The pathogenesis of cerebral small vessel disease (SVD) is incompletely understood. Pathological studies have suggested that there may be 2 types of SVD that can be differentiated on brain imaging. A diffuse arteriopathy of the brain microvasculature, referred to as lipohyalinosis (diffuse-SVD) and localized small vessel dysfunctions may play an important role in the pathogenesis of cerebral small vessel disease, and of cerebrovascular amyloid in the related condition of cerebral amyloid angiopathy. Plaque amyloid fibrils are the predominant constituents of senile plaques, one of the pathological hallmarks of Alzheimer disease, and of cerebrovascular amyloid in the related condition of cerebral amyloid angiopathy. Plaque amyloid is primarily comprised of Aβ1-42, whereas vascular amyloid is formed by the Aβ1-40 species. Recent evidence suggests that amyloid precursor protein overexpression and Aβ accumulation impair cerebral circulation. In vitro studies have suggested direct physiological or toxic effects of Aβ1-40 in the regulation of cerebral circulation by endothelial cells. In humans, recent studies have demonstrated an association among plasma Aβ concentrations, leukoaraiosis, and lacunar infarcts. However, the specific relationship between Aβ and SVD subtypes is unknown. We hypothesized that plasma Aβ levels can play a different role in SVD subtypes in patients with acute lacunar stroke.

Background and Purpose—The underlying mechanisms of small vessel disease (SVD) subtypes are diffuse arteriopathy (diffuse-SVD) or microatheroma (focal-SVD). Endothelial dysfunction by β-amyloid peptide (Aβ) deposition has been associated with lacunar infarcts and leukoaraiosis, but its specific relationship with SVD subtypes is unknown. We hypothesized that plasma Aβ levels can play a different role in SVD subtypes in patients with acute lacunar stroke.

Methods—We studied 149 patients with acute ischemic stroke of SVD etiology according to Trial Of Org 10172 In Acute Stroke Treatment criteria and 25 age-matched control subjects. Patients were classified into focal-SVD: 39 patients with isolated lacunar infarct without leukoaraiosis and diffuse-SVD: 110 patients with an isolated lacunar infarct with leukoaraiosis or with multiple lacunar infarcts with or without leukoaraiosis. Baseline data included vascular risk factors and extensive laboratory tests, including plasma Aβ levels.

Results—Median [quartiles] Aβ1-40 levels (40.4 [35.1, 50.5] versus 55.1 [42.3, 69.6] pg/mL), but not Aβ1-42 levels, were significantly higher in the diffuse-SVD group than in focal-SVD group (P<0.001) and control subjects (P<0.001). No differences in Aβ1-40 levels were found between focal-SVD and control subjects. Logistic regression analysis showed that age (OR, 1.06; 95% CI, 1.01 to 1.12), history of hypertension (OR, 3.5; 95% CI, 1.3 to 9.2), and plasma Aβ1-40 levels over the median value (OR, 17.3; 95% CI, 3.0 to 99 for the third quartile and OR, 6.0; 95% CI, 1.6 to 23 for the fourth quartile) were independently associated with the diffuse-SVD subtype.

Conclusions—Plasma β-amyloid1-40 levels are independently associated with the diffuse-SVD subtype. These results are consistent with the pathophysiological role of fraction Aβ1-40 in disrupting endothelial vascular function. (Stroke. 2009; 40:3197-3201.)

Key Words: acute stroke β-amyloid protein leukoaraiosis small vessel disease
Subjects and Methods

Study Population

BasicMar is a stroke register designed as a tool to study epidemiological data in a hospital-based population of a single center in Barcelona serving a population of approximately 300,000.

From January 2005 to November 2007, 1166 consecutive patients with acute stroke were admitted. Of them, 194 were diagnosed as SVD according to Trial Of Org 10172 In Acute Stroke Treatment (TOAST) criteria17 at hospital discharge. We excluded 27 patients without stored blood samples because they did not give their consent to participate in BasicMar register. We also excluded 8 patients whose Trial Of Org 10172 In Acute Stroke Treatment classification changed at the final visit and 10 patients who we lost contact with and were unable to follow-up at 3 months. Finally, 149 patients were eligible for this study; all survived the 3-month follow-up period.

