C-Reactive Protein Is a Strong but Nonspecific Risk Factor of Fatal Stroke in Elderly Persons

Jacobijn Gussekloo, Marianne C.L. Schaap, Marijke Frölich, Gerard J. Blauw, Rudi G.J. Westendorp

Abstract—An elevated level of C-reactive protein is a strong predictor of cardiovascular events in elderly persons. Whether C-reactive protein has direct adverse vascular effects or is a marker of aspecific systemic inflammation remains to be determined. The aim of this study was to investigate the relation between C-reactive protein and the occurrence of fatal strokes in elderly persons. In the Leiden 85-Plus Study, a population-based prospective follow-up study, we studied the levels of C-reactive protein in 80 participants who died from stroke within the first 5 years of follow-up. Levels of C-reactive protein were determined in serum samples at baseline. Levels of C-reactive protein were also determined in 82 control subjects who survived for the first 5 years of follow-up and in 83 participants who died from noncardiovascular causes. Mortality risks were estimated with logistic regression and adjusted for differences in age, sex, smoking, medication, total cholesterol, history of diabetes or hypertension, and previous cardiovascular events. Levels of C-reactive protein at baseline were 2-fold higher in subjects who died from stroke than in control subjects (median 5.7 versus 2.7 mg/L, $P<0.005$). The levels of C-reactive protein in subjects who died from stroke or from noncardiovascular causes were similar (median 5.7 versus 4.9 mg/L, $P=0.7$). The risk of death from stroke as well as from noncardiovascular causes increased linearly up to 10-fold in subjects with the highest levels of C-reactive protein at baseline ($P<0.001$). The levels of C-reactive protein were lower when more time had elapsed between blood sampling and time of death during follow-up ($P=0.01$). C-reactive protein is a strong but nonspecific risk factor of fatal stroke in old persons. The data do not support the idea that C-reactive protein has direct vascular effects that underlie fatal cerebrovascular disease. (Arterioscler Thromb Vasc Biol. 2000;20:1047-1051.)

Key Words: C-reactive protein ▪ cardiovascular diseases ▪ strokes ▪ survival

Elevated levels of C-reactive protein are related to higher risk of first-ever myocardial infarction, stroke, and peripheral vascular disease. This relation holds not only for middle-aged men but also for women and elderly persons. Inflammation may contribute to the progression of cardiovascular disease because inflammatory cells may account for the local weakening of atherosclerotic plaques, leading to rupture and thrombus formation. Moreover, C-reactive protein induces monocytes to express tissue factor, the initiator of the extrinsic pathway of coagulation, further stimulating vascular thrombosis. The critical question is whether C-reactive protein contributes to the vascular damage and coagulation that lead to cardiovascular events. The alternative would be that C-reactive protein is a marker of underlying, nonspecific, systemic inflammation. C-reactive protein is increased in response to various subclinical atherosclerotic diseases or in response to risk factors of cardiovascular disease. The aim of the present study was to investigate the strength and the specificity of C-reactive protein in the prediction of cerebrovascular events in the oldest of the old. Within the Leiden 85-Plus Study, a population-based prospective follow-up study, we analyzed C-reactive protein in participants who died from stroke or from noncardiovascular causes and in those who survived during the 5 years of follow-up. We have chosen to study fatal stroke instead of other cardiovascular causes of death because fatal strokes are accurately recorded on death certificates.

Methods

Patients and Control Subjects

The Leiden 85-Plus Study is a population-based prospective follow-up study of somatic and psychiatric morbidity in the oldest of...
the old. The design of the study is reported in detail elsewhere.\textsuperscript{15} In short, from 1986 to 1988, a total number of 976 inhabitants of the city of Leiden, the Netherlands, aged \( \geq 85\) years were visited at home by a physician (response \( \geq 90\%\)). No exclusions were made on the basis of health or functioning. The baseline visit consisted of an extensive medical history\textsuperscript{15} and a routine blood analysis (\( n=755\)). Smoking habits were recorded by self-report during the home visit. Participants were classified as current smokers, including former smokers who had stopped \(<10\) years ago, or nonsmokers. The use of NSAIDs, including salicylates, ibuprofen, naproxen, and diclofenac, was also recorded by self-report during the home visit. The diagnosis of diabetes was based on patient history, use of antidiabetic medication, or glucose levels in the blood. The Medical Ethics Committee of Leiden University approved the study, and informed consent was obtained from all participants.

