Prognostic Value of Plasma β-Amyloid Levels in Patients With Acute Intracerebral Hemorrhage

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Background and Purpose—It has been proposed that the deposition of the β-amylloid peptide (Aβ) in the brain parenchyma and brain blood vessels has deleterious effects. We tested the hypothesis that the levels of plasma Aβ are related to the outcome in patients with intracerebral hemorrhage.

Methods—In a multicenter study, we prospectively included patients with spontaneous intracerebral hemorrhage within the first 24 hours after onset. At admission, we measured plasma Aβ40 and Aβ42 levels using ELISA techniques. Also, we recorded age, sex, vascular risk factors, National Institutes of Health Stroke Scale score, presence of intraventricular hemorrhage, localization, cause, and volume of the hematoma. We obtained the modified Rankin scale and defined a unfavorable outcome as modified Rankin scale >2 at 3 months. Bivariate and multivariate regression analyses were performed.

Results—We studied 160 patients (mean age, 73.8±11.3 years; 59.4% of them were men). A favorable outcome was observed in 64 (40%) of the patients. In the bivariate analyses, unfavorable outcome was associated with high age, female sex, diabetes mellitus, presence of intraventricular hemorrhage, high blood glucose, high National Institutes of Health Stroke Scale score, high volume, and high plasma levels of Aβ42 and Aβ40. The multivariate analysis showed that increased age (odds ratio, 1.07; 95% confidence interval, 1.035–1.21; P<0.0001), high admission National Institutes of Health Stroke Scale score (odds ratio, 1.29, 95% confidence interval, 1.17–1.42; P<0.0001), presence of diabetes mellitus (odds ratio, 4.15; 95% confidence interval, 1.21–14.1; P=0.02), and Aβ42 levels >9.7 pg/mL (odds ratio, 4.11; 95% confidence interval, 1.21–14.1; P=0.02) were independently associated with an increased likelihood of an unfavorable outcome.

Conclusions—High levels of plasma Aβ42 in patients with acute intracerebral hemorrhage are associated with a poor functional prognosis. (Stroke. 2014;45:413-417.)

Key Words: amyloid ■ cerebral hemorrhage ■ prognosis

Intracerebral hemorrhage (ICH) carries a poor prognosis. Mortality at 30 days is 40% and only 12% to 39% of the patients reach functional independence.1 Several studies have reported that age, volume of the hematoma at admission, and severity of the neurological deficit at admission are the most important predictors of outcome in ICH.2 In addition, hematoma growth (HG) is the main cause of neurological deterioration and it is associated with increasing mortality and poor functional outcome.5

Cerebral amyloid angiopathy (CAA) consists of the deposition of amyloid β (Aβ) peptide in the walls of small- to medium-sized arteries, arterioles, and capillaries in the cerebral cortex, leptomeninges, and cerebellum.6 In as many as 15% of patients with spontaneous ICH, the pathogenesis of ICH is attributed to CAA.7 However, the deposition of amyloid in cerebral vessels is common, and the extent of amyloid deposition is age related.8 CAA may be confirmed from the autopsy of 10% to 50% of the elderly population, in 80% of patients with Alzheimer disease9 and in 50% of patients with ICH.10

Aβ deposition leads to the loss of integrity of the vessel wall and has direct toxic effects that impair blood vessel and neuronal function.11 This loss of integrity may facilitate the expansion of the hematoma and may be involved in the mechanisms of brain injury during the acute phase of ICH. Thus, Aβ deposition may be associated with the outcome even if CAA is not related causally to the ICH. It is likely that the disruption of the blood–brain barrier (BBB) during the acute
stage of ICH allows for an acute release of Aβ peptides from the brain to the blood, as occurs in acute ischemic stroke. Moreover, circulating Aβ may cross the BBB in both directions, and therefore circulating Aβ may reach the brain. We hypothesize that plasma levels of Aβ in the acute stage of ICH have prognostic value in ICH in combination with other prognostic variables and may facilitate HG. To our knowledge, there are no studies that have evaluated the association between Aβ levels and outcome.

Material and Methods
We prospectively included patients with spontaneous ICH within the first 24 hours after onset in a multicenter study (6 hospitals). We excluded patients if they had a tumor or an arteriovenous malformation. The hospital Ethics Committee’s approved the study. Patients or their legal representative signed a consent form to participate.

