Serum matrix metalloproteinase-9 levels and prognosis of acute ischemic stroke

Chongke Zhong, MD*
Jingyuan Yang, MD*
Tan Xu, MD, PhD
Tian Xu, MD, PhD
Yanbo Peng, MD, PhD
Aili Wang, MD, PhD
Jinchao Wang, MD
Hao Peng, MD, PhD
Qunwei Li, MD
Zhong Ju, MD, PhD
Deqin Geng, MD
Yonghong Zhang, MD, PhD
Jiang He, MD, PhD
For the CATIS Investigators

Correspondence to Dr. Zhang: yhzhang@suda.edu.cn or Dr. He: jhe@tulane.edu

ABSTRACT

Objective: To examine the association between serum matrix metalloproteinases-9 (MMP-9) levels and prognosis of acute ischemic stroke.

Methods: We measured serum MMP-9 levels in 3,186 participants (2,008 men and 1,178 women) from the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS). Study outcome data on death, major disability (modified Rankin Scale score ≥3), and vascular disease were collected at 3 months after stroke onset.

Results: During 3 months of follow-up, 767 participants (24.6%) experienced major disability or died. Serum MMP-9 was significantly associated with an increased risk of death and major disability after adjustment for age, sex, time from onset to randomization, current smoking, alcohol drinking, admission NIH Stroke Scale score, diastolic blood pressure, plasma glucose, white blood cell counts, use of antihypertensive medications, and history of hypertension, coronary heart disease, and diabetes mellitus. For example, 1-SD (0.32 ng/mL) higher log-MMP-9 was associated with an odds ratio (95% confidence interval) of 1.16 (1.06–1.28) for the combined outcome of death and major disability, 1.12 (1.01–1.23) for major disability, and 1.29 (1.01–1.66) for death. The addition of serum MMP-9 to conventional risk factors improved risk prediction of the combined outcome of death or major disability (net reclassification index 9.1%, $p = 0.033$; integrated discrimination improvement 0.4%, $p = 0.004$).

Conclusions: Higher serum MMP-9 levels in the acute phase of ischemic stroke were associated with increased risk of mortality and major disability, suggesting that serum MMP-9 could be an important prognostic factor for ischemic stroke. ***Neurology*® 2017;89:805–812**

GLOSSARY

BP = blood pressure; **CATIS** = China Antihypertensive Trial in Acute Ischemic Stroke; **CI** = confidence interval; **MMP** = matrix metalloproteinase; **mRS** = modified Rankin Scale; **NIHSS** = NIH Stroke Scale; **OR** = odds ratio.

Matrix metalloproteinases (MMPs), a group of proteolytic zinc-dependent enzymes, are implicated in various pathophysiology processes such as systemic inflammation, atherosclerosis, and CNS diseases.[1,2] Among MMPs, MMP-9 (gelatinase B, 92-kDa collagenase) is the most widely investigated enzyme in acute ischemic stroke, and its expression is rapidly upregulated after cerebral ischemia.[3,4] Aberrant MMP-9 activity may weaken the plaque fibrous cap and play a pivotal role in the proteolytic degradation of the blood-brain barrier.[5,6] Some clinical studies have suggested that circulating levels of MMP-9 had a significant correlation with disease severity and infarct volume in the hyperacute phase[7,8] and late hemorrhagic infarction between days 5 and 7 after stroke onset.[9] Despite this, there is limited research on the effect of serum MMP-9 in the acute phase of ischemic stroke on functional outcome or mortality.

Supplemental data at Neurology.org

*These authors contributed equally to this work.

From the Department of Epidemiology (C.Z., J.Y., T.X., Tan Xu, Tian Xu, A.W., H.P., Y.Z., J.H.), School of Public Health and Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Medical College of Soochow University, Suzhou, China; Department of Epidemiology (C.Z., Tan Xu, Y.Z., J.H.), Tulane University School of Public Health and Tropical Medicine, New Orleans, LA; Department of Epidemiology (J.Y.), School of Public Health, Guizhou Medical University, Guiyang; Department of Neurology (Tian Xu), Affiliated Hospital of Nantong University, Jiangsu; Department of Neurology (Y.P.), Affiliated Hospital of North China University of Science and Technology, Hebei; Department of Neurology (J.W.), Yutian County Hospital, Hebei; Department of Epidemiology (Q.L.), School of Public Health, Taishan Medical College, Shandong; Department of Neurology (Z.J.), Kerqin District First People's Hospital of Tongliao City, Inner Mongolia; and Department of Neurology (D.G.), Affiliated Hospital of Xuzhou Medical College, Jiangsu, China.

