Association of Molecular Markers With Perihematomal Edema and Clinical Outcome in Intracerebral Hemorrhage

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Background and Purpose—Perihematomal edema contributes to secondary brain injury in intracerebral hemorrhage (ICH). Increase of matrix metalloproteinases (MMPs) and growth factors is considerably involved in blood–brain barrier disruption and neuronal cell death in ICH models. We therefore hypothesized that increased levels of these molecular markers are associated with perihematomal edema and clinical outcome in ICH patients.

Methods—Fifty-nine patients with spontaneous ICH admitted within 24 hours of symptom onset were prospectively investigated. Noncontrast CT was performed on admission for diagnosis of ICH and quantification of initial hematoma volume. MRI was performed on day 3 to evaluate perihematomal edema. Concentrations of MMP-3, MMP-9, as well as vascular endothelial growth factor and angiopoietin-1 on admission were determined by enzyme-linked immunosorbent assays. Clinical outcome was assessed by modified Rankin Scale at 90 days.

Results—Increased MMP-3 levels were independently associated with perihematomal edema volume (P<0.05). Cytotoxic edema surrounding the hematoma was seen in 36 (61%) cases on 3-day MRI. Cytotoxic edema did not correlate with the level of any of the biomarkers studied. Levels of MMP-3 ≥12.4 ng/mL and MMP-9 ≥192.4 ng/mL but not vascular endothelial growth factor and angiopoietin-1 predicted poor clinical outcome at 90 days (modified Rankin Scale >3) independent of stroke severity and hematoma volume at baseline (odds ratio, 25.3, P=0.035; odds ratio, 68.9, P=0.023; respectively).

Conclusions—MMPs 3 and 9 seem to be significantly involved in secondary brain injury and outcome after primary ICH in humans, and thus should be further evaluated as targets for therapeutic strategies in this devastating disorder. (Stroke. 2013;44:658-663.)

Key Words: brain edema ■ diffusion-weighted MRI ■ ICH ■ inflammation ■ magnetic resonance imaging ■ matrix metalloproteinases ■ outcome
Methods

Study Population
All patients with spontaneous ICH admitted to the Neurological Department of Beijing Tiantan Hospital from January 2011 to December 2011 were screened for this study. Inclusion criteria were time from symptom onset to admission <24 hours, age <80 years, and absence of coma. Exclusion criteria were secondary ICH (hemorrhage resulting from aneurysm, vascular malformation, hemorrhagic infarction, tumor, or impaired coagulation), history of acute or chronic infection, malignant diseases and immunosuppressive treatment, contraindication for MRI, undergoing a surgical procedure, or refusal of participation. A total of 59 patients were prospectively included after informed consent from patients or their relatives. The study has been approved by the local ethics committee.

Clinical data of patients were collected on admission. The variables included sex, age, body mass index, alcohol and tobacco use, a detailed history of vascular risk factors and concomitant medications, body temperature, systolic and diastolic blood pressure, and laboratory tests. Stroke severity was evaluated by Glasgow Coma Scale and Modified Rankin Scale at admission. Poor clinical outcome was defined as modified Rankin Scale >3 assessed at 90-day follow-up.

Radiological Protocol
Non-contrast cerebral computed tomography (NCCT) scans were performed on admission, according to the protocol of the neuroradiological department with an image matrix of 512×512, 4.5-mm-wide slices for posterior fossa, and 9-mm-wide slices for medium and anterior fossae. Cerebral MRI was performed by 3.0 Tesla scanners (Trio-Tim, Siemens, Erlangen, Germany) on 3±1 days. MRI included the following sequences: conventional T1-weighted and T2-weighted images, gradient-recalled echo imaging (T2*), diffusion-weighted images (DWI) using 2 levels of diffusion sensitization (b=0 and 1000 s/mm²), and apparent diffusion coefficient (ADC) maps created from DWI images by the image analysis system. Additionally, in 50 patients, a follow-up NCCT was done at 24 hours as a clinical routine.

Investigators who analyzed the images were blinded to clinical and biomarker information. Hematoma location (supratentorial deep location versus others, including lobar and infratentorial locations), and the presence of intraventricular hemorrhage (IVH) were recorded. Hematoma volume and total lesion area were calculated by the summation-of-area method on each slice multiplied by the interslice thickness on both NCCT and 3-day MRI. Hematoma expansion was defined as an increase of hematoma volume >12.5 mL from symptom onset to baseline NCCT. Hematoma expansion was defined as an increase of hematoma volume >12.5 mL from symptom onset to baseline NCCT. Hematoma expansion was defined as an increase of hematoma volume >12.5 mL from symptom onset to baseline NCCT. Hematoma expansion was defined as an increase of hematoma volume >12.5 mL from symptom onset to baseline NCCT.

