Association Between Serum Ferritin Level and Perihematoma Edema Volume in Patients With Spontaneous Intracerebral Hemorrhage

Manu Mehdiratta, MD; Sandeep Kumar, MD; David Hackney, MD; Gottfried Schlaug, MD, PhD; Magdy Selim, MD, PhD

Background and Purpose—Preclinical evidence indicates that iron plays a key role in mediating neuronal injury and edema formation after intracerebral hemorrhage (ICH). However, the clinical role of iron in patients with ICH has not been well studied. We undertook this exploratory study to investigate the association of serum ferritin, as an indicator of body iron load, with perihematoma edema after ICH.

Methods—We retrospectively reviewed prospectively-collected clinical and laboratory data from 23 consecutive patients with acute spontaneous ICH who had a CT scan and serum ferritin checked on admission, and a follow-up CT scan 3 to 4 days afterward. We measured hematoma and edema volumes on admission and follow-up scans, and calculated the relative edema volume to correct for hematoma volume. We used Spearman correlation coefficient to determine the association of various variables with relative perihematoma edema volume.

Results— Whereas the median hematoma volume increased by approximately 28% from baseline to day 3 to 4, the relative edema volume almost doubled during this time period. We observed a significant positive correlation between serum ferritin and relative perihematoma edema volume on day 3 to 4 (r = 0.78; P = 0.002), but not at baseline; and little correlation between the changes in hematoma volumes and corresponding relative edema volumes (r = 0.14). There was a trend for a positive correlation between body temperature and relative edema volumes.

Conclusions—Our findings support the notion that delayed iron toxicity plays a role in causing brain injury and edema formation after ICH. These findings are preliminary and need to be further investigated in future studies. (Stroke. 2008; 39:1165-1170.)

Key Words: ferritin ■ edema ■ hematoma ■ intracerebral hemorrhage

Hematoma expansion and the development of perihematoma edema are 2 of the major factors that contribute to the high morbidity and mortality of intracerebral hemorrhage (ICH).1–3 The edema volume could exceed that of the original hematoma and lead to elevated intracranial pressure or hydrocephalus with subsequent neurological deterioration or death.2–4 Several mechanisms contribute to the development of brain edema after ICH. There is an early phase during the first several hours after ictus involving hydrostatic pressure during hematoma formation and clot retraction; a second phase during the first 24 hours resulting from thrombin production and activation of the coagulation cascade; and a delayed phase involving hemolysis of red blood cells (RBCs) and hemoglobin-mediated toxicity.5–6 Animal studies demonstrate that brain edema peaks on the third or fourth day after ICH; and that the neurotoxicity of hemoglobin is, at least in part, iron-mediated.6–11 In vivo studies in animal models of ICH show that iron accumulates in the brain after ICH, starting at day 1 and peaking by day 3, and that iron-mediated neurotoxicity contributes to neuronal injury over many days after ICH.8–9 In addition, iron-modifying agents can reduce hemoglobin-induced neurotoxicity and brain edema in experimental models of ICH.12 These links between iron and the pathogenesis of neuronal injury after ICH have not been well studied in the clinical setting. We hypothesized that increased body iron is associated with increased perihematoma edema formation, and consequently undertook this exploratory study to investigate the association of serum ferritin, as an indicator of body iron load and iron availability in the hemolyzed RBCs, and relative brain edema after ICH.

Subjects and Methods

Study Design and Patient Selection
We retrospectively reviewed our prospectively collected stroke database for consecutive patients with ICH admitted to our service...
from August 2006 to February 2007. All patients had a baseline computerized axial tomography (CT) scan and serum ferritin level checked on admission. We identified patients with acute spontaneous ICH who had follow-up CT scans at days 3 to 4 for inclusion into this analysis. Patients with secondary causes of ICH, such as anticoagulant use, underlying aneurysm vascular malformation, or tumor, head trauma, or hemorrhagic transformation of ischemic infarcts were excluded. In addition, we excluded patients with initial or subsequent intraventricular and infratentorial hemorrhages to avoid technical flaws regarding accurate edema volume measurements in these situations. We also excluded patients who died, received antiedema treatments, or underwent surgical intervention before having a repeat scan on day 3 to 4 of hospitalization.

**Data Collection**

We retrieved demographic, clinical, laboratory, and radiological data. These included: age, sex, hemorrhage onset-to-imaging time, and temperature, mean arterial blood pressure (MAP; calculated as $1/3 \text{systolic} + 2/3 \text{diastolic blood pressure}$), serum glucose, complete blood counts (hemoglobin, hematocrit, and white cell count), and serum ferritin level at presentation. We also retrieved the length of hospitalization (LOH), discharge destination (home versus extended care or rehabilitation), modified Rankin Scale (mRS), and National Institute of Health Stroke Scale (NIHSS) scores on admission, discharge, and follow-up visits in the outpatient clinic at approximately 2 months after discharge to assess the degree and rapidity of neurological and functional recovery after ICH. Neurological improvement was defined as any improvement in NIHSS score points at follow-up compared to the NIHSS score on admission.

