Emerging Risk Factors for Recurrent Vascular Events in Patients With Embolic Stroke of Undetermined Source

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Background and Purpose—Underlying embolic causes diagnosed by transesophageal echocardiography could be implicated in mechanisms of embolic stroke of undetermined source. We aimed to explore factors, including underlying embolic causes, related to recurrent vascular events in embolic stroke of undetermined source.

Methods—Patients who fulfilled the diagnostic criteria for embolic stroke of undetermined source and whose potential embolic sources were examined by transesophageal echocardiography were included. Recurrent vascular events, including ischemic stroke, cardiovascular and peripheral artery diseases, and vascular death, were retrospectively analyzed. Cox proportional hazards regression analysis was used to explore factors, including clinical characteristics, embolic causes on transesophageal echocardiography, and the Calcification in the Aortic Arch, Age, Multiple Infarction score (CAM), based on the degree of aortic arch calcification on chest radiograph (0–3 points), age (≥70 years; 1 point), and multiple infarctions on magnetic resonance imaging (multiple infarcts in 1, 2, or ≥3 territories of large intracranial arteries, 1, 2, or 3 points) associated with recurrent vascular events.

Results—A total of 177 patients (age, 64.1±14.2 years; 127 men) were enrolled. Thirty-one patients had recurrent vascular events (follow-up, 3.5±2.7 years; annualized rate, 5.0% per person-year). Among embolic causes on transesophageal echocardiography, incidence of recurrent vascular events was high in patients with large aortic arch plaques (7.5% per person-year). Diabetes mellitus (hazard ratio, 2.56; 95% confidence interval, 1.23–5.32; \( P = 0.012 \)) and CAM score grade (hazard ratio, 2.29; 95% confidence interval, 1.11–4.72; \( P = 0.026 \)) predicted recurrent vascular events.

Conclusions—History of diabetes mellitus and the CAM score could be novel risk factors for recurrent vascular events in embolic stroke of undetermined source. (Stroke. 2016;47:2714-2721. DOI: 10.1161/STROKEAHA.116.013878.)

Key Words: aorta, thoracic ◼ echocardiography, transesophageal ◼ infarction ◼ recurrence ◼ stroke

Embolic stroke of undetermined source (ESUS) is a new clinical entity with specific diagnostic criteria including (1) nonlacunar stroke on neuroradiological imaging, (2) no arterial stenosis >50% or occlusion in a corresponding large artery, (3) lack of major cardioembolic source, and (4) lack of other determined stroke causes.1 Diagnostic modalities to investigate cardioembolic sources in the ESUS criteria involved 12-lead ECG, cardiac monitoring for ≥24 hours, and transthoracic echocardiography. Transesophageal echocardiography (TEE) has presumably not been required to standardize the diagnosis of ESUS. In contrast, TEE clearly detected underlying embolic causes, including patent foramen ovale (PFO), atrial septal aneurysm (ASA), and aortic arch plaques, in unexplained stroke.2–5 Several studies using TEE have shown that these embolic causes increased the risk of recurrence in stroke patients.4–4 In ESUS, these embolic sources could also be latent, and thus, it is suggested that the identification of potential embolic causes could be critical in the prognosis for patients with ESUS.

To date, no studies have investigated the potential embolic sources using TEE and long-term prognosis in association with potential embolic sources on TEE in an ESUS population. The present study was conducted on ischemic stroke patients who fulfilled the diagnostic criteria for ESUS and were undergoing TEE examinations, and we investigated the factors, including clinical characteristics and potential embolic sources on TEE related to recurrent ischemic stroke, as well as cardiovascular events in ESUS patients, in a retrospective manner.
Methods

