Plasma CXCL12 Levels as a Predictor of Future Stroke

Robert C. Schutt, MD, MS; Marie D. Burdick, BS; Robert M. Strieter, MD; Borna Mehrad, MD; Ellen C. Keeley, MD, MS

Background and Purpose—The chemokine ligand CXCL12 is constitutively expressed in the bone marrow and other tissues including the brain endothelium and is responsible for regulating the trafficking of bone marrow progenitor cells. CXCL12 has been shown to play a significant role in animal models of ischemic stroke but its role in human stroke is unclear. The aim of this study was to test the hypothesis that elevated circulating baseline CXCL12 levels are associated with subsequent stroke.

Methods—We prospectively collected demographic and angiographic data from consecutive patients referred for elective coronary angiography. Before coronary angiography a peripheral blood sample was collected for subsequent measurement of CXCL12. One-year stroke risk was calculated using the Framingham Risk Profile. Clinical follow-up was performed at 6 months and 1 year.

Results—Of 206 subjects enrolled, 10 (4.9%) sustained an ischemic stroke over the 1-year follow-up. There were no significant differences in baseline clinical characteristics or angiographic findings. However, median CXCL12 levels were significantly higher in those who sustained an ischemic stroke compared with those who did not (10 856 pg/mL versus 2241 pg/mL; \( P=0.007 \)). The time to stroke distribution between subjects with baseline CXCL12 levels \( \geq 10 421 \) pg/mL and those with baseline CXCL12 levels \( <10 421 \) pg/mL was significantly different (log rank \( P=0.001 \)). The weighted Cox proportional hazard model demonstrated that baseline CXCL12 levels \( \geq 10 421 \) pg/mL were significantly associated with ischemic stroke at follow-up (hazard ratio, 15.29; 95% CI, 3.05–76.71).

Conclusions—Plasma CXCL12 levels may represent a novel biomarker of future ischemic stroke. (Stroke. 2012;43:3382–3386.)

Key Words: biomarker ■ chemokine ■ stroke

With an estimated 610 000 new cases per year, stroke is the third leading cause of death in the United States and the second leading cause of death worldwide. Stroke risk is determined by using traditional risk factors and risk assessment tools. To improve stroke risk assessment, investigators have studied the predictive value of fasting lipids, lipoprotein (a), and high sensitivity c-reactive protein as biomarkers. Biomarkers have been shown to improve risk stratification when used in conjunction with stroke risk assessment tools and are considered a Class IIb indication (level of evidence B) according to the American Heart Association/American Stroke Association primary prevention of stroke guidelines. Discovery of novel biomarkers that identify subjects at risk for future stroke could significantly improve stroke prevention.

The CXC chemokine ligand-12 (CXCL12) is a member of the CXC chemokine subfamily that is constitutively expressed in the bone marrow and other tissues including the brain endothelium and is responsible for regulating the trafficking and localization of bone marrow progenitor cells under steady state and stress conditions. After ischemic stroke, CXCL12 mediates the inflammatory response by recruitment of neural progenitor cells and the mobilization of bone marrow-derived progenitor cells for tissue regeneration and neovascularization. Although it has been shown to play a significant role in acute stroke in animal models, its role in acute stroke in humans is unclear. The prognostic value of CXCL12 levels as a predictor of future stroke has not been tested. The aim of this study was to test the hypothesis that elevated baseline CXCL12 levels are associated with subsequent stroke.

