Long-Term Effects of Inflammation-Sensitive Plasma Proteins and Systolic Blood Pressure on Incidence of Stroke

G. Engström, MD, PhD; P. Lind, MD; B. Hedblad, MD, PhD; L. Stavenow, MD, PhD; L. Janzon, MD, PhD; F. Lindgärde, MD, PhD

Background and Purpose—The present study investigated the relationships between inflammation-sensitive plasma proteins (ISPs) and systolic blood pressure (SBP), as well as the joint long-term effects of ISP and SBP on incidence of stroke.

Methods—BP and 5 ISPs (fibrinogen, α1-antitrypsin, haptoglobin, ceruloplasmin, orosomucoid) were assessed in 6071 healthy men 28 to 61 years of age. All-cause mortality and incidence of stroke were monitored over a mean follow-up of 18.7 years in men defined by SBP (<120, 120 to 139, ≥140 mm Hg) and ISP (0 to 1 or 2 to 5 ISPs in the top quartile).

Results—SBP and diastolic BP were significantly and positively associated with the number of ISPs in the top quartile. As expected, elevated SBP was associated with an increased incidence of stroke. Among men with SBP ≥140 mm Hg, there were, however, significant differences between those with high and low ISP levels. After risk factor adjustment, men with SBP ≥140 mm Hg and high ISP levels had a relative risk of stroke of 4.3 (95% CI, 2.3 to 7.8) compared with men with SBP <120 mm Hg and low ISP levels. In the absence of high ISP levels, the risk associated with SBP ≥140 was 2.5 (95% CI, 1.4 to 4.6). Men with high ISP levels had a significantly increased risk of stroke also after exclusion of the events from the first 10 years of follow-up.

Conclusions—High ISP levels are associated with elevated BP. These proteins are associated with an increased risk of stroke among men with high BP and provide information on stroke risk even after many years of follow-up. (Stroke. 2002;33:2744-2749.)

Key Words: blood pressure ■ epidemiology ■ inflammation

Hypertension is a major risk factor for stroke. However, many men with hypertension remain free of disease even after many years of follow-up, and the prognosis may differ substantially between individuals with similar blood pressure (BP). Whether this variation in individual susceptibility could be related to a low-grade inflammation has not been studied extensively.

Cross-sectional studies have reported associations between BP and markers of inflammation.2,3 High levels of various inflammation-sensitive plasma proteins (ISPs), eg, fibrinogen, α1-antitrypsin, haptoglobin, ceruloplasmin, orosomucoid, and C-reactive protein, have been associated with increased incidences of myocardial infarction4–9 and stroke.8–12 A recent nested case-control study of myocardial infarction concluded that the risk associated with hypertension is marginal without simultaneous occurrence of high C-reactive protein.13 It has been reported that high fibrinogen levels increase the risk of stroke in men with elevated BP.12 However, few have studied whether plasma markers of inflammation modify the risk of stroke in hypertensive subjects.

Studies of myocardial infarction have reported that the increased risk in subjects with high levels of fibrinogen or C-reactive protein is limited to the first years of follow-up.14–17 Data from cohort studies with long follow-up periods, however, are uncommon. Whether elevated ISP levels are associated with an increased long-term risk of stroke is largely unknown.

In a previous study from the present cohort, we demonstrated that plasma levels of fibrinogen, α1-antitrypsin, haptoglobin, ceruloplasmin, and orosomucoid modify the risk of stroke among men with hypercholesterolemia independently of BP and other potential confounders.9 The purpose of this study was to explore the relationships between ISPs and BP, as well as the joint effects of ISPs and elevated BP on the incidence of stroke. We also sought to study whether the increased risk associated with high ISP levels remains after a long follow-up.

Methods

Between 1974 and 1983, 22 444 men participated in a screening program for detection of individuals at high risk of cardiovascular
diseases. Participation rate was 71%. Determination of the plasma proteins was part of the program for 6193 men (mean age, 46.8 ± 3.7 years; range, 28 to 61 years) selected at random and corresponding to 30% of the cohort. Men with a history of myocardial infarction, stroke, or cancer (according to a questionnaire) were excluded. Of the remaining 6075 men, information on BP was available for 6071. The baseline characteristics of the study cohort have been presented previously.

Baseline Examinations
Subjects were categorized into daily smokers and nonsmokers. Tobacco consumption was categorized into daily consumption of <10, 10 to 19, and ≥20 cigarettes.

BP, taken in subjects in the supine position, was measured twice in the right arm after a 10-minute rest. The average of 2 measurements was used. A sphygmomanometer and an appropriately sized rubber cuff were used.

Blood samples were taken after an overnight fast and analyzed at the Department of Clinical Chemistry at Malmö University Hospital. Plasma cholesterol and triglyceride concentrations were analyzed with standard methods at the laboratory.

