New Grading System Based on Magnetic Resonance Imaging in a Mouse Model of Subarachnoid Hemorrhage

Yusuke Egashira, MD, PhD; Hajime Shishido, MD, PhD; Ya Hua, MD; Richard F. Keep, PhD; Guohua Xi, MD

Background and Purpose—A grading system for experimental subarachnoid hemorrhage (SAH) that does not require animal euthanasia is currently unavailable. We proposed a new grading system based on MRI and evaluated the feasibility of this method in a mouse model of SAH.

Methods—SAH was induced by endovascular perforation in adult male C57BL/6 mice. Mice underwent MRI 24 hours after SAH and were categorized into the following 5 grades based on T2*-weighted imaging: Grade 0, no visible SAH or intraventricular hemorrhage (IVH); Grade 1, minimal/localized SAH without IVH; Grade 2, minimal/localized SAH with IVH; Grade 3, thick/diffuse SAH without IVH; and Grade 4, thick/diffuse SAH with IVH. Neurological deficits were then assessed and the mice euthanized for conventional SAH grading.

Results—Among a total of 47 mice, 4% were scored as grade 0, 30% as grade 1, 11% as grade 2, 30% as grade 3, and 26% as grade 4. This MRI grading had excellent interobserver reliability (weighted $\kappa$ value = 0.94), and there were strong correlations between the MRI grading and the conventional grading ($r=0.85$; $P<0.001$) or between MRI grade and neurological scores ($r=-0.46$; $P<0.01$).

Conclusions—The new MRI grading correlated well with conventional grading and enabled in vivo evaluation of SAH severity. This grading system may offer advantages in future studies of experimental SAH.

Key Words: grading system • mice • MRI • subarachnoid hemorrhage

The number of publications using rodent models of subarachnoid hemorrhage (SAH) has been rapidly increasing.1 The endovascular perforation method for inducing SAH is frequently used in rodents and is considered to be the closest imitation of human SAH. In that model, there are greater inter-animal variations in the extent and volume of subarachnoid clot compared with other SAH models.4 A method established by Sugawara et al5 is commonly used to evaluate the SAH severity in perforation models of rodent SAH, but this is performed after euthanasia by visualizing clot extension and thickness at the base of the brain. A grading system that does not require animal euthanasia is currently unavailable. In this study, we developed a new SAH grading system for the mouse endovascular perforation model based on MRI.

Materials and Methods

All animal protocols were approved by the University of Michigan Committee on the Use and Care of Animals. A total of 58 male C57BL/6 mice (Charles River Laboratories, Portage, MI) weighing 22 to 30 g were used. Food and water were available to all animals ad libitum before and after surgery. SAH was induced by single surgeon (Y. Egashira) using endovascular perforation technique as previously described.6 MRI was performed 24 hours after SAH using a 7.0-T Varian MR scanner (Varian Inc, Palo Alto, CA) with acquisition of T2 fast spin-echo and T2* gradient-echo sequences with a field of view of 20×20 mm, matrix of 256×256 mm, and 25 coronal slices (0.5 mm thick).7 We categorized animals into the following 5 grades according to the thickness of SAH clot and the presence of intraventricular hemorrhage (IVH) in lateral ventricle detected by T2*-weighted imaging (T2*WI): grade 0 indicates no visible SAH or IVH; grade 1 indicates minimal/localized SAH with no IVH; grade 2 indicates minimal/localized SAH with IVH; grade 3 indicates thick/diffuse SAH (hematoma exceeding 0.5 mm thickness is visible in >2 slices of T2*WI) with no IVH; and grade 4 indicates thick/diffuse SAH with IVH. Neurological scores were also evaluated at 24 hours after SAH by a blinded observer as previously described.8 Mice were given a score of 3 to 18 in 1-number steps (higher scores indicate greater function). Mice were then euthanized and the brains examined. The severity of SAH was assessed using a conventional grading system.9 The basal brain, including brain stem, was divided into 6 segments. Each segment was assigned a grade from 0 to 3 depending on the amount of SAH as follows: 0, no SAH; 1, minimal SAH; 2, moderate SAH with recognizable arteries; and 3, SAH covering the cerebral arteries. The animals were assigned a total score ranging from 0 to 18 by summing the scores from all 6 segments.

Data in text are expressed as the mean±SD. Commercially available software (JMP 7; SAS Institute Inc, Cary, NC) was used for all statistical analysis. The Cohen $\kappa$ ($K_1$) and weighted $\kappa$ ($K_w$) values were calculated to examine reproducibility of the scale between 2 investigators.10 The correlation between MRI grade and either conventional SAH severity score or neurological score was examined.
Results
The mortality rate was 19% (11 of 58) at 24 hours after SAH induction. Among a total of 47 remaining mice, 2 (4%) were scored as grade 0 by MRI grading system, 14 (30%) were grade 1, 5 (11%) were grade 2, 14 (30%) were grade 3, and 12 (26%) were grade 4 (Table). $K_w$ value for the thickness of the subarachnoid clot and $K_c$ value for the presence of IVH in the lateral ventricle were 0.94 and 0.82, respectively (Tables I and II in the online-only Data Supplement). $K_w$ value for the MRI grading scale was 0.94 (Table III in the online-only Data Supplement). Thus, it was considered that this system has excellent reproducibility. Figure 1 shows the representative T2- and T2*-weighted MR images and corresponding postmortem photos of basal brain in each MRI grade. The conventional SAH severity scores were 3.5±0.7 in grade 0, 6.4±1.4 in grade 1, 8.6±1.8 in grade 2, 10.5±2.0 in grade 3, and 12.8±1.9 in grade 4. There was a strong correlation between MRI grade and conventional SAH score ($r = 0.85; P < 0.001; $ Spearman’s rank correlation test; Figure 2A). There was also a significant correlation between MRI grade and neurological score ($r = -0.46; P < 0.01; $ Spearman’s rank correlation test; Figure 2B). The neurological scores were 13.5±3.5 in grade 0, 14.0±2.8 in grade 1, 11.4±2.4 in grade 2, 12.8±3.0 in grade 3, and 9.4±2.8 in grade 4. As expected, there was also a significant correlation between SAH severity score and neurological score ($r = -0.39; P < 0.01; $ Spearman’s rank correlation test; data not shown).

