C-Reactive Protein and the Future Risk of Thromboembolic Stroke in Healthy Men

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Background—Evidence suggests that C-reactive protein (CRP) is related to thromboembolic (TE) stroke. Whether associations are altered in the presence of other risk factors is unclear. The purpose of this study was to additionally assess the relation between CRP and TE stroke.

Methods and Results—On the basis of 20 years of follow-up after CRP measurement, 259 cases of TE stroke were identified and compared with 1348 controls. Subjects were aged 48 to 70 years when CRP was measured. Levels of CRP were positively associated with TE stroke throughout the 20 years of follow-up. Although associations were modest within 5 years of CRP measurement, the odds of stroke in the top versus bottom CRP quartile increased over time to a 3.8-fold excess by 10 to 15 years into follow-up (P<0.001). For men without hypertension or diabetes, the overall corresponding odds were 1.6 to 1.7 (P<0.05). In men ≤55 years of age, the odds increased to a 3-fold excess (P=0.006), and in nonsmokers, there was a 5.8-fold excess (P<0.001). Associations in past and current smokers, in men >55 years of age, and in those with hypertension or diabetes were not significant.

Conclusions—Findings suggest that elevated CRP in middle adulthood and in men with healthier risk factor profiles may be important as a risk factor for TE stroke. Use of CRP levels as a clinical screen to identify an increased risk of cardiovascular disease in otherwise healthy men warrants consideration. (Circulation. 2003;107:2016-2020.)

Key Words: stroke ■ inflammation ■ epidemiology

C-reactive protein (CRP) is an acute-phase protein thought to be a measure of inflammatory processes in cardiovascular disease.1-12 Although evidence suggests that CRP is associated with stroke,7-10 it is uncertain whether the association has similar meaning in different age groups and in those at high and low risk of cardiovascular disease. The purpose of this study was to examine the relation between levels of CRP and the development of thromboembolic (TE) stroke over a 20-year period in largely middle-aged and healthy Japanese-American men in the Honolulu Heart Program. The association of CRP levels to early versus late disease will also be assessed, as will its relation with disease that may be poorly explained by age, hypertension, diabetes, and other cardiovascular risk factors. Effects will also be assessed in the absence of intervening coronary heart disease.

Methods

Baseline Examination
From 1965 to 1968, the Honolulu Heart Program began following 8006 men of Japanese ancestry through a series of physical examinations and comprehensive follow-up for cardiovascular disease.13-15 At the time of study enrollment, subjects were aged 45 to 68 years. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

Because the inventory of frozen sera from the time of study enrollment was largely depleted, measurement of CRP for this report is based on samples that were available at examinations given from 1967 to 1970. Other risk factors included diabetes, hypertension, total cholesterol, body mass index, and the use of cigarettes. Data on alcohol intake and physical activity were taken from examinations that were given at the time of study enrollment (1965 to 1968). Atrial fibrillation was not included among the risk factors, because it was rarely observed in this age group of Japanese-American men. Statistical adjustment for atrial fibrillation also failed to alter the findings in the present report.

Among the collected risk factors, diabetes was diagnosed on the basis of a medical history or the use of insulin or oral hypoglycemic therapy. Hypertension was defined as systolic or diastolic blood pressures ≥160 and 95 mm Hg, respectively, or when high blood pressure was being medically treated. Measurement of physical activity was based on the physical activity index, a commonly used index for quantifying overall metabolic output during a typical 24-hour period.16,17

TE Stroke Cases and Controls
Assessment of the effects of CRP on the risk of future stroke is based on a nested case-control design. There were 7498 men (≈95% of the...
surviving cohort) who were available for follow-up at the time of CRP measurement. Among this group, cases of TE stroke and controls were selected from 5686 subjects in whom serum samples were available. Sera from 1812 participants were not obtained because of depletion from random selection for special sub studies or, in a few instances, for use in small studies of rare diseases. After excluding cases of stroke and coronary heart disease that were identified in 20 years of follow-up, 1348 controls were randomly selected from the remaining 4145 men.

During the 20-year course of follow-up, information on cardiovascular events was obtained through a comprehensive system of surveillance of hospital discharges, death certificates, autopsy records, and repeat examinations. For this report, subjects were followed up for the first occurrence of a TE stroke (without distinction between an atherothrombotic infarction and embolic event), on the basis of medical records showing a neurological deficit of sudden or rapid onset that persisted for longer than 24 hours or resulted in death. A review of all suspected stroke outcomes by the Honolulu Heart Program Morbidity and Mortality Review Committee confirmed all diagnoses. There were 259 cases of stroke that were identified in the course of follow-up. All were selected without a history of coronary heart disease.

