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Multiple biomarkers covering several pathways improve predictive ability for cognitive impairment among ischemic stroke patients with elevated blood pressure

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HIGHLIGHTS

- The first study using multiple biomarkers to examine improvement in risk prediction for post-stroke cognitive impairment.
- This prospective study is by far the largest biomarker study of post-stroke cognitive impairment.
- Combination of RF, MMP-9 and tHcy might improve the risk prediction of post-stroke cognitive impairment.
- RF, MMP-9 and tHcy should be measured simultaneously among ischemic stroke patients in future clinical practice.
- Further studies are warranted to explore their roles as potential therapeutic targets.

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ABSTRACT

Background and aims: We aimed to evaluate the ability of multiple novel biomarkers representing several pathophysiological pathways to improve risk prediction of post-stroke cognitive impairment.

Methods: We conducted a prospective multicenter study in 638 ischemic stroke patients with elevated blood pressure based on a random subsample from China Antihypertensive Trial in Acute Ischemic Stroke and measured 12 circulating biomarkers in these participants. Cognitive impairment was assessed at 3 months after stroke with definitions of Mini-Mental State Examination (MMSE) score < 27 or Montreal Cognitive Assessment (MoCA) score < 25.

Results: According to MMSE score, 1 SD increase of rheumatoid factor (odds ratio [OR] 1.22, 95% confidence interval [CI] 1.02–1.46), matrix metalloproteinase-9 (OR 1.47, 95% CI 1.22–1.77) and total homocysteine (OR 1.22, 95% CI 1.01–1.49) after log transformation was significantly associated with the risk of post-stroke cognitive impairment. The ORs associated with their simultaneously high levels were 4.89 (95% CI, 2.31–10.35; $p_{trend} < 0.001$) and 3.09 (95% CI, 1.60–5.98; $p_{trend} < 0.001$) for cognitive impairment and the severity of cognitive impairment, respectively. Adding these 3 biomarkers to conventional model significantly improved the risk prediction of cognitive impairment (C statistic 0.729 *vs.* 0.688, $p = 0.004$; net reclassification

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improvement = 33.67%, $p < 0.001$; integrated discrimination index = 4.61%; $p < 0.001$). Similar significant findings were observed when cognitive impairment was defined by MoCA score.
Conclusions: Combination of rheumatoid factor, matrix metalloproteinase-9 and total homocysteine can improve the risk prediction of cognitive impairment among ischemic stroke patients with elevated blood pressure. Further studies are warranted to validate our findings and explore their roles as potential therapeutic targets.

1. Introduction

Stroke is the leading cause of death and long-term disability worldwide [1], and ischemic stroke is by far the most common kind of stroke (nearly 80%) [2]. Due to the similar pathophysiologic mechanisms between vascular cognitive impairment and cerebrovascular disease [3], cognitive impairment and dementia are major complications of ischemic stroke [4]. For patients with ischemic stroke, cognitive impairment has adverse effects on adhering to treatment regimens and initiating activities after stroke onset based on individual's health condition [5]. Given that there is a time window (3 months or more) in the development of post-stroke cognitive impairment [4], an early and accurate risk assessment will be of great clinical value if it can identify patients who are at high risk of post-stroke cognitive impairment and who can then be targeted for aggressive monitoring and preventive interventions.

The predictive capacities of the traditional risk factors of post-stroke cognitive impairment have been well established [6], but these established risk factors could not fully explain the development of post-stroke cognitive impairment. Cognitive impairment is a complex disease and there are several different pathophysiological pathways involved in its progression. Therefore, joint assessments of comprehensive biomarkers covering various pathways may be more effective to improve the risk prediction of post-stroke cognitive impairment, but little is known about this so far. With this background, we systematically explored which of the multiple circulating biomarkers involving different pathways were associated with cognitive impairment after acute ischemic stroke, and investigated the combined effects of them on the improvement for risk prediction of post-stroke cognitive impairment based on a large prospective multicenter study of the CATIS (China Antihypertensive Trial in Acute Ischemic Stroke).

2. Patients and methods

2.1. Study patients

In this prospective study, we used datasets from a pre-planned ancillary study in CATIS. Details on the study design, data collection, and major results of both studies have been published previously [7,8].

CATIS is a multicenter, single-blind, blinded end-points randomized clinical trial conducted in 26 hospitals across China to evaluate whether moderate blood pressure (BP) reduction within the first 48 h after onset will improve prognosis of patients with acute ischemic stroke [7]. After admission, patients were randomly assigned to receive antihypertensive treatment (aimed at lowering systolic BP by 10%–25% within the first 24 h, achieving BP less than 140/90 mm Hg within 7 days, and maintaining this level during hospitalization) or to discontinue all antihypertensive medications (control) during hospitalization. From August 2009 to May 2013, a total of 4071 patients aged over 22 years who had first-ever ischemic stroke confirmed by computed tomography or magnetic resonance imaging of the brain within 48 h of symptom onset, and with an elevated systolic BP between 140 mmHg and 220 mmHg were recruited.

The pre-planned ancillary study was designed to investigate the effects of immediate BP reduction on cognitive function after acute ischemic stroke among a random subsample of CATIS [8]. From August 2009 to November 2012, 660 participants of CATIS were systemically selected prior to randomization from seven participating hospitals for cognitive function assessment at 3 months after acute ischemic stroke. At the 3-month visit, 15 patients were lost to follow-up and 7 patients were deceased. A total of 638 patients completed the cognitive function tests at 3 months (Supplemental Fig. 1).

