Soluble Vascular Cell Adhesion Molecule 1 and N-terminal Pro–B-Type Natriuretic Peptide in Predicting Ischemic Stroke in Patients With Cerebrovascular Disease

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**Background:** Patients with stroke or transient ischemic attack are at high risk of another stroke, and there is need for improved strategies to predict recurrent stroke.

**Objective:** To assess the prognostic value of levels of soluble vascular cell adhesion molecule 1 (sVCAM-1), N-terminal pro–B-type natriuretic peptide (NT-proBNP), C-reactive protein, homocysteine, renin, and lipids and lipoprotein particle concentration and size in patients with previous stroke or transient ischemic attack.

**Design, Setting, and Participants:** A nested case-control study of participants of the Perindopril Protection Against Recurrent Stroke Study was performed. The Perindopril Protection Against Recurrent Stroke Study was a placebo-controlled trial of a perindopril erbumine–based, blood pressure–lowering regimen that reduced ischemic stroke risk by 24% among individuals with previous stroke or transient ischemic attack. Each of 252 patients who experienced ischemic stroke during a mean follow-up of 3.9 years was matched to 1 to 3 control patients. Matching variables were age, sex, treatment allocated, region, and most recent qualifying event at randomization.

**Main Outcome Measures:** Risk of ischemic stroke predicted by baseline levels of sVCAM-1, NT-proBNP, C-reactive protein, homocysteine, renin, and lipids and lipoprotein particle concentration and size.

**Results:** Levels of sVCAM-1 and NT-proBNP predicted recurrent ischemic stroke. The odds ratio for patients in the highest, as compared with the lowest, quarter was 2.24 (95% confidence interval, 1.35-3.73) for sVCAM-1 level and 1.62 (95% confidence interval, 0.98-2.69) for NT-proBNP level, after adjustment for matching and other risk factors. Patients in the highest quarters for both sVCAM-1 and NT-proBNP levels had 3.6 times the risk of recurrent ischemic stroke compared with patients in the lowest quarters for both biologic markers. Level of sVCAM-1 was similarly predictive of ischemic stroke in patients allocated to placebo and perindopril-based therapy. Baseline plasma levels of C-reactive protein, homocysteine, renin, and lipids and lipoprotein particle concentration and size did not predict recurrent ischemic stroke risk.

**Conclusion:** Measurement of sVCAM-1 and NT-proBNP levels provides prognostic information for recurrent ischemic stroke beyond traditional risk factors.

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STROKE IS A LEADING CAUSE OF death and disablement, with a risk of 21% for those living beyond 55 years of age. For many individuals, their initial stroke is the first indication of their stroke risk. Among those who survive a stroke or transient ischemic attack (TIA), as many as one third have another stroke within 5 years of the index event. Recurrent strokes are associated with higher risk and greater degree of disability than initial strokes, and prevention of subsequent stroke in patients with cerebrovascular disease is an important objective of treatment. The objective of this study was to evaluate the prognostic value of a range of potential risk factors for recurrent ischemic stroke by conducting a nested case-control study of participants in the Perindopril Protection Against Recurrent Stroke Study (PROGRESS). A multicenter, randomized, double-blind, placebo-controlled study, PROGRESS was designed to determine the effects of active therapy with a perindopril erbumine–based, blood pressure–lowering regimen on the risk of stroke and other major vascular events among individuals with a stroke or TIA within the previous 5 years. This regimen substantially reduced the risk of total stroke by 28% and ischemic stroke by 24%. The potential risk factors we examined were markers of endothelial dysfunction and inflammation (soluble vas-
cular cell adhesion molecule 1 [sVCAM-1] and C-reactive protein [CRP] levels), cardiac dysfunction (N-terminal pro-B-type natriuretic peptide [NT-proBNP] level), oxidative stress (homocysteine level), renin-angiotensin system activity (active renin level), and dyslipidemia (lipids levels and lipoprotein particle concentration and size). We also examined whether any of these potential risk factors predicted ischemic stroke in subjects receiving perindopril-based therapy.

**STUDY POPULATION**

The design and major outcomes of PROGRESS are described in detail elsewhere. Briefly, 6105 participants were recruited from 172 collaborating centers in 10 countries from Australasia, Europe, and Asia. Participants were randomized to either placebo (n=3054) or active therapy (n=3051), consisting of a flexible regimen based on the angiotensin-converting enzyme inhibitor perindopril erbumine (4 mg/d) with the addition of the diuretic indapamide at the discretion of treating physicians. The institutional ethics committee of each collaborating center approved the trial, and all participants provided written informed consent to participate in the study, including the collection of blood samples for laboratory tests and the measurement of factors that may be linked to an increased risk of stroke. Congestive heart failure was a study exclusion criterion.

**LABORATORY METHODS**

Collection of blood samples, storage of plasma, and measurement of lipids, renin, NT-proBNP, CRP, and creatinine levels are described elsewhere. Lipoprotein particle concentration and size were measured on first-thaw EDTA plasma by nuclear magnetic resonance by LipoScience, Inc, Raleigh, NC. Homocysteine level was measured by high-performance liquid chromatography with fluorometric detection, and sVCAM-1 level was measured by immunoassay using reagents from R&D Systems, Inc, Minneapolis, Minn, on second-thaw EDTA plasma.