Study Subjects

The present case series involved 1158 patients with acute ischemic stroke who had presented within 7 days of onset, and baseline data were derived from the Inpatient Registry of the Department of Neurology at Juntendo University Hospital, a secondary referral center, from April 2008 to March 2014. The Inpatient Registry included the patients’ characteristics (age, sex, atherosclerotic risk factors, and previous history of stroke); brain computed tomographic and magnetic resonance imaging findings involving the size, number, and locations of infarcts on diffusion-weighted imaging; periventricular hyperintensity and deep and subcortical white matter hyperintensity on fluid-attenuated inversion recovery imaging; stenosis of intracranial arteries >50% on magnetic resonance angiography; chest radiograph involving the degree of aortic arch calcification; 12-lead ECG; and carotid ultrasonography. Transthoracic echocardiography was also performed to obtain cardiac function and to screen for the presence of intracardiac thrombus, and continuous electrocardiographic monitoring for at least 24 hours was started during admission. On the basis of the aforementioned initial stroke examinations, whether patients fulfilled the diagnostic criteria of ESUS was retrospectively analyzed, and patients with ESUS who had undergone TEE were enrolled.1 Patients were excluded if paroxysmal atrial fibrillation or other stroke causes or advanced cancer was detected during hospitalization. Survey questionnaires were distributed in the outpatient department and mailed to patients or families, or results were obtained by telephone in the follow-up study period from January 2015 to November 2015. The questionnaire included inquiries about recurrent vascular events (ischemic stroke, cardiovascular and peripheral artery diseases, and vascular death) that had occurred since discharge from our hospital and information on the use of antiplatelet or anticoagulant agents, statins, and other medications during the observation period. Those treatments were performed in our hospital or by their referring physicians according to our or their best medical judgment and were not randomized. Functional performance using the modified Rankin scale was assessed at follow-up. This study was conducted in accordance with the Declaration of Helsinki. The independent ethics committee of Juntendo University Hospital approved this study. Patients or their relatives received full explanations of the study, and their written informed consent was obtained during the follow-up study period but before enrollment.

Risk Factors

At baseline, atherosclerotic vascular risk factors were defined according to the previous literature.2,9

CAM Score

The Calcification in the Aortic Arch, Age, Multiple Infarction score (CAM) is based on the degree of aortic arch calcification on chest radiograph (no visible calcification, 0 points; small spots or a single thin area of calcification, 1 point; 1 or more areas of thick calcification, 2 points; and circumferential calcification, 3 points), age (≥70 years, 1 point), and diffusion-weighted imaging findings (single infarct, 0 points; multiple infarcts in 1 vascular territory, 1 point; 2 vascular territories, 2 points; ≥3 vascular territories, 3 points).6,10 A maximum CAM score is 7 points, and the CAM scores were stratified into 3 grades (0–2, low CAM; 3–4, intermediate CAM; and 5–7, high CAM grade).7

TEE Study

TEE was conducted using a Vivid S6 system equipped with a multiplane 5.0-MHz transducer (GE Medical Systems, Milwaukee, WI) according to the previous protocol.2,3,0

Statistical Analysis

Numeric values are reported as means±SD. Data were analyzed using the χ2 test for categorical variables and the Mann-Whitney U test for nonparametric analyses. Periods between stroke onset and the day of outcome were calculated, and overall survival and presence of recurrent vascular events were calculated from stroke onset to the date of last follow-up available or until the date of death, referring to the method in the previous literature.11 The incidence of recurrent vascular events was estimated per 100 person-years of observation among the potential embolic causes. The Cox proportional hazards model was used to explore the factors, including clinical characteristics, as well as embolic causes on TEE, associated with recurrent vascular events. After univariate analysis of all clinically relevant covariates, those with a P<0.05 were included in the multivariable Cox model. The Kaplan-Meier method and log-rank test were used to estimate cumulative event rates of recurrent vascular events. A 2-sided P<0.05 was considered significant. All data were analyzed using SPSS statistical software version 15.0 for Windows (SPSS, Chicago, IL).

