Prognostic value of intrathecal heme oxygenase–1 concentration in patients with Fisher Grade III aneurysmal subarachnoid hemorrhage

Oversight

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Object. Experimental studies have demonstrated the crucial role of posthemorrhagic erythrocyte catabolism in the pathogenesis of subarachnoid hemorrhage (SAH). The authors of this study aimed to investigate the prognostic value of a series of CSF biomarkers linked to heme metabolism in SAH patients.

Methods. Patients with Fisher Grade III aneurysmal SAH undergoing early aneurysm obliteration were enrolled. The levels of heme oxygenase–1 (HO-1), oxyhemoglobin, ferritin, and bilirubin in intrathecal CSF were measured on the 7th day posthemorrhage. The associations of functional outcome with clinical and CSF parameters were analyzed.

Results. The study included 41 patients (mean age 59 ± 14 years; 16 male, 25 female), 17 (41.5%) of whom had an unfavorable outcome (Glasgow Outcome Scale score ≤ 3) 3 months after SAH. In terms of the clinical data, age > 60 years, admission World Federation of Neurosurgical Societies Grade ≥ III, and the presence of acute hydrocephalus were independent factors associated with an unfavorable outcome. After adjusting for clinical parameters, a higher level of HO-1 appeared to be the most significant CSF parameter related to an unfavorable outcome among all tested CSF molecules (OR 0.934, 95% CI 0.883–0.989, p = 0.018). Further analysis using a generalized additive model identified a cutoff HO-1 value of 81.2 μM, with higher values predicting unfavorable outcome (82.4% accuracy).

Conclusions. The authors propose that the level of intrathecal CSF HO-1 at Day 7 post-SAH can be an effective outcome indicator in patients with Fisher Grade III aneurysmal SAH.

KEY WORDS • subarachnoid hemorrhage • cerebrospinal fluid • bilirubin • HO-1 • lactate • vascular disorders

Abbreviations used in this paper: DIND = delayed ischemic neurological deficit; GAM = generalized additive model; GOS = Glasgow Outcome Scale; HO-1 = heme oxygenase–1; IVH = intraventricular hemorrhage; SAH = subarachnoid hemorrhage; WFNS = World Federation of Neurosurgical Societies.

* Drs. Wang and Tang contributed equally to this work.
SAH and investigated their associations with the 3-month functional outcome in patients with Fisher Grade III aneurysmal SAH.

Methods

Patients

This study was approved by the National Taiwan University Hospital Committee of Human Research and conducted in accordance with human ethics regulations. The study recruited 18- to 80-year-old patients with Fisher Grade III aneurysmal SAH (the appearance of SAH on CT with hemorrhage of more than 1 mm thickness). Patients were excluded if they had obstructive hydrocephalus caused by intraventricular hemorrhage (IVH) in the third or fourth ventricle, arterial dissection, infectious aneurysm, massive intracerebral hemorrhage (defined as a hematoma volume > 30 ml), uncal brain herniation, meningitis, brain tumor, end-stage renal disease, or spinal cord tumor. Written informed consent was obtained from the patient or from the next of kin of patients with decreased consciousness.

Study Design

The patients recruited in this study received standard treatment, which was provided by an integrated team including neurosurgeons, neurointensivists, and interventional neuroradiologists. The management protocol consisted of resuscitation, early surgical or endovascular obliteration of the aneurysm, standard management of intracranial pressure and neurointensive care, and aggressive medical or endovascular therapy for vasospasm if present. In some cases of acute hydrocephalus with impaired consciousness, an external ventricular drain was inserted before angiography.

After surgery, patients were monitored in the neurointensive care unit. Head CT angiography or conventional angiography was performed when vasospasm was suspected or if neurological deterioration was noted. Chronic hydrocephalus requiring ventriculoperitoneal CSF shunting was defined as a clinical deterioration with no detectable cause other than hydrocephalus occurring after day 14 posthemorrhage, with progressive ventricular size increase and Evans index greater than 0.30. Delayed ischemic neurological deficit (DIND) was defined as clinical deterioration (i.e., a new focal deficit, decrease in the level of consciousness, or both) and/or a new infarct on a CT scan that was not visible on the admission or immediate postoperative scans. Other potential causes of clinical deterioration or hypodensity on CT, such as rebleeding, cerebral edema, retraction injury, ventriculitis, metabolic derangements, and seizures, were rigorously excluded. The functional outcome was evaluated using the Glasgow Outcome Scale (GOS) at 3 months after onset. A GOS score ≤ 3 was defined as an unfavorable functional outcome.

