Progression of Symptomatic Intracranial Large Artery Atherosclerosis Is Associated With a Proinflammatory State and Impaired Fibrinolysis

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Background and Purpose—The molecular pathways involved in the progression of intracranial large artery atherosclerosis (ILA) are largely unknown. Our objective was to prospectively study the relationship between circulating levels of inflammatory markers and fibrinolysis inhibitors, and the risk of progression of symptomatic ILA.

Methods—Seventy-five consecutive patients with first-ever symptomatic intracranial atherostenosis were studied. Blood levels of C-reactive protein (CRP), E-selectin, monocyte chemoattractant protein-1, intercellular adhesion molecule-1, matrix metalloproteinases 1, 2, 3, 8, 9, 10, and 13, plasminogen activator inhibitor-1 (PAI-1), and lipoprotein(a) were measured 3 months after the qualifying stroke or transient ischemic attack. Thereafter, patients underwent long-term transcranial Doppler follow-up to detect progression of ILA.

Results—During a median follow-up time of 23 months, 25 (33%) patients showed ILA progression. Multivariable adjusted Cox regression models and Kaplan–Meier curves showed that high baseline level of CRP, E-selectin, intercellular adhesion molecule-1, matrix metalloproteinase 9, PAI-1, and lipoprotein(a) predicted ILA progression independently of vascular risk factors. Of them, only CRP (CRP \( >5.5 \) mg/L; HR, 5.4 [2.3 to 12.7]; \( P = 0.0001 \)) and PAI-1 (PAI-1 \( >23.1 \) ng/mL; HR, 2.4 [1.0 to 5.8]; \( P = 0.05 \)) predicted ILA progression also independently of the other studied molecules.

Conclusion—Progression of symptomatic ILA is associated with a proinflammatory state, as reflected by high levels of inflammatory markers, and with defective fibrinolysis, as indicated by raised concentrations of endogenous fibrinolysis inhibitors. (Stroke. 2008;39:1456-1463.)

Key Words: fibrinolysis • inflammation • intracranial atherosclerosis • intracranial stenosis • progression

Intracranial large artery atherosclerosis (ILA) is a major cause of ischemic stroke worldwide.\(^1\)\(^2\) The patients affected by this disease are exposed to an especially high vascular risk, as reflected by the elevated annual stroke recurrence rates (\( \approx 14\% \)) observed despite best antithrombotic treatment.\(^3\)

ILA is a dynamic disease, because atherosclerotic lesions located in intracranial large arteries may progress over time.\(^4\)

Moreover, progression of symptomatic intracranial atherosclerosis has been shown to determine an increased risk of recurrent ischemic stroke.\(^5\)\(^6\) However, the basic mechanisms responsible for the progression of ILA are still poorly understood. In addition, the atherosclerotic process in intracranial arteries may have differential characteristics derived mainly from the anatomic and hemodynamic peculiarities of the intracranial territory.\(^7\) Therefore, specific research may be needed to elucidate to which extent the molecular pathways implicated in the development of extracranial atherosclerosis are also involved in the progression of intracranial atherosclerosis.

Among the pathogenic mechanisms involved in atherogenesis, we will focus on inflammation and endogenous fibrinolysis. At extracranial arterial territories, inflammation plays a crucial role mediating all the stages of the atherosclerosis process.\(^8\) Similarly, thrombosis and defective fibrinolysis may also contribute to the progression of atherosclerotic lesions.\(^9\) Interestingly, both mechanisms might have a relevant role in the pathogenesis of ILA, as suggested by our
previous studies showing that C-reactive protein (CRP)\textsuperscript{10} and lipoprotein (a) [Lp(a)]\textsuperscript{11} were associated with an increased risk of clinical recurrence in symptomatic ILA patients and with a greater extent of the disease, respectively. Nevertheless, whereas circulating inflammatory markers and fibrinolysis inhibitors have been shown to predict atherosclerosis progression at extracranial territories,\textsuperscript{12–14} their role in the progression of ILA remains unknown. Therefore, our aim was to study the relationship between the blood level of different inflammatory molecules and endogenous fibrinolysis inhibitors, and the risk of progression of symptomatic ILA.