Results

In 1158 patients with ischemic stroke, 866 patients were categorized into groups with small vessel disease, large artery atherosclerosis, cardioembolic stroke, or other determined cause according to the Trial of Org 10172 in Acute Stroke Treatment criteria.15 The remaining 292 patients fulfilled the diagnostic criteria for ESUS, and TEE was performed for 213 patients in whom follow-up study was performed. Of those patients, complete therapy data could not be obtained in 6 patients; 23 patients were lost during the follow-up period; 5 patients declined to participate in the study; 2 patients developed other neurological diseases, includingencephalitis and chronic subdural hematoma; and thus, final data were successfully obtained in 177 patients (mean age 64.1±14.2 years; 127 men; Figure I in online-only Data Supplement). During the follow-up period of 3.5±2.7 years, 31 patients had recurrent vascular events: 24 patients had recurrent ischemic stroke and 7 had major cardiovascular events including coronary artery disease (n=5), aortic dissection (n=1), and arteriosclerosis obliterans (n=1). Eight patients died during the follow-up. The incidence of recurrent vascular events was 5.0% per person-year.

Clinical Characteristics of Patients With Recurrent Vascular Events

Baseline characteristics for all patients with and without recurrent vascular events are summarized in Table 1. Previous history of ischemic stroke was more common in patients with recurrent vascular events than in those without recurrent vascular events (P=0.012). Hypertension and diabetes mellitus (DM) were more common in patients with recurrent vascular events (P=0.021 and P<0.001, respectively). Multiple infarcts and cortical infarcts on diffusion-weighted imaging were more frequent in patients with recurrent vascular events (P=0.046 and P=0.045, respectively). Degrees of deep and subcortical white matter hyperintensity were higher in patients with recurrent vascular events (P=0.040). On TEE examinations, patients with recurrent vascular events had a significantly higher frequency of large aortic arch plaques ≥4 mm compared with patients without recurrent vascular events (P=0.025), whereas no significant differences were observed in frequencies of PFO, ASA, coexistence of PFO and ASA, or valvular abnormalities between groups. Frequency of low, intermediate, and high CAM grades were 71%, 25%, and 4% in patients without recurrent vascular
Table 1. Baseline Characteristics and Radiological and TEE Findings According to Classification by Recurrent Ischemic Stroke or Vascular Events