Results

Baseline Clinical, Neuroimaging, and Laboratory Findings
Baseline variables are shown in Table 1 (n=59). Median time from symptom onset to baseline NCCT was 3.5 (1.6–7.5) hours. Twenty-four–hour follow-up CT was performed in 50 patients. The median hematoma volume on baseline and 24-hour follow-up CT was 9.9 (5.4–21.3) ml and 10.9 (5.6–25.8) ml, respectively (n=50). Hematoma expansion was found in 3 patients who had follow-up CT and in none of the
remaining 9 patients who only underwent follow-up MRI. MMP-3, MMP-9, VEGF, and Ang-1 levels did not correlate with hematoma volume at baseline. No correlation between these molecular markers and the absolute increase of hematoma volume was found ($P>0.05$).

**Association of PHE Volume With Hematoma Volume and Molecular Markers**

Median time to follow-up MRI was 76 (69–88) hours. PHE was present in all patients on 3-day T2-weighted images. A high correlation was found between absolute PHE and hematoma volume at baseline and day 3 ($r=0.922$ and $r=0.959$, respectively; both $P<0.001$; Figure 2). Absolute PHE volume was positively correlated with MMP-3 ($r=0.311$; $P=0.017$), but not with MMP-9, VEGF and Ang-1. Hematoma volume on day 3 was positively correlated with MMP-3 ($r=0.311$; $P=0.017$) and negatively correlated with Ang-1 ($r=-0.281$; $P=0.031$), but did not correlate with MMP-9 nor with VEGF. The linear stepwise regression model revealed that MMP-3 was independently associated with absolute PHE volume ($\beta=0.370$; $P=0.004$), irrespective of age and gender. MMP-3 ($\beta=0.138$; $P=0.043$) remained independently associated with absolute PHE volume when baseline hematoma volume ($\beta=0.868$; $P<0.001$) was also considered. However, only hematoma volume on day 3 ($\beta=0.942$; $P<0.001$) remained as an independent predictor of PHE volume when it was included in the multivariate model instead of hematoma volume on baseline.

**Table 1. Baseline Clinical, Radiological, and Laboratory Characteristics of All Patients and Patients Grouped by 90-Day Outcome**

