Temporal Profile of Matrix Metalloproteinases and Their Inhibitors After Spontaneous Intracerebral Hemorrhage
Relationship to Clinical and Radiological Outcome

José Álvarez-Sabín, MD, PhD; Pilar Delgado, MD; Sònia Abilleira, MD, PhD; Carlos A. Molina, MD, PhD; Juan Arenillas, MD; Marc Ribó, MD; Esteban Santamarina, MD; Manolo Quintana; Jasone Monasterio, MD, PhD; Joan Montaner, MD, PhD

**Background and Purpose**— Matrix metalloproteinases (MMPs) are related to blood–brain barrier disruption, and some members of this family have been recently involved in brain bleedings. We aimed to investigate the temporal profile of MMPs and their natural inhibitors (TIMPs) after acute intracerebral hemorrhage (ICH) and to study its influence on neuroimaging and clinical outcome.

**Methods**— MMP-2, MMP-9, and MMP-3, as well as TIMP-1 and TIMP-2, were serially determined by enzyme-linked immunosorbent assay on admission (<12 hours), and at 24 hours, 48 hours, 7 days, and 3 months in 21 ICH patients. ICH and perihematomal edema (PE) volumes were serially measured on baseline and follow-up computed tomography (48 hours, 7 days, and 3 months), just at the time of neurological assessment.

**Results**— Deep ICH was found in 62% patients. Baseline ICH volume did not influence MMP-TIMP level. Highest levels of MMP-2 and TIMP-2 were found at baseline, for MMP-9 and TIMP-1 at 24 hours, and for MMP-3 at 24 to 48 hours. Baseline MMP-9 was positively correlated to PE volume (r = 0.67, P = 0.004) and, conversely, its inhibitor TIMP-1 was negatively correlated to PE (r = -0.51, P = 0.04). Mortality reached 35% and MMP-3 was the only MMP/TIMP related to mortality (7.5 versus 2.4 ng/mL; P = 0.035) and its most powerful baseline predictor (odds ratio = 22, confidence interval: 1.5 to 314.2). Both MMP-9 and MMP-3 correlated to the residual scar volume at 3 months (r = 0.68, P = 0.01 for baseline MMP-9, and r = 0.86, P < 0.001 for 24-hour MMP-3).

**Conclusions**— A characteristic temporal profile of MMP/TIMP release exists in ICH. Increased MMP-9 is associated with PE, and increased MMP-3 is associated with mortality. Both molecules are related to residual cavity volume. (Stroke. 2004;35:1316-1322.)

**Key Words:** brain edema ■ intracerebral hemorrhage ■ metalloproteinases ■ stroke

Intracerebral hemorrhage (ICH) accounts for 10% to 15% of cerebrovascular diseases. Even though it is associated with higher morbidity and mortality than other stroke subtypes, no effective treatment has been developed.1 Perihematomal edema (PE), together with ICH enlargement, has been shown to be responsible for poor outcome in some patients who survived to early mass effect.2,3 Therefore, a better understanding of molecular changes underlying these complications is required.

Matrix metalloproteinases (MMPs) are involved in extracellular matrix remodeling under normal conditions, although they have also been reported in a wide range of pathological processes. In particular, their ability to disrupt the blood–brain barrier allowing water and some deleterious molecules to get into the cerebral parenchyma is of interest.4–5 Despite a growing body of evidence on detrimental effects of the MMP families in ischemic stroke,6–7 data on their role in human ICH are still scarce.

Infusion of a bacterial collagenase into the caudate nucleus is a reproducible animal model for intracerebral hemorrhage and brain edema in rats.8 In this collagenase-induced ICH model, endogenous MMP-9 is increased when brain edema is maximal.9,10 In humans, an increased expression of MMP-9 within the first 24 hours of ICH has been reported,11 and, recently, in a mouse ICH model, the MMP expression profile has been described.12

In this study, we sought to investigate the temporal profile of several MMPs and their natural inhibitors (TIMPs) after ICH, together with the influence of these molecules on neuroimaging parameters (PE and residual scar volume) and neurological outcome over the time.