<table>
<thead>
<tr>
<th>Variables</th>
<th>All</th>
<th>Recurrent Vascular Events</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=177</td>
<td>n=146, 82%</td>
<td>n=31, 18%</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age, mean±SD</td>
<td>64.1±14.2</td>
<td>63.0±14.8</td>
<td>69.3±9.3</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>127 (72)</td>
<td>101 (69)</td>
<td>26 (84)</td>
</tr>
<tr>
<td>Previous history of stroke, n (%)</td>
<td>18 (10)</td>
<td>11 (8)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>117 (66)</td>
<td>91 (62)</td>
<td>26 (84)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>56 (32)</td>
<td>38 (26)</td>
<td>18 (58)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>103 (58)</td>
<td>86 (59)</td>
<td>17 (55)</td>
</tr>
<tr>
<td>Cigarette smoking, current</td>
<td>54 (31)</td>
<td>45 (31)</td>
<td>9 (29)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>26 (15)</td>
<td>21 (14)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>NIHSS score on admission, mean±SD</td>
<td>3.1±2.9</td>
<td>3.0±2.8</td>
<td>3.4±3.8</td>
</tr>
<tr>
<td>mRS on follow-up, mean±SD</td>
<td>1.1±1.4</td>
<td>1.0±1.4</td>
<td>1.5±1.6</td>
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<tr>
<td>Brain MRI</td>
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<tr>
<td>Number of infarcts, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple lesions</td>
<td>69 (39)</td>
<td>52 (36)</td>
<td>17 (55)</td>
</tr>
<tr>
<td>Size of infarcts, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;15 mm</td>
<td>98 (55)</td>
<td>79 (54)</td>
<td>19 (61)</td>
</tr>
<tr>
<td>Location of infarcts, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical lesions</td>
<td>91 (51)</td>
<td>70 (48)</td>
<td>21 (68)</td>
</tr>
<tr>
<td>White matter abnormality, grade 0–3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVH</td>
<td>1.0±0.9</td>
<td>0.9±0.9</td>
<td>1.3±0.9</td>
</tr>
<tr>
<td>DSWMH</td>
<td>0.8±0.9</td>
<td>0.8±0.8</td>
<td>1.1±0.9</td>
</tr>
<tr>
<td>Intracranial stenosis, n (%)</td>
<td>50 (28)</td>
<td>41 (28)</td>
<td>9 (29)</td>
</tr>
<tr>
<td>Chest radiograph</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Aortic arch calcification, grade 0–3</td>
<td>0.9±0.9</td>
<td>0.8±0.9</td>
<td>1.3±0.9</td>
</tr>
<tr>
<td>TEE findings</td>
<td></td>
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<tr>
<td>Embolic sources, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFO</td>
<td>102 (58)</td>
<td>88 (60)</td>
<td>14 (45)</td>
</tr>
<tr>
<td>ASA</td>
<td>47 (27)</td>
<td>38 (26)</td>
<td>9 (29)</td>
</tr>
<tr>
<td>PFO with ASA</td>
<td>41 (23)</td>
<td>36 (25)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Aortic arch plaques</td>
<td>71 (40)</td>
<td>53 (36)</td>
<td>18 (58)</td>
</tr>
<tr>
<td>Valvular abnormality, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral annular calcification</td>
<td>12 (7)</td>
<td>9 (6)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>43 (24)</td>
<td>39 (27)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Trivial to mild</td>
<td>104 (59)</td>
<td>85 (58)</td>
<td>19 (61)</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>30 (17)</td>
<td>22 (15)</td>
<td>8 (26)</td>
</tr>
<tr>
<td>Aortic valvular calcification</td>
<td>23 (13)</td>
<td>17 (12)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Aortic regurgitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>132 (75)</td>
<td>112 (77)</td>
<td>20 (65)</td>
</tr>
</tbody>
</table>

(Continued)
events and 35%, 52%, and 13% in patients with recurrent vascular events, respectively, which reached statistical significance ($P<0.001$). There were no significant differences in histories of treatment with antithrombotic therapies or statins between the groups.

### Potential Embolic Sources and Recurrent Vascular Events

PFO, PFO with ASA, and aortic arch plaques ≥4 mm were found in 102, 41, and 71 patients, respectively. The incidences of recurrent vascular events in patients with PFO, PFO with ASA, and aortic arch plaques ≥4 mm were 3.4%, 3.0%, and 7.5% per person-year, respectively.

### Factors Associated With Recurrent Vascular Events

In Table 2, age, previous history of stroke, hypertension, DM, aortic arch calcification, and aortic arch plaques were linked with recurrent vascular events, whereas PFO was negatively related to recurrent vascular events in a univariate Cox regression analysis ($P<0.05$). A higher CAM score grade was associated with recurrent vascular events ($P<0.001$). Age, previous history of stroke, hypertension, DM, aortic arch calcification, PFO, aortic arch plaques, and CAM score grade were entered into the multivariate Cox regression analysis as covariates to explore the predictors for recurrent vascular events. The CAM score exhibited significant association with recurrent vascular events (hazard ratio, 2.29; 95% confidence interval, 1.11–4.72; $P=0.026$). DM was also predictive of recurrent vascular events (hazard ratio, 2.56; 95% confidence interval, 1.23–5.32; $P=0.012$; Table 3). The Kaplan-Meier survival curves showed that cumulative event-free rates were significantly lower in patients with DM than in patients without DM (log-rank test, $P=0.001$) and lower according to less severe CAM score grade (log-rank test, $P<0.001$; Figure).

### Discussion

In the present study, the longitudinal outcomes of ESUS patients whose embolic sources were documented by TEE were investigated, and the factors associated with recurrent vascular events were explored in a single-center retrospective study. The major findings of the present study were (1) the incidence of recurrent vascular events was 5.0% per person-year, and (2) DM and the CAM score predicted recurrent vascular events in ESUS. Although patients with large aortic arch plaques exhibited a high incidence of recurrent vascular events (7.5% per person-year), no significant association between large aortic arch plaques and recurrent vascular events was shown on multivariate Cox proportional hazards regression analysis.

