Circulating Endothelial Progenitor Cells and Age-Related White Matter Changes

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Background and Purpose—The objective was to evaluate the relationship between circulating endothelial progenitor cells (EPC) and age-related white matter changes (ARWMC). Endothelial dysfunction plays a role in the development of ARWMC. EPC incorporate into sites endothelial damage and are thought to be involved in the repair of vascular risk factor induced endothelial injury. ARWMC can be evaluated using CT or MRI.

Methods—In 172 individuals, circulating EPC were defined by the surface markers CD31 and von Willebrand factor. ARWMC were rated on CT scan using the ARWMC scale and divided into 3 groups based on ARWMC scale score (ARWMC score 0 [none], score 1–10 [mild-to-moderate], score >10 [severe]). Severity of ARWMC was correlated with levels of EPC and vascular risk factors.

Results—On univariate analysis, EPC were found to be significantly lower in patients with severe ARWMC (P < 0.01). ARWMC were also associated with hypertension (P < 0.001), age (P < 0.001), creatinine clearance (P = 0.031), C-reactive protein (P < 0.001), and use of angiotensin-converting enzyme or angiotensin receptor blocker (P = 0.004). Multiple logistic regression analysis identified EPC level, age, hypertension, and hypertriglyceridemia as significant independent predictors of severe ARWMC.

Conclusions—Levels of circulating EPC were significantly lower in patients with severe ARWMC. Other variables significantly associated with severe ARWMC were age, hypertension, and hypertriglyceridemia. Further study is required to delineate the pathophysiological relationship between EPC, vascular risk factors, and ARWMC. (Stroke. 2009;40:3191-3196.)

Key Words: endothelial progenitor cells ■ endothelium ■ white matter disease
Materials and Methods

Ethics
This study was approved by the Research Ethics Review Board of the University of Alberta. Written informed consent was obtained from all subjects.

Study Patients
Patients presenting to an ambulatory neurology clinic at the University of Alberta hospital with a history of mild stroke or transient ischemic attack were prospectively recruited from June 2003 to October 2006. Subjects were recruited on average 2 months after their stroke or TIA. By nature of recruitment from an ambulatory clinic population, subjects were high-functioning without major stroke deficits. NIH stroke scores and stroke subtypes were not systematically recorded. Vascular risk factors were defined according to international guidelines and were prospectively identified using all available information including medical chart, laboratory results, patient interview, and examination. Hypertension was defined as a history of hypertension, or the use of antihypertensive medications, or a measured blood pressure consistently >140/90 mm Hg. Hyperlipidemia was defined as a cholesterol >200 mg/dL, LDL >150 mg/dL, or triglycerides >150 mg/dL, or history of hyperlipidemia. Diabetes was defined as a history of diabetes, or use of medications for diabetes, or an elevated fasting blood glucose >7.1 mmol/L.

Endothelial Progenitor Cell Measurement
EPC were measured according to methods previously described. Peripheral blood samples were collected at the time of assessment in the ambulatory clinic. Mononuclear cells were separated using a Ficoll density gradient and plating performed with 1 million cells cultured in each well of a 24-well fibronectin-coated plate. To confirm endothelial progenitor cell lineage, indirect CD31 and von Willebrand factor immunostaining was performed using mouse antihuman CD31, von Willebrand factor primary antibodies, and biotinylated rabbit antimouse IgG secondary antibody. Antibody localization was determined using ABC peroxidase elite kit. EPC colonies were counted manually in each well of an individual sample after 7 days. An EPC colony consists of a central cluster of rounded colonies were counted by 2 different observers blinded to clinical data.

