Population-Based Study of Cerebral Microbleeds in Stroke-Free Older Adults Living in Rural Ecuador
The Atahualpa Project

Victor J. Del Brutto, MD; Mauricio Zambrano, BS; Robertino M. Mera, MD, PhD; Oscar H. Del Brutto, MD

Background and Purpose—Prevalence of cerebral microbleeds (CMB) in white and Asian populations range from 4% to 15%. However, there is no information from indigenous Latin American people. We aimed to assess prevalence and cerebrovascular correlates of CMB in stroke-free older adults living in rural Ecuador.

Methods—Of 311 Atahualpa residents aged ≥260 years identified during a door-to-door survey, 258 (83%) underwent brain magnetic resonance imaging. Twenty-one were further excluded for a diagnosis of overt stroke. Using multivariate logistic regression models, adjusted for demographics and cardiovascular risk factors, we evaluated whether CMB were independently associated with silent strokes, white matter hyperintensities, and global cortical atrophy.

Results—Twenty-six (11%) of 237 participants had CMB, which were single in 54% of cases. CMB were deep in 11%, small vessel disease in 11%, and cortical in 9, and located both deep and cortical in 6. In univariate analyses, CMB were associated with age, systolic blood pressure, moderate-to-severe white matter hyperintensities, silent lacunar infarcts, and cortical atrophy. Mean (±SD) values for systolic blood pressure were 155±27 mm Hg in patients who had CMB versus 142±26 mm Hg in those who did not (P=0.017). In the adjusted models, moderate-to-severe white matter hyperintensities (P=0.009), silent lacunar infarcts (P=0.003), and global cortical atrophy (P=0.04) were independently associated with CMB.

Conclusions—Prevalence of CMB in stroke-free older adults living in Atahualpa is comparable with those reported from other ethnic groups. There is a strong relationship between CMB and increased age, high systolic blood pressure, silent markers of cerebral small vessel disease, and cortical atrophy. (Stroke. 2015;46:00-00. DOI: 10.1161/STROKEAHA.115.009594.)

Key Words: cerebral small vessel disease • Ecuador • ethnic groups • microbleed

Cerebral microbleeds (CMB) represent foci of hemosiderin-laden macrophages resulting from extravasation of blood components that have been related to fibrohyalinosis of small penetrating vessels, amyloid angiopathy, and other vasculopathies.1 CMB are associated with imaging markers of cerebral small vessel disease and with cognitive decline, stroke recurrence, and vascular mortality.2 Therefore, recognition of CMB is important for the prompt implementation of preventive strategies. The prevalence of CMB in apparently healthy community dwellers range from 4% to 15% and is markedly influenced by age.3–6 These studies have been conducted in white and Asian populations, but there is no information from indigenous inhabitants of Latin America. We aimed to assess prevalence and correlates of CMB in stroke-free older adults living in rural Ecuador.

Methods
The Atahualpa Project is a population-based cohort study designed to reduce the increasing burden of stroke in rural Ecuador. The village was selected because it is representative of the region. More than 95% of the population belong to the Native/Mestizo ethnic group, and their living characteristics are homogeneous, as detailed elsewhere.7 The neuroimaging substudy enrolled all Atahualpa residents aged ≥260 years with no contraindications for magnetic resonance imaging (MRI) who signed the informed consent.8 The Institutional Review Board of Hospital-Clinica Kennedy, Guayaquil (FWA 0006667), approved the study. MRIs were performed with a Philips Intera 1.5T machine (Philips Medical Systems, Best, the Netherlands) at Hospital-Clinica Kennedy, Guayaquil, after a well-defined protocol detailed elsewhere.8 In brief, MRI included 2-dimensional multislice turbo spin echo T1-weighted, fluid attenuated inversion recovery, T2-weighted, and gradient-echo sequences in the axial plane, as well as a T1-weighted sequence oriented in the sagittal plane. We used the pre-established brain imaging package delivered by the manufacturer to homogenize applicability by technicians; slice thickness was 5 mm with 1-mm gap between slices.

Two readers, blinded to clinical data, independently reviewed all neuroimaging studies after the standards for research into small vessel disease proposed by Wardlaw et al.9 In particular, CMB were identified and rated according to the Microbleed Anatomical Rating System (MARS).10

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Scale,\textsuperscript{10} white matter hyperintensities (WMH) of presumed vascular origin were defined as lesions appearing hyperintense on T2-weighted images that remained bright on fluid attenuated inversion recovery (without cavitation) and graded according to the modified Fazekas scale.\textsuperscript{11} and lacunar infarcts were defined as fluid-filled cavities measuring 3 to 15 mm located in the territory of a perforating arteriole.\textsuperscript{9} Global cortical atrophy was rated according to Pasquier et al.\textsuperscript{15}

Cardiovascular risk factors—smoking status, physical activity, body mass index, blood pressure, fasting glucose, and total cholesterol levels—were assessed through interviews and procedures previously described in the Atahualpa Project.\textsuperscript{7} To recognize—and further exclude—patients with overt strokes, rural doctors screened all patients with the use of a validated field instrument, and then, certified neurologists confirmed the diagnosis.

Data analyses are performed using STATA version 13 (College Station, TX). In the univariate analysis, continuous variables were compared by linear models and categorical variables by $\chi^2$ or Fisher exact test as appropriate. Using multivariate logistic regression models, we evaluated whether CMB were associated with demographics, cardiovascular risk factors, WMH, silent lacunar infarcts, and global cortical atrophy, after adjusting for the other variables.