**Subjects and Methods**

**Patient Selection**

We conducted a prospective, long-term follow-up study. Our target group consisted of first-ever TIA or ischemic stroke patients with symptomatic intracranial atherostenosis, detected by transcranial Doppler (TCD) and confirmed by MRA or CTA, who survived until blood sampling conducted 3 months after the qualifying event. Main exclusion criteria belonged to the following categories: (1) presence of other potential causes of cerebral ischemia; (2) nonatherosclerotic origin of intracranial stenoses; (3) existence of conditions known to modify the levels of the studied molecules; and (4) impossibility to perform TCD long-term follow-up attributable to stroke-related disability or the lack of an adequate acoustic window.

Between June 2001 and January 2004, 196 consecutive TIA or ischemic stroke patients admitted to our Stroke Unit showed intracranial stenoses potentially responsible for the cerebral ischemic event on TCD recordings. Our diagnostic protocol during admission has been reported in detail elsewhere.\textsuperscript{14} After diagnostic work-up, 121 patients had to be excluded because of the following reasons: absence of angiographic confirmation (n = 13); nonsymptomatic intracranial stenosis (n = 7); presence of severe ipsilateral cervical internal carotid artery stenoses (n = 7); embolic cardiopathy (n = 22); nonatherosclerotic causes of intracranial stenosis such as Sneddon syndrome, moyamoya disease, and vasculitis (n = 7); placement of stents (n = 3); neoplasia (n = 7); chronic renal failure (n = 4); chronic inflammatory diseases (n = 9); stroke-related death or severe disability (n = 10); lack of an adequate acoustic window (n = 21); and denial of informed consent (n = 1). At the inclusion visit, performed 3 months after the qualifying event, informed consent and blood samples were obtained from 75 patients with symptomatic intracranial atherostenosis. Sixty-four of these 75 patients had participated in a previous cross-sectional study by our group.\textsuperscript{11} All of them underwent TCD follow-up during a minimum of 1 year to detect ILA progression. This study was approved by the local ethics committee.

**Clinical Variables**

Cigarette smoking and medical history of hypertension, hypercholesterolemia, and type 2 diabetes mellitus were recorded at the inclusion visit. Stroke severity was assessed with the maximum NIHSS score during admission. Functional status at day 90 was assessed by means of the modified Rankin scale score. Secondary prevention therapies were established in an individualized manner, following the recommendations of the American Heart Association guidelines available during the study period, to optimize antithrombotic treatment and vascular risk factor control in all patients.\textsuperscript{15} The use of acenocoumarol, aspirin, clopidogrel, triflusal (an antiplatelet agent structurally related to aspirin approved for stroke prevention in Spain), statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers was registered. After inclusion, clinical visits were conducted every 6 months by a stroke neurologist (J.F.A.) who remained unaware of TCD and biochemical data throughout the study period. The following vascular events were recorded during follow-up: acute ischemic stroke; certainly diagnosed TIA; acute myocardial infarction or angina requiring hospitalization; and vascular death.

**Ultrasound Protocol**

TCD recordings were performed using a Multi-Dop-X/TCD (DWL Elektronische Systeme GmbH) device, with a hand-held transducer in a range-gated, pulsed-wave mode at a frequency of 2 MHz. We used a standard method of insonation through the temporal, occipital, and orbital windows without compression testing, as previously described.\textsuperscript{3,16} According to validated criteria, intracranial stenoses were diagnosed if the mean blood flow velocity at a circumscribed insonation depth was > 80 cm/sec, with side-to-side differences > 30 cm/sec and signs of disturbed flow.\textsuperscript{17} Maximum mean flow velocity values for each stenosis recorded at the inclusion visit were used as baseline values for the progression study. TCD long-term follow-up was conducted in our Cerebral Hemodynamics Laboratory with a 6-month periodicity by stroke neurologists (C.A.M. M.Rubiera) with expertise in detection and monitoring of intracranial stenoses and who were blinded to clinical and biochemical data. Progression of ILA was defined either as the appearance of new stenoses during follow-up or as the sonographic progression of preexisting stenoses, as defined by: (1) an increase\textsuperscript{3} in maximum mean velocity values > 30 cm/sec; or (2) an evolution of the TCD flow signal from a stenotic pattern to a dampened pattern or to a chronic occlusion pattern.\textsuperscript{6} If the changes in velocity values remained below this threshold, ILA was considered to be stable. ILA regression was diagnosed when a decrement > 30 cm/sec in maximum mean velocity values was observed. Coexistent extracranial internal carotid artery atherosclerosis was evaluated and categorized in our laboratory as previously described.\textsuperscript{11}

