Reduced Response to Activated Protein C Is Associated with Increased Risk for Cerebrovascular Disease

Johanna G. van der Bom, MD; Michiel L. Bots, MD, PhD; Frits Haverkate, PhD; P. Eline Slagboom, PhD; Piet Meijer, BSc; Paulus T.V.M. de Jong, MD, PhD; Albert Hofman, MD, PhD; Diederick E. Grobbee, MD, PhD; and Cornelis Kluft, PhD

Background: Resistance to activated protein C (APC), which results from various factors, including a mutation in the gene for coagulant factor V, has been associated with increased risk for venous thrombosis. However, its relation to arterial disease is still not well defined.

Objective: To investigate the association of both response to APC and the factor V Leiden mutation with arterial disease.

Design: Population-based case–control study.

Setting: A district of Rotterdam, the Netherlands.

Participants: 115 patients with a history of myocardial infarction; 112 patients with a history of stroke, transient ischemic attack, or both; and 222 age-matched controls without arterial disease chosen from among 7983 persons in the Rotterdam Study cohort. Patients using anticoagulant drugs were excluded.

Measurements: Response to APC was determined in double-centrifuged platelet-poor plasma. Patients were genotyped for the Arg 506 to Gln mutation in the gene for coagulant factor V.

Results: The prevalence of cerebrovascular disease increased gradually and corresponded to a decreasing response to APC (odds ratio per 1-unit decrease of response to APC, 1.43 [95% CI, 1.12 to 1.81], adjusted for age and sex). Adjustment for the factor V mutation did not change the findings. We found no association between response to APC and myocardial infarction or between factor V mutation and cerebrovascular disease or myocardial infarction.

Conclusions: Low response to APC is associated with an increased risk for cerebrovascular disease but not with an increased risk for myocardial infarction, independent of the factor V Leiden mutation. The association between the factor V Leiden mutation and cerebrovascular disease or myocardial infarction remains to be determined.

Resistance to activated protein C (APC) is a recently described coagulation abnormality (1) that is associated with increased risk for venous thromboembolism (2, 3). The balance of procoagulant and anticoagulant factors undoubtedly plays a critical role in determining the risk for coronary (4, 5) and cerebral thromboembolism (6, 7). Therefore, some studies (8, 9) have suggested that resistance to APC may also be associated with increased risk for arterial disease. Other studies (10–13), however, have not found evidence to support this conclusion.

An extremely low response to APC is often caused by the single-base, Arg 506 to Gln mutation of the factor V gene (14, 15). This mutation affects the site of cleavage of activated factor V by APC, rendering it relatively resistant to inactivation; the resistance, in turn, leads to increased thrombotic tendency. Accordingly, a functional test for response to APC is used to screen for the factor V mutation. Patients with values below an arbitrarily chosen point are considered potential carriers of the mutation. However, a low response to APC can be caused by other factors; not all patients with low values are carriers of the factor V mutation (16).

We studied whether response to APC is associated with arterial disease by comparing levels of response to APC and prevalence of the factor V Leiden mutation in patients with and without a history of stroke, transient ischemic attack, or myocardial infarction. We also examined other determinants of the level of response to APC.

Methods

Population

We did a case–control study of participants in the Rotterdam Study, which is a prospective study of 7983 men and women 55 years of age and older. The rationale and design of the Rotterdam Study have been described elsewhere (17). Between March 1990 and July 1993, all men and women 55 years of age and older living in Ommoord, a district of Rotterdam, the Netherlands, were invited to participate (n = 10,275). The overall response rate was 78%. The study was approved by the ethics committee of


From the Erasmus University Medical School, Rotterdam, the Netherlands; TNO Gauvis Institute for Cardiovascular Research, Leiden, the Netherlands; and the Netherlands Ophthalmic Research Institute, Amsterdam, the Netherlands. For current author addresses, see end of text.

©1996 American College of Physicians
Erasmus University, and written informed consent was obtained from all participants.

**Selection**

Patients with a history of myocardial infarction ($n = 115$) were selected if they had an infarction shown by electrocardiography. Selection was made using the diagnostic classification system of the Modular Electrocardiogram Analysis System (MEANS) (18, 19), independent of a history of chest pain. Patients with a definite or probable history of transient ischemic attack ($n = 55$) were selected if they had a positive medical history of transient ischemic attack. Four screening questions were asked about temporary visual, locomotor, sensory, or speech disturbances; when responses were affirmative, a detailed history of symptoms was obtained. Symptoms were classified by a neurologist as indicating that the patient had definitely, had probably, or had not had a transient ischemic attack, using methods described elsewhere (20). Patients with a history of stroke ($n = 62$) were selected by the question, “Did you ever suffer from stroke, diagnosed by a physician?” Five patients had a history of both transient ischemic attack and stroke. Therefore, 112 patients were classified as having cerebrovascular disease, which was defined as a stroke, a transient ischemic attack, or both. Controls ($n = 222$) were selected from among those persons with a normal electrocardiogram, an ankle-to-arm systolic pressure ratio greater than 0.9 (the ankle-to-arm systolic pressure ratio is the ratio of the systolic blood pressure at the posterior tibial artery to the systolic blood pressure at the arm), and no arterial disease (that is, no history of myocardial infarction, stroke, or transient ischemic attack) (21). Controls were matched in 5-year age strata to persons who had had myocardial infarction. Patients using anticoagulant drugs were excluded.