**Radiological Measurements**

We measured the hematoma and edema volumes on admission and follow-up CT scans, done between 3 and 4 days after admission. A single evaluator (M.M.) experienced in the interpretation of CT and blinded to patients’ clinical and biochemical data analyzed all CT scans to conduct volumetric measurements of ICH and edema lesion volumes. All images were processed off-line with the use of an imaging processing software running on an AW4.2 GE Advantage workstation. The ICH and edema volumes were calculated using a semiautomated process. The examiner manually drew regions-of-interest (ROI) by tracing the perimeter of appropriate high- and low-attenuation zones in each slice throughout the lesion (Figure 1A and 1C). Automated threshold values, based on Hounsfield unit measurements, were then applied to differentiate hematoma from skull and brain parenchyma from perihematoma edema. Using the threshold values to differentiate hematoma from edema, contiguous voxels were automatically summed to yield a hematoma volume and absolute edema volume (the volume of the hematoma and surrounding edema; Figure 1B and 1D). The blinded observer drew the ROI on an initial subset of 8 patients twice, at an interval of 3 weeks apart. The test/retest agreement was $r=0.95$ for edema volume and $r=0.99$ for hematoma volume measurements. Another investigator (M.S.) independently drew the ROI on the same subset of 8 patients. The interobserver agreement was $r=0.90$ for edema volume and $r=0.99$ for hematoma volume measurements. Because the intra- and interobserver reliability was extremely high, only one measurement by one observer (M.M.) was used for the remainder of the ROIs.

To control for hematoma volume, we subtracted the hematoma volume from that of the absolute edema, and divided the product by the hematoma volume, to express perihematoma edema volume as a ratio of the associated hematoma volume (relative edema volume). When very small, nonmeasurable, amounts of edema were present; the relative edema volume was assigned a zero value.

**Statistical Analysis**

We used Spearman correlation coefficient to determine the presence or absence of a correlation between variables, including ferritin level, and relative edema volume on days 3 to 4. Statistical significance was set at probability value of $p<0.05$. A corrected probability value
Table 1. A Summary of Hematoma and Edema (Absolute and Relative) Volumes at Admission and 3 to 4 Days Afterwards

<table>
<thead>
<tr>
<th>Volume</th>
<th>Baseline</th>
<th>Day 3 to 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoma, cm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>15.87 (2.89 to 94.6)</td>
<td>20.32 (2.84 to 186.58)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>31.45±30.57</td>
<td>42.61±35.76</td>
</tr>
<tr>
<td>Absolute edema, cm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>61.97 (11.66 to 242.12)</td>
<td>78.94 (11.43 to 351.34)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>71.15±64.53</td>
<td>98.75±87.85</td>
</tr>
<tr>
<td>Relative edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.32 (0.22 to 4.54)</td>
<td>2.38 (0.81 to 7.54)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1.39±1.04</td>
<td>2.93±1.78</td>
</tr>
</tbody>
</table>

Table 2. Correlation Coefficients of Admission Clinical and Laboratory Data, Including Serum Ferritin, With the Relative Edema Volume at Baseline and Day 3 to 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Admission Data</th>
<th>P Value</th>
<th>Day 3 to 4</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset-to-imaging time</td>
<td>−0.002</td>
<td>0.96</td>
<td>0.08</td>
<td>0.54</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.23*</td>
<td>0.04</td>
<td>0.25*</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.09</td>
<td>0.51</td>
<td>0.06</td>
<td>0.66</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.03</td>
<td>0.77</td>
<td>0.08</td>
<td>0.56</td>
</tr>
<tr>
<td>White cell count</td>
<td>0.06</td>
<td>0.62</td>
<td>0.04</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>0.05</td>
<td>0.67</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>Baseline serum glucose</td>
<td>0.09</td>
<td>0.51</td>
<td>0.10</td>
<td>0.32</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>0.13</td>
<td>0.13</td>
<td>0.78 †</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*P value ≤0.05.
†Statistically significant correlation (corrected for multiple comparisons; P≤0.003).