CATIS Coinvestigators are listed at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Table 1 Characteristics of participants according to quartiles of serum MMP-9

Characteristics[a]	MMP-9, ng/mL					p Value for trend
	Total	<414.7	414.7–671.8	671.8–1,025.8	≥1,025.8	
Patients, n	3186	796	797	796	797	
Age, y	62.4 ± 10.8	63.1 ± 10.4	62.2 ± 10.6	62.3 ± 10.8	61.9 ± 11.2	0.03
Male sex, n (%)	2,008 (63.0)	455 (57.2)	488 (61.2)	512 (64.3)	553 (69.4)	<0.001
Current cigarette smoking, n (%)	1,154 (36.2)	254 (31.9)	267 (33.5)	286 (35.9)	347 (43.5)	<0.001
Current alcohol drinking, n (%)	987 (31.0)	212 (26.6)	239 (30.0)	245 (30.8)	291 (36.5)	<0.001
Time from onset to randomization, h	10.1 (4.5–24.0)	12.0 (5.0–24.0)	11.0 (5.0–24.0)	10.0 (4.3–24.0)	9.0 (4.0–24.0)	<0.001
Admission systolic BP, mm Hg	166.4 ± 17.0	167.3 ± 17.0	165.5 ± 16.1	166.0 ± 16.8	167.0 ± 18.1	0.86
Admission diastolic BP, mm Hg	96.5 ± 11.0	96.1 ± 10.9	96.0 ± 10.9	97.1 ± 11.0	96.9 ± 11.0	0.03
Body mass index, kg/m^2	24.9 ± 3.0	25.0 ± 3.0	25.0 ± 3.0	24.8 ± 2.8	25.0 ± 3.0	0.71
Triglycerides, mmol/L	1.8 ± 1.2	1.9 ± 1.3	1.8 ± 1.2	1.8 ± 1.3	1.8 ± 1.2	0.25
Total cholesterol, mmol/L	5.1 ± 1.1	5.1 ± 1.1	5.1 ± 1.2	5.1 ± 1.0	5.1 ± 1.0	0.63
LDL cholesterol, mmol/L	2.9 ± 0.9	3.0 ± 1.0	2.9 ± 0.9	3.0 ± 0.9	2.9 ± 1.0	0.58
HDL cholesterol, mmol/L	1.3 ± 0.5	1.3 ± 0.4	1.3 ± 0.5	1.3 ± 0.4	1.3 ± 0.5	0.81
Plasma glucose, mmol/L	6.7 ± 2.7	6.7 ± 2.6	6.6 ± 2.6	6.6 ± 2.6	6.8 ± 2.9	0.29
White blood cell counts, 10^9/L	6.7 (5.5–8.3)	6.1 (5.1–7.6)	6.4 (5.3–7.8)	6.8 (5.7–8.4)	7.6 (6.3–9.2)	<0.001
Admission NIHSS score	4.0 (3.0–8.0)	4.0 (2.0–7.0)	4.0 (2.0–7.0)	4.0 (3.0–8.0)	5.0 (3.0–9.0)	<0.001
Admission mRS score	3.0 (2.0–4.0)	2.0 (2.0–4.0)	3.0 (2.0–4.0)	3.0 (2.0–4.0)	3.0 (2.0–4.0)	<0.001
History of hypertension, n (%)	2,504 (78.6)	625 (78.5)	623 (78.2)	631 (79.3)	625 (78.4)	0.90
History of hyperlipidemia, n (%)	229 (7.2)	57 (7.2)	61 (7.7)	61 (7.7)	50 (6.3)	0.52
History of diabetes mellitus, n (%)	561 (17.6)	146 (18.3)	148 (18.6)	130 (16.3)	137 (17.2)	0.35
History of coronary heart disease, n (%)	336 (10.6)	95 (11.9)	78 (9.8)	78 (9.8)	85 (10.7)	0.44
Family history of stroke, n (%)	593 (18.6)	150 (18.8)	141 (17.7)	141 (17.7)	161 (20.2)	0.51
Use of antihypertensive medications, n (%)	1,553 (48.7)	372 (46.7)	393 (49.3)	388 (48.7)	400 (50.2)	0.22
Use of lipid-lowering medications, n (%)	110 (3.5)	28 (3.5)	24 (3.0)	31 (3.9)	27 (3.4)	0.87
Ischemic stroke subtype, n (%)						
Thrombotic	2,430 (76.3)	622 (78.1)	620 (77.8)	584 (73.4)	604 (75.8)	0.09
Embolic	159 (5.0)	19 (2.4)	28 (3.5)	49 (6.2)	63 (7.9)	<0.001
Lacunar	678 (21.3)	170 (21.4)	168 (21.1)	186 (23.4)	154 (19.3)	0.56

Abbreviations: BP = blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MMP-9 = matrix metalloproteinase-9; mRS = modified Rankin Scale; NIHSS = NIH Stroke Scale.
[a] Continuous variables are expressed as mean ± SD or median (interquartile range). Categorical variables are expressed as number (percent).

Risk stratification among patients with acute ischemic stroke may be useful in helping to select therapeutic strategies. Large sample sizes and well-designed prospective studies on the potential relationship between serum MMP-9 levels and clinical outcomes of acute ischemic stroke are needed. The objectives of this study were to prospectively investigate the association between serum MMP-9 levels and prognosis in patients with acute ischemic stroke using data from the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS).

