Does Impaired Cerebrovascular Reactivity Predict Stroke Risk in Asymptomatic Carotid Stenosis?
A Prospective Substudy of the Asymptomatic Carotid Emboli Study

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Background and Purpose—Improved methods are required to identify the subgroup of patients with asymptomatic carotid stenosis who will have stroke develop. It has been suggested that impaired cerebral reactivity (CVR) may predict high risk, but no multicenter studies have examined this.

Methods—In a preplanned substudy of Asymptomatic Carotid Emboli Study, 106 patients were recruited with ≥70% asymptomatic carotid stenosis. Transcranial Doppler was used to measure CVR with a vasodilatory response of carbon dioxide or acetazolamide. A meta-analysis of the data with previously published studies was performed.

Results—Thirty-two of 106 (30.2%) had severely impaired CVR ipsilateral to the study artery. Mean follow-up was 680 days. There were no ipsilateral strokes. There was a nonsignificant trend to more secondary end points of any stroke/TIA in patients with severely impaired CVR. Three of 32 (9.4%) had impaired CVR compared with 2 of 74 (2.7%) without (hazard ratio, 2.54; 95% CI, 0.61–21.74; \( P = 0.158 \)). On meta-analysis of the Asymptomatic Carotid Emboli Study data with previous studies, impaired CVR was associated with increased risk of ipsilateral stroke alone (OR, 6.14; 95% CI, 1.27–29.5; \( P = 0.02 \)), ipsilateral stroke or TIA (OR, 4.76; 95% CI, 1.86–12.16; \( P = 0.001 \)), and any stroke (OR, 4.66; 95% CI, 1.69–12.85; \( P = 0.003 \)).

Conclusions—In this international multicenter study, we found no association between impaired CVR and recurrent events, but the study was underpowered because of the low event rate. Meta-analysis of available data suggested an association between impaired CVR and future risk. However, currently there are insufficient data to justify the routine clinical use of CVR to stratify risk in patients with asymptomatic carotid stenosis for selection for carotid endarterectomy. (Stroke. 2011;42:1550-1555.)

Key Words: asymptomatic carotid stenosis cerebral hemodynamics transcranial Doppler

Carotid stenosis accounts for 15% to 20% of all strokes.¹ Some of these strokes are preceded by TIA or minor stroke, but the majority occur without warning symptoms. Screening for asymptomatic carotid stenosis (ACS) and performing carotid endarterectomy (CEA) before stroke occurs has been suggested as a prevention strategy.² Large randomized trials have shown that CEA results in a highly significant reduction in stroke risk in ACS.³⁴ However, the cost-effectiveness of this approach has been questioned,⁵ particularly because the trial data demonstrated that 32 patients needed to undergo operation to prevent 1 disabling stroke or death over a 5-year follow-up.³⁴ The benefit may be even less with recent advances in medical management.⁶⁷ The large number of patients needed to treat to prevent 1 event is a reflection of the low annual risk of ipsilateral stroke in ACS, which is between 1% and 2%.⁶–⁸ The cost-effectiveness and risk-to-benefit ratios of CEA would be greatly improved if it were possible to identify patients with ACS who are at particularly high risk for stroke.

Emboli is thought to be the major mechanism causing stroke in ACS. However, hemodynamic compromise could also play a role. This could be either via hypoperfusion directly resulting in stroke or via interaction of hemodynamic responses with embolism resulting in impaired clearance of emboli.⁹ Hemodynamic reserve can be studied by determining the increase in blood flow in response to vasodilatory stimuli, such as increased inspired carbon dioxide, or an intravenous injection of the carbonic anhydrase inhibitor acetazolamide. Blood flow can be measured using imaging techniques such as single photon emission computed tomography and positron emission tomography, but more commonly transcranial Doppler (TCD) ultrasound is used to measure middle cerebral artery (MCA) blood flow velocity as a marker of cerebral blood flow.¹⁰ A significant minority of

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patients with ACS have markedly reduced reactivity when tested by this method.\textsuperscript{11–13} In patients with carotid artery occlusion, impaired cerebrovascular activity (CVR) has been shown to predict subsequent ipsilateral stroke risk.\textsuperscript{12,14,15} However, in such patients the etiology of ischemia is thought to be primarily hemodynamic rather than embolic. The predictive value of impaired CVR in asymptomatic carotid stenosis, as opposed to occlusion, is less clear. Cross-sectional studies have demonstrated impaired CVR in a subgroup of patients, but there is limited prospective data on whether this impairment predicts future stroke risk.\textsuperscript{11–13}