ARWMC Rating
ARWMC were rated visually according to the standardized ARWMC scale previously described. This rating scale has been shown to rate ARWMC on CT scan comparable to those identified on MRI. The ARWMC scale defines white matter changes on CT scan as well-defined areas of attenuation >2 mm. Areas of nonlacunar infarction when present were not recorded as white matter changes. The ARWMC scale divides ARWMC into 5 different brain regions: frontal, parieto-occipital, temporal, basal ganglia, and infratentorial. Each region is graded separately, with grade 0 indicating no lesions, grade 1 indicating focal lesions, grade 2 indicating beginning confluence of lesions, and grade 3 indicating diffuse involvement of the entire region. For the basal ganglia, grade 0 indicates no lesions, grade 1 indicates 1 focal lesion (>5 mm), grade 2 indicates >1 focal lesion, and grade 3 indicates confluent lesions. The total score is the sum of the grade for each region (range, 0–30). Based on the total ARWMC scale score, patients were divided into 3 groups: no ARWMC (score 0), mild-to-moderate ARWMC (score 1–10), and severe ARWMC (score >10). The same rater read all CT scans. Rater reliability was assessed on a random sample of 20 of the 172 scans, which were rated by another experienced rater and showed good-to-excellent agreement (retest reliability k = 0.88).

Statistical Analysis
Results were expressed as means ± SE and number (percentage). Univariate analysis were performed using ANOVA, Kruskal-Wallis H, Pearson χ², or Fisher exact test to examine the association of all demographic and clinical factors among ARWMC severities (none, mild-to-moderate, and severe). Multiple comparisons among the 3 groups of ARWMC was performed using post-hoc Sidak test. Multiple logistic regression analysis was used to identify significant independent predictors for ARWMC after adjusting for known confounders. P < 0.05 was considered statistically significant and all P values were 2-sided. SPSS 15.0 for windows was used for all data analysis.

Results
Of the 172 subjects studied, 95 (55.2%) were male and the mean age was 63.77 years (SE, 0.96; Table 1). Mild stroke

Table 1. Descriptive Statistics

<table>
<thead>
<tr>
<th>Factor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>63.77 ± 0.96</td>
</tr>
<tr>
<td>Male</td>
<td>95 (55.2%)</td>
</tr>
<tr>
<td>Acute stroke/TIA &lt;15 days</td>
<td>18 (10.5%)</td>
</tr>
<tr>
<td>Time stroke/TIA to EPC measurement, days</td>
<td>55.4 ± 6.6</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>14 (8.3%)</td>
</tr>
<tr>
<td>White matter change severity</td>
<td></td>
</tr>
<tr>
<td>No ARWMC</td>
<td>n = 50 (29.1%) mean ARWMC score 0</td>
</tr>
<tr>
<td>Mild-to-moderate ARWMC</td>
<td>n = 101 (58.7%) mean ARWMC score 3.9 (1–10)</td>
</tr>
<tr>
<td>Severe ARWMC</td>
<td>n = 21 (12.2%) mean ARWMC score 14.6 (11–22)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>109 (63.4%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>38 (22.1%)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>116 (67.4%)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.57 ± 0.09</td>
</tr>
<tr>
<td>LDL</td>
<td>3.45 ± 0.075</td>
</tr>
<tr>
<td>HDL</td>
<td>1.29 ± 0.032</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.88 ± 0.087</td>
</tr>
<tr>
<td>Smoker</td>
<td>32 (18.6%)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>64 (37.2%)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>6 (3.5%)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.95 ± 0.47</td>
</tr>
<tr>
<td>Hyperhomocysteine</td>
<td>67 (39.0%)</td>
</tr>
<tr>
<td>Fasting homocysteine level</td>
<td>12.84 ± 1.01</td>
</tr>
<tr>
<td>Creatinine</td>
<td>94.45 ± 2.812</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>77.86 ± 2.58</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>6.14 ± 1.03</td>
</tr>
<tr>
<td>Aspirin</td>
<td>94 (54.7%)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>36 (20.9%)</td>
</tr>
<tr>
<td>Stations</td>
<td>77 (44.8%)</td>
</tr>
<tr>
<td>ACEi – ARB</td>
<td>64 (37.2%)</td>
</tr>
<tr>
<td>EPC</td>
<td>9.19 ± 0.67</td>
</tr>
</tbody>
</table>

ACEi indicate angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Results are expressed as mean ± SE and N (percentage).