This study was approved by the institutional review boards at Soochow University in China and Tulane University in the United States, as well as ethical committees at the participating hospitals. Written consent was obtained from all study participants or their immediate family members. The CATIS trial was registered at clinicaltrials.gov (NCT01840072).

2.2. Data collection

Baseline data on demographic characteristics, lifestyle risk factors, medical history, clinical features, and imaging data were collected at the time of enrollment. The National Institutes of Health Stroke Scale (NIHSS) was used to evaluate stroke severity [9]. According to the symptoms and imaging data of the patients, ischemic stroke was classified as large artery atherosclerosis (thrombotic), cardiac embolism (embolic) and small artery occlusion lacunae (lacunar) [10].

2.3. Biomarkers measurement

Blood samples were collected after at least 8 h of fasting within 24 h of hospital admission. All serum samples were separated and frozen at −80 °C in the Central Laboratory of School of Public Health in Soochow University until laboratory testing. We systematically measured 12 circulating biomarkers from different pathophysiological pathways involved in the progression to cognitive impairment after ischemic stroke onset. Serum high-sensitivity C-reactive protein, lipoprotein-associated phospholipase A2, matrix metalloproteinase-9 (MMP-9), hepatocyte growth factor (R&D Systems, Minneapolis, USA); and antiphosphatidylserine antibodies, anticardiolipin antibodies, beta (2)-glycoprotein I-dependent anticardiolipin antibodies (QUANTA Lite kits, INOVA Diagnostics, San Diego, USA); and N-terminal pro-brain natriuretic peptide (Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria) were determined using commercially available enzyme-linked immunosorbent assay kits. Serum rheumatoid factor (RF) was measured with the immunological transmission turbidimetry, plasma total homocysteine (tHcy) was determined by enzymatic cycling assay, serum creatinine was measured using the Jaffe method, and serum uric acid was tested with urate oxidase reagent on the Cobas c 501 analyzer (Roche Diagnostics, Basel, Switzerland). Serum creatinine was used to assess renal function based on estimated glomerular filtration rate (eGFR) calculated by Chronic Kidney Disease Epidemiology Collaboration creatinine equation with adjusted coefficient of 1.1 for the Chinese population [11]. The intra- and interassay coefficients of variations for all biomarkers were below 5.6% and 8.4%, respectively. Laboratory technicians who performed these measurements were blinded to the clinical characteristics and outcomes of patients.

2.4. Outcome assessment

Participants were followed up in person at 3 months after acute ischemic stroke by trained neurologists unaware of treatment assignment. The study outcome was cognitive impairment at 3 months assessed by the Mini-Mental State Examination (MMSE) [12] and the Montreal Cognitive Assessment (MoCA) [13] in Chinese. Both MMSE

and MoCA have been validated as a screening tool for cognitive impairment and dementia in the Chinese population, and the maximum score is 30 for them. The MMSE is a 20-item test that tests cognitive performance in domains including orientation, registration, attention and calculation, recall, language, and visual construction [12]. The MoCA contains 30 items that evaluate the following seven cognitive domains: visuospatial/executive functions, naming, memory, attention, language, abstraction, and orientation [13]. Given that people with 12 years of education or less tended to have worse performance on the MoCA, one additional point was added to the MoCA score (if < 30) for participants with education < 12 years to correct for education effects [13]. According to the recommended cutoffs, cognitive function was categorized as follows: 0–22 (severe cognitive impairment), 23–26 (mild cognitive impairment), and 27–30 (no cognitive impairment) for MMSE scores; 0–19 (severe cognitive impairment), 20–24 (mild cognitive impairment), and 25–30 (no cognitive impairment) for MoCA scores [14,15]. In this analysis, cognitive impairment was defined as MMSE score < 27[14, 15] or MoCA score < 25[14, 15].

2.5. Statistical analysis

In the protocol of pre-planned ancillary study based on CATIS, we assumed 70% of patients in the control group would have MoCA-cognitive impairment and 5% of participants would be lost to follow-up at 3 months based on our pilot data. We estimated that 660 patients would provide 80% statistical power to detect a 15% reduction in MoCA cognitive impairment at a two-sided significance level of 0.05.

Baseline characteristics were compared between patients with cognitive impairment and those without cognitive impairment at 3 months after stroke using chi-squared test, Student's t-test, or Wilcoxon

Table 1
Baseline characteristics of the study participants according to cognitive impairment[a] at 3 months.