Demographic data, stroke risk factors, acute phase clinical and biological data, stroke severity at hospital admission, and functional outcome at 3 months after stroke were prospectively recorded. SVD subtype was classified according to the Trial Of Org 10172 In Acute Stroke Treatment criteria17 using in-hospital and outpatient data and defined as a clinical lacunar syndrome with a compatible acute lesion on MRI or CT. Exclusion criteria included the presence of subcortical infarction >15 mm in diameter or cortical infarction of any size; carotid or vertebral artery stenosis >50%; and potential cardiac sources of embolism constituting high or moderate risk under Trial Of Org 10172 In Acute Stroke Treatment criteria.17 Patients were re-evaluated at 3 months after stroke onset to assess morbidity and mortality rates and confirm the initial Trial Of Org 10172 In Acute Stroke Treatment classification.17 We also included 25 age-matched control subjects without SVD. Control subjects were subjects with stroke mimic events that were included in the BasicMar register (seizures=5, hypoglycemia=4, conversion disorder=8, migraine with aura=2, peripheral neuropathy=3, and dizziness=3). None of them had a final diagnosis of stroke and all these patients had a normal neuroimaging study. Informed consent was obtained from the patients or relatives, and the study was approved by the Ethics Committee of Hospital del Mar, Barcelona.

Data Collection

Patients completed the extensive BasicMar clinical protocol, including demographic data (age and sex) and determination of the following vascular risk factors: arterial hypertension (defined as evidence of at least 2 blood pressure measurements >140/90 mm Hg recorded on different days before stroke onset, a physician’s diagnosis, or use of antihypertensive medication), diabetes (fasting serum glucose level ≥7.0 mmol/L, a physician’s diagnosis, or use of diabetic medication), hyperlipidemia (serum cholesterol concentration >12.2 mmol/L or serum triglyceride concentration >11.1 mmol/L, a physician’s diagnosis, or use of medication), alcohol overuse (>60 g/day), and current smoking status. We also recorded weight, height, waist circumference, systolic and diastolic blood pressure, axillary temperature, severity of symptoms based on National Institutes of Health Stroke Scale score18 at admission, and prestroke antplatelet and statins use. Biological determinations included leukocyte count and plasma creatinine levels. All patients had brain imaging, electrocardiogram, and Duplex or Doppler ultrasound study of the intra- and extracranial vessels. When clinical suspicion was high for a cardioembolic source of stroke, echocardiography was performed. The control group (stroke mimics) was evaluated by using the same clinical protocol, but ultrasound studies and echocardiography were performed only in doubtful cases.

Plasma Amyloid β Levels

Blood samples were collected on admission in glass test tubes, centrifuged at 3000 g for 10 minutes, and immediately frozen and stored at −80°C. Serum Aβ1-42 and Aβ1-40 levels were measured with commercially available quantitative enzyme-linked immunoabsorbent assay kits obtained from Biosource Europe SA, Belgium. Determinations were performed in an independent laboratory blinded to clinical and neuroimaging data.

Subtyping of Lacunar Stroke

Patients were retrospectively classified into 2 groups based on neuroimaging findings: focal-SVD group, including 39 patients with isolated lacunar infarct without leukoaraiosis, and diffuse-SVD group, including 110 patients with an isolated lacunar infarct with leukoaraiosis or with multiple lacunar infarcts (≥3) with or without leukoaraiosis.

Lacunar Brain Infarcts and Leukoaraiosis

We obtained axial T₁-, T₂-, and proton density-weighted scans on 1.5-T MRI scanners (GE Sigma) using 5-mm slice thickness. Slice thicknesses for CT scans varied from 5 to 8 mm.

Leukoaraiosis was defined as ill-defined hyperintensities ≥5 mm on both T2 and proton density/fluid-attenuated inversion recovery MRI images without prominent hypointensities on T1-weighted MRI scans and as ill-defined and moderately hypodense areas of ≥5 mm on CT. Leukoaraiosis was quantified on MRI by using the Fazekas scale.19 This method yields 2 separate scores for subcortical and deep white matter lesions and periventricular lesions. The 4-point Fazekas scale of increasing severity was used to classify each score with 0 indicating a patient without leukoaraiosis.

Lacunes were defined as well-defined hypodense areas of >2 mm and <15 mm on CT and as hyperintensities >2 mm and <15 mm on both T2 and proton density with hypointensities on T1-weighted and fluid-attenuated inversion recovery MRI images. If lesions with these characteristics were ≤2 mm, they were considered perivascular spaces, except around the anterior commissure, where perivascular spaces can be larger. Patients with both CT and MRI studies were classified in the diffuse or focal SVD group according to the MRI assessment.