All participants of the baseline study were followed up for mortality over a 10-year period. We assessed the primary and secondary causes of death by linking the death certificate numbers, obtained from the civic registries, to the causes of death recorded by a physician of the Dutch Central Bureau of Statistics. Causes of death were classified according to the ninth version of the International Classification of Diseases (ICD-9). We categorized ICD-9 codes 390 to 459 as cardiovascular disease, codes 430 to 438 as stroke, and codes 140 to 239 as malignancies.\textsuperscript{15} Both the causes of death and follow-up were complete for all but 2 participants.

During the first 5 years of follow-up, 723 participants (74\%) died. More than one third of these subjects (\( n=270\)) died from cardiovascular causes, including 93 subjects (13\%) who died from stroke. For 80 of these 93 subjects, sufficient serum was available for the determination of C-reactive protein in 1998. As a first control group, we randomly selected 82 subjects of the 251 subjects who were still alive 5 years after the baseline visit. As a second control group, we matched the subjects who died from stroke with 83 subjects who died after a similar period of follow-up from noncardiovascular causes (infectious diseases, \( n=11\); malignancies, \( n=29\); and other noncardiovascular causes of death, \( n=43\)).

**Biochemical Measurements**

Blood samples were taken during home visits at baseline (1986 to 1998). Serum samples were kept in storage at a temperature of \(-20^\circ\text{C}\) until 1998. C-reactive protein was measured by use of a sandwich enzyme immunoassay (Kordia) that was based on 2 polyclonal rabbit antibodies against C-reactive protein. The between-assay coefficient of variation was 5.3\% at 0.82 mg/L and 5.1\% at 8.9 mg/L. The sensitivity of the assay was 1.1 \( \mu \text{g/L}\) in our laboratory. All samples were assayed in 1 batch. Normal values are \(<20\) mg/L.

**Statistical Analysis**

Because the distribution of C-reactive protein levels is skewed, the serum levels of C-reactive protein are presented as medians and interquartile ranges, representing the 25th and 75th percentile of the distribution. Comparisons between groups were performed with nonparametric tests that do not assume any underlying distribution of the data. Categorical data were compared by \( \chi^2\) test. Statistical significance was set at \( P\leq0.05\).

The subjects were divided in 4 strata according to the C-reactive protein levels at baseline: \(<5\) mg/L, 5 to 10 mg/L, 11 to 20 mg/L, and \(>20\) mg/L. We estimated the odds ratios (ORs) and the 95\% CIs of developing fatal stroke for various strata of C-reactive protein relative to the stratum with the lowest levels. Logistic regression was used to adjust for unequal distributions of age, sex, current smoking, use of NSAIDs, total cholesterol level, history of hypertension or diabetes, and previous cardiovascular events between the groups. We tested for linear trends to assess the relation between the risk of cause-specific mortality and strata of C-reactive protein by use of the Kruskal-Wallis test.