The Asymptomatic Carotid Emboli Study (ACES)\textsuperscript{16} was an international, multicenter, prospective study examining whether embolic signals (ES) detected on TCD predicted future stroke risk during a 2-year follow-up. In a protocol defined substudy, a proportion of patients also had CVR measurements at baseline. Here, we present the first analysis of the association between CVR and future stroke risk in this population. We also present these data in the context of a meta-analysis of all prospective studies examining the association of impaired CVR on TCD with future stroke risk.

**Patients and Methods**

The ACES was designed to determine whether asymptomatic ES detected with TCD in the ipsilateral MCA of patients with ≥70% ACS can predict stroke and TIA risk. The full protocol has been published previously.\textsuperscript{17} ACES recruited 482 subjects from 26 centers worldwide. The main results have been published recently.\textsuperscript{18} In a subgroup of patients (those recruited in 7 of the centers), CVR was also performed.

**Patients**

Subjects were included if they had carotid stenosis of 70% to 99% (established by carotid duplex ultrasound) that had been asymptomatic for at least 2 years. If patients had previous symptoms in the contralateral carotid artery or vertebral-basilar territory, then they were only eligible if symptoms occurred >2 years ago. Patients with previous contralateral CEA were eligible 1 year after CEA, assuming that they had been asymptomatic in the territory of the endarterectomized artery since the operation.

Exclusion criteria were: other current diseases that were likely to limit life expectancy to <3 years; if the patient, physician, or surgeon was unwilling to manage ACS medically; absence of an acoustic window necessary for TCD; and nonbiological prosthetic heart valves, because these are associated with large numbers of presumed gaseous ES.\textsuperscript{18}

Brain imaging with CT or MRI was performed at baseline. Subjects were followed-up at 6, 12, and 18 months, with final follow-up at 24 months. There was blinded assessment of all of the end points of stroke and TIA. If stroke or TIA occurred during follow-up, then a repeat brain CT/MRI was performed blinded to TCD information.

**Measurement of Hemodynamic Reserve**

TCD was used to measure cerebral blood flow velocity in the MCA (MCAv) ipsilateral to the study carotid artery. All recordings were made using commercially available TCD machines (Multidop ×4, DWL and Pioneer, EME/Nicolet). A baseline recording of MCAv was performed with the subject breathing room air. One of 2 vasodilatory stimuli was then administered according to centers: either 6% carbon dioxide in air or an intravenous bolus injection of acetazolamide at a dose of 1 gram. Continuous MCAv recordings were made for subsequent later blinded analysis. In patients administered CO\textsubscript{2}, continuous end-tidal CO\textsubscript{2} monitoring was performed. All recordings were saved on digital media and analyzed in the coordinating center blinded to patient identity. The CVR was calculated from the percentage increase in MCAv, determined from the MCAv while breathing 6% CO\textsubscript{2} or 20 minutes after acetazolamide, compared with the MCAv at baseline while breathing room air. Severely impaired CVR was defined as <10% increase as described previously.\textsuperscript{14,15,19} Highly significant correlations between CVR determined in this way when using either 6% CO\textsubscript{2} inhalation or acetazolamide injection have been demonstrated.\textsuperscript{20} Calculation of CVR was performed centrally blinded to clinical and other TCD data.

**TCD ES Detection**

A standard TCD recording protocol was followed, which has been previously described.\textsuperscript{17} Briefly, a 2-MHz transducer was used to insonate the MCA ipsilateral to the ACS. All ES data were recorded onto digital audiotape and analyzed centrally by investigators who were masked to clinical information. For central data analysis, the audio signal was played back on the 1 Doppler machine (Pioneer, EME/Nicolet). Standard consensus criteria on ES identification were used in addition to an intensity threshold of 7 dB. ES were identified visually and audibly. All potential ES were then reviewed by a second observer (H.S.M.) to ensure consistency.

**Statistical Analysis**

The \( \chi^2 \) test with Fisher exact was used for comparisons of categorical variables. Logistic regression was used to determine the interaction and relationship between baseline risk factors, demographics, and treatment, and impaired and sufficient CVR. \( P<0.05 \) was considered statistically significant. Nonparametric correlation was used. Kaplan Meier survival analysis (Breslow) and Cox proportional hazard regression models to calculate hazard ratios (HR) and their 95% CI were used. SPSS software (version 16.0) was used for analysis.

