ORIGINAL RESEARCH

Serum Matrix Metalloproteinase-9 and Cognitive Impairment After Acute Ischemic Stroke

Chongke Zhong, MD; Xiaoqing Bu, MD, PhD; Tan Xu, MD, PhD; Libing Guo, MD; Xuemei Wang, MD; Jintao Zhang, MD; Yong Cui, MD; Dong Li, MD; Jianhui Zhang, MD; Zhong Ju, MD, PhD; Chung-Shiuan Chen, MS; Jing Chen, MS, MSc; Yonghong Zhang, MD, PhD; Jiang He, MD, PhD

***Background*—**The impact of serum matrix metalloproteinases-9 (MMP-9) on cognitive impairment after ischemic stroke is unclear. We aimed to investigate the association between serum MMP-9 in the short-term acute phase of ischemic stroke and cognitive impairment at 3 months.

***Methods and Results*—**Our study was based on a subsample from the CATIS (China Antihypertensive Trial in Acute Ischemic Stroke); a total of 558 patients with serum MMP-9 levels from 7 of 26 participating sites of the trial were included in this analysis. Cognitive impairment severity was categorized as severe, mild, or none (Mini-Mental State Examination score, <23, 23–26, or ≥27, respectively; Montreal Cognitive Assessment score, <20, 20–24, or ≥25, respectively). Cognitive impairment was defined as a score of <27 for Mini-Mental State Examination or <25 for Montreal Cognitive Assessment. According to Mini-Mental State Examination score, 143 participants (25.6%) had mild cognitive impairment and 153 (27.4%) had severe cognitive impairment at 3 months. After adjustment for age, National Institutes of Health stroke score, education, and other covariates, the odds ratio for the highest quartile of serum MMP-9 compared with the lowest quartile was 3.20 (95% confidence interval, 1.87–5.49) for cognitive impairment. Multiple-adjusted spline regression model showed a linear association between MMP-9 levels and cognitive impairment (P<0.001 for linearity). Sensitivity and subgroup analyses further confirmed these results. Similar significant findings were observed when cognitive impairment was defined by Montreal Cognitive Assessment score.

***Conclusions*—**Increased serum MMP-9 levels in the short-term phase of ischemic stroke were associated with 3-month cognitive impairment, independently of established risk factors. **(*J Am Heart Assoc*. 2018;7:e007776. DOI: 10.1161/JAHA.117.007776.)**

Key Words: cognitive impairment • ischemic stroke • matrix metalloproteinase-9 • Mini-Mental State Examination • Montreal Cognitive Assessment

Cognitive impairment and dementia are common after stroke and can substantially affect patients' quality of life.[1] Approximately 1 in 4 patients with stroke has severe cognitive impairment,[2,3] and 1 in 3 has milder levels of cognitive impairment.[4] Accurate identification of novel risk factors to improve the prediction of cognitive function after an ischemic stroke is highly desirable to rapidly optimize patient care and management.

Matrix metalloproteinase-9 (MMP-9) is a key determinant of extracellular matrix degradation and is the most widely

From the Department of Epidemiology, School of Public Health and Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Medical College of Soochow University, Suzhou, China (C.Z., X.B., T.X., Y.Z.); Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA (C.Z., X.B., C.-S.C., J.C., J.H.); Department of Neurology, Siping Central Hospital, Jilin, China (L.G.); Department of Neurology, Jilin Central Hospital, Jilin, China (X.W.); Department of Neurology, The 88th Hospital of PLA, Shandong, China (J.Z.); Department of Neurology, General Hospital of First Automobile Works, Jilin, China (Y.C.); Department of Internal Medicine, Feicheng City People's Hospital, Shandong, China (D.L.); Department of Neurology, Tongliao Municipal Hospital, Inner Mongolia, China (J.Z.); Department of Neurology, Kerqin District First People's Hospital of Tongliao City, Inner Mongolia, China (Z.J.); and Department of Medicine, Tulane University School of Medicine, New Orleans, LA (J.C., J.H.).

Accompanying Tables S1 through S3 are available at http://jaha.ahajournals.org/content/7/1/e007776/DC1/embed/inline-supplementary-material-1.pdf

Correspondence to Yonghong Zhang, MD, PhD, Department of Epidemiology, School of Public Health and Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Medical College of Soochow University, 199 Renai Rd, Industrial Park District, Suzhou, Jiangsu Province 215123, China. E-mail: yhzhang@suda.edu.cn or Jiang He, MD, PhD, Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112. E-mail: jhe@tulane.edu

Received October 4, 2017; accepted November 30, 2017.

Clinical Perspective

What Is New?

- Increased serum matrix metalloproteinase-9 levels in the short-term phase of ischemic stroke were associated with 3-month cognitive impairment, independently of established risk factors.
- Adding serum matrix metalloproteinase-9 to conventional risk factors improved risk prediction for cognitive impairment.

What Are the Clinical Implications?

- Our findings suggested serum matrix metalloproteinase-9 could provide important predictive information for cognitive impairment after acute ischemic stroke.

investigated enzyme of the MMP family in acute ischemic stroke.[5,6] MMP-9 participates in several normal biological processes, including nerve growth, bone remodeling, wound healing, and angiogenesis.[7,8] Accumulating evidence suggests that elevated MMP-9 is involved in the neuropathological processes, such as inflammation, blood-brain barrier damage, and neuronal cell death, which may lead to cognitive impairment.[9–11] It is reported that the brain tissues and cerebrospinal fluid of patients with vascular cognitive impairment have increased levels of MMP-9.[12,13] However, little is known about the relationship between serum MMP-9 level and subsequent cognitive impairment after acute ischemic stroke.

In this study, we aimed to assess the association between serum MMP-9 in the short-term phase of ischemic stroke and cognitive impairment at 3 months, using data derived from the CATIS (China Antihypertensive Trial in Acute Ischemic Stroke).

Methods

Study Design and Participants

The data and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. This prospective observational study was based on a preplanned ancillary study, which was designed to examine the effect of early blood pressure (BP) reduction on cognitive function at 3 months after randomization among a subsample of CATIS. The methods and main results for both studies have been described previously.[14,15] The CATIS was a multicenter, single-blind, blinded end points randomized clinical trial conducted among 4071 patients with ischemic stroke to test whether moderate lowering of BP within the first 48 hours after the onset of an acute ischemic stroke would reduce death and major disability at 14 days or hospital discharge.[14] Eligible participants were those aged ≥22 years who had ischemic stroke, confirmed by computed tomography or magnetic resonance imaging within 48 hours of symptom onset, and who had an elevated systolic BP between 140 and <220 mm Hg. Participants of the preplanned ancillary study were selected before randomization from 7 participating hospitals.[15] Each of the 7 participating hospitals recruited 80 to 100 patients consecutively. From August 2009 to November 2012, 660 participants were recruited for cognitive function assessment at 3-month follow-up after stroke. At the 3-month visit, 15 patients were unavailable for follow-up and 7 patients were deceased. A total of 638 participants completed the cognitive function tests. Of these participants, some did not offer blood samples and some collected samples were hemolysed in storage or transport; 558 patients were finally included in the present analysis (Figure 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.