Characteristics[b]	Total	MMSE score			MoCA score		
		≥27	< 27	*p* value	≥25	< 25	*p* value
Number of participants	638	298	340		216	422	
Demographics							
Age, years	60.7 ± 10.3	58.2 ± 9.9	62.9 ± 10.2	< 0.001	57.8 ± 9.9	62.2 ± 10.2	< 0.001
Male	448 (70.2)	210 (70.5)	238 (70.0)	0.897	155 (71.8)	293 (69.4)	0.543
Education, years	7.7 ± 4.1	7.8 ± 4.0	7.6 ± 4.2	0.528	7.6 ± 3.8	7.7 ± 4.3	0.776
Current cigarette smoking	241 (37.8)	116 (38.9)	125 (36.8)	0.574	85 (39.4)	156 (37.0)	0.557
Current alcohol drinking	220 (34.5)	117 (39.3)	103 (30.3)	0.017	79 (36.6)	141 (33.4)	0.427
Medical history							
History of hypertension	494 (77.4)	232 (77.9)	262 (77.1)	0.811	169 (78.2)	325 (77.0)	0.726
History of hyperlipidemia	43 (6.7)	24 (8.1)	19 (5.6)	0.215	20 (9.3)	23 (5.5)	0.069
History of coronary heart disease	68 (10.7)	33 (11.1)	35 (10.3)	0.750	21 (9.7)	47 (11.1)	0.584
History of diabetes mellitus	109 (17.1)	47 (15.8)	62 (18.2)	0.410	34 (15.7)	75 (17.8)	0.519
Family history of stroke	102 (16.0)	60 (20.1)	42 (12.4)	0.008	47 (21.8)	55 (13.0)	0.004
Medication history							
Use of antihypertensive drugs	278 (43.6)	142 (47.7)	136 (40.0)	0.052	100 (46.3)	178 (42.2)	0.321
Use of lipid-lowering drugs	21 (3.3)	10 (3.4)	11 (3.2)	0.932	11 (5.1)	10 (2.4)	0.068
Clinical features							
Time from onset to hospitalization, h	10.7 (5.0–24.0)	11.7 (5.0–24.0)	10.0 (4.5–24.0)	0.492	11.8 (5.0–24.0)	10.3 (4.7–24.0)	0.977
Systolic BP, mmHg	167.4 ± 16.7	167.6 ± 16.0	167.2 ± 17.3	0.778	168.9 ± 16.1	166.6 ± 16.9	0.105
Diastolic BP, mmHg	98.2 ± 10.0	98.7 ± 10.2	97.8 ± 9.9	0.264	99.0 ± 10.7	97.8 ± 9.7	0.155
Body mass index, kg/m^2	24.9 ± 3.1	25.1 ± 3.1	24.7 ± 3.0	0.059	25.0 ± 3.2	24.8 ± 3.0	0.479
Fasting plasma glucose, mmol/L	5.7 (5.1–7.2)	5.8 (5.2–7.3)	5.7 (5.0–7.0)	0.095	5.8 (5.2–7.3)	5.7 (5.0–7.2)	0.089
Baseline NIHSS score	4.0 (3.0–7.0)	4.0 (2.0–6.0)	5.0 (3.0–8.0)	< 0.001	4.0 (2.0–7.0)	5.0 (3.0–7.0)	< 0.001
Ischemic stroke subtype[c]							
Thrombotic	423 (66.3)	209 (70.1)	214 (62.9)	0.055	146 (67.6)	277 (65.6)	0.621
Embolic	23 (3.6)	6 (2.0)	17 (5.0)	0.044	5 (2.3)	18 (4.3)	0.211
Lacunar	201 (31.5)	90 (30.2)	111 (32.7)	0.507	71 (32.9)	130 (30.8)	0.595
Receiving immediate BP reduction	314 (49.2)	147 (49.3)	167 (49.1)	0.958	104 (48.2)	210 (49.8)	0.699
Circulating biomarkers							
aPS, GPS (n = 489)	5.1 (3.0–9.0)	4.8 (2.9–8.3)	5.7 (3.2–9.4)	0.154	4.8 (2.9–7.8)	5.3 (3.1–9.5)	0.143
aCL, GPL (n = 489)	5.7 (4.4–7.9)	5.6 (4.2–7.9)	5.8 (4.5–7.6)	0.577	5.5 (4.1–7.4)	5.9 (4.5–8.2)	0.071
β2-GPI, SGU (n = 489)	2.3 (2.0–2.8)	2.3 (2.1–2.8)	2.3 (2.0–2.8)	0.596	2.3 (2.0–2.8)	2.3 (2.0–2.8)	0.798
RF, U/mL (n = 582)	5.1 (1.0–9.0)	4.0 (1.0–7.7)	6.3 (1.0–9.2)	0.011	3.1 (1.0–7.6)	6.0 (1.0–9.1)	0.009
NT-proBNP, pg/mL (n = 553)	147.3 (69.0–376.1)	141.3 (67.0–376.1)	161.8 (70.3–379.2)	0.511	137.0 (67.8–309.7)	164.8 (69.0–402.3)	0.249
hs-CRP, mg/L (n = 582)	1.9 (0.8–4.4)	1.6 (0.7–4.2)	2.1 (0.8–4.8)	0.132	1.6 (0.7–4.0)	2.1 (0.8–4.8)	0.102
Lp-PLA$_2$ mass, ng/mL (n = 571)	152.3 (113.8–200.7)	154.9 (113.0–201.5)	150.1 (114.6–200.7)	0.817	155.7 (117.9–194.7)	150.1 (113.2–202.5)	0.974
MMP-9, ng/mL (n = 558)	574.3 (330.6–895.2)	503.3 (280.4–763.1)	607.0 (392.8–954.8)	< 0.001	512.4 (278.8–838.6)	589.1 (371.7–933.1)	0.023
tHcy, μmol/L (n = 566)	14.6 (11.3–21.9)	13.9 (11.0–20.3)	15.4 (11.7–23.0)	0.017	14.3 (11.1–21.3)	14.9 (11.4–22.4)	0.213
eGFR, mL/min per 1.73 m^2 (n = 576)	106.0 (93.9–114.2)	108.2 (97.5–115.7)	104.8 (92.5–112.4)	0.006	108.5 (96.5–117.5)	105.1 (93.2–112.9)	0.027
Uric acid, μmol/L (n = 378)	298.0 (243.0–354.0)	295.9 (243.0–357.0)	300.0 (244.0–350.1)	0.860	299.5 (246.0–361.0)	297.5 (241.0–350.6)	0.590
HGF, ng/mL (n = 573)	1.4 (1.1–1.7)	1.4 (1.1–1.7)	1.4 (1.1–1.8)	0.426	1.4 (1.1–1.7)	1.4 (1.2–1.8)	0.388

MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; BP, blood pressure; NIHSS, National Institute of Health Stroke Scale; aPS, antiphosphatidylserine antibodies; GPS, IgG antiphosphatidylserine antibodies units; aCL, anticardiolipin antibodies; GPL, IgG anticardiolipin antibodies units; β2-GP1, beta(2)-glycoprotein 1-dependent anticardiolipin antibodies; SGU, IgG beta(2)-glycoprotein 1-dependent anticardiolipin antibodies units; RF, rheumatoid factor; NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; Lp-PLA$_2$, lipoprotein-associated phospholipase A2; MMP-9, matrix metalloproteinase-9; tHcy, total homocysteine; eGFR, estimated glomerular filtration rate; HGF, hepatocyte growth factor.