**MRA and CTA**

Intracranial stenoses were confirmed during admission by MRA or by CTA when it was not possible to perform MRI. Our MRA and CTA protocols have been described in detail previously.\textsuperscript{11} Intracranial stenosis was defined as a focal narrowing > 50% in luminal reduction affecting the main cerebral large arteries. Images were interpreted by the same neuroradiologist (A.R.), who was blinded to sonographic data.

**Blood Sampling and Biomarkers Immunoassays**

Blood samples were drawn at the inclusion visit, performed 3 months after the qualifying ischemic event to avoid acute phase changes, always after overnight fast. The following inflammatory markers were determined: CRP, E-selectin, intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), and matrix metalloproteinase (MMP). Representing endogenous fibrinolysis inhibitors, we measured circulating plasminogen activator inhibitor-1 (PAI-1) and Lp(a). Levels of high-sensitivity CRP, MCP-1, E-selectin, ICAM-1, and Lp(a) were measured in serum. For these determinations, blood was allowed to clot at room temperature for 30 minutes. After centrifugation at 3500 rpm and 4°C for 15 minutes, serum was blind coded and stored at −80°C until analyzed. High-sensitivity CRP levels were obtained with a Behring Nephelometer Analyzer and expressed in mg/L. The remaining serum biomarkers were measured using commercially available enzyme-linked immunosorbent assays as follows: MCP-1 (R&D Systems Inc), expressed in ng/mL; ICAM-1 (Bender MedSystems Diagnostics GmbH), in ng/mL; E-selectin (Bender MedSystems Diagnostics GmbH), in ng/mL; and Lp(a) (Macra; Trinity Biotech), in mg/dL. PAI-1 and MMPs were determined in plasma. EDTA tubes were used to collect the blood, and plasma was immediately separated by centrifugation at 3500 rpm for 15 minutes and stored at −80°C. PAI-1 was measured with a commercial enzyme-linked immunosorbent assays (Biopool Menarine) and expressed in ng/mL. A SearchLight Human MMP Array ( Pierce) was used to measure MMPs; this assay consists of multiplexed sandwich enzyme-linked immunosorbent assays for the quantitative measurement of gelatinases (MMP-2 and MMP-9), collagenases (MMP-1, MMP-8, and MMP-13), and stromelysin (MMP-3 and MMP-10). MMPs are expressed in ng/mL. For each marker, all determinations were performed in duplicate, and the mean value of both determinations was used. The mean intra-assay coefficients of variation were < 15% in all cases.
Statistical Analysis

Analyses were performed with the SPSS statistical package (Chicago, Ill), version 12.0. Statistical significance for intergroup differences was assessed by the χ² test for categorical variables and the Student t and Mann–Whitney U tests for continuous variables. Levels of CRP, ICAM-1, Lp(a), and MMPs were not normally distributed (Kolmogorov-Smirnov test). Sample size was estimated when the study was designed. Assuming an α error of 0.05, considering an expected progression rate of 33%, 5 a sample of 75 patients was granted a statistical power >80% to detect statistically significant associations with disease progression for the studied prognostic factors. Univariate analyses were performed to detect variables associated with ILA progression. Differences in the proportion of patients showing progression with increasing tertiles of the population distribution of the studied biomarkers were evaluated. Receiver operating characteristic curves were configured for each biomarker to establish cutoff points that optimally predicted the occurrence of ILA progression. Cox proportional hazards multivariate analyses were used to identify predictors of ILA progression, in which age, sex, hypertension, diabetes, hypercholesterolemia, and variables showing P<0.05 on univariate testing were included. Results were expressed as adjusted HR and corresponding 95% CI. Finally, cumulative event-free rates for the time to disease progression were estimated by the Kaplan–Meier product limit method (log-rank test). The relationship between disease progression and the occurrence of further major vascular events was evaluated by means of a Kaplan–Meier curve (log-rank test). P<0.05 was considered significant.