Methods

The study design was a case–cohort study. The study was approved by the Institutional Review Board, and all patients provided written informed consent. We prospectively collected demographic and angiographic data from consecutive patients referred for coronary angiography at the University of Virginia from November 1, 2007, to November 1, 2010. All patients undergoing elective coronary angiography for assessment of symptoms consistent with angina and/or noninvasive testing consistent with myocardial ischemia who were >21 years old and able to provide informed consent were eligible for enrollment. Exclusion criteria were: (1)

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One-year stroke risk was calculated using the Framingham Risk Profile and included the following variables: sex, age, systolic blood pressure, treated hypertension, diabetes mellitus, tobacco use, cardiovascular disease, and atrial fibrillation. Clinical follow-up was performed by telephone interview at 6 months and at 1 year. Subjects were called on 3 separate occasions at both the 6-month and 1-year time points. If there was no response a letter was sent to the subject’s home asking them to contact the investigators to provide clinical follow-up information. In patients who sustained a stroke, medical records from the stroke admission were reviewed by the investigators. As part of the case–cohort analysis, CXCL12 levels were measured in all cases of stroke and in a subset of nonstroke cases from consecutively enrolled subjects.

Statistical Analysis

Categorical variables were analyzed using the Fisher exact test and continuous variables were analyzed using the Wilcoxon rank-sum test. Time-dependent receiver operator curve analysis was used to identify the level of CXCL12 that may have prognostic significance for future stroke. The study cohort was divided using this value to create 2 survival distributions for time to stroke, and the log rank test was used to compare distributions. Survival analysis using a weighted Cox proportional hazards model was performed to analyze the relationship between elevated CXCL12 levels and stroke using the NestCohort package in R. The logistic regression model used to weight cases for the proportional hazards model included the following variables: sex, age, systolic blood pressure, treated hypertension, diabetes mellitus, tobacco use, cardiovascular disease, and atrial fibrillation. Clinical follow-up was performed by telephone interview at 6 months and at 1 year. Subjects were called on 3 separate occasions at both the 6-month and 1-year time points. If there was no response a letter was sent to the subject’s home asking them to contact the investigators to provide clinical follow-up information. In patients who sustained a stroke, medical records from the stroke admission were reviewed by the investigators. As part of the case–cohort analysis, CXCL12 levels were measured in all cases of stroke and in a subset of nonstroke cases from consecutively enrolled subjects.

Table. Baseline Characteristics of Patients Stratified by the Occurrence of Stroke at 1-y Follow-Up

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total (N=206)</th>
<th>(+) (n=10)</th>
<th>(-) n=196</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD</td>
<td>60±12</td>
<td>57±11</td>
<td>61±12</td>
<td>0.225</td>
</tr>
<tr>
<td>Men</td>
<td>138 (67%)</td>
<td>8 (80%)</td>
<td>130 (66%)</td>
<td>0.502</td>
</tr>
<tr>
<td>Race (white)</td>
<td>181 (88%)</td>
<td>8 (80%)</td>
<td>173 (88%)</td>
<td>0.064</td>
</tr>
<tr>
<td>Clinical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>168 (82%)</td>
<td>6 (60%)</td>
<td>162 (83%)</td>
<td>0.208</td>
</tr>
<tr>
<td>SBP, mm Hg, mean±SD</td>
<td>130±20</td>
<td>124±18</td>
<td>130±20</td>
<td>0.567</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>62 (30%)</td>
<td>4 (40%)</td>
<td>58 (30%)</td>
<td>0.458</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>182 (88%)</td>
<td>9 (90%)</td>
<td>173 (88%)</td>
<td>0.602</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>135 (66%)</td>
<td>7 (70%)</td>
<td>128 (65%)</td>
<td>0.721</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>48 (23%)</td>
<td>3 (30%)</td>
<td>45 (23%)</td>
<td>0.440</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>19 (9%)</td>
<td>1 (10%)</td>
<td>18 (9%)</td>
<td>0.591</td>
</tr>
<tr>
<td>Prior stroke</td>
<td>22 (11%)</td>
<td>2 (20%)</td>
<td>20 (10%)</td>
<td>0.249</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>69 (33%)</td>
<td>3 (30%)</td>
<td>66 (34%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Prior CABG</td>
<td>31 (15%)</td>
<td>1 (10%)</td>
<td>30 (15%)</td>
<td>0.693</td>
</tr>
<tr>
<td>Coronary angiographic data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CAD</td>
<td>35 (17%)</td>
<td>2 (20%)</td>
<td>33 (17%)</td>
<td></td>
</tr>
<tr>
<td>Nonobstructive CAD</td>
<td>35 (17%)</td>
<td>1 (10%)</td>
<td>34 (17%)</td>
<td></td>
</tr>
<tr>
<td>Obstructive CAD*</td>
<td>136 (66%)</td>
<td>7 (70%)</td>
<td>129 (66%)</td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>167 (81%)</td>
<td>9 (90%)</td>
<td>158 (81%)</td>
<td>0.366</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>47 (23%)</td>
<td>1 (10%)</td>
<td>46 (23%)</td>
<td>0.688</td>
</tr>
<tr>
<td>Statin</td>
<td>166 (81%)</td>
<td>8 (80%)</td>
<td>158 (81%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>132 (64%)</td>
<td>3 (30%)</td>
<td>129 (66%)</td>
<td>0.069</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>87 (42%)</td>
<td>4 (40%)</td>
<td>83 (42%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>34 (17%)</td>
<td>2 (20%)</td>
<td>32 (16%)</td>
<td>0.648</td>
</tr>
<tr>
<td>Framingham Stroke Risk†</td>
<td>0.8%</td>
<td>0.9%</td>
<td>0.8%</td>
<td>0.949</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD, median 25%–75% interquartile range, or as no. (percentage). SBP indicates systolic blood pressure; CABG, coronary artery bypass graft; CAD, coronary artery disease; ACE, angiotensin-converting enzyme; CXCL12, CXC chemokine ligand-12.