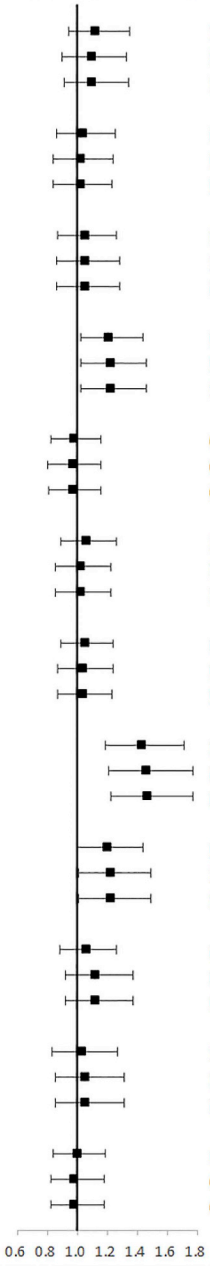
[a] MMSE score < 27 or MoCA score < 25 indicate cognitive impairment.

[b] Continuous variables are expressed as mean ± standard deviation, or as median (interquartile range). Categorical variables are expressed as frequency (percent).

[c] One patient with both thrombotic and embolic subtypes; 9 patients with thrombotic and lacunar subtypes; 1 patient with embolic and lacunar subtypes; 1 patient with all 3 subtypes.

rank-sum test when appropriate. Logistic regression models were used to assess the independent association between each baseline circulating biomarker and the risk of subsequent cognitive impairment assessed by MMSE or MoCA scores, and odds ratio (OR) and 95% confidence interval (CI) was calculated for per SD increment of each log-transformed circulating biomarker. We performed 3 multiple-adjusted logistic regression models. Model 1 adjusted for age, sex, and education. Model 2 included the factors in model 1 as well as time from onset to hospitalization, current smoking, alcohol consumption, body mass index, National Institutes of Health Stroke Scale score, blood glucose and systolic BP at baseline, history of hypertension, history of coronary heart disease, history of diabetes mellitus, history of hyperlipidemia, family history of stroke, and ischemic stroke subtypes. In the Model 3, we adjusted for the factors in Model 2 and further adjusted for the

Biomarkers[a] (per SD increment)	Cognitive impairment (MMSE < 27) Odds Ratio (95% CI)	*p* value	Cognitive impairment (MoCA < 25) Odds Ratio (95% CI)	*p* value
aPS				
Model 1	1.12 (0.94-1.35)	0.213	1.12 (0.93-1.35)	0.241
Model 2	1.10 (0.90-1.33)	0.356	1.11 (0.91-1.36)	0.292
Model 3	1.10 (0.91-1.34)	0.337	1.11 (0.91-1.36)	0.299
aCL				
Model 1	1.04 (0.86-1.25)	0.690	1.20 (0.98-1.46)	0.079
Model 2	1.02 (0.84-1.24)	0.851	1.21 (0.98-1.49)	0.074
Model 3	1.02 (0.84-1.23)	0.862	1.22 (0.98-1.50)	0.071
β2-GPI				
Model 1	1.05 (0.87-1.26)	0.628	1.07 (0.88-1.31)	0.496
Model 2	1.05 (0.86-1.28)	0.650	1.05 (0.85-1.30)	0.661
Model 3	1.05 (0.86-1.28)	0.645	1.05 (0.85-1.30)	0.667
RF				
Model 1	1.21 (1.02-1.44)	0.031	1.25 (1.04-1.50)	0.019
Model 2	1.22 (1.02-1.46)	0.029	1.25 (1.03-1.51)	0.021
Model 3	1.22 (1.02-1.46)	0.030	1.25 (1.03-1.51)	0.021
NT-proBNP				
Model 1	0.98 (0.82-1.16)	0.783	1.02 (0.85-1.22)	0.821
Model 2	0.97 (0.80-1.16)	0.699	1.03 (0.85-1.24)	0.790
Model 3	0.97 (0.81-1.16)	0.700	1.03 (0.85-1.24)	0.790
hs-CRP				
Model 1	1.06 (0.89-1.26)	0.513	1.05 (0.88-1.26)	0.583
Model 2	1.02 (0.85-1.22)	0.849	1.04 (0.86-1.25)	0.698
Model 3	1.02 (0.85-1.22)	0.846	1.04 (0.86-1.25)	0.698
Lp-PLA_2 mass				
Model 1	1.05 (0.89-1.24)	0.577	1.02 (0.86-1.22)	0.820
Model 2	1.04 (0.87-1.24)	0.681	1.02 (0.85-1.23)	0.819
Model 3	1.04 (0.87-1.23)	0.695	1.02 (0.85-1.23)	0.813
MMP-9				
Model 1	1.43 (1.19-1.71)	<0.001	1.35 (1.12-1.61)	0.001
Model 2	1.46 (1.21-1.77)	<0.001	1.36 (1.12-1.64)	0.002
Model 3	1.47 (1.22-1.77)	<0.001	1.36 (1.12-1.64)	0.002
tHcy				
Model 1	1.20 (0.99-1.44)	0.058	1.12 (0.93-1.36)	0.243
Model 2	1.22 (1.01-1.49)	0.044	1.15 (0.94-1.41)	0.175
Model 3	1.22 (1.01-1.49)	0.044	1.15 (0.94-1.41)	0.175
eGFR				
Model 1	1.06 (0.88-1.26)	0.553	1.10 (0.91-1.32)	0.332
Model 2	1.12 (0.92-1.37)	0.249	1.12 (0.91-1.38)	0.267
Model 3	1.12 (0.92-1.37)	0.252	1.13 (0.92-1.39)	0.263
Uric acid				
Model 1	1.03 (0.83-1.27)	0.788	1.08 (0.88-1.34)	0.460
Model 2	1.05 (0.85-1.31)	0.655	1.10 (0.88-1.37)	0.389
Model 3	1.05 (0.85-1.31)	0.640	1.11 (0.89-1.39)	0.356
HGF				
Model 1	1.00 (0.84-1.19)	0.996	1.06 (0.89-1.27)	0.524
Model 2	0.98 (0.82-1.18)	0.854	1.03 (0.86-1.25)	0.742
Model 3	0.98 (0.82-1.18)	0.854	1.03 (0.86-1.25)	0.737