**Measurements**

Information on current health status, medical history, drug use, and smoking was obtained by using a questionnaire. We measured height and weight and calculated body mass index. We measured blood pressure at the right upper arm while patients were seated by using a random-zero sphygmomanometer, and we used the average of two measurements obtained on one occasion. The electrocardiogram was coded using the MEANS computerized coding system (18, 19). The methods we used for blood sampling and storage have been described elsewhere (22). Blood was collected in tubes containing 0.129 mol/L sodium citrate. Platelet-poor plasma was obtained by two-stage centrifugation: Samples were centrifuged at 1600 g and 4°C for 10 minutes; after the plasma midlayer was carefully transferred, a second centrifugation was done at 10,000 g and 4°C for 10 minutes. Plasma was immediately frozen in liquid nitrogen and stored at −80°C for a mean of 2 years. Plasma from 30 healthy volunteers was centrifuged for 30 minutes at 2000 g and 4°C and was pooled to serve as reference plasma for the test of response to APC. The response to APC for the reference plasma was 3.27.

The response of the plasma-activated partial thromboplastin time to APC was determined using the Coatest APC resistance test of Chromogenix (kit 0548-51, Mölndal, Sweden) and is expressed as the ratio of the activated partial thromboplastin time with the addition of APC to the activated partial thromboplastin time without the addition of APC. Serum total and high-density lipoprotein (HDL) cholesterol levels were measured with an automated enzymatic procedure.

Whole blood that was collected and stored at baseline was thawed for DNA extraction. Genotype assay using polymerase chain reaction was done by laboratory personnel who were blinded to case or control status. The Arg 506 to Gln mutation was detected by amplification of a 220-base pair fragment of exon 10–intron 10 of the factor V gene, followed by digestion with the restriction enzyme Mnl I. The primers and conditions that we used have been described elsewhere (14, 23).

**Statistical Analysis**

We calculated means and proportions for potential determinants for five categories of response to APC and adjusted for a history of myocardial infarction or cerebrovascular disease (two dummy variables in the regression model) using linear regression analysis. Logistic regression was used to assess the association of response to APC and the factor V Leiden mutation with cerebrovascular disease and myocardial infarction. Odds ratios with corresponding 95% CIs estimated from the logistic model were used as the measure of association. With myocardial infarction or cerebrovascular disease as the outcome variable, we compared levels of response to APC and genotypes of the factor V mutation adjusted for age and sex. By adding current smoking, total cholesterol level, and activated partial thromboplastin time as covariates in the logistic regression model, we evaluated whether these potentially confounding factors affected the estimates of the odds ratios. In addition, logistic regression was used to explore the association of disease status with response to APC as a continuous variable. Information on factor V mutation was missing for five participants for whom no blood cells were available. In the regression models with factor V as a confounder, the indicator method for missing data was used (24). The results
were similar to analyses done without these participants.

### Results

#### Response to Activated Protein C

The response to APC ranged from 1.5 to 9.5. Mean responses (± SD) were 4.3 ± 1.1 among controls, 3.9 ± 1.0 among patients with a history of cerebrovascular disease, and 4.3 ± 1.1 among patients with a history of myocardial infarction. Mean response to APC was 2.5 ± 0.6 in participants with the factor V mutation and 4.3 ± 1.0 in those without the mutation. The response to APC was higher in men (n = 202; response, 4.5 [CI, 4.4 to 4.7]) than in women (n = 247; response, 3.9 [CI, 4.8 to 4.1]).

Several cardiovascular risk factors were compared across five levels of response for men and women (Table 1). In men, response to APC decreased with increasing age by 0.18 (CI, 0.01 to 0.35) per decade. In women, a trend of 0.08 (CI, −0.06 to 0.23) per decade was seen toward an increase in response to APC with advancing age. Men who smoked had a mean response that was 0.47 (CI, 0.16 to 0.78) higher than that of men who did not smoke. Response to APC of women who smoked did not differ from that of women who did not. Increased cholesterol levels were associated with a decreased response to APC in men but not in women. In men, an increase in cholesterol level of 1 mmol/L was associated with a decrease in response to APC of 0.14 (CI, 0.01 to 0.27).