Assessment of Serum MMP-9 and Potential Covariates

Blood samples were collected after at least 8 hours of fasting within 24 hours of hospital admission. Serum samples were separated and frozen at −80°C in the Central Laboratory of the School of Public Health at Soochow University until testing. Serum MMP-9 concentrations were measured using a

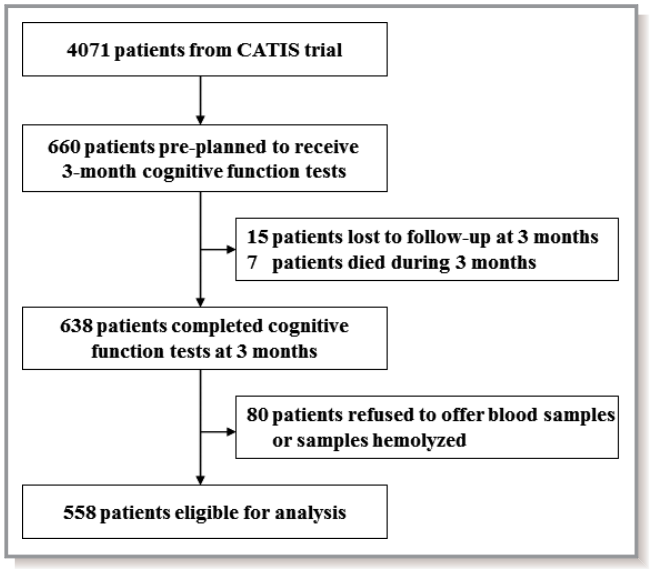

Figure 1. Flow chart of participants' selection. CATIS indicates China Antihypertensive Trial in Acute Ischemic Stroke.

Table 1. Characteristics of Participants According to Serum MMP-9 Quartiles

Characteristics*	MMP-9, ng/mL					*P* Value for Trend
	Total	<330.1	330.1–567.6	567.6–893.3	≥893.3	
No. of subjects	558	138	141	139	140	. . .
Age, y	60.7±10.3	62.4±10.0	61.4±10.1	59.1±10.5	59.9±10.6	0.01
Male sex	385 (69.0)	83 (60.1)	99 (70.2)	101 (72.7)	102 (72.9)	0.02
Education, y	6.9±3.6	7.2±3.6	6.7±3.7	7.1±3.5	6.6±3.6	0.28
Current cigarette smoking	209 (37.5)	39 (28.3)	45 (31.9)	61 (43.9)	64 (45.7)	0.001
Current alcohol drinking	190 (34.1)	32 (23.2)	46 (32.6)	54 (38.9)	58 (41.4)	0.001
Time from onset to randomization, h	10.3 (5.0–24.0)	12.0 (5.0–24.0)	12.0 (5.0–24.0)	10.0 (5.0–24.0)	8.0 (4.0–23.0)	0.01
Baseline systolic BP, mm Hg	167.3±16.6	167.4±15.0	166.8±16.8	166.0±15.7	169.0±18.6	0.52
Baseline diastolic BP, mm Hg	98.1±9.9	96.2±9.6	98.4±10.1	98.4±9.9	99.5±10.1	0.01
Body mass index, kg/m^2	24.8±3.1	24.8±3.2	24.6±3.1	24.9±2.9	25.0±3.0	0.50
Baseline NIHSS score	4.0 (3.0–7.0)	4.0 (2.0–7.0)	4.0 (3.0–7.0)	4.0 (3.0–7.0)	4.0 (3.0–7.0)	0.06
History of hypertension	434 (77.8)	111 (80.4)	115 (81.7)	106 (76.3)	102 (72.9)	0.08
History of hyperlipidemia	40 (7.2)	13 (9.4)	9 (6.4)	6 (4.3)	12 (8.6)	0.64
History of diabetes mellitus	96 (17.2)	25 (18.1)	30 (21.3)	26 (18.7)	15 (10.7)	0.08
History of coronary heart disease	61 (10.9)	20 (14.5)	12 (8.5)	11 (7.9)	18 (12.9)	0.65
Family history of stroke	92 (16.5)	24 (17.4)	19 (13.7)	21 (14.9)	28 (20.0)	0.52
Use of antihypertensive drugs	251 (45.0)	66 (47.8)	67 (47.5)	65 (46.8)	53 (37.9)	0.10
Use of lipid-lowering drugs	20 (3.6)	5 (3.6)	5 (3.6)	4 (2.9)	6 (4.3)	0.85
Ischemic stroke subtype						0.01†
Thrombotic	348 (62.4)	88 (63.8)	93 (66.0)	93 (66.9)	74 (52.9)	
Embolic	21 (3.8)	1 (0.7)	3 (2.1)	5 (3.6)	12 (8.6)	
Lacunar	189 (33.9)	49 (35.5)	45 (31.9)	41 (29.5)	54 (38.6)	
Receiving immediate BP reduction	271 (48.6)	72 (52.2)	72 (51.1)	58 (41.7)	69 (49.3)	0.34

BP indicates blood pressure; MMP-9, matrix metalloproteinase-9; and NIHSS, National Institutes of Health Stroke Scale.
*Continuous variables are expressed as mean±SD or as median (interquartile range). Categorical variables are expressed as frequency (percentage).
†*P* value for overall variation.

commercially available ELISA kit (R&D Systems, Minneapolis, MN). Intra-assay and interassay coefficients of variation were 2.0% and 6.9%, respectively. MMP-9 determinations were performed by laboratory technicians blinded to the clinical characteristics and outcomes.

Data on demographic characteristics, lifestyle risk factors, and medical history were collected at enrollment. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) by trained neurologists at admission.[16] Three BP measurements were obtained at baseline by trained nurses, according to a common protocol adapted from procedures recommended by the American Heart Association.[17]

Assessment of Outcomes

The study outcome was cognitive impairment at 3 months, assessed by trained neurologists using the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA).[18,19] The MMSE contains 20 items that test cognitive performance in domains including orientation, registration, attention and calculation, recall, language, and visual construction.[18] The MoCA is a 30-item test that evaluates the following 7 cognitive domains: visuospatial/executive functions, naming, memory, attention, language, abstraction, and orientation.[19] Previous studies found that people with 12 years of education or less tended to have worse performance on the MoCA; to correct for education effects, 1 point was added for participants with education <12 years on their total MoCA score (if <30).[19] Both MMSE and MoCA have been translated into Chinese and validated as a screening tool for cognitive impairment and dementia in the Chinese population; the maximum score is 30 for these 2 measurements. According to the recommended cutoffs, cognitive function was categorized as follows: 0 to 22 (severe cognitive