METHODS

Study participants. This study was conducted among patients from the CATIS trial, a multicenter randomized clinical trial conducted in 26 hospitals across China from August 2009 to August 2013. The design and results of CATIS have been described previously.[10] Briefly, 4,071 patients ≥22 years of age who had first-ever ischemic stroke, confirmed by CT or MRI of the brain within 48 hours of symptom onset, and who had an elevated systolic blood pressure (BP) between 140 and <220 mm Hg were recruited. Patients with a BP ≥220/120 mm Hg, severe heart failure, acute myocardial infarction or unstable angina, atrial fibrillation, aortic dissection, cerebrovascular stenosis, or resistant hypertension; those in a deep coma; and those who were treated with intravenous thrombolytic therapy were excluded from the CATIS trial. Because several patients refused to offer blood samples, some collected samples were hemolyzed in storage or transport, or we failed to determine

Table 2 ORs (95% CIs) of clinical outcomes according to quartiles of serum MMP-9 in the acute phase of ischemic stroke

	MMP-9, ng/mL				p Value for trend	Each 1-SD (0.32 ng/mL) increase in log-MMP-9
	<414.7	414.7–671.8	671.8–1,025.8	≥1,025.8		
Median, ng/mL	271.0	541.5	835.0	1343.0		
Death or major disability (mRS score 3–6)						
Cases, n (%)	165 (21.2)	170 (21.8)	201 (25.8)	231 (29.6)		767 (24.6)
Unadjusted OR	1.00	1.06 (0.83–1.35)	1.32 (1.04–1.67)	1.57 (1.25–1.98)	<0.001	1.21 (1.11–1.32)
Age- and sex-adjusted OR	1.00	1.11 (0.87–1.43)	1.39 (1.10–1.77)	1.71 (1.35–2.17)	<0.001	1.26 (1.15–1.38)
Multiple-adjusted OR[a]	1.00	1.08 (0.82–1.42)	1.26 (0.97–1.65)	1.35 (1.04–1.76)	0.02	1.16 (1.06–1.28)
Major disability (mRS score 3–5)						
Cases, n (%)	152 (19.5)	151 (19.4)	178 (22.8)	202 (25.9)		683 (21.9)
Unadjusted OR	1.00	1.02 (0.79–1.31)	1.25 (0.98–1.59)	1.45 (1.14–1.84)	0.001	1.18 (1.08–1.29)
Age- and sex-adjusted OR	1.00	1.06 (0.82–1.36)	1.30 (1.02–1.67)	1.55 (1.21–1.98)	<0.001	1.22 (1.11–1.33)
Multiple-adjusted OR[a]	1.00	1.01 (0.77–1.33)	1.16 (0.89–1.53)	1.20 (0.92–1.57)	0.12	1.12 (1.01–1.23)
Death						
Cases, n (%)	13 (1.7)	19 (2.4)	23 (3.0)	29 (3.7)		84 (2.7)
Unadjusted OR	1.00	1.45 (0.71–2.97)	1.79 (0.90–3.55)	2.28 (1.18–4.42)	0.01	1.35 (1.05–1.73)
Age- and sex-adjusted OR	1.00	1.56 (0.76–3.20)	1.90 (0.95–3.80)	2.46 (1.26–4.81)	0.01	1.39 (1.08–1.79)
Multiple-adjusted OR[a]	1.00	1.55 (0.75–3.20)	1.76 (0.87–3.56)	2.04 (1.04–4.03)	0.05	1.29 (1.01–1.66)
Vascular events						
Cases, n (%)	19 (2.4)	23 (3.0)	26 (3.3)	26 (3.3)		94 (3.0)
Unadjusted OR	1.00	1.20 (0.65–2.22)	1.38 (0.76–2.51)	1.38 (0.76–2.52)	0.29	1.18 (0.94–1.48)
Age- and sex-adjusted OR	1.00	1.23 (0.67–2.29)	1.40 (0.77–2.55)	1.40 (0.77–2.56)	0.28	1.19 (0.95–1.49)
Multiple-adjusted OR[a]	1.00	1.21 (0.65–2.25)	1.34 (0.73–2.46)	1.29 (0.70–2.38)	0.44	1.16 (0.92–1.45)

Abbreviations: CI = confidence interval; MMP-9 = matrix metalloproteinase-9; mRS = modified Rankin Scale; OR = odds ratio.
[a] Adjusted for age, sex, time from onset to randomization, current smoking, alcohol drinking, admission NIHSS score, diastolic blood pressure, plasma glucose, white blood cell counts, use of antihypertensive medications, and history of hypertension, coronary heart disease, and diabetes mellitus.

serum MMP-9 levels, a total of 3,186 patients were included in this study (table e-1 at Neurology.org).

Standard protocol approvals, registrations, and patient consents. The CATIS protocol was approved by the institutional review boards or ethics committees at Soochow University in China, Tulane University in the United States, and all participating hospitals. Written consent was obtained from all study participants or their immediate family members. The CATIS trial was registered at clinicaltrials.gov (NCT01840072).