**Subjects and Methods**

**Study Population**

Our target population was any patient with confirmed supratentorial spontaneous ICH attended to in the emergency department within the
first 12 hours after onset. A detailed history of vascular risk factors, drug abuse, alcoholism, and concomitant medication was obtained from each patient. Patients were excluded from this study if the hemorrhage was secondary to vascular malformation, impaired coagulation, head trauma, hemorrhagic infarction, or those with ICH caused by bleeding into a tumoral lesion. Thus, a total of 21 patients were included after informed consent was obtained from all patients or relatives. None of these patients underwent a surgical procedure.

Computed Tomography Scan Protocol
Four cranial computed tomography (CT) scans were performed: at baseline visit (<12 hours) and at follow-up visits (48 hours, 7 days, and 3 months). At 3 months, Barthel index and modified Rankin scale (mRS) were obtained. Death and neurological worsening (an increase in NIHSS score by ≥4 points) were also used as poor outcome parameters. Blood samples, arterial blood pressure, and temperature values were obtained on admission and at follow-up visits.

Immunassay Methods
Venous blood samples were drawn from each patient on admission and follow-up visits. EDTA and citrate tubes were used to collect the blood. Plasma was immediately separated by centrifugation at 3000 rpm for 15 minutes and stored at −80°C until analysis was finished. The levels of MMP-9, MMP-3, and MMP-2 and their natural inhibitors (TIMP-1 and TIMP-2) at each time point are shown in Figure 1. Highest levels for MMP-2 and TIMP-2 were found at baseline, whereas for MMP-9 and TIMP-1 they were higher at 24 to 48 hours. According to the reference values of our laboratory, all these molecules tended to be overexpressed during the acute phase of ICH (P<0.05). Conversely, TIMP-1 concentration increased during the first 24 hours (1024.4

<table>
<thead>
<tr>
<th>TABLE 1. Demographic Data, Risk Factor Profile, and Clinical Variables Among the Studied Patients</th>
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<tbody>
<tr>
<td>Gender, male</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Previous high blood pressure</td>
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<tr>
<td>Smokers</td>
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<tr>
<td>Alcoholism</td>
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<td>Previous stroke</td>
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<tr>
<td>Baseline GCS</td>
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<tr>
<td>Baseline NIHSS</td>
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<tr>
<td>Systolic blood pressure</td>
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<tr>
<td>Diastolic blood pressure</td>
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<tr>
<td>Temperature</td>
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</tbody>
</table>

Baseline blood pressure (BP) is expressed as systolic BP/diastolic BP (mm Hg); baseline temperature is given in °C. Data are expressed in n (%), median (interquartile range), and mean±SD.

Results
Clinical and Neuroimaging Findings
Demographic data, risk factor profile, and clinical variables are shown in Table 1, and radiological data from baseline and follow-up CT scans are provided in Table 2.

ICH enlargement was found in 35% of patients within the first 48 hours. PE showed a significant increase (P<0.0004) during the first 48 hours and then remained stable during the first week. Neurological worsening appeared in 10 (48%) patients within the first 48 hours, and the mortality rate among our patients reached 9.5% at that time point, 23% during the first week, and 35% of the sample at 3 months. Patients who survived (n=14) were severely disabled at 3 months (median mRS=3).

MMPs and TIMPs Temporal Profile
Mean and median values of MMPs and their natural inhibitors (MMP-2, MMP-3, MMP-9, TIMP-1, and TIMP-2) at each time point are shown in Figure 1. Highest levels for MMP-2 and TIMP-2 were found at baseline, whereas for MMP-9 and TIMP-1 they were higher at 24 hours, and for MMP-3 they were higher at 24 to 48 hours. According to the reference values of our laboratory, all these molecules tended to be overexpressed during the acute phase of ICH (P<0.05 for all time points, except for baseline and 3 months for MMP-9 and except for 7 days and 3 months for MMP-3), with the exception of TIMP-2 concentration, which showed very low levels compared with those obtained from healthy controls (P<0.05). Regarding the temporal profile of each molecule, MMP-2 was found significantly decreased during the first 24 hours with respect to baseline concentration (901.4 versus 740.1 ng/mL; P=0.002). Conversely, TIMP-1 concentration increased during the first 24 hours (1024.4
versus 1189.3 ng/mL; \( P=0.036 \)). Other significant variations are also shown in Figure 1.