Statistical Analysis

Quantitative variables are presented as mean±SD or median [interquartile range], as appropriate, and qualitative variables are presented as percentages. The t test or Mann-Whitney U test was used to assess differences in continuous variables, and the χ² test was used to compare proportions. The association between Aβ1-40 levels and the variables listed in Table 1 was analyzed by using the Mann-Whitney U test and Spearman correlation.

The relationship between Aβ levels and the SVD subtype was analyzed by logistic regression analysis (enter method). Due to a lack of linearity of the ORs, Aβ1-40 was entered in the logistic model classified in quartiles according to these 3 cutoff values, 39, 53, and 68 pg/mL. It has been suggested that the ratio of Aβ1-42 to Aβ1-40 may be more important than separate levels, at least for Alzheimer disease.20,21 Therefore, we also analyzed the association between the ratio of plasma Aβ1-42/Aβ1-40 and the diffuse SVD subtype. Potential confounders of the SVD subtype were included in the logistic models; we selected those factors that have been associated with SVD in previous reports or those associated with Aβ1-40 in the present work in bivariate analyses with a probability value <0.05. Statistical analyses were performed with the SPSS 13.0 software package.

Results

SVD subtype was diagnosed according to findings on acute MRI (n=104 [69.8%]) or CT alone (n=45 [30.2%]). A total of 39 patients were classified in the focal-SVD group (26 by MRI); none of them had leukoaraiosis. Two patients in the focal-SVD group had 2 lacunar infarcts in the MRI, whereas the other 37 had only one. The 110 participants classified in the diffuse-SVD group (78 by MRI) included patients with an isolated lacunar infarct with leukoaraiosis (n=41), with
Table 1. Medical History and Baseline Characteristics by SVD Subtype Groups

<table>
<thead>
<tr>
<th></th>
<th>Focal-SVD (n=39)</th>
<th>Diffuse-SVD (n=110)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years</td>
<td>66.3±10.8</td>
<td>73.6±9.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Males</td>
<td>69.2</td>
<td>63.6</td>
<td>0.33</td>
</tr>
<tr>
<td>Weight, kg (n=143)</td>
<td>73.2±13.6</td>
<td>74.3±15.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Height, cm (n=143)</td>
<td>163.7±9.2</td>
<td>162.4±9.8</td>
<td>0.47</td>
</tr>
<tr>
<td>Waist circumference (n=117)</td>
<td>100.2±16.1</td>
<td>100.8±17.3</td>
<td>0.86</td>
</tr>
<tr>
<td>History of arterial hypertension</td>
<td>46.2</td>
<td>73.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>25.6</td>
<td>40.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>33.3</td>
<td>33.6</td>
<td>0.56</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>7.7</td>
<td>6.4</td>
<td>0.80</td>
</tr>
<tr>
<td>Current smoking</td>
<td>53.8</td>
<td>32.4</td>
<td>0.016</td>
</tr>
<tr>
<td>Alcohol overuse</td>
<td>35.9</td>
<td>40</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>145.7±88.2</td>
<td>136.4±58.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Leukocyte count, ×10^9</td>
<td>7942.2±2438.7</td>
<td>7912.3±2475</td>
<td>0.94</td>
</tr>
<tr>
<td>Cholesterol, mg/dL (n=118)</td>
<td>215.6±43.3</td>
<td>195.2±49.2</td>
<td>0.047</td>
</tr>
<tr>
<td>Low-density lipoprotein, mg/dL (n=94)</td>
<td>153.5±33.9</td>
<td>143.9±44</td>
<td>0.32</td>
</tr>
<tr>
<td>High-density lipoprotein, mg/dL (n=94)</td>
<td>52.8±16.3</td>
<td>47.8±16.8</td>
<td>0.19</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.8 [0.7–1]</td>
<td>0.9 [0.7–1]</td>
<td>0.31</td>
</tr>
<tr>
<td>Triglycerides, mg/dL (n=118)</td>
<td>156.6±107.1</td>
<td>134.6±62.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Aβ_1-40 pg/mL</td>
<td>60.6 [35.6, 76.7]</td>
<td>61.3 [37.7, 64.8]</td>
<td>0.83</td>
</tr>
<tr>
<td>Aβ_1-40 mg/mL</td>
<td>40.4 [35.1, 50.5]</td>
<td>55.1 [42.3, 69.6]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aβ_1-42/Aβ_1-40 pg/mL</td>
<td>1.29 [0.89, 1.79]</td>
<td>0.92 [0.74, 1.27]</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>156.7±26.2</td>
<td>159.8±27.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83.2±17.2</td>
<td>83.2±14.9</td>
<td>0.97</td>
</tr>
<tr>
<td>Temperature, °C (n=124)</td>
<td>36.2±0.2</td>
<td>36.1±0.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Premorbid modified Rankin Scale score &gt;2</td>
<td>2.6</td>
<td>3.6</td>
<td>0.043</td>
</tr>
<tr>
<td>National Institutes of Health Stroke Scale</td>
<td>3 [1, 4]</td>
<td>3 [2, 4]</td>
<td>0.86</td>
</tr>
<tr>
<td>Antiplatelet pretreatment</td>
<td>20.5%</td>
<td>34.5%</td>
<td>0.07</td>
</tr>
<tr>
<td>Statins pretreatment; (n=143)</td>
<td>16.2%</td>
<td>23.8%</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Values are percentages, mean (SD) or median [quartiles] (n) no. of patients with available data.