**Results**

The baseline characteristics of the subjects who died from stroke, those who died from noncardiovascular causes, and those who survived during the first 5 years of follow-up are presented in Table 1. For this elderly group, the proportion of women was high, as was the use of NSAIDs; there was also a high representation of subjects with a history of hypertension. The prevalence of diabetes was lowest in those who survived (\( P=0.05\)). Consistent with our previous findings, the serum levels of cholesterol were highest in those who survived compared with those who died from strokes or noncardiovascular causes (\( P<0.01\)).\textsuperscript{15} The proportion of current smokers and the number of subjects with a previous cardiovascular event were low.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Death From Stroke (( N=80))</th>
<th>Noncardiovascular Deaths (( N=82))</th>
<th>Control Subjects (( N=83))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>89.7 (85.6–97.7)</td>
<td>90.4 (85.9–99.8)</td>
<td>88.8 (86.1–97.9)</td>
</tr>
<tr>
<td>Females, n</td>
<td>59 (74%)</td>
<td>65 (79%)</td>
<td>61 (73%)</td>
</tr>
<tr>
<td>Current use of NSAIDs, n</td>
<td>10 (12%)</td>
<td>11 (13%)</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>14 (18%)</td>
<td>13 (16%)</td>
<td>13 (16%)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.4 (3.1–8.6)*</td>
<td>5.1 (3.2–9.5)*</td>
<td>5.9 (3.3–9.8)*</td>
</tr>
<tr>
<td>History of diabetes, † n</td>
<td>14 (18%)</td>
<td>12 (16%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>History of hypertension, n</td>
<td>19 (25%)</td>
<td>18 (24%)</td>
<td>21 (26%)</td>
</tr>
<tr>
<td>Previous cardiovascular event, n</td>
<td>7 (8%)</td>
<td>8 (10%)</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median CRP, mg/L</td>
<td>5.7 (2.2–19.4)*</td>
<td>4.9 (1.8–16.7)*</td>
<td>2.7 (1.3–6.5)</td>
</tr>
<tr>
<td>Subjects with CRP (&lt;5) mg/L, n</td>
<td>31 (39%)</td>
<td>33 (40%)</td>
<td>53 (64%)</td>
</tr>
<tr>
<td>Subjects with CRP 5–10 mg/L, n</td>
<td>20 (25%)</td>
<td>22 (27%)</td>
<td>21 (25%)</td>
</tr>
<tr>
<td>Subjects with CRP 11–20 mg/L, n</td>
<td>10 (13%)</td>
<td>8 (10%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Subjects with CRP (&gt;20) mg/L, n</td>
<td>19 (24%)</td>
<td>19 (23%)</td>
<td>4 (5%)</td>
</tr>
</tbody>
</table>

Values are medians (with interquartile ranges) or number of subjects (with percentages). CRP indicates C-reactive protein.

\* \( P\leq0.01\) vs those who survived during follow-up (Mann-Whitney test); † \( P\leq0.05\) (\( \chi^2\) test, df 2).
The serum levels of C-reactive protein ranged from 0.16 to 282 mg/L (n=245). Compared with control subjects, the median serum levels of C-reactive protein were 2-fold higher in those who died from stroke or from noncardiovascular causes (Table 1). Similar results were found for the noncardiovascular subgroups separately. In total, 42 subjects (17%) had levels of C-reactive protein >20 mg/L, which may indicate an acute phase reaction (eg, a reaction due to infection). Median serum levels of C-reactive protein were higher for men, higher in current smokers, higher in subjects with an elevated total cholesterol level, and higher in those with a history of hypertension. Median serum levels of C-reactive protein were lower in subjects who used NSAIDs (data not shown).

The strength of C-reactive protein to predict specific causes of death is presented in Table 2. The risk of death from stroke gradually increased over the strata of higher serum levels of C-reactive protein at baseline (P<0.001 for trend). A similar gradual increase was observed for risk of death from noncardiovascular causes (P<0.001 for trend). Unequal distributions of age, sex, current smoking behavior, use of NSAIDs, total cholesterol level, history of hypertension or diabetes, and previous cardiovascular events did not explain the findings. After adjustment in a logistic regression model, the relative risks of death from stroke or noncardiovascular causes were similar (Table 2). The mortality risks were similar for women and men. The risk of death from stroke at increased levels of C-reactive protein remained statistically significant when subjects with C-reactive protein levels >20 mg/L were excluded from the analysis (P<0.05 for trend). A similar statistically significant increase was observed for noncardiovascular causes (P<0.05 for trend). Finally, we analyzed the risk of death from noncardiovascular causes after exclusion of deaths from infection (n=11) or deaths from cancer (n=29). The increased mortality risk at higher levels of C-reactive protein remained unaltered.

To further explore the nondiscriminative nature of levels of C-reactive protein at baseline, we compared the levels of C-reactive protein dependent on the time of death during follow-up (Table 3). Levels of C-reactive protein were highest when subjects died from stroke or from noncardiovascular causes during the first year of follow-up. The levels of C-reactive protein were lower when more time had elapsed between the moment of blood sampling and the occurrence of death from stroke during the follow-up period (P=0.01). Similar differences were observed for noncardiovascular death (P=0.05) as well as for the subgroups of noncardiovascular causes separately (data not shown).

