurately Select Acute Stroke Patients for Tissue-Type Plasminogen Activator Treatment Using a Checklist.** *Stroke* 2019**:**STROKEAHA119026948.
27. Mir MA, Maurer MJ, Ziesmer SC, Slager SL, Habermann T, Macon WR, Link BK, Syrbu S, Witzig T, Friedberg JW, et al: **Elevated serum levels of IL-2R, IL-1RA, and CXCL9 are associated with a poor prognosis in follicular lymphoma.** *Blood* 2015, **125:**992-998.
28. Durda P, Sabourin J, Lange EM, Nalls MA, Mychaleckyj JC, Jenny NS, Li J, Walston J, Harris TB, Psaty BM, et al: **Plasma Levels of Soluble Interleukin-2 Receptor alpha: Associations With Clinical Cardiovascular Events and Genome-Wide Association Scan.** *Arterioscler Thromb Vasc Biol* 2015, **35:**2246-2253.
29. Abbate A, Vecile E, Fiotti N, Giansante C, Guarnieri G, Di Sciascio G, Dobrina A: **Plasma concentrations of interleukin-2 soluble receptor in mild ischaemic left ventricular dysfunction.** *Eur J Heart Fail* 2003, **5:**23-25.
30. Limas CJ, Hasikidis C, Iakovou J, Kroupis C, Haidaroglou A, Cokkinos DV: **Prognostic significance of soluble interleukin-2 receptor levels in patients with dilated cardiomyopathy.** *Eur J Clin Invest* 2003, **33:**443-448.
31. Meng H, Zhao H, Cao X, Hao J, Zhang H, Liu Y, Zhu MS, Fan L, Weng L, Qian L, et al: **Double-negative T cells remarkably promote neuroinflammation after ischemic stroke.** *Proc Natl Acad Sci U S A* 2019, **116:**5558-5563.
32. Pikija S, Sztriha LK, Killer-Oberpfalzer M, Weymayr F, Hecker C, Ramesmayer C, Hauer L, Sellner J: **Neutrophil to lymphocyte ratio predicts intracranial hemorrhage after endovascular thrombectomy in acute ischemic stroke.** *J Neuroinflammation* 2018, **15:**319.
33. Warner JJ, Harrington RA, Sacco RL, Elkind MSV: **Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke.** *Stroke* 2019, **50:**3331-3332.

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 ± SD	62.63±11.38	64.16±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-1β	4.35 (2.53-6.14)	5.78 (3.49-9.61)	0.001
MIP-3α	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

HGF, hepatocyte growth factor; IL-10, interleukin-10; IL-1beta, interleukin 1beta; MIP-3alpha, macrophage inflammatory protein 3alpha; IL-2, interleukin-2; IL-2R, interleukin-2 receptor; IL-5, interleukin-5; IL-16, interleukin-16; MMP1, matrix metalloproteinase-1; CD40L, TNF associated activation protein.

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, ×10⁹/L	7.42 (6.18-8.83)	7.18 (5.95-8.47)	8.23 (6.80-9.41)	0.004
Neutrophils, ×10⁹/L	5.07 (3.96-6.45)	4.58 (3.72-5.77)	6.07 (4.30-7.69)	0.000
Lymphocytes, ×10⁹/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	
Biomarkers (as continuous variables)	OR (95% CI)	p value	OR (95% CI)	p value
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-1β, 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-3α, 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, 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

	Continuous NRI		IDI	
Models	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