Blood glucose was analyzed with a hexokinase method. Men with a fasting whole-blood glucose ≥6.7 mmol/L and men who reported treatment for diabetes were considered to have diabetes.

Body mass index (BMI) was calculated as weight divided by height squared.

Inflammation-Sensitive Plasma Proteins
An electroimmunoassay method was used to assess the plasma levels of 5 proteins. The proteins were used in clinical practice at the hospital to estimate inflammatory activity. The analyses were performed consecutively at the time of study entry. The correlation coefficients between the individual proteins range from 0.31 to 0.56. The proteins are weakly correlated with white blood cell count (all r ≤ 0.04). The relationships between ISP and cardiovascular diseases are nonlinear; ie, the risk increases most between the third and fourth quartiles of ISP. The sample was therefore categorized according to the number of proteins in the top quartile (fibrinogen > 4.0 g/L, orosomucoid [α1-glucoprotein] > 0.93 g/L, α1-antitrypsin > 1.42 g/L, haptoglobin > 1.76 g/L, ceruloplasmin > 0.36 g/L). Cronbach’s α was calculated for this composite score (α = 0.64). The α value indicates that this measure had an adequate reliability in terms of internal consistency and that the individual ISP correlated well with the remaining sum score.

Follow-Up
All cases were followed up from the baseline examination until death or December 31, 1997. Stroke was defined as cases coded 430 (subarachnoid hemorrhage), 431 (intracerebral hemorrhage), 434 (ischemic stroke), or 436 (unspecified stroke) according to the International Classification of Diseases, Ninth Revision (ICD-9). The Stroke Register of Malmö (STROMA), which continuously and actively has searched for and validated patients with stroke since 1989, was used for case retrieval. Cases of stroke that occurred before 1989 were retrieved from the patient register of the university hospital and were validated by review of medical records using the same procedure as STROMA. CT scans were available for 172 (of 204) of the strokes that occurred in the city of Malmö. The National Hospital Discharge Register was used for retrieval of patients (n = 34) who moved out of Malmö. These diagnoses were settled by the physician at the time of hospital discharge. The unspecified and ischemic strokes were analyzed together because the number of unspecified strokes was small and it could be assumed that few were hemorrhagic.

Statistical Analysis
Pearson’s correlations were used to study the relationships between BP and ISP levels. Analysis of covariance was used to compare BP in categories of ISP. A Cox proportional-hazards model was used to analyze the event rates in categories of systolic BP (SBP) and ISP and to adjust for potential confounders. Age, BMI, tobacco consumption, cholesterol, and triglycerides were fitted as continuous variables. Diabetes, smoking, angina pectoris, physical inactivity, and SBP and ISP categories were fitted as categorical variables. Logistic regression was used to analyze case fatality rates.

Results
Relationships Between ISP and BP
BP increased with the number of ISPs in the top quartile. Mean ± SD SBP was 128 ± 15 mm Hg among men with no ISP in the top quartile and 131 ± 17 mm Hg for those with 4
Incidence of Stroke and Death

Two hundred thirty-eight men (3.9%) had a stroke; 23 of them died within 28 days. Nine had a subarachnoid hemorrhage; 29 had an intracerebral hemorrhage; 170 experienced an ischemic stroke; and 30 cases were unspecified. A total of 919 men (15.1%) died during follow-up, 378 (41%) from cardiovascular causes (ICD-9 codes 390 to 459).

The age-adjusted relative risks (RRs) for the individual ISPs are presented in Table 2. Except for α1-antitrypsin, all ISPs were significantly associated with stroke. The risk increased with the number of elevated ISPs.

**Event Rates in Categories of SBP and ISP Levels**

The men were categorized by SBP (<120, 120 to 139, ≥140 mm Hg) and number of ISPs in the top quartile (0 to 1 or 2 to 5). Incidence of stroke was significantly higher among men with SBP ≥140 mm Hg and 2 to 5 ISPs in the top quartile than in all other groups (Table 3). The results were virtually identical after exclusion of men with treatment for hypertension (not shown).

The number of intracerebral hemorrhages (n=29) was too small for an extensive multivariate analysis. After adjustment for age and SBP, the RR for intracerebral hemorrhage was 1.7 (95% CI, 0.82 to 3.6; P=0.15) among men with 2 to 5 ISPs in the top quartile.

Among men with SBP ≥140 mm Hg, mean SBP was ≥2 mm Hg higher among those with high ISP levels (Table 3). Further analysis was performed in which men with SBP ≥140 mm Hg were analyzed separately, and SBP, DBP, and treatment for hypertension were included among the covariates. After adjustment for SBP, DBP, treatment, and other potential confounders, men with SBP ≥140 and 2 to 5 elevated ISPs still had significantly higher incidence of stroke (RR, 1.7; 95% CI, 1.1 to 2.6) and all-cause mortality (RR, 1.3; 95% CI, 1.05 to 1.7) than men with SBP ≥140 mm Hg and 0 to 1 ISP in top quartile.