In cryptogenic stroke, large-scale stroke registries and studies have demonstrated that underlying paroxysmal atrial fibrillation was newly diagnosed during follow-up in ≈30% of patients. Likewise, paroxysmal atrial fibrillation was considered latent in ESUS. Stroke recurrence in ESUS was as high as that in cardioembolic stroke and was significantly higher than in other ischemic stroke subtypes. In the current study, paroxysmal atrial fibrillation was detected in some patients, but cardiac monitoring was not done during the follow-up period in all patients. However, the potential embolic causes demonstrated on TEE immediately after stroke onset could be fundamental factors related to the pathogenesis of ESUS. To date, no studies have been performed to evaluate the association of recurrent vascular events with underlying embolic causes in ESUS. Although the design of this study is retrospective in nature, the emphasis of the current study is that we were able to clearly diagnose potential embolic sources using TEE in ESUS patients, and we first investigated the factors associated with recurrent vascular events in patients with ESUS.
Table 2. Univariate Analysis of Baseline Characteristics and Radiological and TEE Findings According to Classification by Recurrent Ischemic Stroke or Vascular Events

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate Cox Regression</th>
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<tr>
<td></td>
<td></td>
<td>HR</td>
<td>95% CI</td>
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<tr>
<td><strong>Demographics</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>1.00–1.06</td>
<td>0.041</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.34</td>
<td>0.90–6.12</td>
<td>0.082</td>
</tr>
<tr>
<td>Previous history of stroke</td>
<td>2.61</td>
<td>1.12–6.11</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>2.75</td>
<td>1.06–7.17</td>
<td>0.038</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3.24</td>
<td>1.59–6.62</td>
<td>0.001</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1.06</td>
<td>0.52–2.17</td>
<td>0.871</td>
</tr>
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<td>Cigarette smoking, current</td>
<td>0.79</td>
<td>0.41–1.95</td>
<td>0.899</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>1.44</td>
<td>0.55–3.77</td>
<td>0.456</td>
</tr>
<tr>
<td>NIHSS score on admission</td>
<td>1.04</td>
<td>0.93–1.15</td>
<td>0.510</td>
</tr>
<tr>
<td>mRS score on follow-up</td>
<td>1.23</td>
<td>0.99–1.53</td>
<td>0.067</td>
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<tr>
<td><strong>Brain MRI</strong></td>
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<tr>
<td>Number of infarcts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple lesions</td>
<td>2.01</td>
<td>0.99–4.10</td>
<td>0.054</td>
</tr>
<tr>
<td>Size of infarcts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;15 mm</td>
<td>1.28</td>
<td>0.62–2.64</td>
<td>0.506</td>
</tr>
<tr>
<td>Location of infarcts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical lesions</td>
<td>1.96</td>
<td>0.92–4.17</td>
<td>0.081</td>
</tr>
<tr>
<td>White matter abnormality, grade 0–3</td>
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<td></td>
</tr>
<tr>
<td>PVH</td>
<td>1.40</td>
<td>0.96–2.05</td>
<td>0.082</td>
</tr>
<tr>
<td>DSWMH</td>
<td>1.43</td>
<td>0.98–2.11</td>
<td>0.067</td>
</tr>
<tr>
<td>Intracranial stenosis on MRA</td>
<td>0.74</td>
<td>0.40–1.92</td>
<td>0.878</td>
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<tr>
<td><strong>Chest radiograph</strong></td>
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<tr>
<td>Aortic arch calcification, grade 0–3</td>
<td></td>
<td>1.70</td>
<td>1.19–2.43</td>
</tr>
<tr>
<td><strong>TEE findings</strong></td>
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<tr>
<td>Embolic sources</td>
<td></td>
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<tr>
<td>PFO</td>
<td>0.45</td>
<td>0.22–0.93</td>
<td>0.030</td>
</tr>
<tr>
<td>ASA</td>
<td>1.06</td>
<td>0.49–2.32</td>
<td>0.876</td>
</tr>
<tr>
<td>PFO with ASA</td>
<td>0.56</td>
<td>0.21–1.46</td>
<td>0.232</td>
</tr>
<tr>
<td>Aortic arch plaques</td>
<td>2.20</td>
<td>1.08–4.49</td>
<td>0.031</td>
</tr>
<tr>
<td>Valvaral abnormality, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral annular calcification</td>
<td>2.29</td>
<td>0.68–7.71</td>
<td>0.179</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td>1.70</td>
<td>0.98–2.95</td>
<td>0.059</td>
</tr>
<tr>
<td>Aortic valvular calcification</td>
<td>1.95</td>
<td>0.79–4.78</td>
<td>0.145</td>
</tr>
<tr>
<td>Aortic regurgitation</td>
<td>1.33</td>
<td>0.70–2.52</td>
<td>0.383</td>
</tr>
<tr>
<td>Grade of the CAM score</td>
<td>2.67</td>
<td>1.62–4.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Antiplatelet agents</td>
<td>2.37</td>
<td>0.91–6.20</td>
<td>0.079</td>
</tr>
<tr>
<td>Anticoagulant agents</td>
<td>0.40</td>
<td>0.15–1.03</td>
<td>0.058</td>
</tr>
<tr>
<td>Statins</td>
<td>0.77</td>
<td>0.38–1.56</td>
<td>0.461</td>
</tr>
</tbody>
</table>