multiple lacunar infarcts (n=18), and with multiple lacunar infarcts with leuкоaraiosis (n=51).

Medical history and baseline characteristics of the study participants by SVD subtype are given in Table 1. The diffuse-SVD group was older, had a higher frequency of arterial hypertension and current smoking, but did not differ from the focal-SVD group in other clinical or biochemical factors. Median [quartiles] Aβ_1-40 levels (40.4 [35.1, 50.5] versus 55.1 [42.3, 69.6] pg/mL), but not Aβ_1-42 levels, were significantly higher in the diffuse-SVD group than in the focal-SVD group (P<0.0001). Accordingly, Aβ_1-42/Aβ_1-40 ratio was lower in the diffuse-SVD subtype (P<0.0001). Aβ_1-40 levels have a moderate correlation with creatinine levels (r=0.30; P<0.001) and age (r=0.21; P<0.010) and were higher in patients with a history of arterial hypertension (P<0.002) and current smoking (P<0.019). The control group had similar mean age (68.2±14.6) and comparable vascular risk factors (current smoking in 32%, alcohol overuse in 20%, history of arterial hypertension in 80%, diabetes mellitus in 24%, hyperlipidemia in 48%, and peripheral arterial disease in 12%) and laboratory results (creatinine 0.8 [0.7 to 1] mg/dL and serum glucose 143±91.8 mg/dL) to the patient groups. Median levels of Aβ_1-40 were significantly higher in the diffuse-SVD group than in control subjects (P<0.0001), but not in the focal-SVD group. No differences were found in Aβ_1-42 levels between patients with SVD and control subjects (Table 2).

Logistic regression analysis showed that age (OR, 1.06; 95% CI, 1.01 to 1.12), history of hypertension (OR, 3.5; 95% CI, 1.3 to 9.2), and plasma β-amyloid_1-40 levels over the median value (OR, 17.3; 95% CI, 3.0 to 99 for the third quartile and OR, 6.0; 95% CI, 1.6 to 23 for the fourth quartile) were independently associated with the diffuse-SVD subtype (Table 3).

**Discussion**

This study demonstrates that plasma Aβ_1-40 levels, but not Aβ_1-42 levels, are strongly associated with the diffuse SVD subtype. The effect remained after controlling for relevant factors associated with either Aβ_1-40 or the diffuse SVD subtype such as age, renal function, and history of arterial hypertension.
Table 2. Median Concentration of Aβ1-42 and Aβ1-40 in Focal-SVD, Diffuse-SVD, and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Focal-SVD (n=39)</th>
<th>Diffuse-SVD (n=110)</th>
<th>( \text{P Value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1-42 (pg/mL)</td>
<td>45.8 [41.9, 52.6]</td>
<td>60.6 [35.6, 76.7]</td>
<td>61.3 [37.7, 64.8]</td>
<td>NS</td>
</tr>
<tr>
<td>Aβ1-40 (pg/mL)</td>
<td>43.5 [33.7, 47]</td>
<td>40.4 [35.1, 50.5]</td>
<td>55.1 [42.3, 69.6]</td>
<td>NS</td>
</tr>
</tbody>
</table>

Aβ levels (pg/mL) expressed as median [quartile 25th, quartile 75th]. NS indicates not significant.