Data collection. Fasting blood samples were collected within 24 hours of patients' hospital admission. Serum samples were separated at clinical laboratories of the participating hospitals and immediately frozen at −80°C. Serum MMP-9 concentrations were measured centrally at Soochow University with a commercially available ELISA kit (R&D Systems, Minneapolis, MN). Intra-assay and interassay coefficients of variation were 2.0% and 6.9%, respectively. Laboratory technicians who measured MMP-9 were blinded to the clinical outcomes of patients.

Baseline data on demographic characteristics, lifestyle risk factors, medical history, and use of medications were collected at hospital admission with a standard questionnaire. Stroke severity was assessed with the NIH Stroke Scale (NIHSS) by trained neurologists at admission.[11] Three BP measurements were also obtained at admission by trained nurses using a standard mercury sphygmomanometer according to a standard protocol adapted from procedures recommended by the American Heart Association.[12] In addition, serum lipids, plasma glucose, and other clinical laboratory measurements were obtained at the participating hospitals at admission.

Study outcomes. Participants were followed up in person at 3 months by trained neurologists unaware of treatment assignment. A combination of death and major disability (modified Rankin Scale [mRS] score ≥3) was used to measure stroke prognosis. Scores on the mRS range from 0 to 6, with a score of 0 indicating no symptoms, a score of 5 indicating severe disability (i.e., bedridden, incontinent, or requiring constant nursing care and attention), and a score of 6 indicating death. Additional outcomes include death, major disability, and vascular diseases (e.g., vascular death, nonfatal stroke, nonfatal myocardial infarction, hospitalized and treated angina, hospitalized and treated congestive heart failure, and hospitalized and treated peripheral arterial disease). We further included an ordered 7-level categorical score of the mRS as an outcome of neurologic functional status. Death certificates were obtained for deceased participants, and hospital data were abstracted for all vascular events. The study outcomes assessment committee, blinded to treatment assignment, reviewed and adjudicated subsequent outcomes using the criteria established in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial.

Table 3 Reclassification and discrimination statistics for 3-month clinical outcomes by serum MMP-9 among patients with acute ischemic stroke

	C statistic		NRI (continuous)		NRI (categorical[a])		IDI	
	Estimate (95% CI)	*p* Value	Estimate (95% CI), %	*p* Value	Estimate (95% CI), %	*p* Value	Estimate (95% CI), %	*p* Value
Death or major disability (mRS score 3–6)								
Conventional model	0.807 (0.792 to 0.820)		Reference		Reference		Reference	
Conventional model + MMP-9	0.808 (0.794 to 0.822)	0.25	9.1 (1.0 to 17.2)	0.033	1.4 (0.08 to 2.7)	0.037	0.4 (0.1 to 0.6)	0.004
Major disability (mRS score 3–5)								
Conventional model	0.795 (0.780 to 0.809)		Reference		Reference		Reference	
Conventional model + MMP-9	0.796 (0.781 to 0.810)	0.32	5.9 (−2.5 to 14.4)	0.17	0.1 (−1.0 to 1.2)	0.89	0.2 (0.03 to 0.4)	0.023
Death								
Conventional model	0.769 (0.754 to 0.784)		Reference		Reference		Reference	
Conventional model + MMP-9	0.776 (0.761 to 0.791)	0.16	22.2 (0.5 to 43.8)	0.045	3.9 (−1.3 to 9.2)	0.14	0.3 (−0.1 to 0.6)	0.14

Abbreviations: CI = confidence interval; MMP-9 = matrix metalloproteinase-9; NRI = net reclassification improvement; IDI = integrated discrimination index; mRS = modified Rankin Scale.
Conventional model included age, sex, time from onset to randomization, current smoking, alcohol drinking, admission NIHSS score, diastolic blood pressure, plasma glucose, white blood cell counts, use of antihypertensive medications, and history of hypertension, coronary heart disease, and diabetes.
[a] Patients were divided into 3 risk categories: <5%, 5% to 15%, and >15%.

Statistical analysis. All participants were classified according to quartiles of serum MMP-9 levels, and baseline characteristics were compared among quartiles. Categorical and ordinal logistic regression was used to estimate the risk of clinical outcomes associated with MMP-9 levels. Odds ratios (ORs) and 95% confidence intervals (CIs) for higher quartiles compared to the lowest quartile and for 1-SD increment of log-transformed serum MMP-9 levels were calculated. The covariates included in the multivariable model were age, sex, time from onset to randomization, current smoking, alcohol drinking, diastolic BP, fasting plasma glucose, white blood cell counts, admission NIHSS score, use of antihypertensive medications, and history of hypertension, coronary heart disease, and diabetes mellitus. Tests for linear trends in ORs across MMP-9 quartiles were conducted with the median within each quartile used as the predictor. In addition, we used restricted cubic splines to examine the shape of the association between MMP-9 and stroke prognosis with 4 knots (at the 5th, 35th, 65th, and 95th percentiles).[13] A receiver operating characteristic curve was configured to establish cutoff points of serum MMP-9 levels that optimally predicted clinical outcome. To test the robustness of our findings, we conducted several sensitivity analyses. Randomized treatment was first included in the multivariable models to control for the effect of immediate BP reduction during hospitalization. Because using antihypertensive medications or lipid-lowering drugs before stroke onset might affect MMP-9 levels, we repeated our aforementioned analysis by excluding patients using these drugs before stroke onset. We also performed a sensitivity analysis by multiple imputation of missing MMP-9 values to assess the impact of these missing data.