Clinical and Radiological Data
We recorded the following data: (1) age and sex; (2) vascular risk factors: hypertension, diabetes mellitus, hypercholesterolemia, ischemic heart disease, previous ischemic stroke, and previous ICH; (3) a certified neurologist evaluated at admission the severity of the neurological deficit as measured by the National Institutes of Health Stroke Scale (NIHSS) score; (4) blood glucose, temperature, and blood pressure at admission; (5) presence of intraventricular hemorrhage; (6) localization (deep/lobar); (7) suspected pathogenesis: hypertensive ICH; (8) with oral anticoagulants was considered the cause of ICH in patients who received this medication; (9) volume of the hematoma on the admission computed tomography, measured by the ABC/2 method.

In the subgroup of patients included within the first 6 hours after onset and who had a 24-hour follow-up computed tomography also, we evaluated the association between the levels of plasma Aβ and HG. HG was measured as the difference between the volume at the follow-up computed tomography and the admission computed tomography, in absolute terms, in relative terms [(follow-up volume–admission volume) / admission volume]×100 and dichotomized into HG ≥33% and HG <33%.

We evaluated the functional outcome at 3 months after ICH with the modified Rankin scale. A favorable outcome was defined as modified Rankin scale ≤2.

Plasma Aβ Measurement
At admission, from each patient, 10 mL of blood was collected in 2.5-mL polypropylene sterile plunger tubes containing tubes containing EDTA as anticoagulant. The blood samples were centrifuged at 3300 rpm (1380g) for 15 minutes and aliquoted in 960-μL quantities into polypropylene tubes containing 40 μL of a protease inhibitor cocktail (Complete Mini, Roche, Indianapolis, IN). The samples were stored at −80°C. Plasma Aβ1-40 to 42 levels (expressed in pg/mL) were measured using a highly sensitive ELISA kits from Wako (Osaka, Japan) according to the manufacturer’s instructions. In a subset of patients, we obtained a second blood sample 24 hours after onset of stroke.

Statistical Analyses
Data are expressed as mean±SD or medians (with interquartile ranges). The prognostic value was dichotomized into favorable and unfavorable outcome (Rankin scale score, 0–2 versus 3–6). In the bivariate analyses, categorical variables were compared using contingency tables and the χ² test. Ordinal variables were compared with the Mann–Whitney test, and quantitative continuous variables were compared with the Student t test. A multivariate logistic regression analysis used variables with a P value ≤0.1 in the bivariate analyses and unfavorable outcome was the dependent variable. Because of the wide range in plasma Aβ40 and Aβ42 values, the results were analyzed with logarithmic transformation (log-Aβ40 and log-Aβ42), and cut-off with the best sensitivity and specificity values in the receiver operator characteristic curve was used to dichotomize the variable. A good-fit test (Hosmer and Lemeshow) was used and the discrimination value of the predictive model obtained in the regression analyses was assessed with the area under the curve of the receiver-operator characteristic curve.

For the HG substudy, patients were separated into 2 groups, according to the cut-off of 33%, and the analyses were the same as in the prognostic study. Bivariate analyses also included the Pearson correlation coefficient between plasma Aβ levels and relative or absolute continuous HG. A P value <0.05 was considered statistically significant.

Results
For various reasons we excluded 77 patients from a total of 237 patients: The blood sample was defective or was not obtained for 40 patients; 4 patients were lost to follow-up; the baseline hematoma volume was not measured for 12 patients; for 21 patients the Glasgow coma score instead of the NIHSS score was obtained. Thus, we studied 160 patients whose mean age was 73.8±11.3 years; 95 (59.4%) of them were men. They were studied a mean of 386±433 minutes (6.4±7.2 hours) after the onset of stroke; 40% were included within the first 3 hours and 67% were included within the first 6 hours. The blood samples were obtained after a mean of 10.1±6.8 hours. Median NIHSS score at admission was 8 (interquartile range, 4–14). Pathogenesis was attributed to hypertension in 63%, possible or probable CAA in 16.5%, oral anticoagulants in 7.5%, other diseases in 6.5%, and unknown in 6.5%. Table 1 shows variables in favorable and unfavorable prognostic groups. Patients with unfavorable outcome were older, more likely to be women, and more often have diabetes mellitus. They had higher NIHSS scores, higher blood glucose levels, higher frequency of intraventricular hemorrhage, higher frequency of deep-located ICH, and higher plasma levels of Log-Aβ40 (P=0.02) and Log-Aβ42 (P=0.048). The levels of Log-Aβ40 were 1.542±0.086 (corresponding to 34.8±7.3 pg/mL) in patients with favorable outcomes versus 1.607±0.249 (corresponding to 40.4±1.7 pg/mL) in those with unfavorable outcomes. The levels of Log-Aβ42 were 0.968±0.12207 (corresponding to 9.3±1.3 pg/mL) in patients with favorable outcomes versus 1.020±0.15808 (corresponding to 10.5±1.4 pg/mL) in those with unfavorable outcomes.