Results

Baseline Variables

Baseline characteristics and risk factor profile of the study population are shown in Table 1. The study sample consisted of 55 (73%) men and 20 (27%) women. Mean age was 66.2±8.3 years. The qualifying event attributable to a symptomatic intracranial atherostenosis was an ischemic stroke in 54 (72%) patients and a TIA in the remaining 21 (28%). Median NIHSS score on admission for stroke patients was 2 (interquartile range, 0 to 4). At inclusion visit, 62 (83%) patients showed a modified Rankin scale score of 0 or 1. All studied subjects remained free of ischemic events during the study period. The median time to clinical recurrence from inclusion was 5 months (interquartile range, 3 to 12). All recurrent cerebral ischemic events were attributable to an intracranial atherostenosis. Three patients died during follow-up: 2 because of fatal strokes and 1 because of cancer. A strong relationship between ILA progression and further cerebral ischemic events during follow-up was identified by means of a Kaplan–Meier curve, as illustrated in Figure 1.

Progression of Symptomatic Intracranial Atherosclerosis

During a median follow-up time of 23 months (interquartile range, 17 to 29), ILA progressed in 25 (33%) patients, regressed partially in 5 (7%), and remained stable in 45 (60%) patients. ILA progressed through the aggravation of previously known lesions in 6 patients, by the appearance of new TCD-detectable intracranial stenoses in 9 patients, and by combining both mechanisms in the remaining 10. During the same period of time, 18 (24%) patients experienced a major ischemic event, categorized as follows: 10 ischemic strokes, 3 TIs, and 5 myocardial infarctions. The median time to clinical recurrence from inclusion was 5 months (interquartile range, 3 to 12). All recurrent cerebral ischemic events were attributable to an intracranial atherostenosis. Three patients died during follow-up: 2 because of fatal strokes and 1 because of cancer. A strong relationship between ILA progression and further cerebral ischemic events during follow-up was identified by means of a Kaplan–Meier curve, as illustrated in Figure 1.

Predictors of Disease Progression

Univariate analysis identified the presence of >2 vascular risk factors, a higher number of intracranial stenoses, and raised baseline concentration of CRP, E-selectin, ICAM-1, PAI-1, and Lp(a), as significantly associated with a higher risk for ILA progression, whereas an almost significant trend was observed for diabetes mellitus and high MMP-9 level.
No significant associations were found between the different medical treatments, including statins, and ILA progression. To better explore the relationship between the biomarkers and the risk for disease progression, we compared the proportion of patients showing ILA progression within the increasing tertiles of the studied molecules. Whereas the excess risk associated with high CRP, MMP-9, and Lp(a) levels appeared to be concentrated in their top tertile, a gradual elevation of the risk for ILA progression was observed for increasing tertiles of ICAM-1, E-selectin, and PAI-1, as shown in Figure 2. Thereafter, survival analyses, described in Table 3, were performed to assess the capacity of each individual marker to predict ILA progression. Increased baseline concentrations of CRP, E-selectin, ICAM-1, MMP-9, PAI-1, and Lp(a) were independently associated with a higher risk for ILA progression in multivariable Cox regression models, in which adjustment for age, sex, hypertension, diabetes, hypercholesterolemia, and number of intracranial stenoses was conducted. Finally, once the association of the individual biomarkers with ILA progression had proven to be independent of vascular risk factors and other baseline variables, an additional multivariable Cox regression analysis including all the biomarkers that had shown significant associations in the previous model was performed. Baseline CRP >5.5 mg/L (HR, 5.4 [2.3 to 12.7]; P=0.0001) and PAI-1 >23.1 ng/mL (HR, 2.4 [1.0 to 5.8]; P=0.05) emerged as the most robust predictors of ILA progression during follow-up. The independent predictive value of CRP and PAI-1 is clearly illustrated on Figure 3, which shows how those patients with high levels of both biomarkers at baseline were burdened with a strikingly elevated (92%) risk for ILA progression.