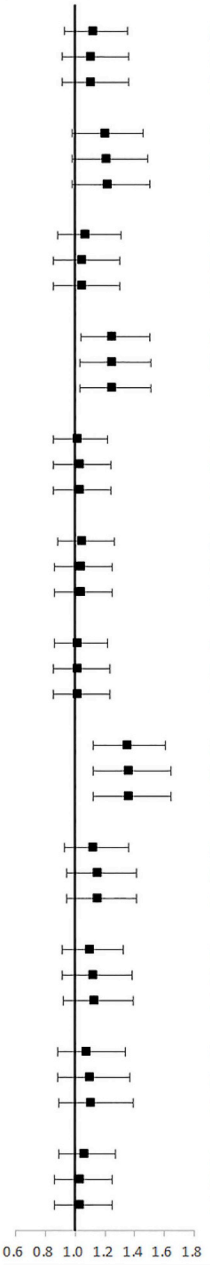


Fig. 1. Multivariate adjusted odds ratios of cognitive impairment at 3 months according to circulating biomarkers in the acute phase of ischemic stroke.
[a] Natural log transformed. Model 1, adjusted for age, sex and education. Model 2, adjusted for Model 1 and further adjusted for time from onset to hospitalization, current smoking, alcohol consumption, body mass index, National Institutes of Health Stroke Scale score, blood glucose and systolic blood pressure at baseline, history of hypertension, history of coronary heart disease, history of diabetes mellitus, history of hyperlipidemia, family history of stroke, and ischemic stroke subtypes. Model 3, adjusted for Model 2 and further adjusted for the difference in systolic blood pressure between baseline and 7 days. MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; aPS, antiphosphatidylserine antibodies; aCL, anticardiolipin antibodies; β2-GP1, beta (2)-glycoprotein 1-dependent anticardiolipin antibodies; RF, rheumatoid factor; NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-CRP, high-sensitivity C-reactive protein; Lp-PLA_2, lipoprotein-associated phospholipase A2; MMP-9, matrix metalloproteinase-9; tHcy, total homocysteine; eGFR, estimated glomerular filtration rate; HGF, hepatocyte growth factor.

difference in systolic BP between baseline and 7 days.

In the secondary analyses, we screened out the novel biomarkers which we found to be significantly associated with post-stroke cognitive impairment after adjustment for traditional risk factors, and a receiver operating characteristic curve was configured to establish cutoff points for them. High level of significantly associated biomarker was defined as circulating risk factor of post-stroke cognitive impairment, and patients were categorized into different groups according to the number of circulating risk factors. Categorical and ordinal logistic regression was used to estimate the effect of circulating risk factors clustering on the cognitive impairment and cognitive impairment severity after adjustment for the aforementioned covariates. In order to assess improvement in the risk prediction of post-stroke cognitive impairment, C statistics [16], net reclassification index (NRI), and integrated discrimination improvement (IDI) [17] were used to compare conventional risk factors model with a model that additionally adding all biomarkers that showed a statistically significant association with cognitive impairment in our main analysis. Two-tailed $p < 0.05$ was considered to be statistically significant. All analyses were conducted using SAS statistical software (version 9.4, Cary, NC, USA).

3. Results

3.1. Baseline characteristics

A total of 638 patients (448 males and 190 females) were included in the present study and the average age was 60.7 ± 10.3 years. According to MMSE score at 3 months after stroke, those with cognitive impairment were more likely to be older; with higher baseline NIHSS score, RF, MMP-9, and tHcy; higher percentage of embolic; lower eGFR; and lower percentage of alcohol drinking and family history of stroke than those with normal cognitive function. According to MoCA score at 3 months after stroke, those with cognitive impairment were more likely to be older; with higher baseline NIHSS score, RF, and MMP-9; lower eGFR; and lower percentage of family history of stroke than those with normal cognitive function (Table 1 and Supplemental Fig. 2).