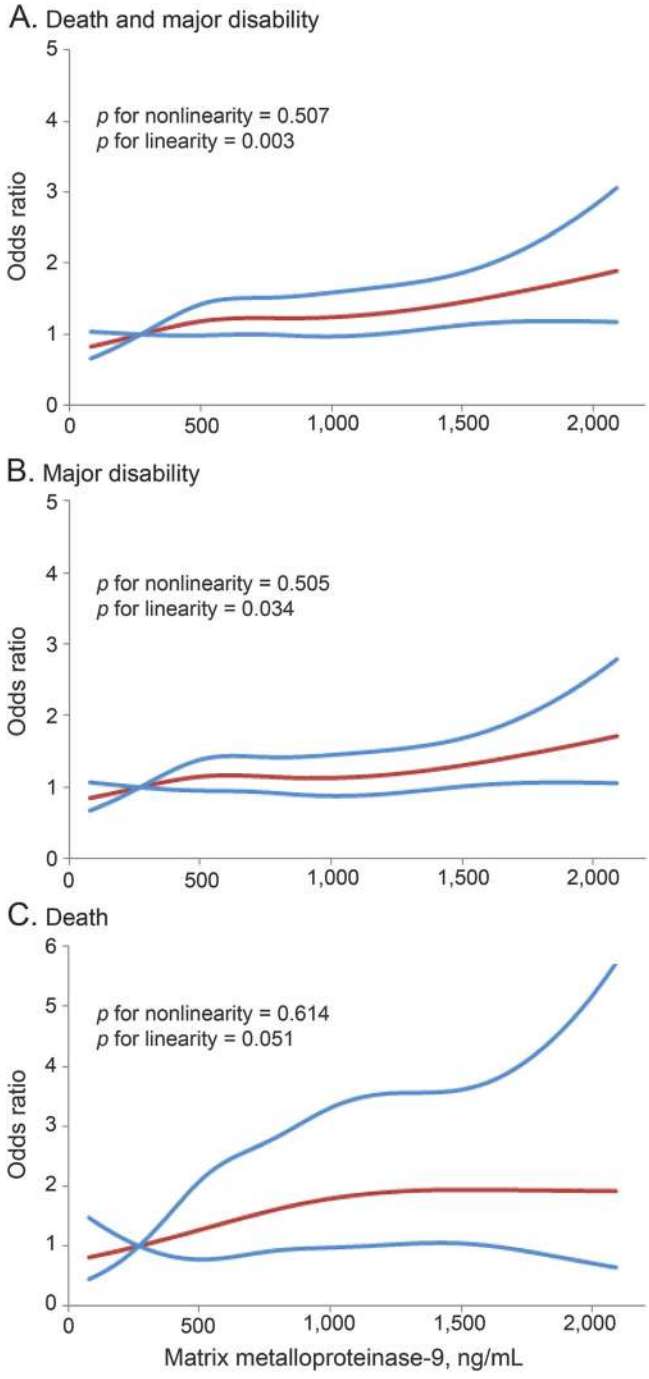
Because previous studies have reported that circulating MMP-9 levels were associated with several stroke-related risk factors (i.e., cigarette smoking, alcohol consumption, hypertension, coronary heart disease, and diabetes mellitus),[14–19] we assessed the potential effect modification by these covariables. Interactions between serum MMP-9 levels and covariables on clinical outcomes were tested by the likelihood ratio test of models with interaction terms, with adjustment for the aforementioned covariates. The effect of immediate BP lowering on prognosis across MMP-9 quartiles was also examined with interaction terms. In addition, C statistics, net reclassification index, and integrated discrimination improvement were used to evaluate the incremental prognostic value of serum MMP-9 levels beyond conventional risk factors.[20,21] All *p* values were 2 tailed, and a significance level of 0.05 was used. Statistical analysis was conducted with SAS statistical software (version 9.2, Cary, NC).

RESULTS

Baseline characteristics. There were 3,186 patients (2,008 men and 1,178 women) with a mean age of 62.4 years included in our study. The median serum MMP-9 concentration was 671.8 ng/mL (interquartile range 414.7–1,025.8 ng/mL). Compared with study participants with lower serum MMP-9 levels, those with higher MMP-9 were more likely to be younger, male, cigarette smokers, and alcohol drinkers; to have a higher prevalence of embolic stroke; to have higher admission diastolic BP, white blood cell count, NIHSS score, and mRS score; and to have shorter time from onset to randomization (table 1).