ASA indicates atrial septal aneurysm; CAM, Calcification in the Aortic Arch, Age, Multiple Infarctions; CI, confidence interval; DSWMH, deep and subcortical white matter hyperintensity; HR, hazard ratio; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; mRS, modified Rankin scale; NIHSS, National Institute of Health Stroke Scale; PFO, patent foramen ovale; PVH, periventricular hyperintensity; and TEE, transesophageal echocardiography.
DM is a strong risk factor for stroke and is associated with stroke recurrence, as well as disability.\textsuperscript{17–20} In particular, DM predicted stroke recurrence in the settings of lacunar stroke and large artery atherosclerosis.\textsuperscript{19,20} Our data first show that DM could be a risk factor for recurrent vascular events in ESUS patients with any underlying embolic causes. On the contrary, we show that the CAM score, based on the degree of aortic arch calcification on chest radiograph, age, and multiple brain infarcts, is independently associated with recurrent vascular events in patients with ESUS. Aortic arch calcification could represent systemic atherosclerosis and has been shown to be related to cardiovascular events, as well as ischemic stroke.\textsuperscript{10,21} Aging is associated with aortic stiffness and atherosclerosis, as well as with recurrent ischemic stroke.\textsuperscript{2,22,23} Multiple brain infarcts indicate a high risk of potential embolic sources.\textsuperscript{1,24} Thus, it is suggested that the CAM score reflects systemic atherosclerosis and is also a relevant indicator of a high risk for embolic stroke. A recent study indicated that a risk stratification score for ischemic stroke in patients with AF was associated with an increase in the risk of recurrent stroke and death in ESUS.\textsuperscript{25} Current data revealed for the first time that the CAM score could be a novel risk factor for recurrent vascular events in patients with ESUS.

Aortic arch plaques ≥ 4 mm, especially those with large plaques with complex morphology, exhibited a higher prevalence of recurrent ischemic stroke or death than plaques < 4 mm in stroke patients, and large aortic arch plaques have been shown to be related to cardiovascular events.\textsuperscript{5,26} In addition, we previously demonstrated that the CAM score was closely linked with the complex morphology of aortic arch plaques in stroke patients.\textsuperscript{9} In the current study, large aortic arch plaques ≥ 4 mm were more frequently found in patients with recurrent vascular events and exhibited a high incidence of recurrent vascular events (7.5% per person-year). However, multivariate Cox proportional hazards regression analysis did not reveal that large aortic plaques predicted recurrent vascular events in ESUS. Although our data in a single-center retrospective study did not indicate statistically significant evidence of linkage between aortic arch plaques and recurrent vascular events, the significance of aortic arch plaques on TEE for recurrent vascular events in ESUS should be investigated in large-scale clinical trials.