*Defined as at least 1 epicardial coronary artery with ≥70% diameter narrowing.
†At 1 y.
the occurrence of stroke within 1 year as the single variable for the weighting model. This was appropriate because cases were purposely oversampled to the probability of a patient being included in the subcohort who had CXCL12 measured was only dependent on the presence of stroke at follow-up. Variables for the proportional hazards model included a dichotomous variable that indicated if the CXCL12 level was above the identified threshold by receiver operator curve analysis, the calculated 1-year Framingham Risk Profile for stroke, and a history of prior stroke. The probability value of <0.05 was considered statistically significant. All statistical analyses were performed using R, Release 2.13.0.

Results

During the study, 206 subjects were followed for an average 320 days after elective coronary angiography. At 1-year follow-up, 10 patients had sustained an ischemic stroke an average of 166 days after angiography (minimum, 9 days; maximum, 340 days). CXCL12 levels were measured in a total of 206 subjects, 10 subjects with stroke and 196 subjects without stroke at follow-up. Complete follow-up was present in 95.1% (196 of 206), 5 subjects were lost to follow-up at Time 0, and 5 subjects were lost to follow-up at 6 months. In all cases of loss of clinical follow-up, neither the subject nor the subject’s family could be reached by telephone on 3 separate occasions at both the 6-month and 1-year time points, and there was no response to a follow-up letter sent to the subject’s home.

Although there were no significant differences in baseline demographic or angiographic data (Table), CXCL12 levels (median [25%–75% interquartile range]) were significantly higher in subjects who had an ischemic stroke at follow-up compared with the nonstroke cohort (10 856 [10 522–48 691] pg/mL versus 2241 [1998–9989] pg/mL, respectively, \( P = 0.007 \); Table; Figure 1). Time-dependent receiver operator curve analysis indicated that a CXCL12 level of 10 421 pg/mL was 79.5% sensitive and 81.3% specific for ischemic stroke within 1 year (area under the curve 74.8%). The time to stroke distribution between subjects with baseline CXCL12 levels ≥10 421 pg/mL and those with baseline CXCL12 levels <10 421 pg/mL were significantly different (log rank \( P < 0.001 \); Figure 2). The weighted Cox proportional hazard model demonstrated that baseline CXCL12 levels ≥10 421 pg/mL were significantly associated with stroke at follow-up (hazard ratio, 15.29; 95% CI, 3.05–76.71; \( P < 0.001 \)). Neither the 1-year Framingham Stroke Risk Profile \( (P = 0.905) \) nor a history of prior stroke \( (P = 0.244) \) reached statistical significance in the model.