Serum MMP-9 levels and clinical outcomes. During 3 months of follow-up, 65 participants (2.0%) were lost to follow-up. Among the remaining participants, 767 participants (24.6%) experienced major disability or died (683 major disability and 84 deaths) (tables 2 and 3). The adjusted OR for the highest vs lowest quartile of MMP-9 was 1.35 (95% CI 1.04–1.76) for the combined outcome after adjustment for age, sex, diastolic BP, fasting plasma glucose, and other covariates. Each 1-SD higher log-transformed MMP-9 was associated with a 16% (OR 1.16, 95% CI 1.06–1.28) increased risk for the combined

Figure 1 Association of serum MMP-9 with risk of death and major disability among patients with acute ischemic stroke



ORs 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. Reference point for serum MMP-9 is the midpoint (271.0 ng/mL) of the reference group from categorical analysis. ORs were adjusted for age, sex, time from onset to randomization, cigarette smoking, alcohol drinking, admission NIH Stroke Scale score, diastolic blood pressure, fasting plasma glucose, white blood cell counts, use of antihypertensive medications, and history of hypertension, coronary heart disease, and diabetes mellitus. (A) Combined outcome, (B) major disability, and (C) death. MMP = matrix metalloproteinase-9; OR = odds ratio.

outcome, 12% (OR 1.12, 95% CI 1.01–1.23) increased risk for major disability, and 29% (OR 1.29, 95% CI 1.01–1.66) increased risk for death. Multivariable-adjusted spline regression models showed a linear association between MMP-9 levels and the combined outcome (P for linearity = 0.003) and major disability (P for linearity = 0.034) (figure 1). An optimal MMP-9 cut point level (812.2 ng/mL) was obtained from the receiver operating characteristic curve, and after adjustment for several covariates, elevated MMP-9 levels were associated with the combined outcome (OR 1.29, 95% CI 1.07–1.56, p = 0.008). There was a dose-dependent relationship between serum MMP-9 levels and 3-month mRS score (p for trend <0.001) (figure 2 and table e-2).

Sensitivity and subgroup analyses. Further adjustment for the randomized treatment in the multivariable model, exclusion of patients using antihypertensive medications or lipid-lowering drugs before stroke onset, and multiple imputation of missing MMP-9 values did not change the association between MMP-9 and clinical outcomes (table e-3). In subgroup analyses stratified by prespecified factors, serum MMP-9 levels were associated with increased risk of the combined outcome in most strata (table e-4). No significant interaction between serum MMP-9 levels and these covariables on the combined clinical outcome was observed (p for interaction >0.05 for all).

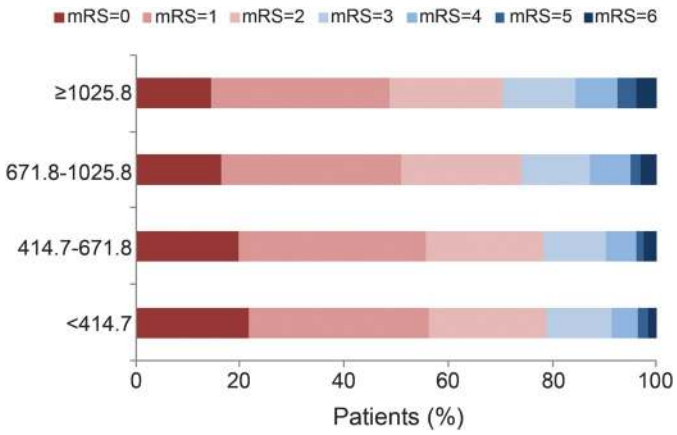
Incremental prognostic value of MMP-9. We examined whether adding serum MMP-9 to conventional risk factors improved the risk prediction of prognosis after acute ischemic stroke. Adding serum MMP-9 to a model containing conventional risk factors did not significantly improve discriminatory power but did significantly improve risk reclassification for the combined outcome (continuous net reclassification index was 9.1% [p = 0.033] and integrated discrimination improvement was 0.4% [p = 0.004]).

Effect of immediate BP reduction. Figure e-1 shows no evidence of heterogeneity in the effects of immediate BP reduction on study outcomes at 3 months across quartiles of serum MMP-9 (p for interaction >0.1 for all).

DISCUSSION In this prospective study among the CATIS patients, we observed dose-response associations between higher MMP-9 levels in acute ischemic stroke and increased risk of major disability and death after adjustment for other established covariates. Sensitivity and subgroup analyses further confirmed these findings. To the best of our knowledge, this is the first study to report that higher serum MMP-9 levels are associated with major disability and death among patients with acute ischemic stroke.

