Serum Matrix Metalloproteinase-9 Is Associated With Depression After Acute Ischemic Stroke

Bizhong Che, MD; Chongke Zhong, MD, PhD; Jinzhuo Ge, BSc; Ruyi Li, BSc; Zhengbao Zhu, MD; Xiaoqing Bu, MD, PhD; Tan Xu, MD, PhD; Zhong Ju, MD; Jiale Liu, MD; Jintao Zhang, MD; Jing Chen, MD; Yonghong Zhang, MD, PhD; Jiang He, MD, PhD

Background: Matrix metalloproteinase-9 (MMP-9), a key determinant of extracellular matrix degradation, might cause cerebral damage after stroke and be involved in the development of depressive symptoms. This study aimed to evaluate the association of serum MMP-9 levels and post-stroke depression (PSD).

Methods and Results: Serum MMP-9 levels were determined in 558 acute ischemic stroke patients from 7 hospitals comprising the China Antihypertensive Trial in Acute Ischemic Stroke. We assessed depression status using the 24-item Hamilton Depression Rating Scale and defined PSD as a cutoff score of 8. Logistic regression was performed to estimate the risk of PSD associated with serum MMP-9. Discrimination and reclassification for PSD by MMP-9 were analyzed. A total of 222 (39.8%) stroke patients were categorized as PSD within 3 months. Serum MMP-9 concentrations were higher among PSD patients than those without PSD (658.8 vs. 485.7 ng/mL; P<0.001). The multiple-adjusted odds ratio (95% confidence interval) for the highest MMP-9 quartile compared with the lowest quartile was 4.36 (2.49–7.65) for PSD, and 1 standard deviation higher log-MMP-9 was associated with 68% (37–106%) increased odds of PSD. Adding MMP-9 to the conventional risk factors model substantially improved discrimination and reclassification for PSD (all P<0.05).

Conclusions: Elevated serum MMP-9 levels in the acute phase of ischemic stroke were associated with increased risk of PSD, suggesting an important prognostic role of MMP-9 for PSD.

Key Words: Acute ischemic stroke; Extracellular matrix; Hamilton Rating Scale for Depression; Matrix metalloproteinase-9; Post-stroke depression

Stroke is the leading common cause of death and disease burden in the developed and some developing countries. Post-stroke depression (PSD) is among the most frequent neuropsychiatric disturbances in stroke survivors, and affects approximately 33% of patients with stroke. Previous studies have reported associations between PSD and poor outcomes of stroke patients, including worse rehabilitation, higher risk of stroke recurrence, and higher mortality. Therefore, novel risk factors to predict PSD early are urgently required to provide clinical evidence for better prevention and intervention of PSD.

The pathophysiology of PSD is complex and multifactorial. Several risk factors of PSD have been established, such as stroke severity, medical history, age, sex and education status. Although the exact mechanisms underlying PSD have not been thoroughly examined, there is considerable evidence supporting the important role of blood–brain barrier dysfunction and consequent inflammation responses in the etiology and course of PSD.
4,071 patients from CATIS study

660 patients pre-planned for cognitive function test at 3 months

15 patients lost to follow-up at 3 months
7 patients deceased during 3 months

638 patients completed cognitive function test at 3 months

80 patients did not offer blood samples or collected samples were hemolyzed or failed to measure serum MMP-9

558 patients eligible for analysis

Matrix metalloproteinase-9 (MMP-9) is the most investigated member of the matrix metalloproteinase family, which normally remodel the extracellular matrix and pathologically attack substrates as part of the neuroinflammatory response. Prior studies have implicated MMP-9 in the proteolytic degradation of the blood-brain barrier and thus might cause cerebral damage in acute ischemic stroke. Meanwhile, increasing evidence has demonstrated a significant association between circulating MMP-9 levels and depressive symptoms. However, whether elevated serum MMP-9 levels increase the risk of subsequent depression after ischemic stroke remains unknown.