We found several links among the studied molecules. MMP-2 was found to be correlated with TIMP-2 at different time points such as baseline (\( r=0.516, P=0.029 \)). Furthermore, baseline MMP-9 was related to MMP-3 at 24 hours (\( r=0.529, P=0.020 \)). In fact, MMP-2 and TIMP-2 showed a very similar pattern of temporal expression, as well as those observed for MMP-9, MMP-3, and TIMP-1 (Figure 1).

### Relationship Between MMPs and TIMPs With Radiological Features

MMP and TIMP release was independent of ICH volume, because we found no correlation between ICH volume and any of the measured molecules. These MMPs were not related to ICH volume increase. Baseline PE volume was positively correlated with baseline MMP-9 (\( r=0.673, P<0.01 \)) and inversely correlated with baseline TIMP-1 (\( r=-0.518, P=0.04 \)). Because of a possible shift toward proteolysis determined by MMP-9 and TIMP-1 interplay, and because the MMP assay measures MMP-TIMP complex, we analyzed MMP-9/TIMP-1 ratio with respect to PE (Figure 2). We came across an even more significant association between MMP-9/TIMP-1 ratio and PE than the relation found when MMP-9 and TIMP-1 were considered individually (\( r=0.73, P<0.01 \)).

Moreover, among baseline concentration of the studied molecules, the only biomarker related to PE enlargement within the first 48 hours was baseline MMP-9 (\( r=0.59, P=0.015 \), and the only one related to day 7 PE enlargement was baseline TIMP-1 (\( r=-0.54, P=0.045 \)).

Residual scar volume was related to all previous ICH and PE volumes, with the strongest association for ICH and PE volumes at 48 hours (\( r=0.825, P=0.002 \) and \( r=0.973, P<0.001 \) respectively). Among studied molecules, only baseline MMP-9 (\( r=0.688, P=0.019 \)) and MMP-3 (\( r=0.864, P<0.01 \)) at 24 hours were associated with residual scar volume.

### Relationship Between MMPs and TIMPs With Neurological Outcome

Among baseline factors, only age, ICH volume, and MMP-3 concentration were related to death (Table 3). After a logistic regression analysis, only baseline MMP-3 level remained as an independent predictor of death (OR=22; 95% CI: 1.54 to 314.29, \( P=0.023 \)) using a cutoff of >6 ng/mL for MMP-3 (Figure 3).

However, no data among clinical, laboratory, or radiological findings obtained at patients’ arrival could predict neurological worsening in the next days (data not shown).

### Discussion

This study shows dynamic changes in blood concentration of some MMPs (MMP-9, MMP-3, and MMP-2) and their natural inhibitors (TIMP-1 and TIMP-2) after a spontaneous supratentorial ICH. At different time points, overexpression of these molecules was found to be related to relevant neuroimaging and clinical endpoints in the setting of ICH. In relation to radiological features, baseline MMP-9 and TIMP-1 were related to PE volume, whereas residual scar volume was related to baseline MMP-9 and 24-hour MMP-3. Interestingly, clinical outcome was influenced by baseline MMP-3, which was a powerful predictor of death.

The temporal profile of some MMPs has been studied in other diseases such as myocardial infarction. Increased MMP-9, MMP-3, and MMP-2 expression was found within the first 24 to 48 hours in ischemic heart regions of a rabbit model of myocardial infarction, contributing to rapid injury and late ventricular dysfunction. In a recent report from Power et al.,12 raised MMP-2, MMP-3, MMP-7, and MMP-9 mRNA levels were found in the periclot area of a rat model of ICH at similar time points as those described in our patients. They found MMP-12, a molecule we did not test, to be the most highly induced MMP in this ICH model. It was expressed in activated monocytod cells surrounding the hematoma. In that study, MMP expression suffered a biphasic increase: MMP-3, -7, and -9 increased immediately after...
Figure 1. Temporal profiles of MMPs and TIMPs after ICH. Median values at each time point are represented by a continuous line. Dashed lines indicate mean values for healthy controls (see Methods). Wilcoxon test: *$P<0.05$, **$P<0.005$. 
ICH; later, a second peak of these molecules was present on day 7.