Data on the effect of serum MMP-9 levels in patients with acute ischemic stroke on poor prognosis

Figure 2 Serum MMP-9 and 3-month mRS score



Adjusted odds ratio of ordinal logistic regression analysis is 1.36 (95% confidence interval 1.13–1.64, p for trend <0.001) for highest vs lowest quartile of serum MMP-9. MMP = matrix metalloproteinase-9; mRS = modified Rankin Scale.

are sparse.[22–24] Ning et al.[22] reported that MMP-9 levels measured in the hyperacute phase were correlated to mRS at 3 months (Spearman $r = 0.58$, $p = 0.0005$) among 52 consecutive ischemic stroke patients. In another study, MMP-9 values at day 7 were positively correlated with mRS score at the 3-month follow-up.[23] A retrospective study based on 844 hemispheric ischemic stroke patients indicated that plasma MMP-9 was associated with poor neurologic outcome (mRS score >3) at 3 months.[24] However, these studies all had small sample sizes or were conducted retrospectively. Our study is a prospective cohort study among participants from the CATIS trial with a large sample size and sufficient statistical power. In addition, standardized protocols and rigid quality control procedures were used for data collection and outcome assessment. Furthermore, comprehensive information about potential confounders was collected and controlled for in the multivariate models. The present study provides a more valid appraisal of the relationship between serum MMP-9 levels and prognosis among patients with acute ischemic stroke.

In the present study, we found that higher serum MMP-9 in acute ischemic stroke was significantly associated with poor outcomes and that the addition of serum MMP-9 to conventional risk factors improves risk prediction for death or major disability. This study has clinical implications for better understanding the etiology of ischemic stroke. Evidence indicates that several inhibitors of MMPs during acute phases of stroke improve neurologic outcomes.[25–27] It is of clinical interest to see whether MMP-9 reduction with specific inhibitors in the acute phase would improve both short- and long-term prognosis of ischemic stroke.

The mechanisms underlying the observed association of serum MMP-9 with poor prognosis are unclear, although several potential pathophysiologic mechanisms have been proposed. Elevated expression of MMP-9 may reduce the capability to withstand high shear stress at the plaque shoulder region, leading to rupture of the plaque cap.[2,5] Moreover, experimental studies suggest that oxidative stress is also implicated in the activation of MMP-9 and blood-brain barrier injury, potentially causing adverse outcome.[6,28] Furthermore, in human studies, circulating MMP-9 has been found to be correlated with stroke severity and infarct volume, as well as being a strong predictor of cerebral edema and hemorrhagic transformation, especially in patients treated with recombinant tissue plasminogen activator.[8,29] However, Zhao et al.[30] suggested that MMP-9 might have beneficial roles during the delayed phase of stroke progression. Their study showed that MMPs participated in delayed cortical responses after focal cerebral ischemia in rats and that treatment with MMP inhibitors at 7 days after stroke suppressed neurovascular remodeling, increased ischemic brain injury, and impaired functional recovery at 14 days. Therefore, studies of the time-dependent effect of MMP-9 on prognosis after stroke are warranted.

Our study has several limitations. First, this study is an observational study among participants from CATIS, a randomized clinical trial. The trial excluded ischemic stroke patients with BP ≥220/120 mm Hg or treated with intravenous thrombolytic therapy at admission. Therefore, generalizability might be a concern. However, the proportion of patients with BP ≥220/120 mm Hg or treated with intravenous thrombolytic therapy is low in China,[10,31] and baseline characteristics of participants in this study were similar to those from the China National Stroke Registry.[32] Second, the possibility of residual confounding cannot be fully eliminated in an observational study, although several important potential confounders have been controlled for in multivariable-adjusted models. Third, because all the patients were from China, the findings should be extrapolated cautiously to other populations. Further prospective studies conducted among different populations are needed to replicate our findings. Moreover, 885 patients were excluded for no MMP-9 measurements. This may further limit the generalizability of our findings. However, most baseline characteristics were well balanced between the 2 groups, and a further sensitivity analysis by multiple imputation of missing MMP-9 values found that the significant associations remained. Finally, serum MMP-9 levels were measured only once in this study. Therefore, we were unable to study the diurnal variation of MMP-9 among patients with acute ischemic stroke or the association between MMP-9 changes and prognosis of acute ischemic stroke.

Our results indicate that higher serum MMP-9 levels in acute ischemic stroke were associated with increased risk of mortality and major disability at 3 months. Serum MMP-9 may act as a potential prognostic marker for initial risk stratification in patients with acute ischemic stroke.

AUTHOR CONTRIBUTIONS

Chongke Zhong: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, statistical analysis. Jingyuan Yang: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, statistical analysis. Tan Xu: study concept or design, accepts responsibility for conduct of research and will give final approval, acquisition of data, study supervision. Tian Xu: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data. Yanbo Peng: study concept or design, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, acquisition of data. Aili Wang: study concept or design, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, study supervision. Jinchao Wang: study concept or design, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, acquisition of data. Hao Peng: analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, acquisition of data, study supervision. Qunwei Li and Zhong Ju: study concept or design, accepts responsibility for conduct of research and will give final approval, acquisition of data. Deqin Geng: study concept or design, accepts responsibility for conduct of research and will give final approval, contribution of vital reagents/tools/patients, acquisition of data. Yonghong Zhang: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, statistical analysis, accepts responsibility for conduct of research and will give final approval, study supervision, obtaining funding. Jiang He: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and will give final approval, study supervision, obtaining funding.