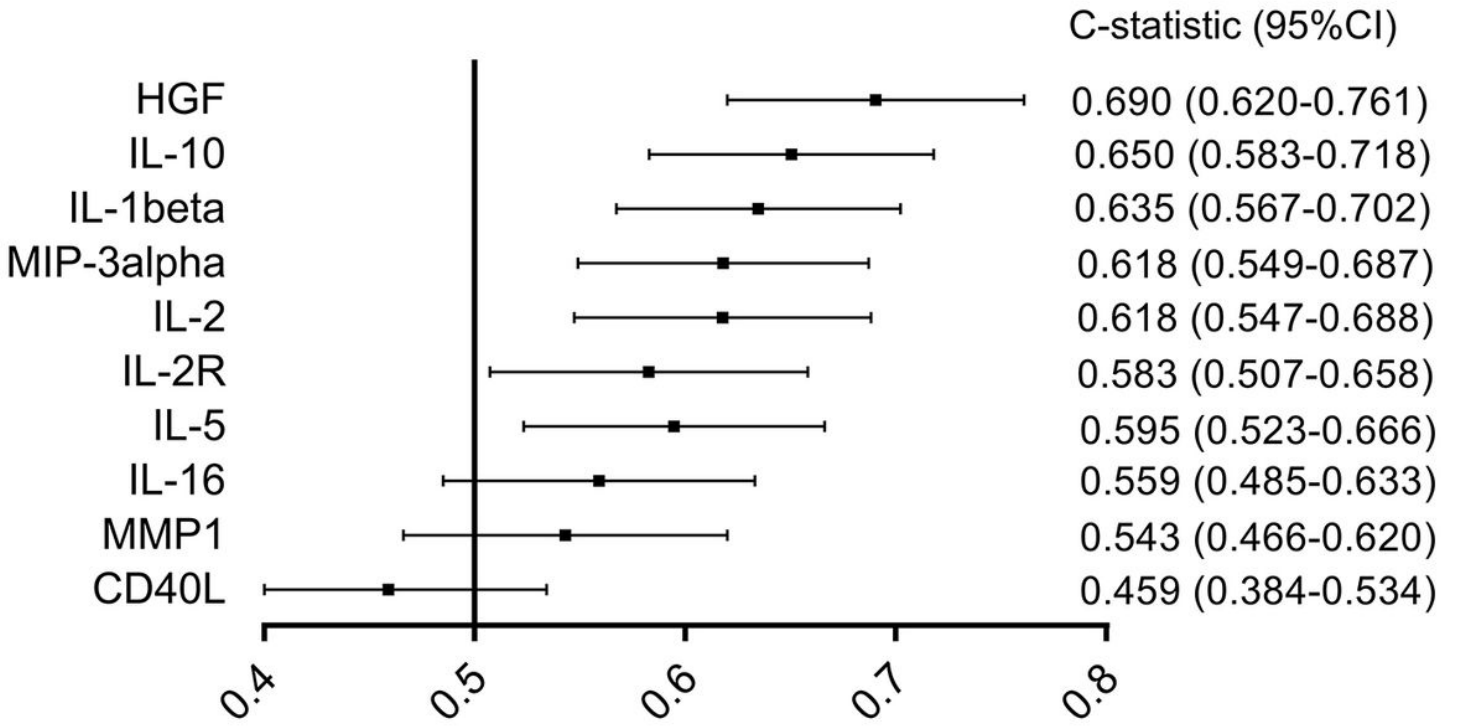

Figure 1

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

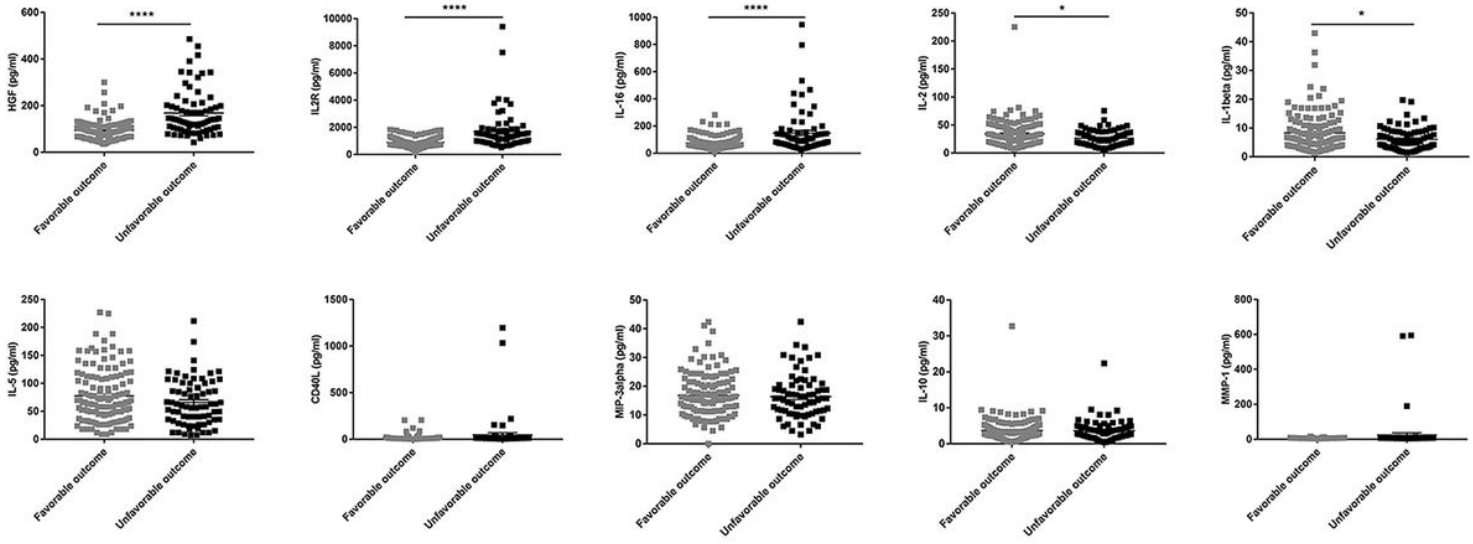

Figure 2

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

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.doc
- SupplementFig1.jpg