The odds ratio of cerebrovascular disease (stroke and transient ischemic attack) increased gradually with decreasing response to APC (odds ratio per 1-unit decrease, 1.43 [CI, 1.12 to 1.81]) after adjustment for age and sex. Separate analyses for stroke (odds ratio, 1.32 [CI, 0.99 to 1.77]) and transient ischemic attack (odds ratio, 1.56 [CI, 1.14 to 2.14]) showed no material difference in their relation to decreasing response to APC. The odds ratios for cerebrovascular disease according to varying levels of response to APC are presented in Table 2. Adjustment for presence of the factor V mutant allele did not substantially change the results; the adjusted

---

Table 1. Cardiovascular Risk Factors in Response to Activated Protein C

<table>
<thead>
<tr>
<th>Variable</th>
<th>Response to Activated Protein C†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;3.5 (n = 108)</td>
</tr>
<tr>
<td>Age, y</td>
<td>73</td>
</tr>
<tr>
<td>Women, %</td>
<td>69</td>
</tr>
<tr>
<td>Body mass index, kg/m²§§</td>
<td>26</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg§§</td>
<td>140</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg§§</td>
<td>73</td>
</tr>
<tr>
<td>Total cholesterol level, mmol/L§§</td>
<td>6.6</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol level, mmol/L§§</td>
<td>1.3</td>
</tr>
<tr>
<td>Current smoking, %§§</td>
<td>20</td>
</tr>
<tr>
<td>Activated partial thromboplastin time, s §§</td>
<td>30</td>
</tr>
</tbody>
</table>

* Values are means or proportions adjusted for case–control status.
† The response of the plasma-activated partial thromboplastin time to APC is expressed as the ratio of activated partial thromboplastin time with the addition of APC to activated partial thromboplastin time without the addition of APC.
‡ Obtained by linear regression with response to activated protein C as a continuous variable.
§§ Adjusted for age and sex.

---

Table 2. Prevalence of Cerebrovascular Disease and Myocardial Infarction by Levels of Response to Activated Protein C

<table>
<thead>
<tr>
<th>Condition</th>
<th>Response to Activated Protein C†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;3.5 (n = 108)</td>
</tr>
<tr>
<td>Controls</td>
<td>48</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>32</td>
</tr>
<tr>
<td>Odds ratio of cerebrovascular disease (95% CI)</td>
<td>2.27 (1.09 to 4.70)</td>
</tr>
<tr>
<td>Adjusted for age and sex (CI)</td>
<td>2.43 (1.13 to 5.22)</td>
</tr>
<tr>
<td>Adjusted for other confounders (CI)§§</td>
<td>2.57 (1.17 to 5.65)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>24</td>
</tr>
<tr>
<td>Odds ratio of myocardial infarction (CI)</td>
<td>1.20 (0.56 to 2.46)</td>
</tr>
<tr>
<td>Adjusted for age and sex (CI)</td>
<td>1.53 (0.72 to 3.24)</td>
</tr>
</tbody>
</table>

* Cerebrovascular disease = stroke and transient ischemic attack.
† The response of the plasma-activated partial thromboplastin time to APC is expressed as the ratio of activated partial thromboplastin time with the addition of APC to activated partial thromboplastin time without the addition of APC.
‡ Reference risk.
§§ Adjusted for age, sex, current smoking, and total serum cholesterol level.

15 August 1996 • Annals of Internal Medicine • Volume 125 • Number 4 267
odds ratio of cerebrovascular disease for each 1-unit decrease in response to APC was 1.43 (CI, 1.12 to 1.81).

Response to APC was not associated with myocardial infarction; the odds ratio for response to APC as a continuous variable was 1.10 (CI, 0.89 to 1.37) (Table 2).

**Factor V Mutation**

Heterozygosity for the factor V mutation was present in 5% of controls (11 of 222), 6% of patients with cerebrovascular disease (6 of 107), and 4% of patients with myocardial infarction (4 of 114).

The factor V mutation was not associated with myocardial infarction (odds ratio, 0.77 [CI, 0.24 to 2.55]) or cerebrovascular disease (odds ratio, 1.12 [CI, 0.40 to 3.15]). Adjustment for age and sex did not change these findings.

**Discussion**

We studied factor V mutation and response to APC in persons with and without a history of cardiovascular disease—particularly stroke, transient ischemic attack, and myocardial infarction. A decreased response to APC was associated with an increased risk for nonfatal stroke and transient ischemic attack, independent of the factor V mutation. The response to APC was not associated with myocardial infarction. Moreover, these initial findings did not show an increased risk for myocardial infarction or cerebrovascular disease in participants with the factor V mutation.