CSF Sample Collection, Preparation, and Analysis

Intrathecal CSF was obtained via lumbar puncture or lumbar drain on the 7th day after SAH. In our treatment protocol, lumbar puncture or lumbar drainage insertion was usually performed at Day 7 because we have found that most events of DIND occur at around 1 week after onset of SAH.

The CSF samples were immediately centrifuged at 900g and 4°C for 20 minutes before being divided into suitable aliquots and snap-frozen at ~80°C within 30 minutes. The concentrations of proteins, glucose, and lactate in the CSF samples were determined using an automatic chemistry analyzer. The levels of iron and ferritin were determined using quickauto-neo-Fe (K) (Toshiba-2000FR), and total bilirubin was determined on a Hitachi 7070 chemistry analyzer (also known as a Hitachi 911). The CSF HO-1 concentration rather than activity was determined using a Human HO-1 ELISA Kit (EKS-800, Stresagen/Assay Designs). The concentration of oxyhemoglobin was determined by means of spectrophotometry. In brief, the CSF samples were measured 3 times at each wavelength (A577 and A630), and the mean values of the measurements were used for calculation of the oxyhemoglobin concentrations.

Statistics

Statistical analysis was performed using R 2.14.1 software (R Foundation for Statistical Computing). In the statistical testing, a 1-sided p value ≤ 0.05 was considered statistically significant. The distributional properties of continuous variables were expressed as the mean ± SD, median, and interquartile range (IQR), whereas categorical variables were represented as frequency and percentage. In univariate analysis, the differences in the clinical and CSF biochemical parameters between good and poor outcomes were examined using the chi-square test, Fisher exact test, 2-sample t-test, 1-way analysis of variance (ANOVA), Wilcoxon rank-sum test, Kruskal-Wallis test, or log-rank test as appropriate. Next, multivariate analysis was conducted via logistic regression with significant variables from the aforementioned univariate analysis. The results were used to adjust the clinical significance (adjusted odds ratio) of CSF biomarkers in association with outcome. Finally, generalized additive models (GAMs) were applied to detect the cutoff value of continuous covariates.

Results

During the period between January 2009 and June 2010, a total of 81 patients with aneurysmal SAH were admitted to our hospital. After exclusion of patients who did not meet the inclusion/exclusion criteria (n = 26) or who did not consent to participation (n = 14), 41 patients were included in the study. Their mean age was 59 ± 14 years, and 39.0% were male (16 male, 25 female). Of these 41 patients, 20 (48.8%) had acute hydrocephalus, 21 (51.2%) had IVH, and 11 (26.9%) had DIND. In 12 cases (29.3%), the patients underwent transarterial embolization of their aneurysms; the other 29 patients underwent surgical clipping. External ventricular drainage was used in 23 cases (56.9%). By the end of the 3-month follow-up period, 17 patients (41.5%) were identified as having an unfavorable outcome.
In terms of the clinical data, univariate analysis showed that the patients with an unfavorable outcome were older and, as a group, had a higher percentage of admission World Federation of Neurosurgical Societies (WFNS) grades ≥ 3, IVH, and acute hydrocephalus than the patients with a favorable outcome (Table 1). Multivariate analysis further revealed that age > 60 years (p = 0.029), WFNS ≥ 3 (p = 0.038), and the presence of acute hydrocephalus (p = 0.021) were independent factors associated with an unfavorable outcome. Regarding the CSF data, univariate analysis showed that the patients with an unfavorable outcome had significantly higher levels of HO-1, oxyhemoglobin, bilirubin, ferritin, and lactic acid, a higher WBC count, and a lower percentage of lymphocytes than those with a favorable outcome. After adjusting for the clinical parameters of age, WFNS (≥ or < 3), and the presence of acute hydrocephalus and IVH, a higher level of HO-1 appeared to be the only independent CSF variable associated with an unfavorable outcome (OR 0.920, 95% CI 0.850–0.995, p = 0.038) (Table 2). There was no significant difference in HO-1 levels for SAH patients with or without DIND (71.1 ± 74.7 vs 99.2 ± 102.8 μM, p = 0.34).