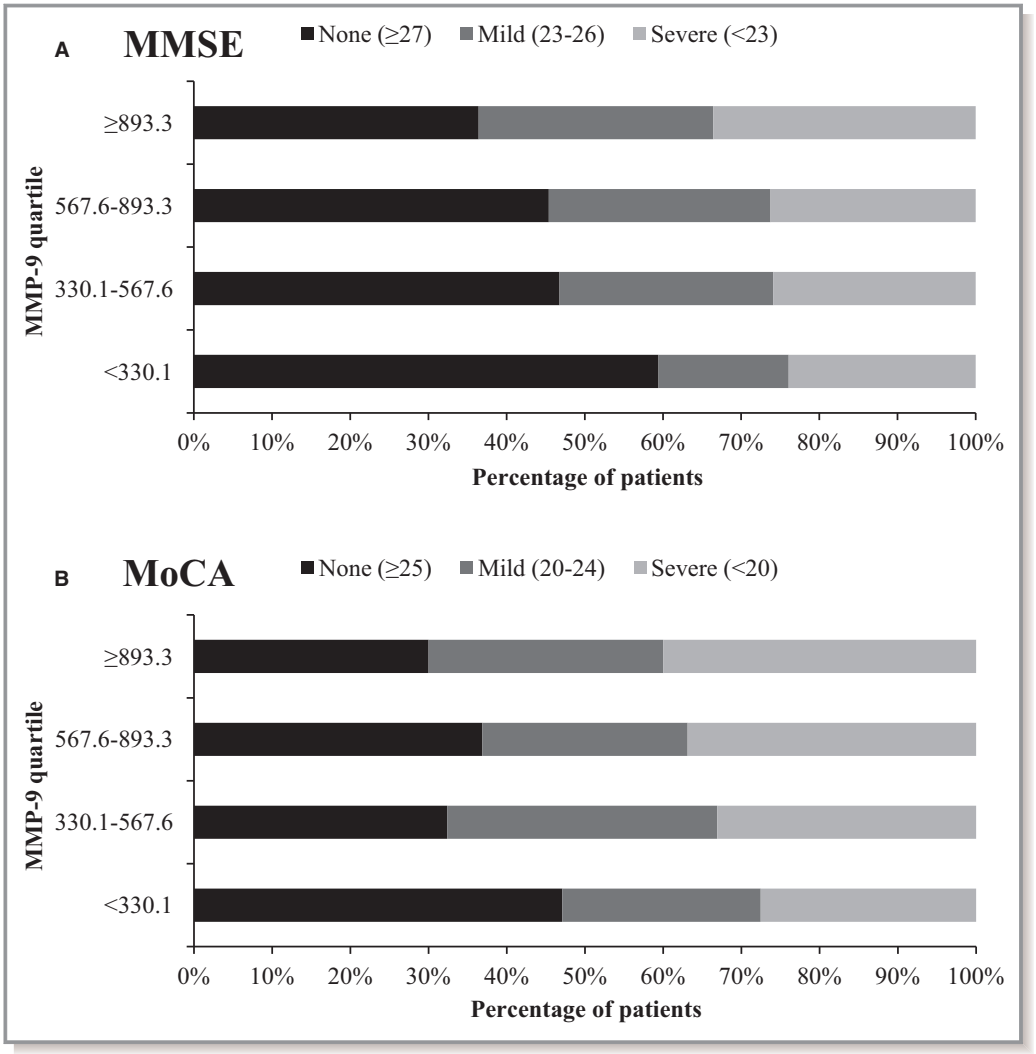

Figure 2. Serum matrix metalloproteinase-9 (MMP-9) and cognitive impairment severity. Adjusted odds ratio of ordinal logistic regression analysis for highest vs lowest quartile of serum MMP-9: 2.55 (95% confidence interval, 1.58–4.12; *P*=0.002 for trend) for Mini-Mental State Examination (MMSE) (A); and 2.08 (95% confidence interval, 1.30–3.33; *P*=0.007 for trend) for Montreal Cognitive Assessment (MoCA).

impairment), 23 to 26 (mild cognitive impairment), and 27 to 30 (no cognitive impairment) for MMSE scores; 0 to 19 (severe cognitive impairment), 20 to 24 (mild cognitive impairment), and 25 to 30 (no cognitive impairment) for MoCA scores.[20,21] In this analysis, a score of <27 on the MMSE[20–22] and <25 on the MoCA[21,23] indicated cognitive impairment.

Statistical Analysis

All participants were divided into 4 subgroups according to quartiles of serum MMP-9. Linear regression analyses were performed to evaluate the relationships of serum MMP-9 levels with MMSE and MoCA scores. Logistic regression analysis was used to estimate the risk of cognitive impairment by calculating odds ratios (ORs) and 95% confidence intervals (CIs). The effect of serum MMP-9 on cognitive impairment severity was analyzed using ordinal logistic regression models. We performed 3 multiple-adjusted logistic regression models. Model 1 adjusted for age, sex, and education level. Model 2 included the factors in model 1 as well as time from onset to randomization, diastolic BP, admission NIHSS score, body mass index, current smoking, alcohol drinking, and hemiparesis. Model 3 included the factors in model 2 as well as medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary heart disease), family history of stroke, use of antihypertensive medications, and ischemic stroke subtype. Potential covariates for cognitive impairment were selected on the basis of prior knowledge. Multiple imputation for missing data was performed using the Markov chain Monte Carlo method. Serum MMP-9 was modeled in terms of OR

Table 2. ORs and 95% CIs for the Risk of Cognitive Impairment According to MMP-9 Quartiles*

Variable	MMP-9, ng/mL				*P* Value for Trend	Each SD (0.32 ng/mL) Increase in Logarithm MMP-9
	<330.1	330.1–567.6	567.6–893.3	≥893.3		
Median	219.0	452.1	681.5	1176.8	. . .	. . .
MMSE score						
Events, n (%)	56 (40.6)	74 (53.2)	77 (54.6)	89 (63.6)	. . .	296 (53.1)
Model 1	1.00	1.87 (1.14–3.08)	2.21 (1.34–3.66)	3.25 (1.94–5.45)	<0.001	1.44 (1.20–1.72)
Model 2	1.00	1.85 (1.12–3.06)	2.19 (1.32–3.65)	3.21 (1.89–5.45)	<0.001	1.44 (1.19–1.73)
Model 3	1.00	1.78 (1.07–2.98)	2.14 (1.27–3.60)	3.20 (1.87–5.49)	<0.001	1.44 (1.19–1.74)
MoCA score						
Events, n (%)	73 (52.9)	94 (67.6)	89 (63.1)	98 (70.0)	. . .	354 (63.4)
Model 1	1.00	2.15 (1.29–3.57)	1.92 (1.16–3.17)	2.65 (1.57–4.47)	0.002	1.37 (1.14–1.65)
Model 2	1.00	2.09 (1.25–3.50)	1.82 (1.09–3.03)	2.49 (1.46–4.24)	0.004	1.35 (1.12–1.62)
Model 3	1.00	2.04 (1.21–3.44)	1.77 (1.05–2.97)	2.56 (1.48–4.41)	0.003	1.36 (1.12–1.64)