**Case Fatality in Relation to ISP Levels**

Case fatality (ie, death within 28 days) after stroke was 10% (23 of 238), 8.5% and 11.1% among patients with 0 to 1 and 2 to 5 ISPs, respectively, in the top quartile (P=0.49). The difference remained nonsignificant after adjustment for type

<table>
<thead>
<tr>
<th>TABLE 1. Correlation Coefficients Between ISPs and BP</th>
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<tbody>
<tr>
<td><strong>Nonsmokers</strong> (n=3151)</td>
</tr>
<tr>
<td>SBP</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td>Fibrinogen</td>
</tr>
<tr>
<td>Haptoglobin</td>
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<tr>
<td>Orosomucoid (α1-glucoprotein)</td>
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For all r<0.03, P<0.05.
For all r>0.06, P<0.001.

or 5 ISPs in the top quartile (P<0.0001 for trend; Figure 1). The corresponding values for diastolic BP (DBP) were 86.5±9.6 and 88.5±11 mm Hg, respectively (P<0.0001 for trend). After adjustments for age, BMI, smoking, tobacco consumption, cholesterol, triglycerides, diabetes, angina pectoris, and physical inactivity, mean SE SBP was 128.0±0.31, 129.3±0.37, 129.5±0.50, 129.9±0.62, and 131.0±0.64 mm Hg for men with 0, 1, 2, 3, and 4 or 5 ISPs, respectively, in the top quartile (P<0.0001 for trend). The corresponding values for DBP were 86.5±0.20, 87.2±0.24, 87.6±0.32, 87.3±0.39, and 88.7±0.40 mm Hg, respectively (P<0.0001 for trend).

With the exception of haptoglobin in smokers, all ISPs were significantly correlated with SBP. The associations were similar for DBP (Table 1).

A total of 280 men reported treatment for hypertension. High ISP levels were more prevalent among men with treatment for hypertension; 39.6% had 2 to 5 ISPs in the top quartile in the group with treatment versus 33.7% for the remaining men. This difference became nonsignificant (P=0.44) after adjustment for age, SBP, cholesterol, triglycerides, BMI, diabetes, smoking, tobacco consumption, angina pectoris, and physical inactivity.

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<tr>
<th>TABLE 2. Incidence of Stroke and All-Cause Mortality Among Men With ISP Levels in the Top Quartile</th>
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<tbody>
<tr>
<td><strong>RR (95% CI)</strong></td>
</tr>
<tr>
<td><strong>Stroke</strong></td>
</tr>
<tr>
<td><strong>All-Cause Mortality</strong></td>
</tr>
<tr>
<td>Fibrinogen &gt;4.0 g/L (yes vs no)</td>
</tr>
<tr>
<td>Haptoglobin &gt;1.76 g/L (yes vs no)</td>
</tr>
<tr>
<td>Ceruloplasmin &gt;0.36 g/L (yes vs no)</td>
</tr>
<tr>
<td>α1-Antitrypsin &gt;1.42 g/L (yes vs no)</td>
</tr>
<tr>
<td>Orosomucoid (α1-glucoprotein) &gt;0.93 g/L (yes vs no)</td>
</tr>
<tr>
<td>No protein in top quartile (reference) (n=2448)</td>
</tr>
<tr>
<td>1 Protein in top quartile (vs none) (n=1563)</td>
</tr>
<tr>
<td>2 Proteins in top quartile (vs none) (n=907)</td>
</tr>
<tr>
<td>3 Proteins in top quartile (vs none) (n=589)</td>
</tr>
<tr>
<td>4 or 5 proteins in top quartile (vs none) (n=564)</td>
</tr>
</tbody>
</table>

*Adjusted for age in a Cox proportional-hazards model.
of stroke (ischemic versus hemorrhagic) and age at the event (odds ratio, 1.5; 95% CI, 0.6 to 3.6).

**Event Rates in Relation to Duration of Follow-Up**

Figure 2 presents incidence of stroke in relation to SBP (<140 and ≥140 mm Hg), ISP levels (0 to 1 versus 2 to 5 in the top quartile), and years of follow-up. The difference between men with high and low ISP levels increased continuously over time. A total of 189 strokes occurred >10 years after the baseline examination. After exclusion of events during the first 10 years of follow-up, high ISP levels were still significantly associated with stroke (RR, 1.5; 95% CI, 1.1 to 2.0) after adjustment for age, smoking, tobacco consumption, cholesterol, triglycerides, physical inactivity, diabetes, and angina pectoris. The adjusted RR for men with SBP ≥140 mm Hg and high ISP levels was similar after exclusion of events during the first 10 years (RR, 3.9; 95% CI, 2.0 to 7.7 versus low ISP levels and SBP <120 mm Hg).