Hence, the present study aimed to investigate the associations between serum MMP-9 levels within a short period of ischemic stroke onset and PSD at 3 months among a population sample from the China Antihypertensive Trial in Acute Ischemic Stroke (CATIS).

Methods
This study was approved by the institutional review boards at Soochow University in China and Tulane University in the USA, as well as the ethical committees at the participating hospitals. Written informed consent was given by all study participants or their immediate family members.

Study Design and Population
The CATIS study was a multicenter, single-blind, blinded endpoints randomized controlled clinical trial performed among 4,071 patients with ischemic stroke. Detailed methods and results of CATIS have been described previously. Eligible participants were ≥22 years who had ischemic stroke, confirmed by computed tomography or magnetic resonance imaging within 48 h of symptom onset, and who had an elevated systolic blood pressure (BP) between 140 and <220 mmHg at admission. The exclusion criteria were BP level ≥220/120 mmHg, severe heart failure, acute myocardial infarction or unstable angina, atrial fibrillation, aortic dissection, cerebrovascular stenosis, resistant hypertension, coma, and receiving intravenous thrombolytic therapy (i.e., intravenous recombinant tissue plasminogen activator). The present prospective study was based on a pre-planned ancillary study of CATIS that was designed to test whether early antihypertensive treatment would reduce cognitive impairment in acute ischemic stroke patients at 3 months after randomization among a subgroup of CATIS participants. In this ancillary study, participants were systematically selected prior to randomization from 7 of 26 participating hospitals for cognitive function and psychological evaluation at their 3-month follow-up visit. The 7 hospitals consecutively recruited 660 patients (17 of the initial 677 patients were excluded because of visual, hearing or psychiatric impairment), and the recruitment was completed by November 2012. During the 3 months of visits, 15 patients were lost to follow-up and 7 patients died. A total of 638 participants completed the cognitive function assessment. Of them, 80 were excluded because they did not offer blood samples, or collected samples were hemolyzed in storage or transport, or failed to determine serum MMP-9 concentrations, leaving 558 participants for final analysis (Figure 1).

Assessment of Serum MMP-9 and Potential Covariates
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 the School of Public Health at Soochow University until laboratory testing. Serum MMP-9 levels were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA). Intra- and interassay coefficients of variation were 2.0% and 6.9%, respectively. Tissue inhibitor of metalloproteinase-1 (TIMP-1), an endogenous inhibitor of MMP-9, was also measured, and the intra- and interassay coefficients of variation were 3.9% and 4.9%, respectively. MMP-9 and TIMP-1 determinations were performed by laboratory technicians unaware of the clinical characteristics of enrolled participants. Cigarette smoking was also measured, and the intra- and interassay coefficients of variation were 2.0% and 6.9%, respectively. Tissue inhibitor of metalloproteinase-1 (TIMP-1), an endogenous inhibitor of MMP-9, was also measured, and the intra- and interassay coefficients of variation were 3.9% and 4.9%, respectively. MMP-9 and TIMP-1 determinations were performed by laboratory technicians unaware of the clinical characteristics and outcomes of participants.

Data on demographic characteristics, lifestyle risk factors, medical history, and medication history were obtained at enrollment using a well-designed questionnaire. Cigarette smokers were those who have smoked at least 1 cigarette per day for 1 year or more.17 Alcohol drinking was defined as consuming any type of alcoholic beverage at least 12 times in the past year. Medical history was defined as follows:18 history of hypertension (patient’s BP ≥140/90mmHg on repeated measurements or on antihypertensive medication before stroke onset), diabetes mellitus (fasting blood glucose level ≥120mg/dL, or use of antidiabetic drugs before stroke onset), dyslipidemia (total cholesterol measurement ≥240mg/dL, high-density lipoprotein measurement <35mg/dL, or use of lipid-lowering agents before stroke onset). Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) score by trained neurologists at admission.19 Ischemic stroke in our study was classified as thrombotic (large artery atherosclerosis), embolic (cardiac embolism), or lacunar (small artery occlusion lacunae) based on the patient’s clinical features combined with imaging data.20 Baseline BP was calculated from 3 measurements obtained by trained nurses according to a common protocol adapted from procedures recommended by the American Heart Association.21