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had occurred in 62.8% of subjects recruited and TIA had occurred in 37.2%. The mean time interval between stroke/TIA onset to EPC measurement was 55.4 days. Hypertension was present in 63.4% of patients, 67.4% had hyperlipidemia, 22.1% had diabetes, 18.6% were smokers, 3.5% had atrial fibrillation, and 8.3% had a history of ischemic heart disease.

To assess the effect of ARWMC on EPC levels, patients were divided into 3 groups based on severity of ARWMC on CT scan. There were 29.1% of patients with no ARWMC, 58.7% with mild-to-moderate ARWMC, and 12.2% with severe ARWMC (Table 1). The mean EPC level in all patients was 9.19 (SE, 0.67). EPC level in the no ARWMC group was 10.36 (SE, 1.47), in the mild-to-moderate ARWMC group it was 9.46 (SE, 0.84), and in the severe ARWMC group it was 5.11 (SE, 1.12; Table 2). Using a multiple comparison test, the level of EPC was compared between each of the ARWMC groups and indicated that EPC were significantly lower in patients with severe ARWMC (P<0.01; Figure, Table 2).

Other factors that were significantly different between patients with no ARWMC and those with severe ARWMC were age (P<0.001), hypertension (P<0.001), creatinine clearance (P=0.031), C-reactive protein (P<0.001), and use of angiotensin-converting enzyme or angiotensin receptor blocker (P=0.004; Table 2). No statistically significant difference was found between the 2 groups for gender, diabetes,
Table 3. Multiple Logistic Regression Model to Identify Independent Predictors of Severe ARWMC (>10; Severe vs None-to-Moderate)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate OR* (95% CI)</th>
<th>Multivariate OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPC (1-cell increase)</td>
<td>0.9 (0.82–0.988)</td>
<td>0.903 (0.816–1.00)</td>
<td>0.049</td>
</tr>
<tr>
<td>Age (1-year increase)</td>
<td>1.081 (1.03–1.134)</td>
<td>1.08 (1.014–1.145)</td>
<td>0.017</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6.44 (1.45–28.65)</td>
<td>4.78 (1.014–22.83)</td>
<td>0.05</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>1.404 (0.988–1.99)</td>
<td>1.718 (1.08–2.741)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

*All variables with P<0.2 on univariate analysis and all biologically important variables were entered into a stepwise multiple regression model with final model shown here. Variables entered were age, gender, hypertension, diabetes, hyperlipidemia, cholesterol, triglyceride, atrial fibrillation, acute stroke/TIA, time stroke/TIA to EPC measurement, body mass index, hyperhomocysteinemia, creatinine clearance, C-reactive protein, ACEi or ARB, and EPC level.

Discussion

In this study we investigated levels of circulating EPC in patients with different severities of ARWMC. EPC were found to be significantly lower in patients with severe ARWMC, independent of measured vascular risk factors. The low EPC level observed in the severe ARWMC patients is in agreement with other studies of ARWMC.12,20

ARWMC and EPC Levels

Low levels of EPC in persons with severe ARWMC are likely a manifestation of vascular risk factors, particularly age and hypertension. This is consistent with previous work indicating age and hypertension are significantly associated with both EPC and ARWMC.10,11 However, in our multivariable regression model, an independent relationship between EPC and ARWMC was found after adjustment for vascular risk factors (P=0.049; Table 3) and after repeat analysis excluding subjects with EPC measured within 15 days of stroke or TIA onset. A possible explanation for this is that EPC reflect vascular risk that was not accounted for. Although we recorded both traditional and nontraditional vascular risk factors based on standard definitions, there may have been additional vascular risk not fully recorded. This is consistent with studies suggesting EPC levels are a reflection of unmeasured vascular risk and, in fact, may be a better marker of vascular risk and cardiovascular disease than the Framingham risk score.8,9,16,18,21 ARWMC, which we found to be associated with low EPC levels, have also been associated with an increased risk cerebrovascular disease not explained by major stroke risk factors.22 Whether low EPC levels can account for this increased risk requires further study; however, previous study23 by our group did find subjects without vascular risk factors to have higher EPC levels (15.53) than subjects in this study.