Results

A total of 87 patients were admitted to our stroke service with ICH during the study period. Sixty-four subjects were excluded from the present analysis; 7 died and 7 underwent subsequent surgical intervention or received antiedema treatment before day 4 of hospitalization, 4 did not have a follow-up CT because of withdrawal of care, and 20 had a secondary cause for ICH, including trauma (n = 3), coagulopathy (n = 10), vascular malformations (n = 4), cerebral venous sinus thrombosis (n = 1), and tumor (n = 2), 11 had intraventricular or infratentorial hemorrhages, and 15 did not have a CT scan on day 3 to 4 after ICH onset.

Data from the remaining 23 subjects were included in this analysis. Each of these 23 patients had a baseline head CT within 12 hours of ICH onset, serum ferritin level drawn within 24 hours of admission, and another head CT at day 3 or 4 of admission. Eight patients (35%) had lobar and 15 (65%) deep ICH. The mean age was 73.3 ± 12.3 years (range 32 to 92). Sixty-five percent were men, and 35% women. The mean NIHSS score on admission was 16 ± 6. The mean onset-to-imaging time was 4.3 ± 1.6 hours for the admission CT scan (range: 70 minutes to 6 hours and 40 minutes), and 70.2 ± 12.6 hours for follow up scan (range: 50 to 90 hours). The median LOH was 9 days (range: 2 to 27; mean±SD: 10.6±6.8). Three patients (13%) were discharged home, 70% to extended-care facilities and rehabilitation, and 17% died during hospitalization. Twelve patients (52%) were lost to follow-up, and 8 of the remaining 11 showed neurological improvement on follow-up. Four of the 11 subjects (36%) had a mRS ≥2 at follow-up. The mean NIHSS score at follow-up was 7 ± 5.

Table 1 lists the median, range, and mean±SD of hematoma and perihematoma edema volumes at admission and 3 to 4 days later. As it shows, whereas the hematoma size increased by approximately 28% from baseline to day 3 to 4, the relative edema volume almost doubled during this time period. There was no significant correlation between the percent change in hematoma volumes between admission and day 3 to 4, and percent change in the corresponding relative perihematoma edema volume (r = 0.14; P = 0.13). Table 2 shows the correlation coefficients of clinical and laboratory variables, including serum ferritin, on admission, with relative edema volume. As it indicates, there was a significant positive correlation between serum ferritin and relative perihematoma edema volume on day 3 to 4 (r = 0.78; P = 0.002), but not at baseline (Figure 2). There was also a positive correlation, albeit not significant when adjusting for multiple comparisons, between body temperature on admission and relative edema volumes on admission (r = 0.23; P = 0.04) and on day 3 to 4 (r = 0.25; P = 0.04).

Discussion

We hypothesized that body iron levels could have a causal relationship with the pathophysiological mechanisms that lead to neuronal injury and edema formation in the perihematoma region after ICH. We found a positive correlation between serum ferritin levels on admission and the relative perihematoma edema volume in this cohort of patients with spontaneous ICH.

Several lines of evidence indicate that brain injury after ICH is attributable in part to the release of hemoglobin and its degradation products; and that iron, a hemoglobin degradation product, plays a key role in mediating neuronal injury and delayed edema formation after ICH by promoting hydroxyl radical formation and oxidative stress.6-11 For example, local injection of lysed red blood cells into the rat’s brain results in marked brain edema formation within 24 hours, in contrast to injections of packed red cells, which causes delayed edema, coinciding with the release of hemoglobin.6 Similarly, infusion of autologous whole blood or ferrous iron into the basal ganglia in rats results in an increase in markers of DNA damage in the perihematoma area 3 days after ICH, suggesting that iron-mediated oxidative stress contributes to DNA damage and brain injury after ICH.11 There is also preclinical evidence that treatment with iron chelators can reduce neurological damage and disability, and provides neuroprotection after experimental ICH.12 To our knowledge, the status and role of iron in the brain after ICH has been examined in only 1 other small clinical study. Savman et al compared the levels of nonprotein-bound iron (NPBI) in the cerebrospinal fluid (CSF) of 20 preterm infants with intraventricular hemorrhage (IVH) and 10 preterm control infants.
They found NPBI in 75% of infants with IVH and in 0% of control infants, a difference that could not be explained by hemolysis alone. The NPBI was found frequently and at high levels in CSF from infants with IVH who had white matter lesions and subsequent disability, linking NPBI to brain damage after hemorrhage. Our findings lend further support to the notion that delayed iron toxicity plays a role in causing brain injury and might contribute to edema formation and enlargement after ICH.