A possible explanation for this biphasic pattern in MMP expression would be the contribution of different pathogenic mechanisms underlying edema formation. Early edema is thought to be the result of release and accumulation of osmotically active serum proteins from the clot,\textsuperscript{16} and delayed edema is produced by the blood–brain barrier disruption (vasogenic edema) and cytotoxic edema that follows neuronal death. This late edema peaks between days 5 and 6 after

\[ \text{Baseline Edema Volume (cc)} \]

**Figure 2.** MMP-9/TIMP-1 ratio correlation with PE within 12 hours from symptom onset.

**Table 3.** Clinical, Radiological, and Laboratory Findings on Arrival Profile at the Emergency Department of Patients Who Died or Survived During the Follow-up Study Period

<table>
<thead>
<tr>
<th></th>
<th>Death (n=7)</th>
<th>Survival (n=14)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male</td>
<td>6</td>
<td>8</td>
<td>0.35</td>
</tr>
<tr>
<td>Age, y</td>
<td>80 (68–85)</td>
<td>65 (54–74)</td>
<td>0.026*</td>
</tr>
<tr>
<td>Previous high blood pressure</td>
<td>3</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>4</td>
<td>0.24</td>
</tr>
<tr>
<td>Liver disease</td>
<td>2</td>
<td>0</td>
<td>0.098</td>
</tr>
<tr>
<td>Undergoing antiplatelet therapy</td>
<td>0</td>
<td>3</td>
<td>0.51</td>
</tr>
<tr>
<td>Baseline GCS</td>
<td>15 (14–15)</td>
<td>15 (14–15)</td>
<td>0.96</td>
</tr>
<tr>
<td>Baseline NIHSS</td>
<td>14 (11–18)</td>
<td>13 (4.5–15.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>190 (166–230)</td>
<td>179 (170–210)</td>
<td>0.75</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>90 (81–110)</td>
<td>104 (90–110)</td>
<td>0.41</td>
</tr>
<tr>
<td>Temperature</td>
<td>36.5 (36–36.95)</td>
<td>36.4 (36.1–36.7)</td>
<td>0.75</td>
</tr>
<tr>
<td>ICH volume</td>
<td>74.3 (8–115.5)</td>
<td>10.3 (3.8–22.8)</td>
<td>0.043*</td>
</tr>
<tr>
<td>PE volume</td>
<td>3.7 (0–27.7)</td>
<td>6 (0.6–15.7)</td>
<td>0.968</td>
</tr>
<tr>
<td>Leucocyte count</td>
<td>11.9 (9.7–12.4)</td>
<td>10.4 (7.7–11.7)</td>
<td>0.341</td>
</tr>
<tr>
<td>Platelet count</td>
<td>226 (100–248)</td>
<td>199 (163.5–245)</td>
<td>0.990</td>
</tr>
<tr>
<td>INR</td>
<td>1.18 (1.06–1.20)</td>
<td>1.22 (1.10–1.29)</td>
<td>0.294</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>2.8 (2.4–3.0)</td>
<td>2.8 (2.4–3.9)</td>
<td>0.633</td>
</tr>
<tr>
<td>Glucose</td>
<td>132 (114–152)</td>
<td>124 (104–156)</td>
<td>0.428</td>
</tr>
<tr>
<td>MMP-2</td>
<td>963.6 (846.0–1248.8)</td>
<td>878.4 (695.1–1043.7)</td>
<td>0.399</td>
</tr>
<tr>
<td>MMP-3</td>
<td>7.5 (2.8–40.6)</td>
<td>2.4 (2.4–4.0)</td>
<td>0.035*</td>
</tr>
<tr>
<td>MMP-9</td>
<td>131.7 (42.4–165.5)</td>
<td>46.8 (14.0–124.7)</td>
<td>0.190</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>1167.5 (854.7–3867.4)</td>
<td>1049.8 (918.0–1329.6)</td>
<td>0.574</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>15.5 (4.7–79.7)</td>
<td>7.7 (3.2–11.3)</td>
<td>0.158</td>
</tr>
</tbody>
</table>

For continuous variables, medians are shown.