Figure 1 demonstrates the association of the levels of oxyhemoglobin, ferritin, and bilirubin with individual GOS scores. Among the 4 CSF parameters, HO-1 had the best fit with GOS grade, because HO-1 decreased gradually as the GOS score increased. Furthermore, a GAM plot identified a clinically useful HO-1 cutoff value of 81.2 μM in identifying patients with an unfavorable outcome (64.7% sensitivity, 100% specificity, 100% positive predictive value, 80.0% negative predictive value, and 82.4% accuracy for HO-1 > 81.2 μM as a predictor of unfavorable outcome) (Fig. 2).

Discussion

Several clinical studies have shown that older patient age, poor neurological grade on admission, greater SAH on CT, and the existence of IVH or the occurrence of vasospasm or DIND are commonly associated with a poor outcome.2,3,20,21 However, using clinical scales such as the Fisher grade for the amount of blood on head CT, initial Glasgow Coma Scale score, or WFNS grade at admission often provides a crude estimation of the outcome. It is also difficult to precisely measure the amount of blood in the subarachnoid space based on routine imaging programs. In addition, the occurrence of vasospasm may be difficult to detect if not associated with obvious neurological deterioration. Therefore, it would be valuable to identify an appropriate biochemical marker to assist in predicting outcome in the early stages of SAH.

Recently, experimental studies have suggested that posthemorrhagic erythrocyte catabolism is crucially involved in the pathophysiological mechanisms of both early brain injury and delayed vasospasm after SAH.6,11,12,16 Therefore, the activation of heme metabolism may theoretically reflect the degree of neurological damage and correlate with outcome after SAH. Review of the previous literature shows that ferritin and bilirubin are the 2 most commonly investigated targets among the molecules involved in the heme metabolic pathway in clinical studies. One study showed that the CSF ferritin level peaked between Day 7 and Day 11 after SAH and was significantly correlated with Fisher CT score.25 Another study reported that level of CSF bilirubin was detectable in the 1st week but then became undetectable in the 2nd week.16 In contrast, the CSF ferritin level rose gradually and reached a peak between 1 and 2 weeks after SAH.

Regarding the association between the heme metabolic pathway and SAH-mediated neurological complications, one study in which CSF samples were collected from 70 SAH patients showed that patients with shunt-dependent hydrocephalus (SDHC) had significantly higher levels of CSF ferritin than those without SDHC in acute SAH.26 Another study included 39 patients with Fisher Grade III aneurysmal SAH, 20 of whom developed asymptomatic or symptomatic vasospasm.32 That study showed that the levels of ferritin and bilirubin were significantly higher in patients with no vasospasm than in patients with vasospasm on Day 5 to Day 7, and thus it was concluded that induction of HO-1 may be responsive for an intrinsic mechanism against vasospasm after the occurrence of SAH. In contrast, a study that included 12 SAH patients and focused on a similar topic showed that patients with vasospasm had higher CSF concentrations of HO-1 than patients without vasospasm.19 Moreover, most of the aforementioned studies evaluated only 1 or 2 parameters or recruited a relatively small sample size.