3.2. Association between circulating biomarkers and post-stroke cognitive impairment

According to MMSE score at 3 months after stroke, a total of 340 patients had cognitive impairment (Table 1). Among all 12 biomarkers tested, only RF, MMP-9, and tHcy were found to be associated with subsequent cognitive impairment after adjustment for potential confounders. Per one SD increase of log-transformed RF, log-transformed MMP-9, and log-transformed tHcy was associated with a 22% (95% CI, 2%–46%; $p = 0.030$), 47% (95% CI, 22%–77%; $p < 0.001$), and 22% (95% CI, 1%–49%; $p = 0.044$) increased risk of cognitive impairment, respectively (Fig. 1).

The MMSE score at 3 months after stroke decreased with the number of circulating risk factors clustering (Supplemental Fig. 3), and the patients with 3 circulating risk factors had the highest incidence of cognitive impairment according to MMSE at 3 months after stroke (Supplemental Table 1). Moreover, the severity of cognitive impairment also increased with the number of circulating risk factors clustering (χ [2] = 24.82, $p < 0.001$; Fig. 2 and Supplemental Table 1). After adjustment for potential confounders, the ORs associated with 3 circulating risk factors were 4.89 (95% CI, 2.31–10.35; $p_{trend} < 0.001$) and 3.09 (95% CI, 1.60–5.98; $p_{trend} < 0.001$) for cognitive impairment and the severity of cognitive impairment, respectively (Fig. 3). In addition, the MMSE scores of most cognitive subdomains were lower in patients with more circulating risk factors clustering (Supplemental Table 2). Similar findings were observed when cognitive function was measured by MoCA score.

3.3. Incremental predictive value of multiple biomarkers for post-stroke cognitive impairment

As shown in Table 2, adding all 3 biomarkers (RF, MMP-9 and tHcy) to the conventional model significantly improve discriminatory power (MMSE score < 27: C statistic 0.729 vs. 0.688; MoCA score < 25: C statistic 0.717 vs. 0.692; all p value < 0.05), and significantly improve reclassification for the risk of cognitive impairment (MMSE score < 27: NRI = 33.67%, IDI = 4.61%; MoCA score < 25: NRI = 24.92%, IDI = 2.67%; all p value < 0.05).

4. Discussion

To our knowledge, this is the first prospective multicenter study to use a comprehensive set of circulating biomarkers involving different pathways to examine the improvement in the risk-predictive ability for post-stroke cognitive impairment beyond traditional risk factors. Among the 12 blood biomarkers measured in the present study, RF, MMP-9 and tHcy were found to be independently associated with post-stroke cognitive impairment. The risk of cognitive impairment increased with the number of circulating risk factors clustering, and

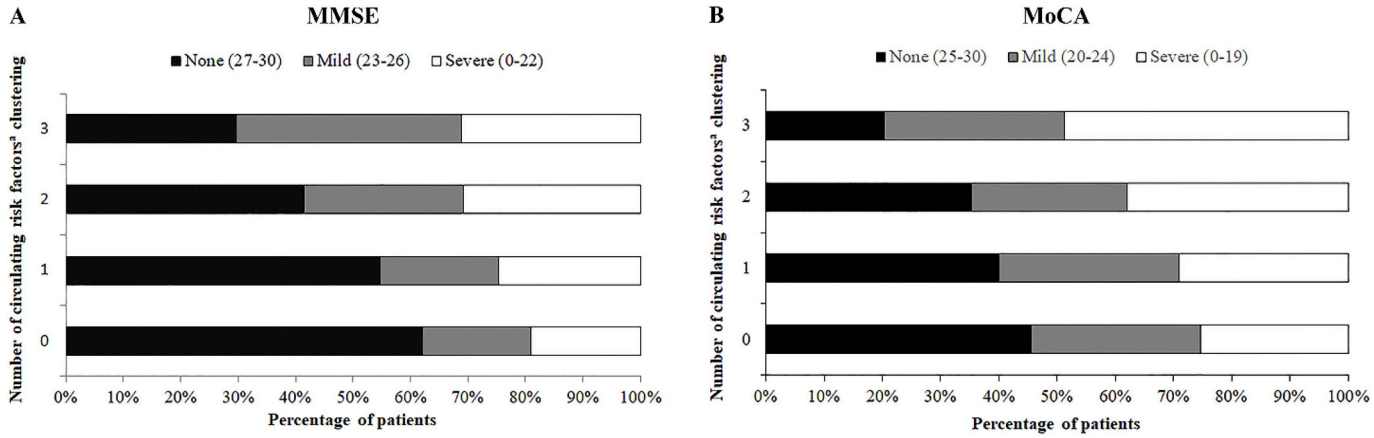


Fig. 2. Number of circulating risk factors[a] clustering and cognitive impairment severity.
(A) The distribution of cognitive impairment severity according to MMSE is different across the number of circulating risk factors (χ [2] = 24.82, $p < 0.001$). (B) The distribution of cognitive impairment severity according to MoCA is different across the number of circulating risk factors (χ [2] = 17.17, $p = 0.009$). [a] Circulating risk factors include high serum rheumatoid factor, matrix metalloproteinase-9, and plasma total homocysteine according to Fig. 1. High serum rheumatoid factor, matrix metalloproteinase-9, and plasma total homocysteine were defined as ≥6.47 U/mL, 462.6 ng/mL and 14.8 μmol/L, respectively (optimal cut point obtained from the ROC curve).