Model 1, adjusted for age, sex, and education level; model 2, adjusted for model 1 and further adjusted for time from onset to randomization, diastolic blood pressure, baseline National Institutes of Health Stroke Scale scores, body mass index, current smoking, and alcohol drinking; model 3, adjusted for model 2 and further adjusted for medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary heart disease), family history of stroke, use of antihypertensive medications, and ischemic stroke subtype. CI indicates confidence interval; MMP-9, matrix metalloproteinase-9; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; and OR, odds ratio.
*MMSE score of <27 or MoCA score of <25 indicates cognitive impairment.

per 1-SD increment of logarithm-transformed MMP-9 level and also across discrete categories. Tests for linear trends in ORs across MMP-9 quartiles were conducted using the median within each quartile as the predictor. In addition, net reclassification index and integrated discrimination improvement were calculated to evaluate the predictive value of adding MMP-9 to conventional risk factors model.[24]

Spline regression models were used to provide more precise estimates and explore the shape of association between serum MMP-9 and cognitive impairment, fitting a restricted cubic spline function with 4 knots (at the 5th, 35th, 65th, and 95th percentiles).[25] In stratified analysis, we explored the potential effect modification by age, sex, education, body mass index, admission NIHSS score, smoking status, alcohol consumption, history of hypertension, and receiving immediate BP reduction. The interaction between MMP-9 and interested factors was tested by the likelihood ratio test of models with interaction terms. Several sensitivity analyses were conducted to test the robustness of our findings. Randomized treatment was first included in the multivariable models to control the effect of immediate BP reduction during hospitalization. We repeated our aforementioned analysis by excluding patients using antihypertensive medications or lipid-lowering drugs before stroke onset to eliminate the potential effects of these drugs on MMP-9 levels. A receiver operating characteristic curve was configured to establish cutoff points of serum MMP-9 that optimally predicted cognitive impairment. All *P* values were 2 tailed, and a significance level of 0.05 was used. Statistical analysis was conducted using SAS statistical software, version 9.2 (SAS Institute Inc, Cary, NC).

Results

Baseline Characteristics

Most baseline characteristics of enrolled and excluded patients in this analysis were well balanced (Table S1). Among 558 patients (173 women and 385 men; mean age, 60.7±10.3 years) in our study, the median serum MMP-9 concentration was 574.3 ng/mL (interquartile range, 330.6–895.2 ng/mL). Compared with study participants with lower serum MMP-9 levels, those with a higher MMP-9 level were more likely to be younger, men, cigarette smokers, and alcohol drinkers; have higher baseline diastolic BP and proportion of thrombotic and embolic infarcts; and have lower time from onset to randomization (Table 1). Baseline characteristics of participants according to randomized treatment were presented in Table S2, and all baseline characteristics were comparable between intervention group and control group, except for significant difference of education.

Association Between Serum MMP-9 and Cognitive Impairment

Median (interquartile range) scores of MMSE and MoCA were 26 (22–29) and 22 (18–26), respectively. After adjustment for age, sex, education level, admission NIHSS score, and other

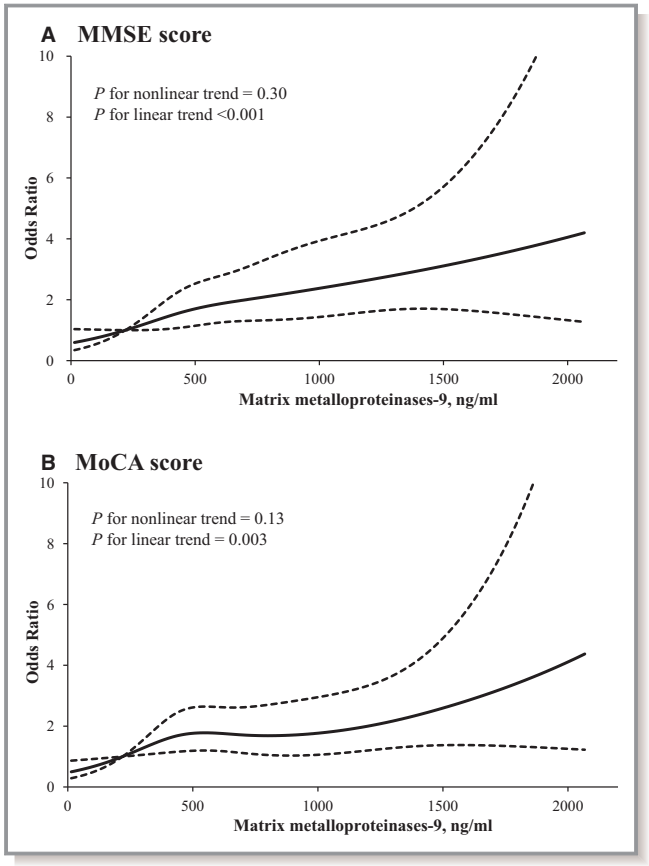

Figure 3. Association of serum matrix metalloproteinase-9 (MMP-9) with risk of cognitive impairment after acute ischemic stroke. Odds ratios and 95% confidence intervals derived from restricted cubic spline regression, with knots placed at the 5th, 35th, 65th, and 95th percentiles of the distribution of serum MMP-9. The reference point for serum MMP-9 is the midpoint (219.0 ng/mL) of the reference group from categorical analysis. Odds ratios were adjusted for the same variables as model 3 in Table 2. A, Mini-Mental State Examination (MMSE) score of <27. B, Montreal Cognitive Assessment (MoCA) score of <25.

covariates in model 3, the linear regression models found serum MMP-9 levels were associated with both MMSE and MoCA scores (P<0.001 for MMSE, and P=0.002 for MoCA). According to MMSE categories, 143 participants (25.6%) had mild cognitive impairment and 153 (27.4%) had severe cognitive impairment; the ordinal analysis showed a significant association between serum MMP-9 and impairment severity (OR, 2.55; 95% CI, 1.58–4.12; P=0.001 for trend, when 2 extreme quartiles were compared; Figure 2). The adjusted OR (95% CI) for the highest quartile of MMP-9 was 3.20 (1.87–5.49) for cognitive impairment compared with the lowest quartile (Table 2). Per 1-SD increase of logarithm MMP-9 was associated with a 44% (95% CI, 19%–74%) increased odds of cognitive impairment. Multiple-adjusted spline regression model further confirmed the dose-response relationships between MMP-9 levels and 3-month cognitive impairment (P<0.001 for linearity; Figure 3). Adding MMP-9 to a model containing conventional risk factors significantly improved risk reclassification for cognitive impairment (category-free net reclassification index, 0.277 [95% CI, 0.113–0.442] [P=0.001]; integrated discrimination improvement, 0.024 [95% CI, 0.011–0.037] [P<0.001]) (Table 3).