**Study Outcomes**

The principle outcome was depression at 3 months after stroke onset, which was assessed by a trained neurologist using the validated version of the Hamilton Rating Scale for Depression (HRSD-24).22 24 The HRSD-24 has been translated into Chinese and validated as a screening instrument for depression in the Chinese population. It is widely accepted that a total HRSD score of 8 is the cutoff point for diagnosing depressive symptoms.25 26 Furthermore, depression severity was categorized as follows: scores ≤7 do not indicate the presence of depression, scores between 8 and 19 indicate mild depression and scores ≥20 indicate severe depression.27

**Statistical Analysis**

All participants were classified into 4 groups according to quartiles of serum MMP-9 levels, and baseline characteristics were compared among quartiles. Baseline characteristics were also compared between enrolled and excluded patients. Categorical variables were compared using Chi-square tests, and Student’s t-test or Wilcoxon rank-sum test was applied for continuous variables. Serum MMP-9 levels were compared between patients with and without PSD using Wilcoxon rank-sum tests. Linear regression analyses were performed to assess the relationships between serum MMP-9 levels and HRSD scores. Logistic regression
analysis was used to estimate the risk of PSD associated with serum MMP-9 levels. Odds ratios (ORs) and 95% confidence intervals (CIs) for higher quartiles compared with lowest quartile and for 1 standard deviation increment of log-transformed serum MMP-9 levels were computed. The effect of MMP-9 on depression severity was analyzed using ordinal logistic regression models. We performed 3 multiple-adjusted logistic regression models. Model 1 adjusted for age, sex, education status, current smoking, and alcohol drinking. Model 2 included the factors in model 1 as well as systolic BP, baseline NIHSS score, time from onset to randomization and ischemic stroke subtype. Model 3: adjusted for model 2 and further adjusted for medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary artery disease) and use of antihypertensive medications. *Severity of depression categorized as normal (HRSD score: 0–7), mild depression (HRSD score: 8–19) and severe depression (HRSD score ≥20). HRSD, Hamilton Rating Scale for Depression; MMP-9, matrix metalloproteinase-9.

## Results

### Baseline Characteristics

The 558 participants in this analysis had a mean age of 60.7±10.3 years, and 385 (69.0%) were men. The median serum MMP-9 level was 567.6 ng/mL (interquartile range: 330.1–893.3 ng/mL). Table 1 presents the baseline sociodemographic and clinical characteristics of the participants. Compared with participants with lower serum MMP-9 levels, those with higher MMP-9 were more likely to be younger, male, cigarette smokers, and alcohol drinkers, have higher baseline diastolic BP and proportion of thrombotic and embolic infarcts, and have a shorter time from onset to randomization. As shown in Supplementary Table 1, most of the baseline characteristics of the enrolled and excluded cases were well balanced, except for significant differences in age, sex, education level, diastolic BP and use of antihypertensive medications.

### Association Between Serum MMP-9 and Post-Stroke Depression

At 3 months after ischemic stroke, a total of 222 (39.8%) patients were categorized as having PSD. The MMP-9 levels were higher among patients with PSD than those without PSD [658.8 (452.3–1,009.6) vs. 485.7 (287.4–737.9) ng/mL; P<0.001]. The median score of the HRSD-24 was 219.0 (452.1–681.5) in those without PSD and 452.1 (681.5–1,176.8) in those with PSD. The adjusted OR (95% CI) of PSD without PSD was 1.58 (1.30–1.93). Probability of PSD increased 1.58 (1.30–1.93) times with each SD increase of serum MMP-9 (logarithm).