We only observed a positive correlation between serum ferritin level and relative edema volume 3 to 4 days after ICH, but not at baseline. This is concordant with earlier studies showing that both brain edema and iron accumulation in the perihematoma region peak 3 to 4 days after experimental ICH, and that this coincides with the timing for hemoglobin hemolysis. Studies regarding the pathophysiological mechanisms and temporal evolution of perihematoma edema formation indicate that early edema formation within the first 24 hours after hemorrhage onset involves activation of the coagulation cascade, clot retraction, complement activation, and thrombin production; and that late edema is linked to erythrocyte lysis and toxicity from hemoglobin and its degradation products, including iron. The lack of an observed correlation between serum ferritin and relative edema volume on admission likely reflects the pathophysiological evolution of perihematoma edema.

We also found little correlation between the changes in hematoma volumes and corresponding relative edema volumes between admission and day 3 to 4. This result is in line with previous investigations and indicates that perihematoma edema formation cannot be solely considered as an epiphenomenon of hematoma growth.

We observed that the volume of edema was overall larger than the hematoma volume on admission in our patients, contrary to common anecdotal clinical experience where the volume of perihematoma edema is generally modest compared to the hematoma during the first 24 hours. This could be an artifact of our small sample size.

We measured serum ferritin, as an indicator of body iron load because other measurements, such as serum iron concentrations, total iron binding capacity, and transferrin saturation, have considerable analytic and day-to-day variability compared to that for ferritin. MRI using T2*-weighted gradient echo sequence and field-dependent relaxation rate increase (FDRI) methodology has been used to measure in vivo brain ferritin in a variety of neurological disorders. However, the ability of available MRI techniques to reliably distinguish the susceptibility effect of the hematoma from that of ferritin iron in the perihematoma tissue is yet to be optimized. Although we could not directly assess ferritin level in the vicinity of the hemorrhagic brain tissue, serum ferritin might be a suitable indicator of the availability of ferritin and iron in the area of ICH. After ICH, where the blood leaks out of the ruptured vessel, ferritin at the hemorrhage site is partly derived from the serum as blood serum separates from clotted blood following clot retraction, thereby releasing its ferritin content into the hematoma site. There is also indirect evidence to suggest that iron release from hemolyzed RBCs is likely to be influenced by serum ferritin levels. Comparative studies indicate good correlation between iron content in the bone marrow, where RBCs are stored, and serum ferritin levels.
Ferritin aggregates “siderotic granules” can be seen in developing RBCs when bone marrow smears are stained with Prussian blue. These granules are found in normal erythroblasts, but are absent in erythroblasts obtained from subjects with iron deficiency anemia. Furthermore, indirect indices of iron content in RBCs, including mean corpuscular volume and content of hemoglobin in the cell, positively correlate with serum ferritin levels, whereas the percentage of hypochromic erythrocytes with low hemoglobin content and erythrocyte protoporphyrin, which incorporates iron into the heme moieties of hemoglobin, are inversely correlated with serum ferritin.24–26

Lastly, a previous study in patients with ischemic stroke reported a positive correlation between ferritin concentration in the serum and CSF.27

We also found a trend for a positive correlation between body temperature and relative edema volumes. This finding and the pathophysiological links between body temperature and edema formation require further investigation, in view of similar results from other studies where higher body temperature on admission was associated with early neurological deterioration in patients with spontaneous ICH28; and emerging interest in the application of hypothermia to improve the outcome of patients with ischemic stroke.

Our study has limitations, and its small sample size does not allow us to arrive at firm conclusions. Therefore, it should be considered preliminary and hypothesis-generating at this stage. We had limited data on patients’ functional and neurological outcomes, as several of our patients were lost-to-follow up. We also do not know whether serum ferritin in our patients is a reflection of the body’s iron stores or is secondary to stress response after ICH. It is conceivable that the serum values obtained within hours of ICH onset mirror the upregulation of ferritin as part of an acute phase response.29 However, there is evidence against an early increase of ferritin secondary to stress response in patients with ischemic stroke, where serum ferritin levels remain stable during the first 48 hours after stroke and are unrelated to other biochemical markers of stress reaction.30 We also found that white cell count, a marker of stress response, did not correlate with edema formation or ferritin level after ICH in the present study.

Although recent therapeutic trials have focused on preventing hematoma expansion, there has been relatively less attention placed on the importance of perihematoma edema volume and its impact on neurological deterioration after ICH.31–32 Reducing perihematoma edema enlargement could be an important adjunctive therapeutic target to improve the outcome of patients with ICH, and in contrast to hematoma expansion which often occurs within the first few hours of onset,33 the delayed time window of iron-mediated injury may facilitate treatment. Despite the acknowledged limitations of our study, accumulating evidence warrants further investigations to assess whether iron-modifying agents could be of therapeutic value in ICH.

In conclusion, this study provides evidence that serum ferritin levels correlate with the relative perihematoma edema volume after ICH. This could be of potential therapeutic importance. Our findings are preliminary and need to be further investigated in larger-scale prospective studies.

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Disclosures
None.

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