Discussion
In the present study, we developed a new reproducible SAH grading system based on T2*-weighted MRI. In endovascular perforation models of SAH in rodents, brain injury is related to the SAH bleeding (severity). In addition, it has been reported that SAH-related conditions, such as acute hydrocephalus and white matter injury, are also affected by SAH severity. It is expected that SAH severity may also relate to delayed SAH-induced complications, such as chronic hydrocephalus, as in humans. The MRI grading system enables in vivo evaluation of SAH severity without animal euthanasia and might offer advantages especially in long-term experimental studies. Thus, for example, it may be used to (a) examine the extent to which SAH severity affects long-term outcome, (b) reduce the model variability in therapeutic trials by ensuring that animals have a similar initial SAH severity, and (c) determine whether the effects of therapeutic interventions vary with SAH severity.

Our scale imitated the human SAH scale using computed tomography established by Claassen et al. Interestingly, the proportion of each grade in mice is comparable to that in human. Mice with IVH in the lateral ventricle tended to have worse neurological scores than those without IVH. In humans, SAH with IVH also tends to lead to worse clinical outcomes. MRI makes it possible to detect IVH, and the effect of IVH in SAH-induced brain injury should be determined in future experimental studies.

Although the present study has used T2*-weighted images to examine degree and location of SAH, the utility of other forms of imaging (susceptibility weighted imaging, quantitative susceptibility mapping) merits investigation. T2-weighted MR images can also be used to examine SAH-induced injury, allowing the relationship between T2WI abnormalities and SAH grading to be examined in future studies.

Table. Proposed MRI Grading System and Proportion of Animals in Each Grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Criteria</th>
<th>Number, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visible SAH or IVH</td>
<td>2 (4.3)</td>
</tr>
<tr>
<td>1</td>
<td>Minimal/thin SAH, no IVH in lateral ventricle</td>
<td>14 (29.8)</td>
</tr>
<tr>
<td>2</td>
<td>Minimal/thin SAH, with IVH in lateral ventricle</td>
<td>5 (10.6)</td>
</tr>
<tr>
<td>3</td>
<td>Thick SAH*, no IVH in lateral ventricle</td>
<td>14 (29.8)</td>
</tr>
<tr>
<td>4</td>
<td>Thick SAH*, with IVH in lateral ventricle</td>
<td>12 (25.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>47 (100)</td>
</tr>
</tbody>
</table>

IVH indicates intraventricular hemorrhage; and SAH, subarachnoid hemorrhage.

*Thick (>0.5 mm) SAH is visible in >2 slices in T2*-weighted MRI.

Figure 1. Representative T2- and T2*-weighted MR images (T2WI and T2*WI, respectively) and corresponding photos of basal brain in each MRI grade. Arrows indicate basal subarachnoid hemorrhage (SAH) clot and arrowheads indicate intraventricular hemorrhage in the lateral ventricle.

Figure 2. Relationships between MRI grading and conventional subarachnoid hemorrhage (SAH) score (A) and MRI grading and neurological score (B). There are significant correlations between MRI grades and SAH severity and neurological scores (Spearman’s rank correlation test). Circles indicate means.
Conclusions
Our new MRI grading system correlated well with conventional grading system and had excellent reproducibility. This system may offer advantages in future studies using rodent SAH models.

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Disclosures
None.

References
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SUPPLEMENTAL MATERIAL
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Cover title: In-vivo grading system for experimental SAH

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**SUPPLEMENTAL TABLES**

**Supplemental Table I: Frequency of response of two investigators for the thickness of SAH**

<table>
<thead>
<tr>
<th>Investigator 2</th>
<th>Investigator 1</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No SAH</td>
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<tr>
<td>No SAH</td>
<td>1</td>
</tr>
<tr>
<td>Minimal/localized SAH</td>
<td>1</td>
</tr>
<tr>
<td>Thick/diffuse SAH</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
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</table>

* Thick (> 0.5mm) subarachnoid hematoma (SAH) is visible in more than 2 slices in T2*-weighted MRI. In all cases of animals with thick/diffuse SAH, such hemorrhage was found on at least two adjacent sections; i.e. the hemorrhage was not sporadically located.

**Supplemental Table II. Frequency of response of two Investigators for the presence of intraventricular hemorrhage in the lateral ventricle**

<table>
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<th>Investigator 2</th>
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<th>Total</th>
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</thead>
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<tr>
<td>Absent</td>
<td>27</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Present</td>
<td>3</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>17</td>
<td>47</td>
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</table>

**Supplemental Table III. Frequency of response of two investigators for the MRI grades**

<table>
<thead>
<tr>
<th>Investigator 2</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>15</td>
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<tr>
<td>Total</td>
<td>2</td>
<td>14</td>
<td>5</td>
<td>14</td>
<td>12</td>
<td>47</td>
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