Elevated C-Reactive Protein and Long-Term Mortality After Ischaemic Stroke

Relationship With Markers of Endothelial Cell and Platelet Activation

Saran Shantikumar, MBChB; Peter J. Grant, MD; Andrew J. Catto, PhD; John M. Bamford, MD; Angela M. Carter, PhD

Background and Purpose—Inflammatory biomarkers predict development of atherothrombotic events. In the present study we examined the relationships between C-reactive protein (CRP), complement C3, and long-term mortality after acute ischemic stroke.

Methods—CRP and C3 were analyzed by in-house enzyme-linked immunosorbent assay in 394 subjects with acute ischemic stroke who survived for >30 days, followed-up for a median of 7.4 years.

Results—CRP was higher in subjects who died (10.8 mg/L; 95% CI, 9.1–12.8) compared with survivors (3.8 mg/L; 95% CI, 3.1–4.7), whereas C3 was similar in both groups (P=0.26). CRP remained predictive for mortality after adjusting for conventional clinical and demographic risk factors (the adjusted hazard ratio for those with CRP in the highest compared with the lowest quartile was 2.0; 95% CI, 1.3–3.1). However, CRP was no longer independently predictive of mortality after additionally adjusting for β-thromboglobulin or von Willebrand factor.

Conclusions—Our data suggest that the relationship between CRP and poststroke mortality may in part reflect inflammation-induced endothelial cell dysfunction and platelet activation. (Stroke. 2009;40:00-00.)

Key Words: C-reactive protein ▪ cerebral infarction ▪ endothelial cell dysfunction ▪ mortality ▪ platelet activation

Chronic inflammation plays a key role in the pathophysiology of atherosclerosis and ischemic stroke. Levels of C-reactive protein (CRP) are associated with the risk of future cardiovascular thrombotic events in healthy people and in those with preexisting coronary artery disease. Furthermore, CRP has been found to predict risk of stroke1 and, in a limited number of studies involving relatively small cohorts or short follow-up times, CRP has been shown to predict mortality after acute ischemic stroke.2-4 The role of CRP as a contributing factor in atherogenesis is yet to be established, and other inflammatory proteins have also been shown to predict cardiovascular events, including complement C3.5 In the present study we determined the relationships between CRP and complement C3 and all-cause mortality after acute ischemic stroke in a cohort of 394 patients.

Subjects and Methods

Subjects

The recruitment and characteristics of patients have been fully described elsewhere.6 Briefly, ischemic stroke was subclassified according to Oxfordshire Community Stroke Project classification: lacunar infarction, total and partial anterior circulation infarction, posterior circulation infarction. Subjects were classified as current, former, or nonsmokers. A medical history of previous stroke or TIA, ischemic heart disease, and peripheral vascular disease was documented. Atrial fibrillation at stroke presentation was confirmed by 12-lead ECG. Diabetes and hypertension were determined from case notes and current use of hypoglycemic and antihypertensive agents. Subjects gave informed consent according to a protocol approved by the Leeds Teaching Hospitals Research Ethics Committee. All patients included in this study were flagged with the Office for National Statistics for notification of death.6 Only patients who survived for >30 days after the acute event with plasma available for analysis of CRP and complement C3 were included in the present study (n=394).

Laboratory Analyses

Biochemical, hematologic, and hemostatic factors were analyzed as previously described. CRP and C3 were determined by in-house enzyme-linked immunosorbent assays using antibodies from Dako-Cytomation: CRP, intra-assay coefficient of variation=6.26%, interassay coefficient of variation=1.71%, interassay coefficient of variation=3.93%; C3, intra-assay coefficient of variation=2.29%, interassay coefficient of variation=6.26%.

Statistical Analyses

The outcome measure was all-cause long-term mortality after acute ischemic stroke. Surviving patients were censored on January 19, 2002.6 Univariate associations between quartiles of CRP and C3 and mortality were assessed using Kaplan-Meier survival analysis with significance determined using the logrank test. The association between inflammatory markers and mortality was determined using univariate and multivariate Cox regression analyses, with data...
presented as hazard ratios (95% CI). Log-minus-log plots confirmed the validity of the proportionality of hazards assumption over time.