### Discussion

The findings from this population-based prospective follow-up study among elderly subjects are 2-fold. First, the

**TABLE 2. Relative Risk of Death From Stroke or Noncardiovascular Death According to Serum Levels of CRP**

<table>
<thead>
<tr>
<th>Serum Level of CRP</th>
<th>&lt;5 mg/L</th>
<th>5–10 mg/L</th>
<th>11–20 mg/L</th>
<th>&gt;20 mg/L</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death from stroke</td>
<td>OR (95% CI)</td>
<td>1.0</td>
<td>1.6 (0.8–3.5)</td>
<td>3.4 (1.1–11)</td>
<td>8.1 (2.5–26)</td>
</tr>
<tr>
<td>Corrected OR (95% CI)</td>
<td>1.0</td>
<td>2.1 (0.9–5.4)</td>
<td>3.4 (0.9–13)</td>
<td>11 (2.4–45)</td>
<td></td>
</tr>
<tr>
<td>Noncardiovascular death</td>
<td>OR (95% CI)</td>
<td>1.0</td>
<td>1.7 (0.8–3.5)</td>
<td>2.6 (0.8–8.5)</td>
<td>7.6 (2.4–24)</td>
</tr>
<tr>
<td>Corrected OR (95% CI)</td>
<td>1.0</td>
<td>1.9 (0.8–4.7)</td>
<td>2.5 (0.6–11)</td>
<td>10 (2.3–44)</td>
<td></td>
</tr>
<tr>
<td>Death from stroke vs noncardiovascular death</td>
<td>OR (95% CI)</td>
<td>1.0</td>
<td>1.0 (0.4–2.1)</td>
<td>1.3 (0.5–3.8)</td>
<td>1.1 (0.5–2.4)</td>
</tr>
<tr>
<td>Corrected OR (95% CI)</td>
<td>1.0</td>
<td>0.9 (0.4–2.2)</td>
<td>1.0 (0.3–3.6)</td>
<td>0.8 (0.3–2.3)</td>
<td></td>
</tr>
</tbody>
</table>

Corrected ORs are adjusted for age at baseline, sex, current smoking status, use of NSAIDs, total cholesterol level, history of hypertension or diabetes, and previous cardiovascular events; logistic regression was used.

**TABLE 3. Levels of CRP in Subjects Who Died From Stroke or Noncardiovascular Causes Dependent on Time of Death During Follow-Up**

<table>
<thead>
<tr>
<th>Time During Follow-Up</th>
<th>First Year</th>
<th>Second Year</th>
<th>Third Year</th>
<th>Fourth Year</th>
<th>Fifth Year</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death from stroke</td>
<td>No. of subjects</td>
<td>32</td>
<td>13</td>
<td>15</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Median, mg/L</td>
<td>16</td>
<td>5.3</td>
<td>5.8</td>
<td>2.7</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Interquartile range, mg/L</td>
<td>3.8–26</td>
<td>2.1–13</td>
<td>1.9–19</td>
<td>1.4–9.1</td>
<td>1.7–10</td>
</tr>
<tr>
<td>Noncardiovascular death</td>
<td>No. of subjects</td>
<td>32</td>
<td>12</td>
<td>14</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Median, mg/L</td>
<td>7.2</td>
<td>6.5</td>
<td>7.5</td>
<td>2.9</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Interquartile range, mg/L</td>
<td>1.8–40</td>
<td>3.0–19</td>
<td>2.8–17</td>
<td>0.9–6.2</td>
<td>1.2–7.7</td>
</tr>
</tbody>
</table>

* Differences between median levels of CRP were estimated by Kruskal-Wallis test.
baseline levels of C-reactive protein were 2-fold higher in subjects who died from stroke during the 5-year follow-up period than the baseline levels in control subjects. The risk of fatal stroke increased linearly up to 10-fold in subjects with the highest levels of C-reactive protein. Second, a similar significant relation was observed between baseline levels of C-reactive protein and death from noncardiovascular causes. Taken together, these findings indicate that C-reactive protein is a strong, but nonspecific, risk factor of fatal stroke in elderly subjects.