The association of serum MMP-9 with risk of cognitive impairment was similar across subgroups stratified according to age, sex, education, body mass index, admission NIHSS score, smoking status, alcohol consumption, history of hypertension, and receiving immediate BP reduction (P>0.05 for interaction for all; Figure 4). In the sensitivity analyses, further adjustment for randomized treatment or exclusion of patients using antihypertensive medications or lipid-lowering drugs before stroke onset did not change the association between MMP-9 and cognitive impairment (Table 4). An optimal MMP-9 cut point (462.6 ng/mL) was obtained from the receiver operating characteristic curve; after adjustment for several covariates, high MMP-9 levels were significantly associated with cognitive impairment (OR, 2.15; 95% CI, 1.47–3.15; P<0.001). The MMSE scores of most cognitive subdomains were lower in patients with higher serum MMP-9 (≥462.6 ng/mL) (Table S3). Similarly, significant findings were observed when cognitive function was measured by MoCA score.

Discussion

To our knowledge, this is the first study to investigate the prospective association of serum MMP-9 with cognitive impairment after acute ischemic stroke. Our study documented a significantly increased risk of cognitive impairment after stroke in patients with high MMP-9, even after adjustment for several potential confounders. The findings were consistent across different subgroups. Furthermore, adding serum MMP-9 to conventional risk factors improved risk prediction for cognitive impairment. These findings suggested serum MMP-9 could provide important predictive information for cognitive impairment after acute ischemic stroke.

Experimental studies showed that the expression of circulating MMP-9 is rapidly upregulated after cerebral ischemia,[7] and our previous study found higher serum MMP-9 levels in the short-term phase of ischemic stroke were associated with increased risk of mortality and major disability.[26] However, limited studies have investigated the relationship between serum MMP-9 and cognitive impairment, especially in patients with acute ischemic stroke.[12,13,27] A case-control study of 15 patients with vascular dementia and 8 neurologically normal controls showed that MMP-9 levels were significantly elevated in the cerebrospinal fluid in the cases compared with controls.[12] Similarly, in an analysis based on Religious Orders Study, the MMP-9 activity of brain

Table 3. Reclassification Statistics (95% CI) for Cognitive Impairment by Serum MMP-9 Among Patients With Acute Ischemic Stroke*

	NRI (Category Free)		IDI	
Variable	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value
MMSE score				
Conventional model				
Conventional model+MMP-9 (continuous)†	0.277 (0.113–0.442)	0.001	0.024 (0.011–0.037)	<0.001
Conventional model+MMP-9 (quartiles)	0.360 (0.196–0.523)	<0.001	0.030 (0.016–0.044)	<0.001
MoCA score				
Conventional model				
Conventional model+MMP-9 (continuous)†	0.192 (0.021–0.363)	0.03	0.016 (0.006–0.028)	0.005
Conventional model+MMP-9 (quartiles)	0.214 (0.043–0.385)	0.02	0.015 (0.004–0.026)	0.001

Conventional model included age, sex, education level, time from onset to randomization, diastolic blood pressure, baseline National Institutes of Health Stroke Scale scores, body mass index, current smoking, alcohol drinking, medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary heart disease), family history of stroke, use of antihypertensive medications, and ischemic stroke subtype. CI indicates confidence interval; IDI, integrated discrimination index; MMP-9, matrix metalloproteinase-9; MoCA, Montreal Cognitive Assessment; and NRI, net reclassification improvement.

*MMSE score of <27 or MoCA score of <25 indicates cognitive impairment.

†Logarithm transformed.

samples was significantly higher in patients with mild cognitive impairment than those with normal cognitive function, and inverse correlations between MMP-9 activity with Global Cognitive Score and MMSE score were found.[13] However, another study conducted in 60 patients with suspected vascular cognitive impairment failed to show a significant increase of MMP-9 in patients with subcortical ischemic vascular disease compared with the nonneurological impairment controls.[27] These studies were conducted with small sample size and could not clarify the prospective association of MMP-9 with cognitive impairment. On the basis of a subset in the CATIS randomized clinical trial, we found that serum MMP-9 in the highest quartile was associated with ≈2.5-fold increased odds of subsequent cognitive impairment, as measured by MMSE or MoCA score. Moreover, when considering subdomains of cognitive function, the MMSE scores of all subdomains were lower in patients with high MMP-9 levels, except for registration.

The mechanisms by which serum MMP-9 affects cognitive function after ischemic stroke are unclear, but several potential pathophysiological pathways have been suggested. The blood-brain barrier is likely to represent an important link between these 2 conditions. Aberrant MMP-9 activity plays a pivotal role in the proteolytic degradation of the blood-brain barrier, and a blood-brain barrier dysfunction may cause damage of the white matter and be related to a progression of cognitive impairment.[28–31] Other possible pathways include causing neuroinflammation, damaging endothelial cells, increasing oxidative stress, activating glia cells, and depositing amyloid β or τ protein.[32–34] Previous studies indicated that MMP-9 level is a strong predictor of brain hemorrhages after human cardioembolic stroke or in the setting of tissue-type plasminogen activator,[35,36] and brain hemorrhages have been associated with cognitive decline or dementia.[37] This may be a potential pathway of the relationship between serum MMP-9 and cognitive impairment. Further studies are needed to clarify this potential mechanism.

Our study provided further evidence about the potential role of serum MMP-9 on cognitive impairment, and had important clinical implications for early prevention and intervention. Given the higher incidence of cognitive impairment after stroke and heavy economic burden, it is urgent for us to identify novel and effective risk factors for cognitive impairment. Higher serum MMP-9 at short-term phase was significantly associated with subsequent cognitive impairment. The addition of serum MMP-9 to a conventional risk factors model would improve risk prediction for cognitive impairment, suggesting that serum MMP-9 in the short-term phase of an ischemic stroke could be a potential predictive marker for cognitive impairment. Further studies with a larger sample size and long-term follow-up are needed to verify our findings. Prior evidence indicated that several inhibitors of MMPs during short-term phases of stroke could improve neurologic function.[38,39] It is of clinical interest to see whether MMP-9 reduction with specific inhibitors in the short-term phase would reduce the risk of cognitive impairment. If these hypotheses are confirmed, serum MMP-9 could be used to identify patients with a higher future risk of cognitive impairment.

This prospective study was from the CATIS randomized clinical trial; standardized protocols and rigid quality control procedures were used for data collection and outcome