We based our study on a large population-based cohort of white men and women 55 years of age and older. Response rates were high, controls were randomly sampled, and laboratory analyses were done by blinded investigators. However, some limitations need to be discussed. First, because of its cross-sectional design, our study was restricted to nonfatal cases, and the response to APC was measured after the events (myocardial infarction, stroke, or transient ischemic attack). Second, stroke was defined on the basis of a questionnaire; the possible misclassification of cases of stroke is probably random, which may lead to an underestimation of the true association. Third, no information was available on the nature of the strokes (hemorrhage or infarction). In the Netherlands, hemorrhagic strokes constitute about 15% of total strokes (25). Therefore, assuming that a decreased response to APC is associated with an increased risk for ischemic stroke, our empiric risk estimate is again more likely to be an underestimate than an overestimate of the true association.

Levels of response to APC in our study are higher than those reported by others. This disparity is probably a result of our methods of processing blood. We measured response to APC in double-centrifuged, platelet-poor plasma. Absence of platelets in plasma has been associated with an increased response to APC (26).

A unique feature of our study is that, instead of dichotomizing response to APC, we chose to analyze it as a continuous variable. When we analyzed it in this manner, it became clear that decreased response was associated with an increased risk for cerebrovascular disease, independent of the factor V mutation. This suggests that response to APC may serve as a continuous measure of risk for cerebrovascular disease.

Cerebrovascular and coronary disease differ from one another with regard to several other risk factors. For example, in industrialized western countries, elevated cholesterol levels are generally more strongly associated with coronary disease than with cerebrovascular disease (27). Similarly, our results suggest that decreased response to APC is associated with increased risk for cerebrovascular disease but not for myocardial infarction. Cerebral arteries seem to be more sensitive than coronary arteries to an imbalance of the protein C–protein S system, perhaps because the protein C–protein S system exerts different pathophysiological effects in brain tissue. Thrombomodulin, an endothelial co-factor protein for thrombin-mediated protein C activation, is present in all human tissues except brain tissue (28).

In the Physicians’ Health Study (10), a large, longitudinal study of healthy men, risks for myocardial infarction and stroke in persons with the factor V mutation were not increased. Our findings agree with those of the Physicians’ Health Study. Although the number of participants in our study who were heterozygous for the mutation was small, our data lend some support to the hypothesis that risks for myocardial infarction and stroke may not be increased in men or women with the factor V mutation.

To date, non-genetic determinants of APC response have not been studied. Our finding that response to APC is associated with other factors, such as age, sex, smoking, and serum total cholesterol levels, may have important implications for use of the response to APC test as a screening test for the factor V mutation. Furthermore, these risk factors should be considered, particularly when they are distributed differently among cases and controls, in studies on response to APC and disease.

In conclusion, a decreased response to APC is associated with an increased risk for cerebral thrombembolism. This association is independent of the factor V Leiden mutation. Response to APC may serve as a risk indicator for cerebrovascular disease. The association of response to APC with age, sex,
smoking, and serum total cholesterol levels should be taken into account in both clinical and in research settings.

Acknowledgments: The authors thank the participants in the Rotterdam Study, P.J. Koudstaal, MD, PhD (University Hospital, Rotterdam), for his contribution in establishing the diagnosis of transient ischemic attacks; and all field workers and laboratory technicians in the Ommoord Research Centre and Bas Heijmans from the Gaubius Laboratory for their enthusiasm and skillful contributions to data collection.

Grant Support: The Rotterdam Study is supported in part by the NESTOR program for geriatric research (Ministry of Health and Ministry of Education), the Netherlands Heart Foundation, the Netherlands Organisation for Scientific Research, the Rotterdam Medical Research Foundation, and the Municipality of Rotterdam. Dr. van der Bom is supported by grant 92.398 from the Netherlands Heart Foundation.

Requests for Reprints: Diederick E. Grobbee, MD, PhD, Department of Epidemiology and Biostatistics, Erasmus University Medical School, PO Box 1738, 3000 DR Rotterdam, the Netherlands.

Current Author Addresses: Drs. van der Bom, Bots, Hofman, and Grobbee: Department of Epidemiology and Biostatistics, Erasmus University Medical School, PO Box 1738, 3000 DR Rotterdam, the Netherlands. Drs. Haverkate, Slagboom, and Kluit and Mr. Meijer: Gaubius Laboratory, TNO Prevention and Health, PO Box 430, 2300 AK Leiden, the Netherlands. Dr. de Jong: Netherlands Ophthalmic Research Institute, PO Box 12141, 1100 AC Amsterdam, the Netherlands.

References