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>All (n=59)</th>
<th>Favorable Outcome (n=50)</th>
<th>Unfavorable Outcome (n=9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>41 (69.5)</td>
<td>35 (70.0)</td>
<td>6 (66.7)</td>
<td>1.000</td>
</tr>
<tr>
<td>Age, y</td>
<td>56±11</td>
<td>56±11</td>
<td>60±14</td>
<td>0.338</td>
</tr>
<tr>
<td>BMI</td>
<td>25.3±3.5</td>
<td>25.4±3.5</td>
<td>24.7±3.8</td>
<td>0.601</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>52 (88.1)</td>
<td>44 (88.0)</td>
<td>8 (88.9)</td>
<td>1.000</td>
</tr>
<tr>
<td>History of diabetes mellitus</td>
<td>11 (18.6)</td>
<td>9 (18.0)</td>
<td>2 (22.2)</td>
<td>0.670</td>
</tr>
<tr>
<td>History of stroke</td>
<td>13 (22.0)</td>
<td>12 (24.0)</td>
<td>1 (11.1)</td>
<td>0.673</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>24 (40.7)</td>
<td>19 (38.0)</td>
<td>5 (55.6)</td>
<td>0.619</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>26 (44.1)</td>
<td>22 (44.0)</td>
<td>4 (44.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td>26 (44.1)</td>
<td>21 (42.0)</td>
<td>5 (55.6)</td>
<td>0.697</td>
</tr>
<tr>
<td>Antiplatelet medication</td>
<td>8 (13.6)</td>
<td>6 (12.0)</td>
<td>2 (22.2)</td>
<td>0.494</td>
</tr>
<tr>
<td>NIHSS</td>
<td>6 [3−10]</td>
<td>5 [2−9]</td>
<td>13 [10−15]</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>168±25</td>
<td>165±24</td>
<td>180±25</td>
<td>0.108</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>97±15</td>
<td>98±15</td>
<td>91±15</td>
<td>0.163</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Imaging data on initial CT</th>
<th>All</th>
<th>Favorable Outcome</th>
<th>Unfavorable Outcome</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoma volume</td>
<td>10.0 [5.2–23.9]</td>
<td>9.5 [4.1–20.1]</td>
<td>25.2 [16.5–38.1]</td>
<td>0.003*</td>
</tr>
<tr>
<td>PHE volume</td>
<td>6.5 [3.0–11.9]</td>
<td>5.7 [2.5–11.7]</td>
<td>10.0 [6.7–22.1]</td>
<td>0.062</td>
</tr>
<tr>
<td>Deep location</td>
<td>49 (83.1)</td>
<td>40 (80.0)</td>
<td>9 (100)</td>
<td>0.322</td>
</tr>
<tr>
<td>Intraventricular hemorrhage extension</td>
<td>13 (22.0)</td>
<td>12 (24.0)</td>
<td>1 (11.1)</td>
<td>0.673</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>All</th>
<th>Favorable Outcome</th>
<th>Unfavorable Outcome</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte, 10E9/L</td>
<td>9.0±2.7</td>
<td>8.9±2.7</td>
<td>9.9±3.1</td>
<td>0.313</td>
</tr>
<tr>
<td>Platelet, 10E9/L</td>
<td>220.7±47.3</td>
<td>218.5±49.1</td>
<td>232.9±34.9</td>
<td>0.406</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>7.0±2.1</td>
<td>7.2±2.2</td>
<td>6.0±0.8</td>
<td>0.144</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>64.4±18.4</td>
<td>64.2±19.6</td>
<td>65.6±10.2</td>
<td>0.844</td>
</tr>
<tr>
<td>INR</td>
<td>0.99±0.06</td>
<td>0.99±0.06</td>
<td>0.99±0.06</td>
<td>0.928</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.6±0.9</td>
<td>2.5±0.8</td>
<td>3.0±0.8</td>
<td>0.118</td>
</tr>
<tr>
<td>MMP-3, ng/mL</td>
<td>10.7 [7.5–21.0]</td>
<td>9.9 [6.9–18.7]</td>
<td>17.0 [10.3–28.1]</td>
<td>0.124</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>140.2 [102.4–217.0]</td>
<td>135.2 [100.4–192.2]</td>
<td>232.7 [97.7–306.8]</td>
<td>0.124</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>324.9 [186.7–516.2]</td>
<td>331.7 [197.2–523.2]</td>
<td>270.6 [164.6–404.9]</td>
<td>0.411</td>
</tr>
</tbody>
</table>

*P<0.05 was considered significant. Data are expressed as n (%), mean±SD, or median [IQR] as appropriate. Chi-square test was used for dichotomizing variables; the Student t test or Mann–Whitney U test was used for continuous variables.

Ang indicates angiopoietin; BMI, body mass index; GCS, Glasgow Coma Scale; INR, international normalized ratio; MMP, matrix metalloproteinase; NIHSS, National Institutes of Health Stroke Scale; PHE, perihematomal edema; and VEGF, vascular endothelial growth factor.
Association of CE With Hematoma Volume and Molecular Markers

Among the 59 included patients, CE was detected in 36 (61%) on 3-day MRI. Mean absolute and relative ADC value of CE were 527.5±83.4×10^{-6}mm²/s and 0.665±0.116, respectively. Absolute and relative ADC value were negatively correlated with baseline hematoma volume (r=-0.337, P=0.044; r=-0.352, P=0.035; respectively). Patients with CE had a significantly larger hematoma volume at baseline and day 3 than those without (baseline: 14.7 [7.7–25.1] vs 6.9 [2.8–12.5] ml, P=0.011; day 3: 21.1 [10.0–41.3] vs 7.6 [2.9–17.5] ml, P=0.001; respectively; Figure 3). CE and ADC values did not correlate with MMP-3, MMP-9, VEGF, or Ang-1 levels. Radiological data and levels of measured markers grouped according to the status of CE are provided in Table 2.