The multivariate analysis indicated that an unfavorable outcome was more likely with a high NIHSS score, old age, presence of diabetes mellitus, and a high level of plasma Aβ42 (dichotomized as greater or lower than 9.7 pg/mL because this cut-off showed the best accuracy in the receiver-operator characteristic curve analysis; Table 2). The Hosmer and Lemeshow test was nonsignificant (P=0.10). The area under the curve of this predictive model was 0.88 (95% confidence interval, 0.82–0.93).

The levels of Log-Aβ40 and Log-Aβ42-amyloid did not correlate with age or with NIHSS score. However, there was a weak correlation between Log-Aβ42 and the hematoma volume at admission (r=0.17; P=0.02), but not with Log-Aβ40. A trend to a higher level of Log-Aβ40 in patients with intraventricular hemorrhage (P=0.07) was also
observed. A blood sample was collected 24 hours after the onset of ICH in 52 patients. In these patients, the level of Aβ40 and Aβ42 did not correlate with hematoma volume and NIHSS score, but increasing age was associated with higher level of plasma Aβ42 at 24 hours (r=0.34; P=0.01). Levels of Aβ40 and Aβ42 were not associated with localization and pathogenesis of ICH.

The level of Log-Aβ40 was lower in patients who had HG (n=22) compared with those (n=88) who did not (1.55±0.19 versus 1.59±0.19; P=0.03). There was a trend also for lower values for Log-Aβ42-amyloid (0.95±0.14 versus 1.02±0.12; P=0.06) in patients with HG. However, there was no correlation between either Log-Aβ40 or Log-Aβ42 and absolute or relative HG as the continuous variable.

Discussion

In our study, we found an association between the level of plasma Aβ40 and Aβ42 peptides at admission and the clinical outcome. A higher level of Aβ was associated with an increased likelihood of an unfavorable outcome, although only the Aβ42 level was significantly associated with the outcome. Importantly, the prognostic value was independent of other predictors of spontaneous ICH. To our knowledge, this is the first study that investigated the role of plasma Aβ during the acute stage of ICH.

Cerebral amyloid peptides are produced by neurons, as well as the vessel walls.8,9 Accordingly, an imbalance between production and clearance results in accumulation of Aβ. 15 Increases in the Aβ40/Aβ42 ratio may promote the deposition of Aβ from the parenchyma to the blood vessels. However, Aβ peptides in the blood may be synthesized outside the brain9 mainly by platelets,16 but also by liver and kidney. Aβ can be found in the blood and cerebrospinal fluid of healthy individuals, and both the cerebral and peripheral pools of Aβ can cross the BBB in both directions.14,17,18 This is particularly relevant when there is a disrupted BBB as in the acute stage of ICH.19,20

The relationship between cerebral Aβ and their levels in plasma and cerebrospinal fluid in patients with stroke is not well understood. In patients with acute ischemic stroke, the levels of plasma Aβ, but not the levels of P-selectin, are

Table 1. Comparison of Demographic Data, Clinicoradiological Variables, and Plasma Log-Aβ40 and Log-Aβ42-Amyloid Values in Patients With ICH According to Clinical Outcome