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Table 2. Variables Associated With the Progression of Intracranial Atherosclerosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Progression (n=50)</th>
<th>Progression (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 65.8±8.6</td>
<td>67±8.1</td>
<td>0.550</td>
<td></td>
</tr>
<tr>
<td>Sex (male) 37 (74)</td>
<td>18 (72)</td>
<td>0.854</td>
<td></td>
</tr>
<tr>
<td>Smoker 24 (48)</td>
<td>11 (44)</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>Hypertension 39 (78)</td>
<td>21 (84)</td>
<td>0.540</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus 23 (46)</td>
<td>17 (68)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia 35 (70)</td>
<td>20 (80)</td>
<td>0.356</td>
<td></td>
</tr>
<tr>
<td>&gt;2 Risk factors 20 (40)</td>
<td>16 (64)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Qualifying event: stroke 37 (74)</td>
<td>17 (68)</td>
<td>0.612</td>
<td></td>
</tr>
<tr>
<td>Extracranial carotid disease 18</td>
<td>10 (40)</td>
<td>0.736</td>
<td></td>
</tr>
<tr>
<td>N of stenoses 2.8±1.2</td>
<td>3.8±2.2</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Treated with statins 32 (64)</td>
<td>21 (84)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Treated with antiplatelets 38</td>
<td>20 (80)</td>
<td>0.754</td>
<td></td>
</tr>
<tr>
<td>hs-CRP, mg/L 2.4 (1.1–4.2)</td>
<td>11.4 (3.3–18.9)</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>E-selectin, ng/mL 13.4±11.5</td>
<td>23.1±11.2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>ICAM-1, ng/mL 168.9 (131.3–223)</td>
<td>215.7 (176.8–248.8)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>MCP-1, ng/mL 389.9±132.6</td>
<td>394.2±128.7</td>
<td>0.895</td>
<td></td>
</tr>
<tr>
<td>MMP-1, ng/mL 12.3 (6.9–19.9)</td>
<td>15.2 (7.9–34–5)</td>
<td>0.301</td>
<td></td>
</tr>
<tr>
<td>MMP-9, ng/mL 50.04 (30.7–89.2)</td>
<td>75.7 (41.2–136)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>PAI-1, ng/mL 21.9±9.2</td>
<td>27.1±9.4</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Lp(a), mg/dL 16 (5–30)</td>
<td>25 (14–44)</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Univariate analysis of variables potentially associated with the progression of intracranial atherosclerosis. Cells express results in mean±SD, N (%), and median (interquartile range), as appropriate. No significant associations were found for the remaining members of the MMP family (data not shown).
Discussion

In the present prospective study, high blood levels of E-selectin, ICAM-1, MMP-9, CRP, PAI-1, and Lp(a), obtained 3 months after the first TIA or stroke caused by an intracranial atherostenosis, were identified as significant predictors of ILA progression, independently of vascular risk factors. Moreover, CRP and PAI-1 predicted ILA progression also independently of the remaining studied molecules, thus emerging as the most robust predictors of disease progression. Finally, progression of intracranial atherosclerosis appeared to be strongly associated with a higher risk of experiencing recurrent ischemic strokes. To our knowledge, this is the first study to address the role of inflammatory molecules and fibrinolysis inhibitors, whose relevance in extracranial atherosclerosis is well known,\(^8\) in the progression of atherosclerosis affecting intracranial arteries.