**Results**

Subjects were followed-up for a median of 7.4 years, and during follow-up 231 patients (59%) died. CRP levels were significantly higher in those who subsequently died (10.8; 95% CI, 9.1–12.8 mg/L) compared with those who survived (3.8; 95% CI, 3.1–4.7 mg/L; \( P < 0.001 \)). In contrast, C3 levels were similar in the patients who died and survivors (\( P = 0.257 \)). Kaplan-Meier analyses indicated a progressive decline in survival with increasing quartiles of CRP, but not with C3 (Figure).

The characteristics of subjects classified according to quartiles of CRP are presented in Table 1. CRP was associated with previously reported independent predictors of mortality,\(^6\) including advancing age, atrial fibrillation, and previous stroke/TIA. CRP was also related to Oxfordshire Community Stroke Project stroke classification, with the highest prevalence of lacunar infarction in those with CRP in the lowest quartile and the highest prevalence of total anterior circulation infarction in those with CRP in the highest quartile. Furthermore, CRP was associated with biochemical and hemostatic predictors of mortality, with a progressive increase in levels of creatinine, \( \beta \)-thromboglobulin (\( \beta \)TG), and von Willebrand Factor (vWF), and progressive decrease

![Figure. Kaplan–Meier survival curves indicating relationship between (A) CRP, (B) C3, and all-cause mortality after ischemic stroke during a median follow-up of 7.4 years. Crosses indicate censored data.](http://stroke.ahajournals.org/)

**Table 1. Characteristics of Patients With Acute Ischemic Stroke Categorized by CRP Quartiles**

<table>
<thead>
<tr>
<th>CRP Quartiles</th>
<th>(&lt;2.48 \text{ mg/mL (n}=99))</th>
<th>(2.48–6.62 \text{ mg/mL (n}=98))</th>
<th>(6.63–22.33 \text{ mg/mL (n}=99))</th>
<th>(&gt;22.33 \text{ mg/mL (n}=98))</th>
<th>( P ) ( (\text{Trend}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>68 (58–76)</td>
<td>70 (61–77)</td>
<td>71 (64–79)</td>
<td>74 (69–81)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Male</td>
<td>51 (0.51)</td>
<td>56 (0.57)</td>
<td>49 (0.49)</td>
<td>54 (0.55)</td>
<td>0.703</td>
</tr>
<tr>
<td>Current smokers</td>
<td>48 (0.48)</td>
<td>45 (0.46)</td>
<td>33 (0.33)</td>
<td>34 (0.35)</td>
<td>0.180</td>
</tr>
<tr>
<td>Former smokers</td>
<td>13 (0.13)</td>
<td>20 (0.20)</td>
<td>21 (0.21)</td>
<td>20 (0.20)</td>
<td></td>
</tr>
<tr>
<td>Stroke subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>LACI</td>
<td>53 (0.54)</td>
<td>37 (0.38)</td>
<td>31 (0.31)</td>
<td>18 (0.18)</td>
<td></td>
</tr>
<tr>
<td>PACI</td>
<td>26 (0.26)</td>
<td>28 (0.29)</td>
<td>40 (0.40)</td>
<td>37 (0.38)</td>
<td></td>
</tr>
<tr>
<td>POCI</td>
<td>14 (0.14)</td>
<td>13 (0.13)</td>
<td>5 (0.05)</td>
<td>8 (0.08)</td>
<td></td>
</tr>
<tr>
<td>TACI</td>
<td>6 (0.06)</td>
<td>20 (0.20)</td>
<td>23 (0.23)</td>
<td>35 (0.36)</td>
<td></td>
</tr>
<tr>
<td>Previous stroke/TIA</td>
<td>27 (0.27)</td>
<td>18 (0.18)</td>
<td>29 (0.29)</td>
<td>38 (0.38)</td>
<td>0.017</td>
</tr>
<tr>
<td>AF</td>
<td>8 (0.08)</td>
<td>21 (0.21)</td>
<td>13 (0.13)</td>
<td>23 (0.24)</td>
<td>0.111</td>
</tr>
<tr>
<td>IHD</td>
<td>14 (0.14)</td>
<td>23 (0.23)</td>
<td>39 (0.39)</td>
<td>35 (0.36)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15 (0.15)</td>
<td>14 (0.14)</td>
<td>16 (0.16)</td>
<td>13 (0.13)</td>
<td>0.949</td>
</tr>
<tr>
<td>Hypertension</td>
<td>44 (0.44)</td>
<td>34 (0.35)</td>
<td>44 (0.44)</td>
<td>41 (0.42)</td>
<td>0.461</td>
</tr>
<tr>
<td>PVD</td>
<td>9 (0.09)</td>
<td>15 (0.15)</td>
<td>17 (0.17)</td>
<td>10 (0.10)</td>
<td>0.260</td>
</tr>
<tr>
<td>Aspirin use at time of study</td>
<td>18 (0.18)</td>
<td>23 (0.24)</td>
<td>23 (0.23)</td>
<td>31 (0.32)</td>
<td>0.172</td>
</tr>
<tr>
<td>Survival time, yr</td>
<td>6.6 (5.1–7.7)</td>
<td>6.0 (5.2–7.6)</td>
<td>3.6 (0.9–7.0)</td>
<td>3.0 (0.9–7.0)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>42.3 (41.3–43.30)</td>
<td>40.7 (39.6–41.80)</td>
<td>39.1 (38.1–40.1)</td>
<td>38.5 (37.5–39.5)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>92.4 (88.3–96.7)</td>
<td>93.3 (88.3–98.5)</td>
<td>112.9 (104.7–121.6)</td>
<td>113.2 (105.1–121.9)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>( \beta )TG, ng/mL</td>
<td>36.8 (33.1–4.10)</td>
<td>46.5 (39.5–65.7)</td>
<td>55.3 (46.5–65.7)</td>
<td>66.4 (58.4–75.4)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>vWF, IU/mL</td>
<td>1.47 (1.33–1.61)</td>
<td>1.61 (1.47–1.77)</td>
<td>1.87 (1.69–2.06)</td>
<td>2.39 (2.23–2.56)</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>