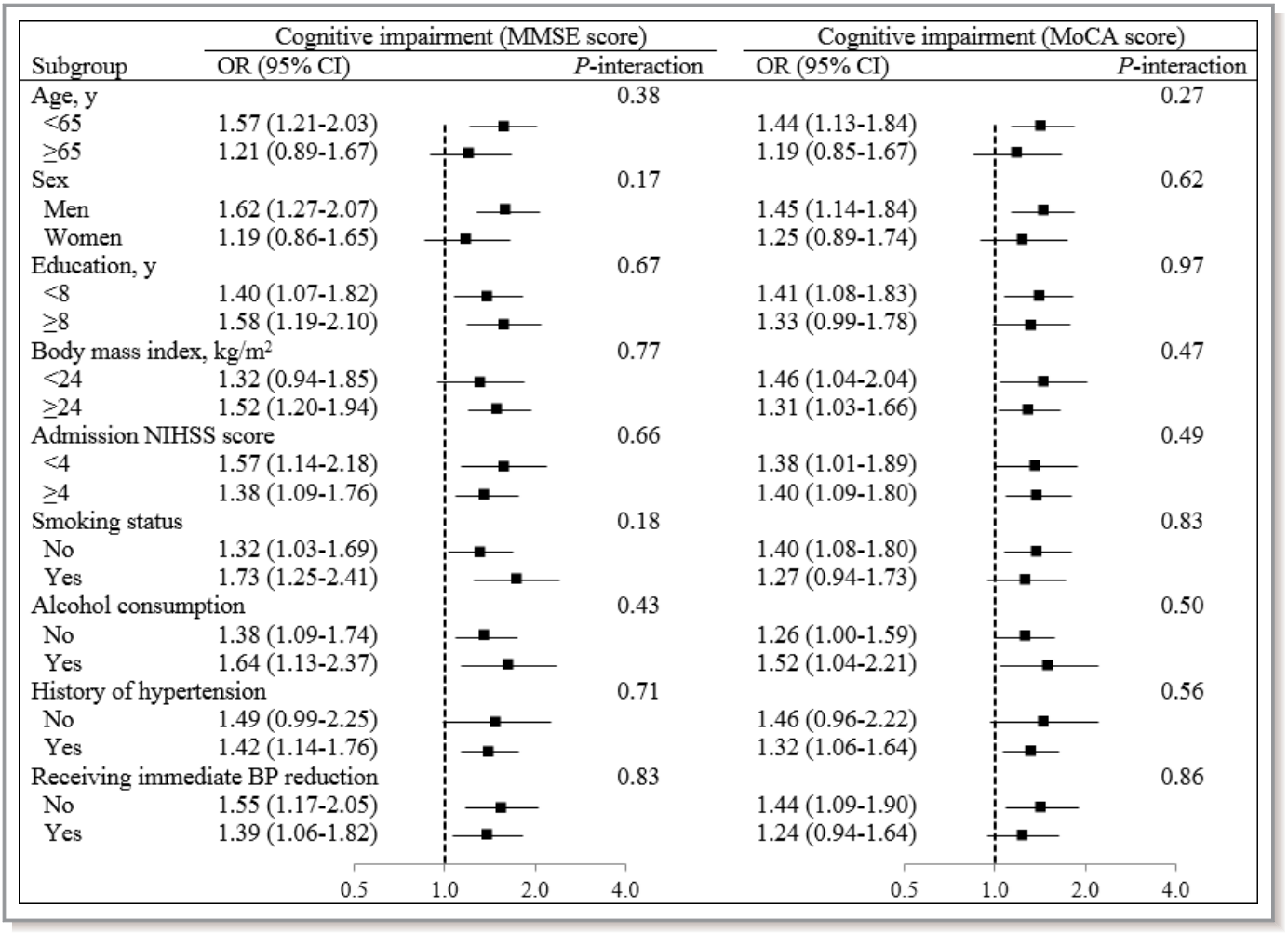

Figure 4. Subgroup analyses of the association between serum matrix metalloproteinase-9 (MMP-9) and cognitive impairment. Odds ratios (ORs) were calculated for each SD (0.32 ng/mL) increase in logarithm MMP-9 after adjustment for the same variables as model 3 in Table 2, except for the stratified variable. Mini-Mental State Examination (MMSE) score of <27 or Montreal Cognitive Assessment (MoCA) score of <25 indicate cognitive impairment. CI indicates confidence interval; and NIHSS, National Institutes of Health Stroke Scale.

assessment. In addition, comprehensive information about relevant covariates was controlled in the multivariable models. Furthermore, both MMSE and MoCA tests were used to assess cognitive performance, and the consistent results validated the significant relationship. Therefore, the present study in method was appropriate and rigorous, providing a more valid appraisal of the association between serum MMP-9 levels and subsequent cognitive impairment after acute ischemic stroke. However, some limitations deserve to be mentioned. First, the participants were from a random sample of CATIS, and patients with BP ≥220/120 mm Hg at admission were excluded. Therefore, a selection bias might be a concern. However, the proportion of patients with BP ≥220/120 mm Hg is low in China, and baseline characteristics of participants in this study were similar to those from the China National Stroke Registry.[40] Second, the CATIS excluded patients with ischemic stroke treated with intravenous thrombolytic therapy; this limited us to exploring the association of serum MMP-9 with cognitive impairment after tissue-type plasminogen activator. Third, we did not conduct serial measurements of serum MMP-9 levels after stroke onset; the association between MMP-9 changes and cognitive impairment after acute ischemic stroke cannot be examined. Finally, we did not collect the data of cognitive function before stroke for the participants, so we could not control the potential confounding of prestroke cognitive impairment.

Conclusions

Elevated serum MMP-9 levels in the short-term phase of ischemic stroke were associated with cognitive impairment, independently of established conventional risk factors. Further prospective studies conducted among different populations are needed to verify our findings.

Table 4. Association of Quartiles of MMP-9 With Cognitive Impairment: Sensitivity Analyses*

Variable	MMP-9, ng/mL				*P* Value for Trend	Each SD (0.32 ng/mL) Increase in Logarithm MMP-9
	<330.1	330.1–567.6	567.6–893.3	≥893.3		
Median	219.0	452.1	681.5	1176.8	. . .	. . .
MMSE score						
Model 1	1.00	1.78 (1.07–2.98)	2.14 (1.27–3.60)	3.20 (1.87–5.49)	<0.001	1.44 (1.19–1.74)
Model 2	1.00	1.78 (1.07–2.98)	2.15 (1.28–3.62)	3.20 (1.87–5.49)	<0.001	1.44 (1.19–1.74)
Model 3	1.00	2.06 (0.99–4.30)	2.50 (1.19–5.23)	4.19 (1.98–8.90)	<0.001	1.53 (1.18–1.98)
Model 4	1.00	1.83 (1.08–3.09)	2.34 (1.37–3.99)	3.65 (2.09–6.37)	<0.001	1.52 (1.25–1.85)
MoCA score						
Model 1	1.00	2.04 (1.21–3.44)	1.77 (1.05–2.97)	2.56 (1.48–4.41)	0.002	1.36 (1.12–1.64)
Model 2	1.00	2.04 (1.21–3.44)	1.76 (1.05–2.97)	2.56 (1.48–4.41)	0.002	1.36 (1.12–1.64)
Model 3	1.00	2.29 (1.07–4.90)	1.79 (0.85–3.75)	3.28 (1.53–7.06)	0.007	1.42 (1.09–1.84)
Model 4	1.00	2.03 (1.20–3.46)	1.76 (1.04–2.99)	2.66 (1.52–4.64)	0.002	1.37 (1.13–1.66)

Data are given as odds ratio (95% confidence interval) unless otherwise indicated. Model 1, adjusted for age, sex, education level, time from onset to randomization, diastolic blood pressure, baseline National Institutes of Health Stroke Scale scores, body mass index, current smoking, alcohol drinking, medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary heart disease), family history of stroke, use of antihypertensive medications, and ischemic stroke subtype; model 2, adjusted for model 1 and further adjusted for randomized treatment; model 3, adjusted for model 1 and further excluded patients using antihypertensive medications before stroke onset; model 4, adjusted for model 1 and further excluded patients using lipid-lowering drugs before stroke onset. MMP-9 indicates matrix metalloproteinase-9; MMSE, Mini-Mental State Examination; and MoCA, Montreal Cognitive Assessment. *MMSE score of <27 or MoCA score of <25 indicates cognitive impairment.