**Discussion**

BP was positively correlated with plasma levels of ISP. As expected, incidence of stroke was increased among men with
elevated BP. However, the risk of stroke among men with elevated BP depended on whether ISP levels were elevated. The results from this relatively young cohort also demonstrate that these proteins are associated with an increased risk >10 years after the examination. These proteins may be useful in clinical practice in assessment of the prognosis among individuals with an elevated BP.

The 5 ISPs in the present study are part of the acute and chronic inflammatory response. Among other things, haptoglobin irreversibly binds hemoglobin and thereby saves iron for the organism, and fibrinogen has a role in thrombogenesis and tissue repair. α1-Antitrypsin is an inhibitor of proteolytic enzymes. The physiological role is still uncertain for ceruloplasmin and orosomucoid. Ceruloplasmin seems to be a regulator of the iron metabolism, and orosomucoid might have an anti-inflammatory activity. Because of the collinearity between the proteins and because ISPs show non-linear relationships with incidence of cardiovascular diseases, the top quartile was used as the cutoff, and the number of elevated ISPs was used in the analysis. BP and incidence of stroke and deaths increased with the number of elevated ISPs. The results suggest that a composite measure of inflammation may be preferable to individual ISPs in studies of cardiovascular disease and in assessment of risk.

Few studies on inflammatory markers have investigated how the cardiovascular risk develops over a long follow-up period. Studies of myocardial infarction have suggested that fibrinogen and C-reactive protein “act out” their roles as risk factors during the first 2 to 4 years of follow-up, although results are not fully consistent. Our results clearly show that the increased risk remains even after many years of follow-up. Whether the conflicting results could be explained by differences in inflammatory markers or study populations remains to be evaluated. Misclassification of exposure, ie, poor reliability of the inflammatory markers, is another factor that could reduce the long-term associations with outcome. We used 5 proteins instead of 1 to estimate the degree of inflammation, and it is possible that this improved the precision.

The reason that high ISP levels are associated with stroke is unclear. It has been suggested that inflammation may reduce plaque stability and increase thrombogenesis. Embolism from rupturing atherosclerotic plaques seems to be more frequent in carotid arteries with a certain degree of inflammation. However, in this relatively young cohort, elevated ISP levels were associated with increased stroke rates 10 to 20 years later, and it seems unlikely that inflammation in destabilized plaque caused the increased ISP levels. An alternative explanation is that inflammation is associated with an accelerated progression of atherosclerosis, particularly in men with hypertension. Yet another possibility is that high ISP levels are associated with the development of other risk factors, eg, future hypertension and an increased age-related elevation of BP. A study from this cohort demonstrates that, over a 16-year follow-up, men with high ISP levels developed a higher BP than those with low ISP levels (unpublished data).

There was no significant association between ISP levels and case fatality after the stroke events. Previous studies have reported associations between inflammatory markers and a worse prognosis for patients with coronary heart disease and stroke. There are no previous studies on ISP levels in healthy subjects in relation to the prognosis after a subsequent stroke. Whether the absence of a significant difference is due to a lack of statistical power or whether the association between ISP and case fatality is limited to the protein levels at the time of the stroke event is another question that remains to be elucidated.

Misclassification of exposure is a potential cause of bias. At the baseline examination, BP was assessed twice at the same occasion. BP is characterized by large variations, and several repeated measurements should be performed to reduce misclassification and diagnose hypertension. However, the relationships were significant even though it is likely that misclassification of BP reduced the associations.

STROMA was the main source for case retrieval. Validation studies have shown that this register identifies more cases than routine registers such as local and national patient registers. Individuals who moved out of the city or region were identified in the National Hospital Discharge Register. The proportion of nonhospitalized cases is very small in Sweden, and the proportion in STROMA is similar compared with other Swedish studies. There is no reason to believe that incomplete retrieval of cases biased the results.

Another question is whether the degree of atherosclerosis differed between those with and without elevated ISP levels at baseline. Men with a history of stroke or myocardial infarction were excluded. Angina pectoris, BP treatment, and the major risk factors for atherosclerosis were taken into account in the analysis. It is still possible, however, that men with high ISP levels could have been hypertensive for a longer time before the baseline examination and that, eg, left ventricular hypertrophy or carotid atherosclerosis could be more common among those with high ISP levels.

Although hypertension is strongly associated with stroke, there are great differences between hypertensive men with similar BP levels. It is our conclusion that high ISP levels are associated with elevated BP. These proteins add to the risk of stroke among men with high BP and provide information on stroke risk even after many years of follow-up.

Acknowledgments
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References


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