With regard to treatments for potential embolic sources, there has been insufficient evidence to establish whether oral anticoagulants are superior to antiplatelet agents for secondary stroke prevention in stroke patients with these underlying embolic causes on TEE, while, in part, oral anticoagulants could reduce the risk of stroke recurrence further than antiplatelet agents in stroke patients with PFO with ASA and in those with aortic arch plaques.\textsuperscript{4,5,8,17,27–29} In the current study, the following therapeutic interventions were applied. Warfarin (target international normalized ratio, 1.6–2.6) was used for patients with coexistence of PFO and ASA and paradoxical brain embolism, as well as for those with the presence of PFO with deep venous thrombosis. Antiplatelet agents were given to patients with PFO alone and aortic arch plaques. This might have affected our data, suggesting that oral anticoagulant therapy apparently reduces the risk of recurrent vascular events. With regard to statin therapy, we recently elucidated that treatment with 5 mg rosuvastatin induced aortic arch

<table>
<thead>
<tr>
<th>Variables</th>
<th>Multivariate Cox Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
</tr>
<tr>
<td>Age</td>
<td>1.00</td>
</tr>
<tr>
<td>Previous history of stroke</td>
<td>2.11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.69</td>
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<tr>
<td>Diabetes mellitus</td>
<td>2.56</td>
</tr>
<tr>
<td>Aortic arch calcification on chest radiograph</td>
<td>1.13</td>
</tr>
<tr>
<td>PFO</td>
<td>0.58</td>
</tr>
<tr>
<td>Aortic arch plaques</td>
<td>0.79</td>
</tr>
<tr>
<td>CAM score grade</td>
<td>2.29</td>
</tr>
</tbody>
</table>

CAM indicates Calcification in the Aortic Arch, Age, Multiple Infarctions; CI, confidence interval; HR, hazard ratio; PFO, patent foramen ovale; and TEE, transesophageal echocardiography.

Figure. Kaplan-Meier curves of freedom from recurrent vascular events during follow-up. The x axis indicates time in days since inclusion in the study. The y axis indicates the proportion of patients surviving free of vascular event or recurrent stroke. Cumulative event–free rates were compared based on the presence of diabetes mellitus (A); and among low-, intermediate-, and high-grade Calcification in the Aortic Arch, Age, Multiple Infarctions (CAM) scores (B); showing P<0.001 and P<0.001 on the log-rank test, respectively.
plaque stabilization together with a 40% reduction of low-density lipoprotein-cholesterol in stroke patients. However, intensity of statin therapy was not determined, and no efficacy of statins for the prevention of recurrent vascular events was shown in the present study. Currently, ongoing clinical trials aim to compare the efficacy and safety of direct oral anticoagulant agents with aspirin. However, antiplatelet agents and statins have been recommended for stroke associated with large aortic arch plaques in the American Heart Association/ American Stroke Association guidelines. Thus, in the ESUS population, TEE is critical for the diagnosis of aortic arch plaques, and future large-scale studies using TEE to explore the efficacy of optimal antithrombotic therapy and intensive statin therapy are warranted.

Some potential limitations of this study must be considered when interpreting the present results. First, this study was retrospective. The diagnosis of ESUS was made according to the data from our registry, which might have influenced the accurate diagnosis of ESUS. Second, it was not possible to obtain detailed information on treatment or daily compliance with medications from the medical records or by survey questionnaire. Third, the data from the current study were derived from a single center, and the number of patients with recurrent vascular events was small. Moreover, TEE examinations were performed in a limited number of patients with ESUS in consideration of the absence of severe stroke, pneumonia, congestive heart failure, or severe dementia, and more than a few patients dropped out during follow-up. Thus, there might have been selection bias in the final analysis of patients and an issue regarding the generalizability of the results to the entire ESUS population.