<table>
<thead>
<tr>
<th>Variable</th>
<th>Favorable Outcome (n=64)</th>
<th>Unfavorable Outcome (n=96)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to diagnosis, min</td>
<td>462.5±577</td>
<td>336.1±294</td>
<td>0.11</td>
</tr>
<tr>
<td>Age, y</td>
<td>69.4±11.9</td>
<td>76.7±10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex, % men</td>
<td>71.9</td>
<td>51</td>
<td>0.009</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>65.6</td>
<td>61.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>7.8</td>
<td>26</td>
<td>0.004</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>18.8</td>
<td>20</td>
<td>0.99</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>7.8</td>
<td>9.5</td>
<td>0.78</td>
</tr>
<tr>
<td>Prior ischemic stroke, %</td>
<td>4.7</td>
<td>6.3</td>
<td>0.74</td>
</tr>
<tr>
<td>Prior intracerebral hemorrhage, %</td>
<td>1.6</td>
<td>3.1</td>
<td>0.65</td>
</tr>
<tr>
<td>Blood glucose at admission, mg/dL</td>
<td>113.5±28</td>
<td>132.7±44</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic blood pressure at admission, mm Hg</td>
<td>173.4±32</td>
<td>169.3±28</td>
<td>0.40</td>
</tr>
<tr>
<td>Diastolic blood pressure at admission, mm Hg</td>
<td>95.9±20.1</td>
<td>89.8±19</td>
<td>0.054</td>
</tr>
<tr>
<td>NIHSS score (median, IQR)</td>
<td>5 (1.25–8)</td>
<td>12 (8–17)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intraventricular hemorrhage, %</td>
<td>10.9</td>
<td>34.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Localization (% deep)</td>
<td>54</td>
<td>74</td>
<td>0.018</td>
</tr>
<tr>
<td>Cause, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>56.2</td>
<td>68.4</td>
<td>...</td>
</tr>
<tr>
<td>CAA</td>
<td>20.3</td>
<td>13.7</td>
<td>...</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>6.2</td>
<td>8.4</td>
<td>0.38</td>
</tr>
<tr>
<td>Other</td>
<td>7.8</td>
<td>5.3</td>
<td>...</td>
</tr>
<tr>
<td>Unknown</td>
<td>9.4</td>
<td>4.2</td>
<td>...</td>
</tr>
<tr>
<td>Volume of the hematoma at admission, mL</td>
<td>12.5±17.4</td>
<td>29.1±33.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log Aβ40-amyloid</td>
<td>1.54±0.08</td>
<td>1.6±0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>Log Aβ42-amyloid</td>
<td>0.97±0.12</td>
<td>1.02±0.15</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Aβ indicates β-amyloid peptide; CAA, cerebral amyloid angiopathy; ICH, intracerebral hemorrhage; IQR, interquartile range; and NIHSS, National Institutes of Health Stroke Scale.

Table 2. Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.07</td>
<td>1.03–1.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NIHSS score at admission</td>
<td>1.29</td>
<td>1.17–1.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aβ42-amyloid &gt;9.7 pg/mL</td>
<td>4.11</td>
<td>1.65–10.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4.15</td>
<td>1.21–14.16</td>
<td>0.023</td>
</tr>
</tbody>
</table>

The dependent variable is unfavorable outcome. Aβ indicates β-amyloid peptide; CI, confidence interval; and NIHSS, National Institutes of Health Stroke Scale.
elevated suggesting that they are synthesized in the brain. They are associated also with the size of the infarct and the NIHSS score.\textsuperscript{12} Also, it is possible for plasma levels of Aβ to be a marker of chronic small-vessel disease (lacunar infarcts and leukoaraiosis) because of either hypertension or CAA.\textsuperscript{20–23} In hemorrhagic stroke, one study reported that patients with chronic lobar ICH attributed to probable or possible CAA had higher concentrations of plasma Aβ when compared with controls and that these findings were unrelated to age.\textsuperscript{24} However, previous studies\textsuperscript{25} concluded that Aβ levels were the same in patients with ICH because of hypertension or CAA. However, patients with CAA exhibited decreased cerebrospinal fluid of Aβ40 and Aβ42 when compared with controls and with patients with Alzheimer disease.\textsuperscript{26} To our knowledge, no studies have investigated the effect of plasma Aβ levels in patients with acute ICH.