Symptomatic intracranial atherosclerosis is a progressive disease burdened with a high risk of clinical recurrence, and the optimal therapeutic strategies aimed to prevent ILA progression and complication remain yet to be determined.\(^3\) Despite the epidemiological importance of this disease, currently considered to be the most frequent cause of ischemic stroke among Asian patients,\(^2\) our knowledge about the basic mechanisms involved in ILA progression, in comparison with extracranial carotid or coronary atherosclerosis, is still very limited. Therefore, there is a need to investigate the molecular
pathways implicated in ILA progression to implement future research in therapies for this disease. In this context, our results suggest that ILA progression is associated with the presence of raised levels of inflammatory markers and fibrinolysis inhibitors. This novel finding is in accordance with the results of previous studies by our group. In addition, proinflammatory state and defective fibrinolysis are 2 main characteristics of the metabolic syndrome, which has been recently shown to increase the risk of recurrent ischemic stroke in patients affected by symptomatic ILA. Taken together, these observations suggest that inflammation and impaired fibrinolysis may play a crucial role in the progression of intracranial atherosclerosis.

Regarding inflammation, we studied molecules involved in different stages of the atherosclerotic process. E-selectin mediates the first step in leukocyte adhesion; ICAM-1 facilitates the firm attachment of leukocytes and their migration into the arterial wall; MCP-1 plays a fundamental role in monocyte recruitment; CRP is a sensitive indicator of systemic inflammation; and MMPs are a family of proteolytic enzymes involved in arterial remodeling and plaque rupture through the degradation of the extracellular matrix components. All of them, except MCP-1, were found to be associated with ILA progression. Among MMPs, MMP-9 appeared as the only member of the family that predicted ILA progression. Interestingly, elevated MMP-9 activity has been detected in unstable carotid plaques, suggesting a crucial role for MMP-9 in plaque rupture. Our results support the hypothesis that MMP-9 might be involved in plaque progression and complication also in intracranial arteries. Regardless of inflammation, we studied molecules involved in different stages of the atherosclerotic process. E-selectin mediates the first step in leukocyte adhesion; ICAM-1 facilitates the firm attachment of leukocytes and their migration into the arterial wall; MCP-1 plays a fundamental role in monocyte recruitment; CRP is a sensitive indicator of systemic inflammation; and MMPs are a family of proteolytic enzymes involved in arterial remodeling and plaque rupture through the degradation of the extracellular matrix components. All of them, except MCP-1, were found to be associated with ILA progression. Among MMPs, MMP-9 appeared as the only member of the family that predicted ILA progression. Interestingly, elevated MMP-9 activity has been detected in unstable carotid plaques, suggesting a crucial role for MMP-9 in plaque rupture. Our results support the hypothesis that MMP-9 might be involved in plaque progression and complication also in intracranial arteries. Whether MCP-1 elevation accompanies, and does not precede, ILA progression, as observed in coronary patients, remains a question for future studies. Finally, it should be considered that investigation with blood biomarkers does not allow the establishing of whether the observed associations are of causal significance.

The major finding of this study was that CRP and PAI-1 predicted ILA progression independently of vascular risk factors and of all the remaining measured biomarkers. Remarkably, the highest risk of ILA progression was observed in those patients with elevated level of both molecules. CRP has been shown to be a powerful predictor of atherosclerosis progression at various sites in the extracranial arterial tree, and recent studies suggest that it may be a direct mediator in atherogenesis. With respect to PAI-1, its increased expression in atherosclerotic vessels may cause an increase in intracranial arterial stiffness and decreased cerebral blood flow. The combination of circulating levels of the 2 most robust predictors identified by multivariate analyses (CRP and PAI-1) significantly increases the risk of ILA progression. The proportion of patients showing progression of symptomatic intracranial atherosclerosis attending the combination of circulating levels of the 2 most robust predictors identified by multivariate analyses (CRP and PAI-1). The risk of disease progression reaches its maximum (92%) when the concentration of both markers is elevated. (P = 0.00001).