**Meta-Analysis**

A meta-analysis was performed of all prospective studies on established ACS that reported CVR measurement estimated using TCD and subsequent risk stroke. Medline, Embase, and PubMed were searched between January 1, 1990 and October 1, 2010. Only articles in English that reported results for humans were included. Search terms were (transcranial Doppler OR TCD OR ultrasound OR ultrasonography) AND (asymptomatic carotid stenosis OR large vessel disease OR atherosclerosis) AND (reactivity OR hemodynamic OR autoregulation OR carbon dioxide OR breath holding OR acetazolamide) AND (stroke OR transient ischemic attack OR transient ischemic attack OR amaurosis fugax OR death). Reference lists of articles fulfilling the inclusion criteria and reviews were also searched for relevant references. Data were only included from prospective studies. Meta-analyses were performed using RevMan5 software by use of a random-effects model. Cut-off values for impaired reactivity were used as reported in individual studies.

**Results**

**Demographics Including Baseline CVR**

One hundred six patients were recruited to the CVR substudy. Baseline characteristics are given in Table 1. Reactivity was assessed using CO\textsubscript{2} inhalation in 79 (75%) and using acetazolamide in 27 (25%). Mean CVR was 23.89% (SD,
Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Exhausted CVR &lt;10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total  Yes  No</td>
</tr>
<tr>
<td>N</td>
<td>106     32  74</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>72.29 (8.079) 71.91 (8.931) 72.46 (7.741)</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>84 (79.2) 21 (65.6)* 63 (85.1)*</td>
</tr>
<tr>
<td>Smoker, no. (%)</td>
<td>41 (38.7) 11 (34.4) 30 (40.5)</td>
</tr>
<tr>
<td>Current</td>
<td>17 (16) 4 (12.5) 13 (17.6)</td>
</tr>
<tr>
<td>Former</td>
<td>48 (45.3) 17 (53.1) 31 (41.9)</td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>82 (77.4) 23 (71.9) 59 (79.7)</td>
</tr>
<tr>
<td>Diabetes, no. (%)</td>
<td>15 (14.2) 5 (15.6) 10 (13.5)</td>
</tr>
<tr>
<td>Atrial fibrillation, no.</td>
<td>7 (6.6) 3 (9.4) 4 (5.4)</td>
</tr>
<tr>
<td>Ischemic heart disease, no. (%)</td>
<td>32 (30.6) 5 (15.6)* 27 (36.5)*</td>
</tr>
<tr>
<td>Previous ischemia, no.</td>
<td>42 (39.6) 15 (46.9) 27 (36.5)</td>
</tr>
<tr>
<td>Degree of carotid stenosis (%)</td>
<td>70–79 55 (51.9) 16 (50) 39 (52.7)</td>
</tr>
<tr>
<td></td>
<td>80–89 26 (24.5) 9 (28.1) 17 (23)</td>
</tr>
<tr>
<td></td>
<td>90–99 25 (23.6) 7 (21.9) 18 (24.3)</td>
</tr>
<tr>
<td>Contralateral carotid disease</td>
<td>23 (21.7) 7 (21.9) 16 (21.6)</td>
</tr>
<tr>
<td>70%–99% stenosis</td>
<td>13 (12.3) 3 (9.4) 10 (13.5)</td>
</tr>
<tr>
<td>Occlusion</td>
<td>10 (9.4) 4 (12.5) 6 (8.1)</td>
</tr>
<tr>
<td>Statin therapy, no. (%)</td>
<td>75 (70.8) 23 (71.9) 52 (70.3)</td>
</tr>
<tr>
<td>Antiplatelet therapy, no. (%)</td>
<td>98 (92.5) 28 (87.5) 70 (94.6)</td>
</tr>
<tr>
<td>Aspirin therapy, no. (%)</td>
<td>85 (80.2) 19 (59.4)† 66 (89.2)†</td>
</tr>
<tr>
<td>Antihypertensive therapy, no. (%)</td>
<td>82 (77.4) 23 (71.9) 59 (79.7)</td>
</tr>
</tbody>
</table>

Hypertension indicates using antihypertensive medication and/or systolic or diastolic blood pressures >140 or >90 mm Hg, respectively. Diabetes mellitus included a clinical diagnosis of type I and type II diabetes. Therapies refer to treatment at baseline. CVR indicates cerebral reactivity; SD, standard deviation. *P<0.05. †P<0.001.