The underlying mechanism that accounts for the association between plasma Aβ levels and clinical outcome is unclear. A brain with a high amyloid content and with a large amount of chronic small-vessel disease may have difficulty compensating for an acute injury, independent of whether the ICH is attributable to CAA. The levels of plasma Aβ might be the result of parenchymal and vessel injury. A recent study\textsuperscript{27} found an association between cerebral atrophy and prognosis in patients with ICH and attributed this finding to a pre-existing neurodegenerative disease. Therefore, the clinical outcome is likely to be worse if the parenchymal or vessel amyloid concentrations are high. It is possible that some amyloid is released from the brain into the blood because of the disrupted BBB. As noted in ischemic stroke,\textsuperscript{12,21–23} plasma levels of Aβ might be an index of the cerebral amyloid content and the existence of chronic small-vessel disease. In agreement with this interpretation, we found a significant correlation between the volume and the levels of plasma Aβ42, the subtype of amyloid that is preferentially deposited in the parenchyma. However, this correlation was weak and was not detectable in the subset of patients in whom we measured Aβ in a 24-hour follow-up sample. Also, we did not find a correlation between volume and level of plasma Aβ40, the amyloid peptide that is preferentially deposited in blood vessels of the brain. Moreover, plasma Aβ levels were unrelated to HG, in all of the patients, and in the subset of patients with a follow-up sample at 24 hours. This finding suggests that if amyloid is involved in the pathogenesis of HG,\textsuperscript{28} this was not evident in the peripheral blood. We must emphasize that we did not find any relation between Aβ42 levels in the peripheral blood and age. This finding is unexpected because amyloid deposition in the brain parenchyma and brain vessels is strongly age related. Specifically, the levels of Aβ in blood increase with age,\textsuperscript{14} and age is strongly related to a worse outcome. However, in agreement with 1 study,\textsuperscript{24} the levels of Aβ in patients with chronic ICH attributable to CAA were unrelated also to age.

After an ICH, amyloid peptides may cause cerebral injury or interfere with the mechanisms of neurorepair, irrespective of the pathogenesis of ICH, and of the origin (cerebral or peripheral) of the amyloid. It is clear that amyloid is toxic for neurons and blood vessels, and it interferes with their structure and function. Some of the toxic effects attributed to amyloid include decrease in the expression of the tight junction proteins,\textsuperscript{9} increase in the expression of matrix metalloproteases,\textsuperscript{6,9,29} impairment of the regulation of cerebral blood flow,\textsuperscript{6,9,30} impairment of endothelial function,\textsuperscript{9,18,29} interference with the general homeostatic mechanisms of the aging brain,\textsuperscript{6,31,32} enhancement of BBB disruption,\textsuperscript{6} promotion of inflammation,\textsuperscript{6} oxidative stress,\textsuperscript{29,32} and antithrombotic effects.\textsuperscript{29,31,33} Aβ can cause abnormal vascular reactivity without vessel deposition or vessel wall dysfunction.\textsuperscript{11}

Our study has some limitations. The patients were included in the acute phase of ICH and were not controlled for the time of blood collection. Because circadian rhythms in plasma and cerebrospinal fluid Aβ have been reported,\textsuperscript{44} this may have influenced our results. However, any error of this type would be balanced in patients with and without a good outcome. Although our study was prospective, the inclusion of patients was not strictly consecutive and therefore some selection bias may have occurred. For instance, it is possible that patients with severe neurological deficits at admission (ie, with large hematomas and coma) or those who underwent a surgical evacuation could have been excluded from our study. Moreover, we excluded 77 patients for various reasons and they may differ from the analyzed population. Because we observed a wide range of values of plasma Aβ, we used logarithmic transformation. However, we cannot exclude the possibility that the outlier values are important markers of a unique phenomenon. We do not have cognitive assessments of patients before, during, or after a stroke. Cognitive impairment would support our hypothesis that a patient with a high amyloid content previous to the ICH is associated with high peripheral concentrations of Aβ during the acute stage of ICH. Finally, because of the lack of previous studies that measured amyloid proteins in patients with ICH, we were unable to calculate precisely whether our sample had sufficient power to test our hypothesis.

Conclusions

We found that a high Aβ42 concentration is associated with a worse clinical outcome after spontaneous ICH, without influencing the risk of HG. Our study is hypothesis-generating and requires confirmation. If confirmed, in addition to their prognostic value, our findings may have therapeutic interest and should encourage the design of studies directed to interfere with the production, accumulation, and release of amyloid in patients at risk of ICH. At risk are patients who are receiving oral anticoagulants; those who have experienced a first ICH related to CAA and those in whom a Pittsburgh compound B-PET demonstrates the accumulation of amyloid. If confirmed, plasma Aβ levels could be used as a prognostic or theragnostic marker in clinical trials in patients with ICH. Finally, the precise mechanisms by which amyloid peptides lead to a worse clinical outcome should be investigated.

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Circulating β-Amyloid and Prognosis in ICH

Disclosures

None.

References


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