*\( P < 0.05 \).
symptom onset. In our study, a relation exists between baseline MMP-9 and early edema, and also with the growth of this edema during the next days.

The presence of ischemia surrounding cerebral bleedings has been largely debated and yet not elucidated. MMP-9 is beginning to be considered as a marker of ischemia in some territories. However, a comparison between those MMP-9 concentrations found among ischemic strokes and those others found among primary ICH reveals a clear difference: mean MMP-9 concentration is higher for those patients with ischemic strokes. We might therefore hypothesize that the lesser MMP-9 concentrations found among patients in this study would reveal hypoperfusion without ischemia rather than a true ischemia supported by others. Similarly, recent PET and MRI studies have shown that periclot hypoperfusion is a consequence of reduced metabolic demand, i.e., diaschisis rather than a sign of ischemia.

In our study, the response of different TIMPs in front of ICH is not homogeneous. TIMP-1 level increases very early after ICH onset; conversely, TIMP-2, which is constitutively expressed in brain parenchyma, decreases. Others have shown that the administration of TIMP-2 results in a reduction of PE by protecting the blood–brain barrier. Whether this is also true in humans and whether the very low TIMP-2 level observed in our study is caused by a TIMP-2 consumption is unknown. However, the role of TIMP-1 has not been properly studied in neurovascular pathologies. It is now assumed that it can exert antiapoptotic properties as well as MMP inhibition. In this context, our study would support a protective effect of baseline TIMP-1, particularly by modulating the balance between matrix synthesis and matrix degradation through MMP-9 inhibition.

It is known that hematoma induces 3 pathological changes in its proximity: cell death, vasogenic edema, and breakdown of blood–brain barrier. Histological sections are characterized by the presence of edema, neuronal damage, macrophages, and neutrophils in the region surrounding the hematoma. In a recent report, Qureshi et al investigated the formation of cell death associated with ICH in the perihematomal region of human specimens, showing that apoptosis seems to be a prominent form of cell death during the first days, whereas after 5 days of symptom onset, necrosis with inflammation seemed to be most prominent. Previous experimental studies in rats showed that apoptotic changes were mostly identified in neurons and astrocytes. The apoptotic pathway in ICH may involve nuclear factor κB, and it is also known that this transcription factor is implicated in the control of some inducible MMPs such as MMP-3, MMP-7, and MMP-9.

In our sample, MMP-3 level at 24 hours correlated with residual scar volume at 3 months. One of the substrates degraded by MMP-3 is laminin, whose degradation leads to neuronal death. We may therefore speculate that MMP-3 could contribute to cell death by several interrelated mechanisms that would have eventually contributed to the mortality of some ICH patients. Poor neurological outcome and residual cavity volume have also been related to other mechanisms such as increased plasma glutamate concentrations.

Several limitations should be noted in the present study. First, the small sample size and the high mortality rates in our population reduced the final number of fully available data on studied subjects at 3 months. Second, we studied just those molecules previously related to edema and blood–brain barrier breakdown. To fully elucidate molecular basis of ICH complications, future studies should include some other molecules theoretically more involved in ICH enlargement, such as hemostatic and fibrinolytic factors. Moreover, some of the studied biomarkers might already be elevated before ICH and related to vascular risk factors, because high concentrations are still found several months after the hemorrhagic event.

In conclusion, a characteristic temporal profile of MMP/TIMP release exists in human ICH. Increased MMP-9 is associated with PE and MMP-3 is associated with mortality. Both molecules are related to the residual scar volume. Whether blocking these molecules might be beneficial for ICH patients remains to be demonstrated.

Acknowledgments

This study was supported by a grant from Spain’s Fondo de Investigación Sanitaria (FIS 01/1389) awarded by the Ministry of Health and Welfare. We thank Pilar Bermúdez, Dorita Quiroga, and the Neuroradiology staff for technical assistance.

References


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Stroke. 2004;35:1316-1322; originally published online April 15, 2004;
doi: 10.1161/01.STR.0000126827.69286.90

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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