Acknowledgments

We thank the study participants and their relatives and the clinical staff at all participating hospitals for their support and contribution to this project.

Sources of Funding

This study was supported by the National Natural Science Foundation of China (grants 81172761 and 81320108026); a Project of the Priority Academic Program Development of Jiangsu Higher Education Institutions, China; and the National Institute of General Medical Sciences of the National Institutes of Health under award P20GM109036.

Disclosures

None.

References

1. Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. *Lancet Neurol*. 2009;8:1006–1018.
2. Sachdev PS, Brodaty H, Valenzuela MJ, Lorentz L, Looi JCL, Wen W, Zagami AS. The neuropsychological profile of vascular cognitive impairment in stroke and TIA patients. *Neurology*. 2004;62:912–919.
3. Desmond DW, Moroney JT, Sano M, Stern Y. Incidence of dementia after ischemic stroke: results of a longitudinal study. *Stroke*. 2002;33:2254–2260.
4. Sachdev PS, Brodaty H, Valenzuela MJ, Lorentz L, Looi JCL, Berman K, Ross A, Wen W, Zagami AS. Clinical determinants of dementia and mild cognitive impairment following ischaemic stroke: the Sydney Stroke Study. *Dement Geriatr Cogn Disord*. 2006;21:275–283.
5. Sundström J, Evans JC, Benjamin EJ, Levy D, Larson MG, Sawyer DB, Siwik DA, Colucci WS, Sutherland P, Wilson PW. Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: the Framingham Heart Study. *Circulation*. 2004;109:2850–2856.
6. Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, Chan PH, Park TS. Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. *Am J Physiol Heart Circ Physiol*. 2005;289:H558–H568.
7. Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem*. 1999;274:21491–21494.
8. Montaner J, Alvarezsabín J, Molina C, Anglés A, Abilleira S, Arenillas J, González MA, Monasterio J. Matrix metalloproteinase expression after human cardioembolic stroke temporal profile and relation to neurological impairment. *Stroke*. 2001;32:1759–1766.
9. Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation*. 2003;107:1579–1585.
10. Brown DL, Hibbs MS, Kearney M, Loushin C, Isner JM. Identification of 92-kD gelatinase in human coronary atherosclerotic lesions: association of active enzyme synthesis with unstable angina. *Circulation*. 1995;91:2125–2131.
11. Wang X, Jung J, Asahi M, Chwang W, Russo L, Moskowitz MA, Dixon CE, Fini ME, Lo EH. Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. *J Neurosci*. 2000;20:7037–7042.
12. Adair JC, Charlie J, Dencoff JE, Kaye JA, Quinn JF, Camicioli RM, Stetler-Stevenson WG, Rosenberg GA. Measurement of gelatinase B (MMP-9) in the cerebrospinal fluid of patients with vascular dementia and Alzheimer disease. *Stroke*. 2004;35:159–162.
13. Bruno MA, Mufson EJ, Wuu J, Cuello AC. Increased matrix metalloproteinase 9 activity in mild cognitive impairment. *J Neuropathol Exp Neurol*. 2009;68:1309–1318.
14. He J, Zhang Y, Xu T, Zhao Q, Wang D, Chen C-S, Tong W, Liu C, Xu T, Ju Z. Effects of immediate blood pressure reduction on death and major disability in patients with acute ischemic stroke: the CATIS randomized clinical trial. *JAMA*. 2014;311:479–489.
15. Bu X, Zhang Y, Bazzano LA, Xu T, Guo L, Wang X, Zhang J, Cui Y, Li D, Zhang F. Effects of early blood pressure reduction on cognitive function in patients with acute ischemic stroke. *Int J Stroke*. 2016;11:1009–1019.

16. Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg V. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. 1989;20:864–870.
17. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Shepp SG, Roccella EJ. Recommendations for blood pressure measurement in human and experimental animals, part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation*. 2005;111:697–716.
18. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189–198.
19. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, Chertkow H. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53:695–699.
20. Delavaran H, Jönsson AC, Lövkvist H, Iwarsson S, Elmståhl S, Norrving B, Lindgren A. Cognitive function in stroke survivors: a 10-year follow-up study. *Acta Neurol Scand*. 2016;136:187–194.
21. Webb AJ, Pendlebury ST, Li L, Simoni M, Lovett N, Mehta Z, Rothwell PM. Validation of the Montreal cognitive assessment versus mini-mental state examination against hypertension and hypertensive arteriopathy after transient ischemic attack or minor stroke. *Stroke*. 2014;45:3337–3342.
22. Pendlebury ST, Cuthbertson FC, Welch SJ, Mehta Z, Rothwell PM. Underestimation of cognitive impairment by Mini-Mental State Examination versus the Montreal Cognitive Assessment in patients with transient ischemic attack and stroke: a population-based study. *Stroke*. 2010;41:1290–1293.
23. Pendlebury ST, Mariz J, Bull L, Mehta Z, Rothwell PM. MoCA, ACE-R, and MMSE versus the National Institute of Neurological Disorders and Stroke-Canadian Stroke Network Vascular Cognitive Impairment Harmonization Standards Neuropsychological Battery after TIA and stroke. *Stroke*. 2012;43:464–469.
24. Pencina MJ, D'Agostino RB, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157–172.
25. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*. 1989;8:551–561.
26. Zhong C, Yang J, Xu T, Xu T, Peng Y, Wang A, Wang J, Peng H, Li Q, Ju Z. Serum matrix metalloproteinase-9 levels and prognosis of acute ischemic stroke. *Neurology*. 2017;89:805–812.
27. Candelario-Jalil E, Thompson J, Taheri S, Grossetete M, Adair JC, Edmonds E, Prestopnik J, Wills J, Rosenberg GA. Matrix metalloproteinases are associated with increased blood-brain barrier opening in vascular cognitive impairment. *Stroke*. 2011;42:1345–1350.
28. Hanyu H, Asano T, Tanaka Y, Iwamoto T, Takasaki M, Abe K. Increased blood-brain barrier permeability in white matter lesions of Binswanger's disease evaluated by contrast-enhanced MRI. *Dement Geriatr Cogn Disord*. 2002;14:1–6.
29. Rosenberg GA, Sullivan N, Esiri MM. White matter damage is associated with matrix metalloproteinases in vascular dementia. *Stroke*. 2001;32:1162–1168.
30. Wardlaw J, Doubal F, Armitage P, Chappell F, Carpenter T, Munoz-Maniega S, Farrall A, Sudlow C, Dennis M, Dhillon B. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann Neurol*. 2009;65:194–202.
31. Wardlaw JM, Sandercock PA, Dennis MS, Starr J. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? *Stroke*. 2003;34:806–812.
32. Leppert D, Lindberg RLP, Kappos L, Leib SL. Matrix metalloproteinases: multifunctional effectors of inflammation in multiple sclerosis and bacterial meningitis. *Brain Res Rev*. 2001;36:249–257.
33. Mcgeer PL, Mcgeer EG. Mechanisms of cell death in Alzheimer disease: immunopathology. *J Neural Transm Suppl*. 1998;54:159–166.
34. Weninger SC, Yankner BA. Inflammation and Alzheimer disease: the good, the bad, and the ugly. *Nat Med*. 2001;7:527–528.
35. Montaner J, Alvarez-Sabín J, Molina CA, Anglés A, Abilleira S, Arenillas J, Monasterio J. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. *Stroke*. 2001;32:2762–2767.
36. Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribó M, Quintana M, Alvarez-Sabín J. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. *Circulation*. 2003;107:598–603.
37. Benedictus MR, Hochart A, Rossi C, Boulouis G, Hénon H, Wm VDF, Cordonnier C. Prognostic factors for cognitive decline after intracerebral hemorrhage. *Stroke*. 2015;46:2773–2778.
38. Fagan SC, Waller JL, Nichols FT, Edwards DJ, Pettigrew LC, Clark WM, Hall CE, Switzer JA, Ergul A, Hess DC. Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. *Stroke*. 2010;41:2283–2287.
39. Malemud CJ. Matrix metalloproteinases (MMPs) in health and disease: an overview. *Front Biosci*. 2006;11:1696–1701.
40. Wang Y, Liao X, Zhao X, Wang DZ, Wang C, Nguyen-Huynh MN, Zhou Y, Liu L, Wang X, Liu G, Li H, Wang Y. Using recombinant tissue plasminogen activator to treat acute ischemic stroke in China: analysis of the results from the Chinese National Stroke Registry (CNSR). *Stroke*. 2011;42:1658–1664.

SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of acute ischemic stroke patients.

Characteristics	Excluded	Enrolled	*P* value
Number of patients	3513	558	
Age, y	60.7 ± 10.3	62.8 ± 11.0	<0.001
Male sex	2219 (63.2)	385 (69.0)	0.008
Education, y	6.9 ± 3.6	6.2 ± 3.7	<0.001
Current cigarette smoking	1276 (36.3)	209 (37.5)	0.61
Current alcohol drinking	1063 (30.3)	190 (34.1)	0.07
Time from onset to randomization, h	10.3 (5.0-24.0)	10.0 (4.5-24.0)	0.92
Baseline systolic BP, mm Hg	167.3 ± 16.6	166.0 ± 16.9	0.08
Baseline diastolic BP, mm Hg	98.1 ± 9.9	96.4 ± 11.3	<0.001
Body mass index, kg/m^2	25.0 ± 3.2	24.8 ± 3.1	0.30
Baseline NIHSS score	4.0 (3.0-7.0)	4.0 (2.0-8.0)	0.24
History of hypertension	2775 (79.0)	434 (77.8)	0.51
History of hyperlipidemia	237 (6.8)	40 (7.2)	0.71
History of diabetes mellitus	623 (17.7)	96 (17.2)	0.76
History of coronary heart disease	383 (10.9)	61 (10.9)	0.98
Family history of stroke	661 (18.8)	92 (16.5)	0.19
Receiving immediate BP reduction	1767 (50.3)	271 (48.6)	0.45

Abbreviations: BP, blood pressure; NIHSS, National Institute of Health Stroke Scale.

Table S2. Characteristics of participants according to randomized treatment.

Characteristics	Antihypertensive Treatment	Control	*P* value
Number of patients	271	287	
Age, y	61.0 ± 9.9	60.4 ± 10.8	0.46
Male sex	193 (71.2)	192 (66.9)	0.27
Education, y	7.2 ± 3.7	6.6 ± 3.5	0.04
Current cigarette smoking	106 (39.1)	103 (35.9)	0.43
Current alcohol drinking	90 (33.2)	100 (34.8)	0.68
Time from onset to randomization, h	12.0 (4.5-24.0)	10.0 (5.0-24.0)	0.96
Baseline systolic BP, mm Hg	168.2 ± 17.1	166.4 ± 16.1	0.21
Baseline diastolic BP, mm Hg	97.5 ± 9.7	98.7 ± 10.1	0.15
Body mass index, kg/m^2	24.9 ± 3.2	24.8 ± 3.0	0.52
Baseline NIHSS score	4.0 (3.0-7.0)	4.0 (3.0-7.0)	0.61
History of hypertension	207 (76.4)	227 (79.1)	0.44
History of hyperlipidemia	18 (6.6)	22 (7.7)	0.64
History of diabetes mellitus	50 (18.5)	46 (16.0)	0.45
History of coronary heart disease	27 (10.0)	34 (11.9)	0.48
Family history of stroke	45 (16.6)	47 (16.4)	0.94
Use of antihypertensive drugs	124 (45.8)	127 (44.3)	0.72
Use of lipid-lowering drugs	10 (3.7)	10 (3.5)	0.90
Serum MMP-9, ng/ml	524.0 (303.3-900.7)	588.5 (343.7-891.4)	0.35
MMSE score	26.0 (22.0-29.0)	26.0 (22.0-29.0)	0.29
MoCA score	22.0 (18.0-27.0)	23.0 (18.0-26.0)	0.53

Abbreviations: BP, blood pressure; NIHSS, National Institute of Health Stroke Scale; MMP-9, matrix metalloproteinase-9; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment.

Table S3. The domain-specific cognitive function according to serum MMP-9 levels.

	High MMP-9*	Low MMP-9	*P* value
Number of patients	340 (60.9)	218 (39.1)	
MMSE assessment			
Orientation	10 (8, 10)	10 (9, 10)	0.01
Registration	3 (3, 3)	3 (3, 3)	0.29
Attention and calculation	4 (3, 5)	5 (3, 5)	0.05
Recall	3 (2, 3)	3 (2, 3)	0.001
Language	7 (6, 8)	7 (6, 8)	0.02
Visuospatial	1 (0, 1)	1 (1, 1)	0.004
MoCA assessment			
Visuospatial/executive	3 (2, 4.5)	4 (2, 5)	0.02
Naming	3 (2, 3)	3 (2, 3)	0.001
Memory	3 (1, 5)	4 (2, 5)	0.003
Attention	4 (3, 6)	4.5 (3, 6)	0.55
Language	1 (0, 2)	1 (0, 2)	0.46
Abstraction	2 (1, 2)	2 (1, 2)	0.68
Orientation	6 (5, 6)	6 (5, 6)	0.04

Abbreviations: MMP-9, matrix metalloproteinase-9; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment.

*High serum MMP-9 was defined as ≥462.6 ng/ml (Optimal cut point obtained from the ROC curve).