In conclusion, this is the first study to elucidate the long-term prognosis for ESUS patients in association with potential embolic sources clearly detected by TEE. The current study shows that DM and the CAM score are predictive of recurrent vascular events in our ESUS patients. Physicians should be aware that the patient history, including age and diabetes mellitus, and evaluation for distribution of infarct lesions on diffusion-weighted imaging and aortic arch calcification on chest radiograph are critical to predict future recurrent vascular events in ESUS.

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Disclosures
None.

References


Emerging Risk Factors for Recurrent Vascular Events in Patients With Embolic Stroke of Undetermined Source
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Supplemental Materials

Methods

Risk factors
Atherosclerotic vascular risk factors were defined as follows: (1) hypertension, history of treatment with antihypertensive agents, systolic blood pressure >140 mmHg, or diastolic blood pressure >90 mmHg at 14 days after stroke onset; (2) diabetes mellitus, use of oral hypoglycemic agents or insulin, or glycosylated hemoglobin ≥6.5%; (3) dyslipidemia, use of antihyperlipidemic agents, serum low-density lipoprotein cholesterol ≥140 mg/dL, high-density lipoprotein cholesterol <40 mg/dL, or triglycerides ≥150 mg/dL; (4) current smoking; and (5) coronary artery disease, defined as a history of angina pectoris or myocardial infarction.

TEE Study
The patients were awake during the study and were not premedicated. Local anesthesia was delivered to the oropharynx with topical lidocaine spray, and the patients were placed in the left lateral decubitus position during the examination. Patent foramen ovale (PFO) was assessed by injecting 10 mL of agitated saline and having the patient perform the Valsalva maneuver. The numbers of microbubbles with and without contrast agent were compared. The numbers of microbubbles transiting from the right atrium to the left atrium were also counted. PFO was diagnosed when microbubbles were visualized in the left atrium during the Valsalva maneuver. At the same time, atrial septal aneurysm was diagnosed when the atrial septum extended ≥11 mm into the left or right atrium, or both. The presence or absence of intracardiac thrombus was noted. Degrees of mitral and aortic regurgitation were also examined. After cardiac screening, the sizes of the plaques in the aortic arch and proximal descending aorta were measured. Mobile and ulcerative aortic plaques were diagnosed as having mobile components swinging on their peduncles and having uneven compositions with a base width and depth of at least 2 mm each in the plaques, respectively. The examinations were performed by at least 2 of 4 experienced sonographers (YU, TK, KH, and NK) and recorded on a DVD.
Supplemental Figure Legend

Figure 1. Flow diagrams of the current study

TOAST=Trial of Org 10172 in Acute Stroke Treatment, ESUS=embolic stroke of undetermined source, TEE=transesophageal echocardiography
<table>
<thead>
<tr>
<th>Patients with Acute ischemic stroke</th>
<th>N=1158</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulfilled the diagnostic criteria of ESUS</td>
<td>N=292</td>
</tr>
<tr>
<td>Categorized into small-vessel disease, large-artery atherosclerosis, cardioembolic stroke, and other determined etiologies based on TOAST criteria</td>
<td>N=866</td>
</tr>
<tr>
<td>Underwent TEE and enrolled to the study</td>
<td>N=213</td>
</tr>
<tr>
<td>Eligible for carrying out TEE: absence of severe stroke severity, pneumonia, congestive heart failure, or severe dementia</td>
<td>N=79</td>
</tr>
<tr>
<td>Final analysis</td>
<td>N=177</td>
</tr>
<tr>
<td>Complete therapy data could not be obtained (6), lost during the follow-up period (23), declined to participate in the study (5); developed other neurological diseases (2).</td>
<td>N=36</td>
</tr>
</tbody>
</table>