### Odds Ratios and 95% Confidence Intervals for the Risk of Post-Stroke Depression According to MMP-9 Quartiles

<table>
<thead>
<tr>
<th>Model</th>
<th>OR (95% CI)</th>
<th>P value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>1.55 (1.29–1.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.60 (1.00–2.84)</td>
<td>2.44 (1.46–4.08)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.66 (0.97–2.84)</td>
<td>2.35 (1.38–3.99)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.58 (0.92–2.71)</td>
<td>2.28 (1.33–3.89)</td>
</tr>
</tbody>
</table>

**Table 2. Odds Ratios and 95% Confidence Intervals for the Risk of Post-Stroke Depression According to MMP-9 Quartiles**

<table>
<thead>
<tr>
<th>Median Post-stroke depression (HRSD score ≥8)</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events, n (%)</td>
<td>35 (25.4)</td>
<td>48 (34.5)</td>
<td>61 (43.3)</td>
<td>78 (55.7)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.55 (0.92–2.61)</td>
<td>2.24 (1.35–3.73)</td>
<td>3.70 (2.23–6.15)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Model 1: adjusted for age, sex, education level, current smoking, and alcohol drinking. Model 2: adjusted for model 1 and further adjusted for systolic blood pressure, baseline National Institutes of Health Stroke Scale scores, time from onset to randomization and ischemic stroke subtype. Model 3: adjusted for model 2 and further adjusted for medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary artery disease) and use of antihypertensive medications. *Severity of depression categorized as normal (HRSD score: 0–7), mild depression (HRSD score: 8–19) and severe depression (HRSD score ≥20). HRSD, Hamilton Rating Scale for Depression; MMP-9, matrix metalloproteinase-9.
Discrimination and Reclassification of Serum MMP-9

Discrimination for PSD by serum MMP-9 was analyzed (Figure 5). The basic model included age, sex, education level, current smoking, alcohol drinking, systolic BP, baseline NIHSS score, time from onset to randomization, ischemic stroke subtype, medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary artery disease) and use of antihypertensive medications. This model displayed a C statistic of 0.698; when we added serum MMP-9 to this model, it displayed a C statistic of 0.734 (P=0.007). In addition, adding MMP-9 to this basic model significantly improved risk reclassification for PSD (Table 3), shown by an increase in category-free NRI of 0.399 (95% CI, 0.234–0.565; P<0.001) and IDI of 0.045 (95% CI, 0.028–0.063; P<0.001).
levels to a model including conventional risk factors substantially improved risk prediction for PSD. Our findings suggested that MMP-9 could provide important predictive information for PSD.

Earlier literature has documented the association between MMP-9 and ischemic stroke. For instance, several experimental studies reported that MMP-9 was overexpressed in the infarcted brain tissue of human stroke cases compared with non-ischemic areas of the brain. Rosell et al demonstrated that MMP-9-positive neutrophil infiltration

### Discussion

As far as we know, this is the first study to examine the association between MMP-9 and PSD in a Chinese population. We demonstrated a significant association between serum MMP-9 level and a higher risk of PSD after adjustment for several confounders. The result of linear association suggested a dose-dependent relationship between MMP-9 and PSD. This finding was consistent across different subgroups. Furthermore, adding high MMP-9

#### Figure 4

Subgroup analyses of the association between serum matrix metalloproteinase-9 (MMP-9) and post-stroke depression. OR (95% CI) was calculated for each standard deviation (0.32 ng/mL) increase in logarithm MMP-9 after adjustment for the same variables as in model 3 in Table 2, except for the stratified variable. CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio.
MMP-9 and Post-Stroke Depression