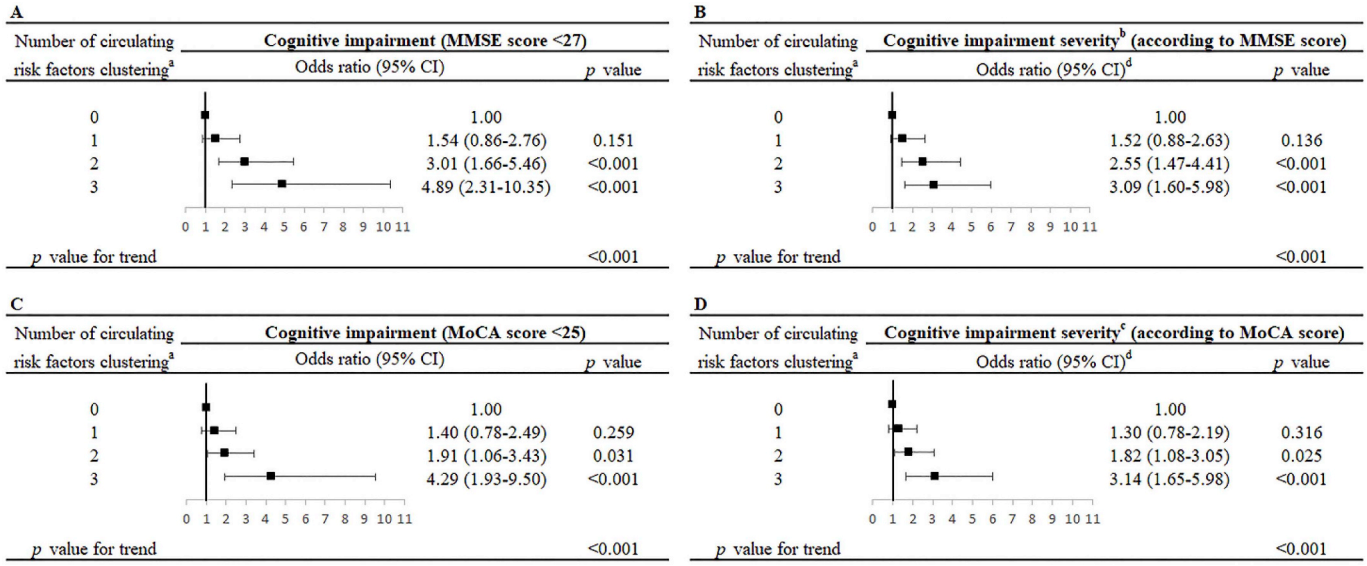


Fig. 3. Odds ratios and 95% confidence interval of 3-month cognitive impairment associated with circulating risk factors[a] clustering among ischemic stroke patients[e]. Odds ratios were adjusted for the same variables as Model 3 in Fig. 1 [a] Circulating risk factors include high serum rheumatoid factor, matrix metalloproteinase-9, and plasma total homocysteine according to Fig. 1. High serum rheumatoid factor, matrix metalloproteinase-9, and plasma total homocysteine were defined as ≥6.47 U/mL, 462.6 ng/mL and 14.8 μmol/L, respectively (Optimal cut point obtained from the ROC curve). [b] Cognitive impairment severity was according to MMSE categories: none (27–30), mild (23–26), severe (0–22). [c] Cognitive impairment severity was according to MoCA categories: none (25–30), mild (20–24), severe (0–19). [d] Odds ratios are derived from ordinal regression. [e] There were 541 patients included in this analysis, who has all data of serum rheumatoid factor, matrix metalloproteinase-9, and total homocysteine.

adding all these three biomarkers to conventional risk factors significantly improved the risk prediction for cognitive impairment. These findings suggested that RF, MMP-9 and tHcy might simultaneously contribute to the pathogenesis of post-stroke cognitive impairment, and their combination could provide additionally predictive information for cognitive impairment among ischemic stroke patients with elevated blood pressure.

RF is an autoantibody targeting the Fc region of IgG antibodies [18]. Animal and human studies have found that autoimmune process may play a role in the development of post-stroke cognitive decline [19], but the effect of RF on the post-stroke cognitive function is unclear. Our study adds new information on the relationship between autoimmune process and the risk of post-stroke cognitive decline, with findings that there is an elevated risk of post-stroke cognitive impairment with increasing levels of RF. The primary harmful mechanism of autoantibodies in the central nervous system is inhibition of signal transduction and interference with neuronal function [20].

MMP-9 is a key determinant of extracellular matrix degradation [21], and it is reported that patients with vascular cognitive impairment have elevated levels of MMP-9 in the brain tissues and cerebrospinal fluid [22,23]. In this study, our findings are consistent with previous studies and extend the association between MMP-9 and cognitive impairment to serum terms. Abnormal MMP-9 expression and activity play a critical role in the proteolytic degradation of the blood-brain barrier, which may induce cognitive impairment [21,24].

Homocysteine is a sulfur-containing amino acid, and it has already emerged as an independent risk factor for dementia in general population [25]. One previous study including 81 stroke patients found that high tHcy could also increase the risk of post-stroke cognitive impairment [26]. In the present study, we investigate the association between them in a larger prospective cohort, and we found a significant association between tHcy and post-stroke cognitive impairment. Elevated plasma tHcy is well known to cause endothelial dysfunction [27] which is associated with hemorrhagic transformation after ischemic stroke [28], and brain hemorrhages have been associated with cognitive decline or dementia [29], which may be a potential pathway of the

Table 2
Reclassification and discrimination statistics for 3-month cognitive impairment by circulating risk biomarkers among acute ischemic stroke patients.[a]

Cognitive impairment	Model	C statistics		Continuous NRI, %		IDI, %	
		Estimate (95% CI)	*p* value	Estimate (95% CI)	*p* value	Estimate (95% CI)	*p* value
MMSE score < 27	Conventional model[b]	0.688 (0.647–0.727)					
	Conventional model[b] + RF[c] + MMP-9[c] + tHcy[c]	0.729 (0.690–0.766)	0.004	33.67 (17.07–50.27)	< 0.001	4.61 (2.86–6.36)	< 0.001
MoCA score < 25	Conventional model[b]	0.692 (0.652–0.731)					
	Conventional model[b] + RF[c] + MMP-9[c] + tHcy[c]	0.717 (0.677–0.754)	0.047	24.92 (7.55–42.29)	0.005	2.67 (1.25–4.09)	< 0.001

MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; RF, rheumatoid factor; MMP-9, matrix metalloproteinase-9; tHcy, total homocysteine; IDI, integrated discrimination index; NRI, net reclassification improvement.