**CRP Determination**

Serum specimens collected at the time of CRP measurement were stored at −20°C until 1980, at which time they were transferred to −70°C. Although specimens in the present study had previously been thawed and refrozen, measurement of CRP is known to be relatively stable under such conditions.18 For each participant, determination of a CRP level was based on an enzyme-linked immunosorbent assay, a colorimetric competitive immunoassay that uses purified protein and polyclonal anti-CRP antibodies. The interassay coefficient of variation was ≈5%.

**Statistical Analysis**

Estimated age-adjusted risk factor comparisons between the cases and controls were derived from analysis of covariance methods after log-transforming the CRP values.19,20 Similar comparisons were made across CRP quartiles. Estimated age-adjusted percent of men with a TE stroke were also derived across CRP quartiles and within separate 5-year periods of follow-up after the baseline examination.19,20 Such percents were not intended to represent the incidence of TE stroke but rather to show how the cases of TE stroke were distributed across the observed CRP quartiles. Beyond the first 5 years of follow-up, cases of TE stroke and deaths among the controls that preceded a follow-up period were deleted from the corresponding calculations. Cases of TE stroke that came after a 5-year follow-up period were treated as controls.

To assess the relation between levels of CRP and the odds of a TE stroke, statistical analysis relied on proportional hazards regression models.21 Use of the proportional hazards regression model, which allows for the modeling of the time to an event among the cases of TE stroke and the time to death among the controls who failed to survive the 20-year period of follow-up. To provide a test for trend, CRP was also modeled as a continuous variable. All reported \( P \) values were based on 2-sided tests of significance.

**Results**

At the time of CRP measurement, the average ages of the cases and controls were 58.1 years (range, 49 to 69) and 55.8 years (range, 48 to 70), respectively (\( P < 0.001 \)). Among the cases, the average age when a TE stroke occurred was 69 years (range, 51 to 87), and the average time to an event was 10.5 years (range, 2 months to 20 years).

Baseline characteristics of the study participants according to case-control status are described in Table 1. As expected, at the time of CRP measurement, risk factor profiles were significantly less favorable in cases than in controls. Frequency of hypertension and diabetes were more than doubled in the TE stroke cases (\( P < 0.001 \)). Cases had higher levels of total cholesterol (\( P < 0.01 \)) and body mass index (\( P < 0.001 \)) as well. Cases were more likely to be current cigarette smokers (\( P < 0.001 \)) and less likely to have stopped smoking than controls (\( P < 0.05 \)). Cases also consumed more alcohol and were less physically active than controls, although differences were not statistically significant.

Risk factor differences across quartiles of CRP are additionally described in Table 2. Here, frequency of hypertension was more than doubled in the top versus bottom quartile of CRP (19.4 versus 8.4%, \( P < 0.001 \)). Although not statistically significant, the percent of men with diabetes also seemed positively related to CRP concentrations. Differences in total cholesterol across quartile strata were modest. Smoking, however, increased with rising CRP levels (\( P < 0.001 \)), with a jump in the use of cigarettes between the third and top CRP quartiles. Although less clear, the percent of men who were past smokers tended to decline with increasing CRP levels (\( P < 0.05 \)). Although body mass index increased consistently with increasing CRP levels, amounts of physical activity declined (\( P < 0.001 \)). There was no clear association between the intake of alcohol and levels of CRP.

Table 3 provides the age-adjusted percent of men with a TE stroke within each quartile of CRP and within separate 5-year periods of follow-up after CRP measurement. Although associations were modest within 5 years of CRP measurement, the relationship increased over time up to 10 to 15 years into

### Table 1. Distribution of CRP, Mean Age, and Age-Adjusted Mean Risk Factor Levels for Controls and Cases of Thromboembolic Stroke

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Controls (n=1348)*</th>
<th>Cases (n=259)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean†</td>
<td>0.60</td>
<td>0.78‡</td>
</tr>
<tr>
<td>Arithmetic mean†</td>
<td>1.16</td>
<td>1.43</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.32</td>
<td>0.44</td>
</tr>
<tr>
<td>Median</td>
<td>0.54</td>
<td>0.73</td>
</tr>
<tr>
<td>75th percentile</td>
<td>1.00</td>
<td>1.39</td>
</tr>
<tr>
<td>Range</td>
<td>0.1 to 79.20</td>
<td>0.1 to 34.19</td>
</tr>
<tr>
<td>Age, y</td>
<td>55.8±5.4§</td>
<td>58.1±5.7‡</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>10.6</td>
<td>27.0†</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>13.3</td>
<td>27.0‡</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.6±0.9 (215±35)</td>
<td>5.8±1.0 (222±40)¶</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.5±3.0</td>
<td>24.3±3.3§</td>
</tr>
<tr>
<td>Past cigarette smoker, %</td>
<td>27.8</td>
<td>21.3#</td>
</tr>
<tr>
<td>Current cigarette smoker, %</td>
<td>37.6</td>
<td>54.4‡</td>
</tr>
<tr>
<td>Alcohol intake, oz/mo</td>
<td>13.2±22.5</td>
<td>15.2±26.6</td>
</tr>
<tr>
<td>Physical activity index</td>
<td>33.0±4.5</td>
<td>32.7±4.6</td>
</tr>
</tbody>
</table>