In our study, the clinical data showed that older age and the occurrence of acute hydrocephalus were 2 inde-

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**TABLE 1: Factors related to outcome in 41 patients with aneurysmal SAH**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Favorable Outcome (n = 24)</th>
<th>Unfavorable Outcome (n = 17)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean age in yrs</td>
<td>53.7 ± 12.7</td>
<td>65.8 ± 11.8</td>
<td>0.007</td>
</tr>
<tr>
<td>male</td>
<td>11 (45.8)</td>
<td>5 (29.4)</td>
<td>0.344</td>
</tr>
<tr>
<td>anterior circulation aneurysm</td>
<td>20 (83.3)</td>
<td>14 (82.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>WFNS grade ≥ III†</td>
<td>7 (29.2)</td>
<td>14 (82.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>IVH</td>
<td>8 (33.3)</td>
<td>13 (76.5)</td>
<td>0.011</td>
</tr>
<tr>
<td>acute hydrocephalus</td>
<td>6 (25.0)</td>
<td>14 (82.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DIND</td>
<td>6 (25.0)</td>
<td>5 (29.4)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

* Values are number of patients (% of outcome category) unless otherwise indicated. Means are presented with SDs. Favorable outcome represents a GOS score of 4 or 5; unfavorable outcome represents a score of 1–3.
† Grade on admission.
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TABLE 2: Relationship between CSF study results and outcome in 41 patients with aneurysmal SAH*

<table>
<thead>
<tr>
<th>CSF Study</th>
<th>Favorable Outcome (n = 24)</th>
<th>Unfavorable Outcome (n = 17)</th>
<th>Unadjusted p Value</th>
<th>Adjusted OR 95% CI p Value</th>
<th>Adjusted p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-1 (ng/ml)</td>
<td>38.56 ± 23.77</td>
<td>138.42 ± 99.82</td>
<td>&lt;0.001</td>
<td>0.920</td>
<td>0.950–0.995</td>
</tr>
<tr>
<td>bilirubin (mg/dl)</td>
<td>0.47 ± 0.49</td>
<td>0.81 ± 0.37</td>
<td>0.002</td>
<td>1.545</td>
<td>0.235–10.151</td>
</tr>
<tr>
<td>ferritin (ng/ml)</td>
<td>2.26 ± 2.75</td>
<td>5.49 ± 3.17</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>0.999–1.000</td>
</tr>
<tr>
<td>OxyHb (μM)</td>
<td>35.93 ± 62.74</td>
<td>244.52 ± 291.41</td>
<td>&lt;0.001</td>
<td>0.991</td>
<td>0.976–1.005</td>
</tr>
<tr>
<td>lactic acid (mmol/L)</td>
<td>5.09 ± 2.01</td>
<td>7.59 ± 2.05</td>
<td>&lt;0.001</td>
<td>0.898</td>
<td>0.513–1.572</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>55.92 ± 27.41</td>
<td>61.24 ± 39.28</td>
<td>0.989</td>
<td>1.000</td>
<td>1.000–1.000</td>
</tr>
<tr>
<td>WBC (per hpf)</td>
<td>1.14 ± 2.04</td>
<td>3.99 ± 4.47</td>
<td>&lt;0.001</td>
<td>1.023</td>
<td>0.979–1.068</td>
</tr>
<tr>
<td>lymphocytes (%)</td>
<td>40.04 ± 33.18</td>
<td>14.82 ± 10.85</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CSF data are adjusted for age, admission WFNS grade, and the presence of acute hydrocephalus and IVH. Outcome was considered favorable if the GOS score was 4 or 5 and unfavorable if it was 1–3. Boldface type indicates statistical significance. WBC = white blood cell count.

Fig. 1. Levels of (A) HO-1, (B) oxyhemoglobin, (C) bilirubin, and (D) ferritin at different GOS scores. The HO-1 levels of patients with GOS scores of 1 and 2 were significantly higher levels than those of patients with higher GOS scores, and the oxyhemoglobin levels of patients with GOS scores of 1 were significantly higher levels than those with the other GOS scores. Importantly, among the 4 parameters, HO-1 showed the best fit with GOS score, as HO-1 decreased gradually as the GOS score increased. **p < 0.01.
Our study not only supported the importance of HO-1 in the pathogenesis of acute SAH, but also proposed a clinically useful cutoff value of CSF HO-1 as a poor outcome indicator among patients with Fisher Grade III aneurysmal SAH.

Conclusions

Our study not only supported the importance of HO-1 in the pathogenesis of acute SAH, but also proposed a clinically useful cutoff value of CSF HO-1 as a poor outcome indicator among patients with Fisher Grade III aneurysmal SAH.

Disclosures

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