ARWMC and Endothelial Dysfunction

The pathogenesis of ARWMC remains not fully understood. Hypertension is a major risk factor for ARWMC but does not account for all the risk.24 Endothelial dysfunction has been suggested to mediate the development of the severe ARWMC phenotype.25 Dysfunctional endothelium is thought to cause ARWMC either by chronic hypoperfusion or breakdown of the blood–brain barrier.26 Genetic studies also indicate that the endothelium plays a role in microvascular disease and the development of ARWMC.27 Our study showing low levels of EPC, which play a role in endothelial repair, are associated with ARWMC lends support to growing evidence that endothelial dysfunction is involved in cerebral small vessel disease.

Lacunar infarction is another manifestation of cerebral small vessel disease. EPC levels have been shown to be lower in lacunar stroke compared cardioembolic ischemic stroke.19 EPC from subjects with lacunar infarction also have reduced angiogenic abilities.19 Lacunar infarction and silent cerebral infarcts have been shown to be associated with lower EPC levels and reduced ability for EPC cluster formation particularly in subjects with a haptoglobin 1-to-1 phenotype.28 Other studies have shown lower EPC in subjects with cerebrovascular disease, although not specifically in the small vessel disease subtype.15,17,29 These findings are consistent with ARWMC being associated with low EPC levels.

EPC levels may show promise as a surrogate marker of dysfunctional cerebrovascular endothelium. This potentially could aid in the identification of patients at risk for the development of ARWMC. Although not specific to ARWMC, levels of EPC have been shown to be a marker cerebrovascular function and outcome after cerebrovascular events.17 Other markers of dysfunctional endothelium have been asso-
associated with white matter changes. Serum levels of intercellular adhesion molecule-1 have been related to progression of white matter disease. Homocysteine causes endothelial dysfunction and is a risk factor for cerebral small vessel disease. Low EPC levels have been associated with elevated levels of homocysteine. The relationship between dysfunctional endothelium, ARWMC, and levels of intercellular adhesion molecule-1, homocysteine, and EPC requires further study.

ARWMC, EPC and Stroke Outcome

Severe ARWMC have been associated with poor outcomes after ischemic stroke independent of vascular risk factors, including age, hypertension, and diabetes. Similarly, low levels of EPC have been associated with worse outcomes after ischemic stroke. Together these 2 observations are consistent with our finding of low EPC levels being associated with severe ARWMC. The mechanisms by which ARWMC and EPC are associated with poor outcomes after ischemic stroke remain unclear and warrant further study. Severe white matter damage may limit neuroplasticity and thus recovery after ischemic stroke. EPC levels may be a reflection of ARWMC severity and possibly cumulative vascular risk. EPC levels may also be a reflection of a patient’s ability to repair damaged endothelium. This is supported by studies in mice indicating CD34+ cells can promote neovascularization of ischemic brain.

Study Limitations

Our study had several limitations. Although we were able to record all major vascular risk factors and several nontraditional vascular risk factors, we would have preferred to obtain more data on cerebrovascular risk, including evaluation of retinal changes, left ventricular hypertrophy, ankle brachial index, cognitive testing, and gait assessment. We did not observe a significant difference between EPC levels in the mild-to-moderate ARWMC group as compared to the no ARWMC group. This may have been the result of insufficient power; there was a smaller point estimate for the difference in EPC levels between these 2 groups as compared to either group and the severe ARWMC group. Our study provides data that can be of assistance in planning future studies. The use of MRI or fully quantitative methods to rate ARWMC may also improve the measurement of ARWMC. Nevertheless, the ARWMC rating scale has been validated for both CT and MRI usage, and severe changes are well-identified by CT and likely to be clinically significant. Finally, the number of patients in the severe ARWMC group was relatively small and a larger study population would improve statistical power.

Conclusions

EPC were found to be significantly lower in patients with severe ARWMC. Other variables identified to be significant independent predictors of severe ARWMC were age, hypertension, and hypertriglyceridemia. This hypothesis-generating study warrants further investigation to clarify the relationship between ARWMC, EPC, and vascular risk factors, particularly age and hypertension.

Disclosures

None.

References


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