*Sample size.
†Age-adjusted.
‡Significantly different from controls (\( P < 0.001 \)).
§Mean±SD.
¶Corresponding units in mg/dL.
#Significantly different from controls (\( P < 0.01 \)).
§Significantly different from controls (\( P < 0.05 \)).
During this time, 8.3% of the men in the top CRP quartile had a stroke compared with 2.3% in the bottom quartile ($P<0.001$). Although changes in the relation between CRP and the risk of TE stroke seemed to occur with time, differences were not statistically significant.

The estimated relative odds of a TE stroke for men in the top versus bottom quartile of CRP, after age and risk factor adjustment, is additionally described for each 5-year period of follow-up in Table 4. Relative odds of a TE stroke are also provided within risk factor strata across the entire 20-year period.

After age and risk factor adjustment, the estimated relative odds of a TE stroke in men in the top versus bottom quartile of CRP were no longer statistically significant within the 5- to 10- and 15- to 20-year periods of follow-up. For the 10- to 15-year period, however, the odds of a TE stroke rose significantly with increasing CRP level when CRP was modeled as a continuous risk factor ($P=0.010$). There was also a 2.6-fold excess in the odds of a TE stroke in the top versus bottom quartile of CRP during this period of follow-up ($P<0.05$).

Although sample sizes were reduced within risk factor strata, the estimated odds of a TE stroke continued to increase significantly with rising CRP levels for middle-aged men (≤55 years, $P=0.018$), in men without hypertension or diabetes ($P=0.019$ and $P=0.006$, respectively), and in those who were never smokers of cigarettes ($P<0.001$). For men without hypertension or diabetes, there was a 1.6- to 1.7-fold excess ($P<0.05$) in the odds of a TE stroke in the top versus bottom CRP quartile. In middle-aged men, the corresponding odds increased to a 3-fold excess ($P=0.006$), and in nonsmokers, there was a 5.8-fold excess ($P<0.001$). Effects were also independent of total cholesterol, body mass index, alcohol intake, physical activity index, and the other risk factors in Table 4. Although associations were weaker in men with hypertension or diabetes, differences in associations from those when either condition was absent were not statistically significant. In contrast, the effect of CRP on the risk of TE stroke was significantly stronger in never smokers compared with past smokers ($P=0.005$) and current smokers ($P=0.010$).
Discussion

Although the longitudinal data in this report provide additional evidence for an association between CRP and the incidence of TE stroke, specific strengths of the present study are worth noting. To date, the Honolulu Heart Program offers the longest and most complete follow-up of the association between CRP and stroke. Follow-up also includes a well-defined acute event without intervening coronary heart disease. Although others have also reported on an association between CRP and cardiovascular disease, documentation of an association with stroke has been equivocal. Earlier reports have combined stroke with other forms of cardiovascular disease as a single event. Recent findings from the Framingham Study are also based on the combination of stroke with poorly defined transient ischemic attacks as a pooled event in an elderly sample that comprised half of surviving cohort members. Although the Physician’s Health Study also showed an increase in the risk of ischemic stroke with increasing levels of CRP, possible differences in the importance of the association between age groups, smoking strata, and groups at high and low risk of cardiovascular disease were not addressed. Although an association between CRP and the risk of stroke was observed in the Third National Health and Nutrition Examination Survey, stroke cases were self-reported, and the timing of events was not recorded. Levels of CRP were also undetectable in 71% of the study participants.

Findings of a stronger relation between CRP levels and the risk of stroke in low- versus high-risk groups in the Honolulu Heart Program are also consistent with other reports. As with stroke, similar effects of CRP on myocardial infarction were also observed in the Honolulu Heart Program. Here, adverse effects were stronger in middle-aged men, in men without hypertension or diabetes, and in those who were nonsmokers. Although small sample sizes could have weakened the capacity to detect effects in higher-risk individuals, others have described strong relations between CRP and cardiovascular disease in nonsmoking women and in women without hypertension or diabetes as well. Findings from the Framingham Study also describe stronger associations in women than in men for the combined events of ischemic stroke and transient ischemic attack.