Major risk factors of stroke and cardiovascular disease, such as smoking, previous history of cardiovascular disease, and the presence of diabetes and hypertension, are associated with higher levels of C-reactive protein.\textsuperscript{13,14} These relations could potentially explain the associations between C-reactive protein and fatal stroke. Adjustment for such risk factors, however, left the associations unaltered. The interpretation would then be that the atherosclerosis underlying clinical cardiovascular events does not explain the relation between C-reactive protein and fatal stroke.

Why is C-reactive protein such a strong risk factor of fatal stroke but also associated with other specific causes of death? It may be considered that the death certificates used in the present study are not sufficiently reliable. For example, the presence of a disease may be unknown to the doctor who certifies death. Also, a disease that is present at the time of death may not be considered to be the underlying cause. Death from circulatory diseases, however, is usually recorded. More than 90\% of the patients who were admitted for circulatory diseases, including stroke, and died within 4 weeks had the condition recorded as the underlying cause of death.\textsuperscript{16,18} Moreover, within the Leiden 85-Plus Study, we have previously been able to demonstrate associations between total cholesterol levels and specific causes of death\textsuperscript{15} and between the methylenetetrahydrofolate reductase gene polymorphism and specific causes of death.\textsuperscript{19} We conclude that it is reasonable to assume that the causes of death on the death certificates are sufficiently reliable to reveal significant associations with the determinants under study.

Stroke is a heterogeneous disorder with different pathogenic mechanisms, eg, intracerebral hemorrhage, atherothrombotic brain infarct, and cardiac emboli. It is not likely that this has grossly affected our data. In the elderly, in contrast to other age groups, ischemic strokes are the main category of stroke, accounting for $>80\%$ of all cerebrovascular events in subjects aged $\geq 70$ years.\textsuperscript{20,21} Because the proportion of hemorrhagic strokes decreases with age, we hypothesized that almost all observed fatal strokes in our study population aged $\geq 85$ years were ischemic strokes.

There was a wide range of serum levels of C-reactive protein in our study population. Almost 20\% of the participants had C-reactive protein levels $>20$ mg/L, which may indicate an acute phase reaction. It may be hypothesized that participants with comorbid conditions at the time of blood sampling, who had high levels of C-reactive protein, disturbed the analysis. Therefore, we repeated all analyses with only those subjects who had C-reactive protein levels $<20$ mg/mL. Because the associations between C-reactive protein and mortality remained statistically significant, it is less likely that our finding can be explained by comorbid conditions.

The present findings are similar to the results in the Iowa 65+ Rural Health Study,\textsuperscript{22} in which high serum levels of C-reactive protein are associated not only with cardiovascular but also with noncardiovascular mortality. These findings are at odds with the original hypothesis that C-reactive protein is a specific marker of subsequent cardiovascular disease.\textsuperscript{11} An explanation for the association of C-reactive protein with several causes of death is that inflammation plays a role in various disease mechanisms. Inflammation is thought to play a causal role not only in vascular damage but also in coagulation, the host defense against pathogens and cancer, and autoimmune diseases, among others. Elevated levels of C-reactive protein may thus identify subjects at higher risk for the development of these diseases.

In the present study, blood was sampled up to 5 years before the occurrence of death. This makes it less likely that the level of C-reactive protein is merely the reflection of imminent disease from which death has not yet occurred. This is different from the situation in which blood is sampled after the event has occurred. The latter methodology makes causal inference difficult, inasmuch as it cannot be excluded that increased levels of C-reactive protein are caused by an ongoing inflammatory reaction triggered by the event itself.

Nonetheless, the time sequence of C-reactive protein and the occurrence of death may shed light on the causal relation between the two. It appeared that C-reactive protein levels were highest in subjects who experienced a fatal event during the first year of follow-up. The levels of C-reactive protein were lower when more time elapsed between the moment of blood sampling and the fatal event. This phenomenon has been reported previously.\textsuperscript{4} Hence, the risks of fatal stroke associated with high levels of C-reactive protein were higher for the short-term than for the long-term follow-up. This time sequence suggests that C-reactive protein is part of, or reflects, a final common pathway of various causes of death.

In conclusion, an elevated level of C-reactive protein is a strong risk factor of fatal stroke. However, the association is not specific and therefore does not provide an argument for low-grade inflammation as a specific causal mechanism of fatal cerebrovascular disease.

Acknowledgment

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References


17. Deleted in proof.


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