Two types of cerebral SVD have been proposed based on neuropathological studies1–3; the first subtype shows single large lacunar infarcts caused by atherosclerosis in the larger perforating arteries, and the second is defined by multiple smaller lacunar infarcts resulting from a diffuse arteriopathy affecting the smaller perforating vessels in which the underlying pathology is lipohyalinosis usually due to hypertension.1–3 MRI studies have shown that the subtype with multiple lacunar infarcts is usually associated with leukoaraiosis.25 Distinct pathology of the subtypes is supported by differences in the risk factor profile.24,26 Therefore, the classification used in this study is justified by reported clinical and pathological findings.1–3

Considerable evidence suggests that endothelial dysfunction may play an important role in the diffuse SVD subtype. Two major mechanisms have been proposed: chronic hypoperfusion27–30 and increased blood–brain barrier permeability with leakage of plasma components into the vessel wall and surrounding brain parenchyma.31 Although we cannot rule out a confounding effect by yet unknown factors, the present results are consistent with the pathophysiological role of Aβ1-40 fraction in disrupting endothelial vascular function.14,15

Endothelial dysfunction by Aβ deposition has been related to lacunar infarcts and leukoaraiosis.14,15 Amyloid precursor protein is cleaved by secretases to produce several peptides such as Aβ1-40 and Aβ1-42. The insoluble Aβ1-42 is more fibrilligenic and deposits early in amyloid plaques. Soluble Aβ1-40 is a normal component of blood and cerebrospinal fluid, and in vitro studies have suggested direct physiological or toxic effects of Aβ1-40 on the blood vessel wall.13,32 Interestingly, these studies in cell cultures and transgenic mouse models13,32 provide evidence that Aβ1-40, but not Aβ1-42, causes reactive oxygen species-mediated cerebrovascular dysfunction.

Plasma Aβ1-40 levels were found to be markedly elevated in patients with ischemic stroke compared with age-matched control subjects in one study.23 Patients with cardioembolic and large artery atherosclerotic infarcts had higher Aβ1-40 levels than patients with SVD infarctions, and levels of Aβ1-40 correlated positively with infract size and stroke severity. However, this study included only 12 patients classified as SVD and the presence of leukoaraiosis was not analyzed. Elevated circulating Aβ1-40 in patients with ischemic stroke may derive from the brain as a consequence of the acute ischemic insult, but it might also be the result of prior chronic vascular insufficiency. In an experimental model in rodents, chronic cerebral hypoperfusion elicited the cleavage of the amyloid precursor protein into Aβ-sized fragments.34 Our results may reasonably rule out a secondary increase of plasma Aβ1-40 levels after an acute lacunar stroke because levels were similar in control and focal-SVD groups. In contrast, our results support the idea that previous Aβ1-40 levels increase as a result of diffuse-SVD disease. This, in turn, supports the pathophysiological role of fraction Aβ1-40 in disrupting endothelial vascular function.

This study has some strengths that should be acknowledged: Stroke-mimic patients with comparable vascular risk factors but without any SVD were studied as control subjects; Aβ plasma levels were measured in an independent laboratory without knowledge of the clinical factors and neuroimaging findings; and neuroimages were also evaluated blinded to all other data. We recognize also some limitations. Aggregation and clearance of Aβ may be influenced by the apolipoprotein E polymorphism, but that was not determined in this study. However, 2 studies have reported different results. In the Rotterdam Scan Study,14 increased plasma Aβ levels were positively associated with lacunar infarcts and leukoaraiosis in apolipoprotein E e4 carriers. This association was not found in the Gurol et al15 study performed on subjects with Alzheimer disease, mild cognitive impairment, and cerebral amyloid angiopathy. Another limitation is the relatively small sample size of the focal-SVD group.

The observation that plasma Aβ1-40 levels are strongly associated with diffuse SVD subtype is novel. Prospective studies are needed to confirm our results.

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Disclosures
None.

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Plasma β-Amyloid 1-40 Is Associated With the Diffuse Small Vessel Disease Subtype

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