**Table 3.** Progression of Intracranial Atherosclerosis: Survival Analyses

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Best ROC Cutoff (Sensitivity-Specificity)</th>
<th>Cox Regression Model</th>
<th>Kaplan-Meier Log-Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>5.5 mg/L (63%–82%)</td>
<td>5.65 (2.4–13.2) 0.0001</td>
<td>0.00001</td>
</tr>
<tr>
<td>E-selectin</td>
<td>24.4 ng/mL (54%–84%)</td>
<td>3.4 (1.5–7.6) 0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>205.8 ng/mL (63%–72%)</td>
<td>2.4 (1.03–5.6) 0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>MMP-1</td>
<td>383.7 ng/mL (50%–54%)</td>
<td>0.8 (0.3–1.9) 0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>MMP-9</td>
<td>74.7 ng/mL (54%–72%)</td>
<td>2.5 (1.1–5.8) 0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>PAI-1</td>
<td>23.1 ng/mL (63%–67%)</td>
<td>2.9 (1.3–6.9) 0.01</td>
<td>0.008</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>26.5 mg/dL (52%–70%)</td>
<td>2.3 (1.1–5.3) 0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

For each biomarker, receiver operating characteristic (ROC) curves were configured. The optimal cutoff values obtained, which were used to include the variables in the analyses, are shown in the second column. In the third column, results of the Cox proportional hazards multivariate analyses for each biomarker, after adjustment for age, sex, hypertension, diabetes, hypercholesterolemia, and variables showing P < 0.05 on univariate testing (Table 2), expressed as hazard ratios (HR), and 95% CI, are shown. Finally, the cumulative progression-free rates for the time to ILA progression were estimated by the Kaplan-Meier product limit method. For each biomarker, the 2 groups of patients with concentrations above and below the cutoff value were compared by the log-rank test, and the resulting P values are shown in the last column.
impaired fibrinolytic response to mural thrombi leading to a greater extent and persistence of thrombi in the arterial lumen and to an increased exposure to clot-associated mitogens in the artery wall (such as platelet-derived growth factor, transforming growth factor-β, and thrombin), both being effects responsible for accelerated atherosclerosis.9,22,23 Moreover, the enhanced expression of PAI-1 by atheromatous arteries has been shown to contribute to the elevated circulating levels observed in patients with type 2 diabetes, metabolic syndrome, and insulin resistance.24 Finally, the strong predictive capacity observed for CRP and PAI-1 may have relevant implications for the clinical management of patients with symptomatic ILA. First, both biomarkers might be useful as prognostic tools in the selection of patients with high-risk ILA who may benefit from more intensive preventive approaches. Second, they could be used to monitor the efficacy of anti-atherosclerotic therapies such as statins, or to help optimize risk factor and metabolic control. In this context, the potential impact of statins and other treatments on these markers of ILA progression may need to be addressed in the setting of a randomized clinical trial. Third, given that both molecules have been proposed as direct mediators of atherogenesis, their therapeutic inhibition may represent a promising new approach for the medical treatment of patients affected by ILA.25

Conclusions

This study has some limitations. First, as a major methodological weakness, following the study design and budget, the biomarkers were measured only once and blood sampling could not be repeated later during the follow-up period. This fact precluded the assessment of intraindividual variation of the molecules during follow-up. Second, the sample size is reduced. However, the patients were highly selected after a careful diagnostic work-up. Third, the follow-up period was not standard for all patients, which may limit the value of the univariate analysis. Nevertheless, the main results of the study derive from survival analyses including Cox proportional hazards models, which correct for differences in follow-up time. Finally, we relied on TCD and MRA or CTA for the diagnosis of intracranial stenoses, and not on conventional angiography, considered the gold standard technique, to avoid invasive procedures for our patients.

In conclusion, high level of E-selectin, ICAM-1, MMP-9, CRP, PAI-1, and Lp(a) predicted ILA progression independently of vascular risk factors, whereas only CRP and PAI-1 predicted ILA progression independently of the other biomarkers. These results suggest that patients who experience progression of symptomatic intracranial atherosclerosis are characterized by a proinflammatory state and impaired endogenous fibrinolysis.

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Disclosures

None.

References


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