**Results**

MRIs were performed in 258 (83\%) of 311 Atahualpa residents aged ≥60 years identified during a door-to-door survey. Reasons for not obtaining MRI included refusal to participate (n=26), severe disability (n=11), claustrophobia (n=8), and implanted pacemaker (n=1); 7 additional patients had died or emigrated between the survey and the invitation. Twenty-one of the 258 participants were further excluded because of a diagnosis of overt stroke. Mean age of the 237 individuals enrolled in this study was 70±8 years, and 140 (59\%) were women. Twenty-six (11\%) individuals had CMB, which were single in 54\% of cases; CMB were deep in 11 cases, cortical in 9, and located both deep and cortical in 6. Moderate-to-severe WMH were noticed in 52 (22\%) patients, silent lacunar infarcts in 28 (12\%), and moderate-to-severe global cortical atrophy in 120 (51\%).

Kappa coefficients for inter-rater agreements of MRI lesions of interest were 0.90 for WMH, 0.76 for deep and 0.53 for cortical CMB, 0.90 for lacunar infarcts, and 0.82 for cortical atrophy. The modest agreement rate for cortical CMB was related to the endemcity of calcified neurocysticercosis in the village because both lesions may appear identical on MRI. As previously described by our group, inter-rater discrepancies were resolved by consensus and by reviewing CT findings\textsuperscript{15} and, for the present study, we only included lesions that were definitive cortical CMB.

Table 1 summarizes clinical and imaging characteristics of participants and across the categories of CMB. In univariate analyses, CMB were associated with age, systolic blood pressure, moderate-to-severe WMH, silent lacunar infarcts, and cortical atrophy. In multivariate adjusted models, moderate-to-severe WMH, silent lacunar infarcts, and global cortical atrophy were independently associated with CMB (Table 2).

**Discussion**

Prevalence of MB in stroke-free older adults living in Atahualpa (11\%) is within the range of that reported from industrialized nations. Our results provide robust evidence for an association between CMB and age, high systolic blood pressure, silent markers of cerebral small vessel disease, and cortical atrophy. Population-based studies conducted in developed countries have presented conflicting results about the association of CMB with cardiovascular risk factors or with markers of small vessel disease in individuals without history of cerebrovascular disease.\textsuperscript{3-6} To our knowledge, no population-based study from Latin America has addressed the prevalence and cerebrovascular correlates of CMB in patients not consulting for a vascular disorder. A potential limitation of the present study is the small sample size, which is counterbalanced by its population-based design and the unbiased selection of participants. Additional studies in other regional communities or in Latin American immigrants to the United States (Hispanics) are needed to confirm our findings, and longitudinal studies would be of value to address the consequences of CMB in this ethnic group.

**Table 1. Characteristics of Stroke-Free Atahualpa Residents Aged ≥60 Years According to the Presence of Cerebral Microbleeds**

<table>
<thead>
<tr>
<th></th>
<th>Total Series (n=237)</th>
<th>Microbleeds (n=26)</th>
<th>No Microbleeds (n=211)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD</td>
<td>70±8</td>
<td>75±9</td>
<td>69±8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>140 (59)</td>
<td>14 (54)</td>
<td>126 (60)</td>
<td>0.718</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>2 (1)</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Poor physical activity, n (%)</td>
<td>16 (7)</td>
<td>4 (15)</td>
<td>12 (6)</td>
<td>0.082</td>
</tr>
<tr>
<td>BMI, kg/m$^2$, mean±SD</td>
<td>27±5</td>
<td>26±5</td>
<td>27±5</td>
<td>0.337</td>
</tr>
<tr>
<td>Systolic BP, mm Hg, mean±SD</td>
<td>143±26</td>
<td>155±27</td>
<td>142±26</td>
<td>0.017</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg, mean±SD</td>
<td>72±11</td>
<td>74±10</td>
<td>72±11</td>
<td>0.378</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL, mean±SD</td>
<td>102±40</td>
<td>110±49</td>
<td>101±39</td>
<td>0.282</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL, mean±SD</td>
<td>212±40</td>
<td>214±40</td>
<td>212±40</td>
<td>0.810</td>
</tr>
<tr>
<td>Moderate-to-severe cortical atrophy, n (%)</td>
<td>120 (51)</td>
<td>19 (73)</td>
<td>101 (48)</td>
<td>0.027</td>
</tr>
<tr>
<td>Moderate-to-severe WMH, n (%)</td>
<td>52 (22)</td>
<td>14 (54)</td>
<td>38 (18)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Silent lacunar infarcts, n (%)</td>
<td>28 (12)</td>
<td>10 (38)</td>
<td>18 (9)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure; and WMH, white matter hyperintensities.
Table 2. Association of Cerebral Microbleeds With Moderate-to-Severe White Matter Hyperintensities of Presumed Vascular Origin, Silent Lacunar Infarctions, and Moderate-to-Severe Global Cortical Atrophy in Multivariate Logistic Regression Models Adjusted for Demographics and Cardiovascular Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silent lacunar infarction</td>
<td>11.82</td>
<td>2.3–60.3</td>
<td>0.003</td>
</tr>
<tr>
<td>White matter hyperintensities</td>
<td>5.67</td>
<td>1.6–20.7</td>
<td>0.009</td>
</tr>
<tr>
<td>Silent lacunar infarction + white matter hyperintensities</td>
<td>11.35</td>
<td>2.51–51.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Global cortical atrophy</td>
<td>2.61</td>
<td>1.04–6.57</td>
<td>0.042</td>
</tr>
</tbody>
</table>

CI indicates confidence interval.

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Disclosures

Dr Mera has stock interests in GlaxoSmithKline. The other authors report no conflicts.

References

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