24.10%). Thirty-two of 106 (30.2%) patients had severely impaired CVR ipsilateral to the study artery.

Analysis was performed to see whether risk factors were related to baseline CVR. On univariate analysis, impaired CVR was associated with being female (P=0.036), absence of ischemic heart disease (P=0.039), and not using aspirin (P<0.001). However, on multivariate analysis the only persisting difference was aspirin therapy; patients with impaired CVR were less likely to be using aspirin (P=0.002).

Ninety-one of the 106 subjects had bilateral CVR measurements, with measurements also from the contralateral MCA. Mean CVR in the contralateral artery was 24.23% (SD, 25.76%). Of these 91, 30 had carotid stenosis >70% in the contralateral artery and, of these, 22 had bilateral impairment and 8 had contralateral impairment only.

Table 2. Number and Type of End Points in Subjects With and Without Impaired Cerebral Reactivity

<table>
<thead>
<tr>
<th>CVR</th>
<th>Ipsilateral TIA</th>
<th>Ipsilateral Stroke</th>
<th>Any Stroke/TIA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired, no. (%)</td>
<td>1 (3)</td>
<td>0</td>
<td>3 (9)</td>
<td>32</td>
</tr>
<tr>
<td>Sufficient, no. (%)</td>
<td>1 (1)</td>
<td>0</td>
<td>2 (3)</td>
<td>74</td>
</tr>
</tbody>
</table>

CVR indicates cerebral reactivity; TIA, transient ischemic attack.

Relationship Between CVR and Recurrent Events

Mean follow-up was 680 days (SD, 162.9 days). There were 5 ischemic events during follow-up: 4 TIA and 1 stroke. Two TIA were in the study artery, and 2 were in the contralateral carotid artery. The 1 stroke was in the vertebrobasilar territory. Both of the patients who experienced ipsilateral ischemia underwent CEA. The patient with vertebrobasilar stroke died from noncerebro/cardiovascular causes. Neither patient with contralateral TIA had >70% stenosis in the carotid artery of the affected territory.

The relationship between impaired CVR and recurrent events is shown in Table 2. There were no ipsilateral strokes in the 106 subjects. There was 1 (3.1%) ipsilateral TIA in the impaired CVR group (n=32) and 1 (1.4%) in the preserved CVR group (n=74; P=0.515). There was a nonsignificant trend to more secondary end points of any stroke/TIA in patients with severely impaired CVR. Three (9.4%) events occurred in those with ipsilateral severely impaired CVR (n=32) compared with 2 (2.7%) events in those without impaired CVR (n=74; HR, 3.62; 95% CI, 0.61–21.74; P=0.158).

ES Detection

One hundred five of 106 patients had baseline ES detection and CVR data. There was no association between the presence of ES and impaired CVR; 7 of 32 (21.9%) patients who had impaired CVR had ES on either of the 2 baseline recordings, compared with 11 of 73 (15%) who were ES-positive (χ²=0.726; P=0.409). However, the median number of ES was higher in those with impaired reactivity (1 versus 4; P=0.022). ES frequency at baseline negatively correlated with CVR (Kendall’s τ_b = −0.170; P=0.03).

Although ES were associated with recurrent events in the whole ACES cohort, in the subgroup in this study there was no association between the presence of ES and either any stroke or TIA or ipsilateral stroke or TIA (HR, 1.17; 95% CI, 1.31–10.53; P=0.888).

Meta-Analysis

Systematic review retrieved 3 articles in addition to ACES that reported CVR measurement in established asymptomatic stenosis with follow-up for future stroke/TIA. Different vasodilatory stimuli were used: acetazolamide alone,11 breath-holding,13 and carbon dioxide.12 Meta-analysis of these studies with the ACES data found that in ACS, impaired CVR was associated with an increased risk of ipsilateral stroke or TIA (OR, 4.76; 95% CI, 1.86–12.16; P=0.001; Figure A), ipsilateral stroke alone (OR, 6.14; 95% CI, 1.27–29.5; P=0.02; Figure B), any stroke (OR, 4.66; 95% CI,
1.69–12.85; P = 0.003; Figure C), and any stroke/TIA (OR, 4.58; 95% CI, 1.93–10.87; P = 0.0006). There was no heterogeneity for any analyses. The annual risk of ipsilateral stroke (and ipsilateral stroke and TIA) in the different studies and the years in which they were performed were: 2.3% (7.9%) during 1994 to 1996; 0.96% (3.84%) during 1996 to 1999; 5.9% (7.9%) during 1996 to 2000; and 1.2% (3%) in ACES patients recruited from 2000 to 2010.