MMP-9 and Post-Stroke Depression

depression onset and coexisting somatic diseases. Our studies have expanded these findings to an ischemic stroke population derived from CATIS, and we found that the highest serum MMP-9 quartile was associated with approximately 3.6-fold increased odds of subsequent PSD. The biological mechanisms of the association between MMP-9 and PSD are not fully understood, but several potential pathophysiological processes have been confirmed. MMP-9 increases in the acute phase of cerebral ischemia and participates in uncontrolled degradation of extracellular basal lamina, resulting in breakdown of the blood-brain barrier, hemorrhagic transformation and a cascaded neuroinflammatory response. Inflammatory pathways through blood-brain barrier damage may contribute to neuronal injury, apoptosis and brain damage. In addition to degradation and remodeling of the extracellular matrix, MMP-9 has been found to modulate inflammatory cytokines and cytokine receptors, such as interleukin-6 and C-reactive protein, which are proven to be associated with depression.

The current study was based on a relatively large sample from the CATIS randomized clinical trial, which used standardized protocols and strict quality control procedures.

Table 3. Reclassification Statistics for Depression by Serum MMP-9 Among Patients With Acute Ischemic Stroke

<table>
<thead>
<tr>
<th></th>
<th>NRI (category free)</th>
<th>IDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Conventional model+MMP-9 (continuous)</td>
<td>0.399 (0.234–0.565)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conventional model included age, sex, education level, current smoking, alcohol drinking, systolic blood pressure, baseline National Institutes of Health Stroke Scale scores, time from onset to randomization, ischemic stroke subtype, medical history (hypertension, diabetes mellitus, hyperlipidemia, and coronary artery disease) and use of antihypertensive medications. CI, confidence interval; IDI, integrated discrimination index; MMP-9, matrix metalloproteinase-9; NRI, net reclassification improvement.
for data collection and outcome assessment. Thus, our study should be a valid evaluation of the association between serum MMP-9 levels and PSD. Risk stratification for subsequent depression in patients with acute ischemic stroke is useful for selecting timely therapeutic strategies. The addition of MMP-9 to a model including conventional factors improved both the discrimination and reclassification for PSD. Couple with our recent findings of the predictive value of MMP-9 in mortality, major disability and cognitive impairment, these results serve as strong evidence supporting the promising prognostic usefulness of serum MMP-9 in patients with ischemic stroke, and have important clinical implications. MMP-9 is a representative member of the MMP family; other members, including MMP-2, MMP-7, and TIMPs, are also implicated in degradation and remodeling of extracellular matrix. However, we did not find a significant association between serum TIMP-1 and PSD in this study. It is reported that TIMP-1 may be involved in other biological activities separate from MMP inhibitory capacities, such as angiogenesis and apoptosis. Further prospective studies with large sample sizes are needed to verify our findings, and to measure TIMPs and other MMP family members to clarify the role of extracellular matrix degradation in the prediction of PSD.

Study Limitations
Several limitations warrant discussion. First, our study was observational using a random subsample of the CATIS, excluding ischemic stroke patients with BP ≥220/120 mmHg or treated with intravenous thrombolytic therapy at admission. A selection bias was inevitable. However, the proportion of patients with BP ≥220/120 mmHg or treated with intravenous thrombolytic therapy is low in China, and the baseline characteristics of participants in this study were similar to those from the China National Stroke Registry. In addition, most of the baseline characteristics of the enrolled and excluded patients in this analysis were well balanced. Serum MMP-9 levels were only determined in the acute phase of stroke onset; therefore, the association of MMP-9 dynamic changes with PSD could not be assessed. Finally, we did not collect data on depression or proxy depression complaints before stroke for the participants, so we could not control the potential confounding of pre-stroke depression.

Conclusions
Higher serum MMP-9 levels in the short term of ischemic stroke were associated with increased risk of PSD, suggesting MMP-9 could be a promising prognostic factor for PSD. Further longitudinal studies conducted among different populations are needed to confirm the clinical value of MMP-9 and to clarify the potential biological mechanisms.

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Disclosures
All authors report no conflicts of interest to disclose.

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References
MMP-9 and Post-Stroke Depression


Supplementary Files
Please find supplementary file(s);