The weaker association in older or less healthy men could have several explanations. Although inflammation may still be important, in the presence of other risk factors, a high risk of stroke could mask any residual (and perhaps weaker) effects of inflammation. Even in the absence of important cardiovascular conditions, stroke can still occur. In such cases, effects of isolated processes, such as inflammation, may be more apparent in disease progression.

Regardless of susceptibility, mechanistic derangements by which CRP is associated with the long-term incidence of atherosclerotic disease are not clear. Inflammatory mediators and products have been associated with cellular proliferation, lipid accumulation, and thrombosis. Markers of low-level inflammation, such as CRP, may reflect activity in any of these systems. Levels of CRP may also be directly related to processes of pathophysiology through complement activation.

| TABLE 4. Estimated Age-Adjusted and Risk Factor–Adjusted Relative Odds of Thromboembolic Stroke for Men in the Top vs Bottom Quartiles of CRP |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|----------------|
| Risk Strata                     | Relative Odds   | Test for Trend (P) | Relative Odds   | Test for Trend (P) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|----------------|
| Follow-up period, y             | Age-Adjusted    | Risk Factor–Adjusted* |
| 0 to 5                          | 1.1 (0.5 to 2.2) | 0.573           | 0.8 (0.4 to 1.7) | 0.861           |
| 5 to 10                         | 2.5 (1.1 to 5.9) | 0.021           | 1.9 (0.8 to 4.6) | 0.124           |
| 10 to 15                        | 3.8 (1.7 to 8.3) | <0.001          | 2.6 (1.1 to 5.8) | 0.010           |
| 15 to 20                        | 2.5 (1.2 to 5.1) | 0.010           | 1.8 (0.9 to 3.8) | 0.144           |
| Overall                         | 2.3 (1.6 to 3.3) | <0.001          | 1.6 (1.1 to 2.4) | 0.008           |
| Age, y                          | 48 to 55        | 3.8 (1.8 to 8.0) | <0.001          | 3.0 (1.4 to 6.4) | 0.018 |
| 56 to 70                        | 1.8 (1.2 to 2.9) | <0.001          | 1.3 (0.8 to 2.0) | 0.076 |
| Hypertension                    | Absent          | 2.1 (1.4 to 3.3) | <0.001          | 1.6 (1.0 to 2.6) | 0.019 |
|                                | Present         | 1.3 (0.6 to 2.7) | 0.128           | 1.3 (0.6 to 2.7) | 0.221 |
| Diabetes                        | Absent          | 2.5 (1.6 to 3.9) | <0.001          | 1.7 (1.0 to 2.7) | 0.006 |
|                                | Present         | 1.5 (0.8 to 3.0) | 0.258           | 1.4 (0.7 to 2.8) | 0.580 |
| Smoking status                  | Never           | 6.1 (2.5 to 14.8)| <0.001          | 5.8 (2.3 to 14.4)| <0.001 |
|                                | Past            | 1.2 (0.5 to 2.6) | 0.508           | 0.8 (0.3 to 1.8) | 0.520 |
|                                | Current         | 1.6 (1.0 to 2.6) | 0.052           | 1.3 (0.8 to 2.2) | 0.152 |

*Adjusted for total cholesterol, body mass index, alcohol intake, physical activity index, and the other risk factors in this table.
†95% confidence interval.
and tissue factor expression. Although reports are conflicting, it is possible that CRP reflects reactions to infectious agents that have been associated with atherosclerotic diseases. Observations in the Honolulu Heart Program that show a greater frequency of smoking cessation in men in the bottom quartile of CRP (where the risk of stroke is low) versus the top quartile additionally suggest that inflammation may be reversible. Smoking cessation in individuals with an elevated CRP may also be an effective strategy for improving CRP levels and reducing the adverse effects of inflammation.

Among the strengths of the present study, there are also limitations. For example, the Honolulu Heart Program is entirely composed of men of Japanese ancestry. Extensions to other groups could be important. Findings in Framingham and the Women’s Health Study suggest that associations in men are likely to apply to women. In general, the relative risk of stroke is similar to the relative risk observed in other population-based samples. Levels of CRP also fall within the range of values reported elsewhere. In some instances, average values are higher, whereas in others they are lower. Overall increases in the risk of stroke and transient ischemic attacks in Framingham men and in men in the Physician’s Health Study in the top versus bottom quartiles of CRP are also similar to those found in Hawaii.

Although elevated levels of CRP seem to be related to an increased risk of TE stroke in middle-aged men and in those free of important cardiovascular risk factors, it remains to be determined if the measurement of CRP in cigarette smokers or in those with hypertension or diabetes can provide additional prognostic information. Whether CRP levels can be used as a clinical screen to identify an increased risk of cardiovascular disease in otherwise healthy adults also warrants additional consideration.

Acknowledgments

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References

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