ACKNOWLEDGMENT

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

STUDY FUNDING

This study was supported by the National Natural Science Foundation of China (grants 81172761 and 81320108026), by a project of the Priority Academic Program Development of Jiangsu Higher Education Institutions, China, and by the National Institute of General Medical Sciences of the NIH under award P20GM109036. Dr. Zhong was supported by a research training grant (D43TW009107) from the NIH Fogarty International Center, Bethesda, MD.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received September 13, 2016. Accepted in final form May 30, 2017.

REFERENCES

1. Creemers EEJM, Cleutjens JPM, Smits JFM, Daemen MJAP. Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? Circ Res 2001;89:201–210.
2. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis the good, the bad, and the ugly. Circ Res 2002;90:251–262.
3. Foerch C, Montaner J, Furie KL, Ning MM, Lo EH. Invited article: searching for oracles? Blood biomarkers in acute stroke. Neurology 2009;73:393–399.
4. Romanic AM, White RF, Arleth AJ, Ohlstein EH, Barone FC. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size. Stroke 1998;29:1020–1030.
5. 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.
6. Asahi M, Wang XY, Mori T, et al. Effects of matrix metalloproteinase 9 gene knockout on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. J Neurosci 2001;21:7724–7732.
7. Rosell A, Alvarez-Sabín J, Arenillas JF, et al. A matrix metalloproteinase protein array reveals a strong relation between MMP-9 and MMP-13 with diffusion-weighted image lesion increase in human stroke. Stroke 2005;36:1415–1420.
8. Demir C, Ulvi H, Özel L, Özdemir G, Güzelcik M, Aygül R. Relationship between plasma metalloproteinase-9 levels and volume and severity of infarct in patients with acute ischemic stroke. Acta Neurol Belg 2012;112:351–356.
9. Montaner J, Alvarez-Sabín J, Molina CA, et al. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. Stroke 2001;32:2762–2767.
10. He J, Zhang Y, Xu T, et al. 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.
11. Brott T, Adams HP, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864–870.
12. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. J Clin Hypertens 2005;7:102–109.
13. Durrleman S, Simon R, Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989;8:551–561.
14. Yasmin, Wallace S, Mceniery CM, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler Thromb Vasc Biol 2005;25:372–378.
15. Blann AD, Beevers DG. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). Am J Hypertens 2004;17:764–769.
16. Wright JL, Tai H, Wang R, Churg A. Cigarette smoke upregulates pulmonary vascular matrix metalloproteinases via TNF-alpha signaling. Am J Physiol Lung Cell Mol Physiol 2007;292:L125–L133.
17. Sillanaukee P, Kalela A, Seppä K, Höyhtyä M, Nikkari ST. Matrix metalloproteinase-9 is elevated in serum of alcohol abusers. Eur J Clin Invest 2002;32:225–229.
18. Kalela A, Koivu TA, Sisto T, et al. Serum matrix metalloproteinase-9 concentration in angiographically assessed coronary artery disease. Scand J Clin Lab Invest 2002;62:337–342.

19. Nakamura T, Ebihara I, Shimada N, Koide H. Effect of cigarette smoking on plasma metalloproteinase-9 concentration. Clin Chim Acta 1998;276:173–177.
20. Delong ER, Delong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–845.
21. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, 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.
22. Ning M, Furie KL, Koroshetz WJ, et al. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. Neurology 2006;66:1550–1555.
23. Lucivero V, Prontera M, Mezzapesa DM, et al. Different roles of matrix metalloproteinases-2 and -9 after human ischaemic stroke. Neurol Sci 2007;28:165–170.
24. Rodríguezyáñez M, Castellanos M, Blanco M, et al. New-onset hypertension and inflammatory response/poor outcome in acute ischemic stroke. Neurology 2006;67:1973–1978.
25. Fagan SC, Waller JL, Nichols FT, et al. Minocycline to Improve Neurologic Outcome in Stroke (MINOS): a dose-finding study. Stroke 2010;41:2283–2287.
26. Lee JH, Lee YK, Ishikawa M, et al. Cilostazol reduces brain lesion induced by focal cerebral ischemia in rats: an MRI study. Brain Res 2003;994:91–98.
27. Malemud CJ. Matrix metalloproteinases (MMPs) in health and disease: an overview. Front Biosci 2006;11:1696–1701.
28. Wang X, Jung JC, Asahi M, et al. Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. J Neurosci 2000;20:7037–7042.
29. Montaner J, Molina CA, Monasterio J, et al. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation 2003;107:598–603.
30. Zhao BQ, Wang S, Kim HY, et al. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006;12:441–445.
31. Wang Y, Liao X, Zhao X, et al. 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.
32. Luo Y, Wang X, Matsushita K, et al. Associations between estimated glomerular filtration rate and stroke outcomes in diabetic versus nondiabetic patients. Stroke 2014;45:2887–2893.