**MAIN OUTCOME MEASURES**

Stroke, either fatal or nonfatal, was defined as an acute disturbance of focal neurological function with symptoms lasting more than 24 hours or resulting in death in earlier studies. Diagnosis of ischemic stroke required a computed tomographic scan (performed within 3 weeks) that either had normal results or showed infarction in the clinically expected area, or autopsy evidence of cerebral infarction. The adjudication committee reviewed source documentation for all deaths and every potential stroke and myocardial infarction recorded during the study follow-up period.

**STATISTICAL ANALYSIS**

To minimize the influence of the qualifying event on the biochemical parameters, and also changes in biochemical parameters following the collection of blood before freezing of plasma, the base population for this study was 2673 patients who were enrolled more than 1 month after their most recent qualifying event and whose plasma was frozen at −80°C within 24 hours of blood collection. Each of 252 cases of ischemic stroke during the mean 3.9 years of follow-up was matched with 1 to 3 controls randomly sampled from patients who were alive and did not have a stroke between randomization and the time of case ascertainment; anyone who became a case after the onset of ischemic stroke in the index case was eligible for selection as a match, and controls could be matched for more than 1 case. Matching variables were age (within 5 years), sex, treatment allocated (perindopril-based or placebo, mono or dual therapy), region, and most recent qualifying event at randomization.

Baseline variables were compared between cases and controls using $\chi^2$ parametric, or nonparametric tests, as appropriate. The case-control data were analyzed using 2 different conditional logistic regression models to obtain odds ratios (ORs) for sVCAM-1, NT-proBNP, and CRP levels, according to equal quarters of the distribution of each plasma marker in the total sample (cases and controls). Model 1 was unadjusted, except for the matching variables. Model 2 was adjusted through matching and also for all baseline predictors and potential predictors of ischemic stroke. These were baseline systolic blood pressure; total cholesterol level; a history of current smoking, diabetes mellitus, and peripheral arterial disease; and antihypertensive medication other than β-blockers, calcium channel blockers, or diuretics. In addition, model 2 for sVCAM-1 level was adjusted for NT-proBNP and CRP levels, model 2 for NT-proBNP level was adjusted for sVCAM-1 and CRP levels, and model 2 for CRP level was adjusted for sVCAM-1 and NT-proBNP levels. All $P$ values were 2 sided, and values $\leq 0.05$ were considered to indicate statistical significance. All analyses were performed using SAS version 8.2 (SAS Institute Inc, Cary, NC).

**BASELINE CHARACTERISTICS**

The baseline clinical and biochemical characteristics of the 252 ischemic stroke cases and 544 controls without ischemic stroke are given in Table 1 and Table 2. More than 90% of cases and controls had previous ischemic stroke and/or TIA. In comparison with controls without ischemic stroke during the period of observation, ischemic stroke cases were more likely to have hypertension on enrollment; to have a history of current smoking, diabetes mellitus, and peripheral arterial disease; and to be taking antihypertensive medication other than β-blockers, calcium channel blockers, or diuretics (Table 1). In addition, ischemic stroke cases had higher baseline levels of sVCAM-1, NT-proBNP, and CRP. There were no differences between cases and controls with respect to levels of plasma lipids, creatinine, homocysteine, and renin and lipoprotein particle concentration and size (Table 2).

**MAIN OUTCOME MEASURES**

The OR for ischemic stroke increased with increasing levels of baseline sVCAM-1, NT-proBNP, and CRP (Table 3) but for none of the other variables listed in Table 2. The OR for patients in the highest, as compared with the lowest, quarter was 2.34 (95% confidence interval [CI], 1.46-3.77) for sVCAM-1 level and 1.90 (95% CI, 1.19-3.04) for NT-proBNP level. Whereas the OR was significantly elevated for only patients in the highest quarter of NT-proBNP level, it was similarly elevated for patients in the second, third, and fourth quarters of sVCAM-1 level.
The OR of 1.32 (95% CI, 0.86-2.03) for CRP level did not achieve statistical significance (Table 3, CRP level model 1). In multiple-variable analysis (Table 3, model 2), the OR for patients in the highest, as compared with the lowest, quarter was 2.24 (95% CI, 1.35-3.73) for sVCAM-1 level, borderline nonsignificant at 1.62 (95% CI, 0.98-2.69) (P = .06) for NT-proBNP level, and nonsignificant for CRP level at 1.00 (95% CI, 0.63-1.58). However, the OR for NT-proBNP level increased to 1.79 (95% CI, 0.98-2.69) (P = .02) when sVCAM-1 level was removed from model 2. Level of sVCAM-1 correlated with NT-proBNP level (r = 0.13; P < .001) and CRP level (r = 0.13;
Levels of sVCAM-1 and NT-proBNP were independent predictors of ischemic stroke risk in this population of patients with established cerebrovascular disease, more than 90% having had previous ischemic stroke or TIA. Level of NT-proBNP correlated with sVCAM-1 level, and the OR for prediction of ischemic stroke by NT-proBNP level was borderline nonsignificant when sVCAM-1 level was included in the multiple-variable model.
den. Angiotensin II induces VCAM-1 expression in vasculature and may therefore contribute to the elevated VCAM-1 levels in patients at increased risk of recurrent ischemic stroke. However, renin level did not predict ischemic stroke in this population.