Association of PHE and Molecular Markers With Clinical Outcome

At 90-day follow-up, 50 patients showed favorable outcome (modified Rankin Scale 0–3) and 9 unfavorable outcome (modified Rankin Scale 4–6). Clinical, radiological, and laboratory data according to outcome are presented in Table 1. Larger PHE volume on day 3 (P<0.001) but not on admission (P=0.062) was observed in those patients with unfavorable clinical outcome compared with those with favorable outcome. No association was found between outcome and presence or absence of CE. Receiver operating characteristic analysis was performed for concentrations of MMP-3, MMP-9, VEGF, and Ang-1. A possible cut-off point for the discrimination of dichotomized clinical outcome was calculated for MMP-3 as 12.4 ng/mL, MMP-9 as 192.4 ng/mL, VEGF as 317.6 pg/mL, and Ang-1 as 27.3 ng/mL. Using these cut-off points, univariate analysis found that MMP-3 ≥12.4 ng/mL and MMP-9 ≥192.4 ng/mL predicted unfavorable 90-day outcome (P=0.029 and P=0.018, respectively), whereas VEGF and Ang-1 did not (P=0.277 and P=0.252, respectively). Initial hematoma volume, Glasgow Coma Scale, and National Institutes of Health Stroke Scale score on admission were determined to be predictors of outcome as well. In addition to all the significant variables in univariate analysis, age, sex, and hematoma expansion were included in a multivariate logistic analysis to determine independent predictors for unfavorable outcome. As a result, MMP-3 ≥12.4 ng/mL (OR, 25.3; P=0.035), MMP-9 ≥192.4 ng/mL (OR, 68.9; P=0.023), and National Institutes of Health Stroke Scale score at baseline (OR, 1.7; P=0.005) were identified as independent predictors of unfavorable 90-day outcome.

Discussion

The main findings of the present study in acute patients with spontaneous ICH are: (1) the circulating levels of MMP-3 are correlated with absolute PHE volume; (2) increased levels of MMP-3 and MMP-9 at admission are independent predictors of poor clinical outcome; and (3) no association of the investigated markers (MMP-3, MMP-9, VEGF, and Ang-1) with the presence of CE was detected.

Our finding that baseline MMP-3 and MMP-9 levels predict poor clinical outcome, independently of stroke severity and baseline hematoma volume, can be explained pathophysiological. After ICH, secondary brain injuries, including BBB disruption, brain edema, and neuronal cell death, are induced by interaction of the coagulation cascade and thrombin production, erythrocyte lysis, and hemoglobin toxicity, together with inflammation. Pro-MMPs can be activated by plasmin, thrombin, hemoglobin products, and reactive oxygen species produced after ICH. Activated MMP-3 can activate MMP-9, which predominantly degrades the components of the extracellular matrix. In ICH animal models, several studies showed that elevated levels of MMP-9 lead to BBB breakdown and brain edema. It was recently demonstrated that MMP-9 and MMP-3 jointly contributed to blood-induced lesions and neuronal cell death. In previous clinical studies, MMP-9 levels significantly correlated with PHE volume in the first 24 hours after symptom onset. In our study, MMP-3 independently predicted PHE volume on day 3 after symptom onset. This phase of PHE is thought to result predominantly from BBB disruption and neuronal injury because of the interplay of hemoglobin toxicity and inflammation. Our study showed a relationship between 3-day PHE volume and clinical outcome. However, this association might be driven by the hematoma volume, and it remains unclear whether PHE independently influences clinical outcome.
Previous studies showed increased MMP-9 levels in patients who developed hematoma expansion and neurological deterioration after ICH. 5,6,21 Increased MMP-3 levels on admission were reported to be associated with mortality in ICH patients, both MMP-3 and MMP-9, with the residual scar volume at 3 months. 27 Our study confirmed a significant role of the MMPs both MMP-3 and MMP-9, with the residual scar volume at 3 months. 27

Our study has some limitations. Overall, the sample size is small. However, considering the fact that MRI was used, and thus the best available method to quantify PHE, 26,34 this study includes a relatively high number of cases. Second, our findings cannot be generalized to patients with coma on admission and to those noneligible for MRI. Particularly in patients with large hematomas who were not eligible for this study according to the exclusion criteria, pronounced CE and larger PHE volume can be expected. Third, we only investigated molecular marker levels on baseline, although after ICH significant changes over time were reported. 27

In conclusion, our data show that MMP-3 levels are associated with perihematomal edema in acute spontaneous ICH patients. Increased levels of MMP-3 and MMP-9 are independently associated with poor clinical outcome. Further investigations are needed to explain the mechanisms behind them, which might lead to treatment options for the prevention of secondary brain damage and unfavorable outcome.

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Disclosures
None.

References
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