Figure. Forest plots showing the results of the meta-analysis associating impaired cerebral reactivity at baseline with events during prospective follow-up for (A) ipsilateral stroke or transient ischemic attack (TIA), (B) ipsilateral stroke alone, and (C) any stroke. CI indicates confidence interval.
Discussion

ACES is the largest prospective study examining the association between impaired CVR and future stroke risk in patients with ACS. Previous smaller single-center studies have suggested an association but ACES is the first multicenter study to test this association. Although a significant proportion (30%) of subjects had impaired CVR, no association with recurrent events was determined, although there were very few events during follow-up. However, when we combined the ACES data with data from previous studies in a meta-analysis, a significant association was found between impaired CVR and both ipsilateral stroke and all stroke.

Previous studies have shown a replicable association between impaired CVR and future stroke risk in patients with carotid occlusion. However, there are less data from prospective studies in patients with asymptomatic stenosis than occlusion. The results of our meta-analysis suggest there is also an association in this group, but the results of ACES suggest the risk of stroke is low in ACS patients with impaired CVR who are treated with current medical therapy, and therefore the technique may not be a useful method to identify individuals for CEA.

A striking feature of this study were the low recurrent stroke and TIA risks, particularly in patients with impaired CVR. The overall ipsilateral stroke and TIA risks in this ACES substudy were lower than that in the overall ACES cohort (ipsilateral stroke 0% versus 1.2%; ipsilateral stroke and TIA 0.9% versus 3%), but this difference is likely to be attributable to chance. Nevertheless, the recurrent event rate in patients with impaired CVR in ACES was much lower than that in previous studies in the 1990s, being only 1.56% compared with 13.9% to 18.9% in previous studies. This may, at least partly, reflect the reduced stroke risk seen in ACS in general over the past two decades that may reflect improving treatment.

Analysis of baseline CVR and its association with other parameters revealed some intriguing findings. First, using low-dose aspirin therapy was associated with better CVR on both univariate and multivariate analyses. This was not a protocol-specified analysis and therefore it should be treated cautiously. However, low-dose aspirin is known to have vasoactive properties that may be through a cyclooxygenase-independent pathway, and it is possible this mechanism of action could underlie this association. Another possible mechanism could be through aspirin decreasing the ES load. We also found an association between CVR and number of embolic signals. Again, this was a secondary analysis, but is interesting in view of the suggested interaction between hypoperfusion and embolism in causing cerebral ischemia.

In contrast to the lack of association with CVR in this substudy, in the whole ACES cohort the presence of ES was a strong predictor of future stroke risk. However, within the subgroup described in this article, there was no association between ES and future risk, reflecting the small number of recurrent events in the 107 patients in this substudy. Taking all the ACES data together, we can conclude that ES are a predictor of future stroke risk but that there are insufficient data to determine whether impaired CVR is also a predictor. It should also be pointed out that our results only apply to asymptomatic carotid stenosis, and not to symptomatic carotid stenosis or occlusion.

Our study has a number of strengths. It is the first multicenter international study examining this question. All analysis of CVR measurements was performed blinded to recurrent clinical events in a central reading laboratory. No subjects were lost to follow-up and all recurrent events were independently adjudicated. However, it also has some weaknesses. Not all patients in ACES had CVR measurements because these were only performed in certain centers. Both acetazolamide and carbon dioxide were used as stimuli to achieve vasodilation. However, it has been shown that impaired CVR using the 2 methods correlates well.

Conclusions

In conclusion, in this international multicenter study, we were unable to confirm an association between impaired CVR and recurrent ischemic events in patients with ACS, but the low recurrent event rate meant that it was underpowered. A meta-analysis of available data did suggest an association between impaired CVR and future risk. However, overall, there are insufficient data to justify the use of CVR in routine clinical practice to stratify risk in patients with ACS for selection for CEA.

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Disclosures

None.

References


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