[a] There were 541 patients included in this analysis, who has all data of serum rheumatoid factor, matrix metalloproteinase-9, and total homocysteine.

[b] Conventional model included age, sex and education, time from onset to hospitalization, current smoking, alcohol consumption, body mass index, National Institutes of Health Stroke Scale score, blood glucose and systolic blood pressure at baseline, history of hypertension, history of coronary heart disease, history of diabetes mellitus, history of hyperlipidemia, family history of stroke, ischemic stroke subtypes, and the difference in systolic blood pressure between baseline and 7 days.

[c] Serum RF, MMP-9, and plasma tHcy were classified into two categories (high or low). The cut-off point was defined as 6.47 U/mL, 462.6 ng/mL and 14.8 μmol/L, respectively (optimal cut point obtained from the ROC curve).

association between plasma tHcy and post-stroke cognitive impairment.

Due to the multifactorial pathophysiology of post-stroke cognitive impairment, it seems reasonable that the better accuracy of risk prediction could be obtained by a panel combining markers from the different pathways. Herein, adding the combination of RF (reflecting autoimmune processes), MMP-9 (reflecting blood-brain barrier damage), and tHcy (reflecting endothelial dysfunction) to the traditional risk factors showed a significant improvement in C statistic, NRI and IDI, illustrating that these biomarkers convey complementary information related to different biological pathways. Moreover, when considering subdomains of cognitive function, the MMSE or MoCA scores of most subdomains (MMSE: orientation, attention and calculation, recall, language, visuospatial; MoCA: visuospatial/executive, naming, memory, language, orientation) were lower in patients with simultaneously high levels of these three biomarkers. Therefore, RF, MMP-9 and tHcy could be measured simultaneously among acute ischemic stroke patients with elevated blood pressure in future clinical practice, and ischemic stroke patients with high levels of them should receive aggressive monitoring to prevent subsequent cognitive impairment. In addition, biomarkers that are predictors of post-stroke cognitive impairment in our study may also suggest novel treatment targets, so further studies are needed to explore their roles as potential therapeutic targets.

This study has several important strengths. First, it focuses on an important clinical issue: identifying patients at the highest risk of post-stroke cognitive impairment. Unlike most blood biomarker studies that focus on one or at most a few biomarkers, we systematically measured 12 biomarkers from several pathophysiological pathways. Second, this prospective study is by far the largest biomarker study of post-stroke cognitive impairment, and it is from the CATIS randomized clinical trial with standardized protocols and rigid quality control procedures in data collection and outcome assessment. Third, comprehensive information about relevant covariates was controlled in the multivariable models. In addition, both MMSE and MoCA tests were used to assess cognitive performance, and the consistent results validated our findings. Therefore, the present study in method was appropriate and rigorous, providing a more valid appraisal of multiple biomarkers on the improvement for risk prediction of cognitive impairment after ischemic stroke. However, some limitations should be discussed here. First, the participants are from a random sample of CATIS trial, a selection bias may exist. However, baseline characteristics of participants in this study were similar to those from the China National Stroke Registry [30], indicating that the selection bias might be minimal. Second, the biomarkers included in this study were selected based on previous reports, other novel biomarkers that were not tested might have additional predictive information. Finally, we did not test baseline cognitive function using MMSE or MoCA score for patients, so we could not control the potential confounding effect of pre-stroke cognitive impairment. However, NIHSS has a subset of cognitive function assessment [31], and baseline cognitive function is also highly correlated with baseline NIHSS score, age, sex, education, diabetes and other baseline characteristics [6,32]. We adjusted all of them (including baseline NIHSS score) in the analysis, indicating that the effect of baseline cognitive function on the results was minimal.

In summary, we found that combination of RF, MMP-9, and tHcy, representing different pathophysiological pathways, could improve the risk prediction of cognitive impairment in ischemic stroke patients with elevated blood pressure. Further studies are required to ensure the validity of our findings and to determine whether these biomarkers could serve as therapeutic targets for post-stroke cognitive impairment.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

Tan Xu, Jiang He, and Yonghong Zhang conceived and designed the study. Tan Xu and Yonghong Zhang coordinated the study. Zhengbao Zhu, Chongke Zhong, Tan Xu, Libing Guo, Jiale Liu, Jintao Zhang, Dong Li, Jianhui Zhang, Zhong Ju, Chung-Shiuan Chen, Jing Chen, Jiang He, and Yonghong Zhang oversaw subject recruitment and monitored gathering of clinical data. Zhengbao Zhu, Chongke Zhong, Daoxia Guo, Xiaoqing Bu, and Yonghong Zhang performed the experiments. Zhengbao Zhu and Yonghong Zhang conducted the statistical analysis and prepared the paper. Jiang He and Yonghong Zhang revised the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.05.028.

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