Blood pressure lowering with perindopril-based therapy reduced ischemic stroke incidence by 24%, indicating that a proportion of ischemic strokes in PROGRESS participants assigned placebo was attributable to mechanisms responsive to perindopril-based therapy; however, a larger proportion of ischemic strokes was attributable to mechanisms unresponsive to this therapy. Our finding that sVCAM-1 level predicted recurrent ischemic stroke in patients receiving perindopril-based therapy suggests that sVCAM-1 level is a marker of mechanisms of ischemic stroke pathogenesis distinct from those responsive to perindopril-based therapy. Identification of these mechanisms may assist development of novel therapies for the prevention of recurrent ischemic stroke.

Our finding that NT-proBNP level predicted ischemic stroke risk is in agreement with previous reports that B-type natriuretic peptide and NT-proBNP levels are associated with an increased risk of death and cardiovascular events, including heart failure, myocardial infarction, atrial fibrillation, and stroke or transient ischemic attack. Whereas previous studies examined initial stroke, to our knowledge, our study is the first to show that B-type natriuretic peptides predict recurrent ischemic stroke. We found that homocysteine level did not predict recurrent ischemic stroke, although other studies reported that homocysteine level predicts recurrent ischemic stroke risk.

Level of CRP is an established risk factor for cardiovascular events, including initial ischemic stroke. However, there are limited data about the association of CRP level with recurrent ischemic stroke risk. The present study was confined to plasma samples frozen within 24 hours of collection, and the OR for recurrent ischemic stroke for patients in the highest, as compared with the lowest, quarter for CRP level was 1.32 (95% CI, 0.86-2.03) in an analysis adjusted only for matching. In a separate analysis of nearly twice the number of cases and controls using PROGRESS plasma samples frozen within 48 hours of collection, the OR for recurrent ischemic stroke for patients in the highest, as compared with the lowest, third for CRP level was 1.52 (95% CI, 1.15-2.00), and CRP level remained a predictor of recurrent ischemic stroke after adjustment for fibrinogen level. By contrast, the present study showed that CRP level did not predict recurrent ischemic stroke after adjustment for sVCAM and NT-proBNP levels.

Plasma lipids levels have only a weak association with ischemic stroke risk. Lipoprotein particle size analysis was proposed to provide better prediction of cardiovascular risk than measurement of plasma lipids levels because it permits specific quantification of the more atherogenic small low-density lipoprotein particles. To our knowledge, this is the first report of the association of lipoprotein particle concentration and size and ischemic stroke risk, and our findings support the lack of association between plasma lipids levels and recurrent ischemic stroke risk.

There are several limitations to our study. Our analyses were based on single baseline determinations that may not accurately reflect risk factor levels before enrollment or over the mean 3.9 years of follow-up. In addition, frozen storage may affect lipoprotein analysis, although plasma samples from cases and controls were treated in an identical and blinded fashion. These sources of variability are unlikely to account for the observed associations between sVCAM-1 and NT-proBNP levels and recurrent ischemic stroke risk because any random misclassification would bias results toward the null hypothesis and systematic differences between cases and controls are unlikely. Another limitation of our study relates to the heterogeneity of ischemic stroke subtypes because these may have different mechanisms of pathogenesis. Ischemic strokes include those due to small-vessel, large-vessel, and cardioembolic causes, and it is possible our grouping of ischemic strokes masked risk factors for specific ischemic stroke subtypes. For example, sVCAM-1 level may be a marker for small- and large-vessel causes of ischemic stroke, whereas NT-proBNP level may be a marker for cardiac dysfunction that contributes to cardioembolic stroke. Further large studies are required to examine whether these and other potential risk factors are stroke subtype–specific.

In conclusion, we performed head-to-head evaluation of a large number of potential risk factors for recurrent ischemic stroke. We found sVCAM-1 and NT-proBNP levels independently predicted ischemic stroke risk in patients with previous stroke or TIA, and sVCAM-1 level was similarly predictive in patients receiving placebo or perindopril-based, blood pressure–lowering treatment. The prognostic information provided by sVCAM-1 and NT-proBNP levels was in addition to that provided by traditional risk factors for ischemic stroke and may enable more effective targeting of prevention strategies. We also showed levels of CRP, homocysteine, renin, and lipoprotein particle concentration and size were not associated with recurrent ischemic stroke risk. Characterization of the mechanisms of ischemic stroke pathogenesis associated with increased sVCAM-1 level may lead to development of therapies that provide benefits additional to those provided by angiotensin-converting enzyme inhibitor–based, blood pressure–lowering therapies.

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**REFERENCES**