Discussion

We found that, in our cohort, elevated CXCL12 levels were strongly associated with future ischemic stroke even after adjusting for traditional risk factors and the Framingham Stroke Risk Profile. CXCL12 may be an important biomarker for stroke risk stratification particularly in patients in whom traditional risk factors are equivocal and may identify individuals in whom more aggressive risk factor modification, diagnostic evaluation, or even intervention is warranted.

To date only 2 publications have reported data regarding CXCL12 levels in patients with stroke, but both studies measured CXCL12 levels during the acute stroke phase.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\)\(^13\) In the first article, there was no significant difference in circulating CXCL12 levels between patients with stroke and normal control subjects.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\)\(^13\) However, the investigators did find a significant correlation between CXCL12 levels and peak C-reactive protein levels. In the second study, investigators found an inverse relationship between plasma CXCL12 levels and acute lesion volumes as determined by brain MRI: high circulating CXCL12 levels were associated with small lesion volumes and low circulating CXCL12 levels were associated with large lesion volumes. This inverse association suggests that low levels of circulating CXCL12 measured during the early phase in patients with large strokes may be secondary to decreased brain neuroregeneration due to extensive tissue damage.\(^\text{12}\)\(^\text{13}\) Our study adds to the literature regarding CXCL12 and stroke in humans and is unique because we were able to use CXCL12 levels measured at baseline to predict a future stroke occurrence.

One potential mechanism for the observed association is that CXCL12 levels may be elevated due to the presence of a brain lesion made “vulnerable” due to subclinical ischemia or inflammation resulting in persistent, low-level tissue damage and the need for influx of endothelial progenitor cells. Along this line of reasoning, investigators have demonstrated increased CXCL12 expression in atherosclerotic plaques obtained from human carotid endarterectomy specimens.\(^\text{18}\) Although greater than half of our overall cohort had traditional risk factors including hypertension, hyperlipidemia, coronary artery disease, and tobacco use, there were no significant differences between subjects who did and did not sustain a follow-up ischemic stroke in these factors in addition to the Framingham Risk Score. The finding that CXCL12 levels alone were predictive of future stroke in this high-risk population is striking and underscores the significance of our findings.

Our study has several limitations. First, the study cohort included only patients undergoing elective coronary angiography; thus, this patient population may not be generalizable to all patients at risk for stroke. It is important to note, however, that all the strokes occurred well outside the periprocedural setting and therefore none were due to a...
complication of coronary angiography. Second, brain imaging scans were not performed before coronary angiography. Third, brain imaging scans performed as part of the workup for stroke were not available for independent review for all patients with stroke; however, in all cases, hospital records were obtained to verify the admission diagnosis as ischemic stroke. Fourth, we do not know if the subjects who sustained a stroke had previous transient ischemic attacks that may have been a trigger for increased CXCL12 expression. Fifth, the Framingham score was not associated with future stroke and is likely due to the small number of outcomes over the 1-year follow-up period. Lastly, we do not have plasma samples from the acute stroke admission to test the hypothesis that CXCL12 levels are low during the acute phase due to neuronal necrosis and astrocyte death.

Conclusions
Elevated circulating CXCL12 levels are strongly associated with the future occurrence of ischemic stroke in patients undergoing elective coronary angiography independent of traditional risk factors (including a prior stroke) and the Framingham Stroke Risk assessment tool. Further studies are needed to confirm this association and define the role for CXCL12 as a novel biomarker for stroke.

Figure 2. Kaplan-Meier curve of subjects with and without stroke over 1-year follow-up. Plasma CXCL12 levels ≥10 421 pg/mL are depicted in blue, and plasma CXCL12 levels <10 421 pg/mL are depicted in orange. CXCL12 indicates CXC chemokine ligand-12.

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Disclosures
None.

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