Age and survival time presented as median (25th and 75th percentiles); categorical data presented as number (frequency); other data presented as mean or geometric mean (95% CI).

AF indicates atrial fibrillation; IHD, ischemic heart disease; LACI, lacunar infarction; PACI, partial anterior circulation infarction; POCI, posterior circulation infarction; PVD, peripheral vascular disease; TACI, total anterior circulation infarction.
in levels of albumin with increasing CRP quartiles (Table 1). Cox regression analyses with varying degrees of adjustment for factors previously shown to be independently predictive of mortality in this cohort are presented in Table 2. The association between CRP and mortality persisted after adjustment for age, atrial fibrillation, previous stroke/TIA, and stroke subtype (model 1). The association between CRP and mortality was lost after adjustment for albumin, creatinine, βTG, or vWF. CRP was not associated with mortality in a final model (model 2) with backwards stepwise selection for albumin, creatinine, βTG, and vWF (Table 2), in which only βTG and vWF were retained in the model with age, atrial fibrillation, previous stroke/TIA, and stroke subtype.

### Discussion

In the present study, we found that a single measurement of CRP within 10 days of ischemic stroke was a predictor of long-term mortality. The relationship between CRP and cardiovascular events has been shown by numerous studies to be independent of conventional cardiovascular risk factors.\(^1\) Consistent with these findings, in the present study CRP remained associated with poststroke mortality after adjusting for age, stroke subtype, previous stroke/TIA, and atrial fibrillation, with CRP levels in the third and fourth quartiles (>6.6 mg/L) predicting mortality. This is in agreement with available evidence from smaller cohorts and cohorts with shorter follow-up.\(^2\) For instance, Muir et al\(^1\) found that levels of CRP >10.1 mg/L were associated with increased mortality over ~3 years of follow-up. We have previously shown that nonclassical risk factors (albumin, creatinine, vWF, and βTG) were independently associated with poststroke mortality,\(^6\) and in the present study CRP was significantly associated with each of these risk factors. We therefore accounted for these nonclassical risk factors and found that CRP was no longer significantly associated with mortality. In contrast, we found that vWF and βTG remained independently predictive of mortality after adjusting for CRP, which may suggest that CRP is upregulated, at least in part, as a consequence of endothelial cell dysfunction and platelet activation. However, it has been shown that infusion of CRP in healthy volunteers (maximal CRP concentration, 29.0 mg/L) results in ~50% increase in plasma vWF and soluble E-selectin,\(^7\) suggesting that CRP may have a direct influence on endothelial function. Further studies are warranted to investigate the relationships between CRP and endothelial cell and platelet activation to clarify the role of CRP in cardiovascular disease.

### Limitations of the Study and Conclusion

The study was not originally designed to assess the effect of inflammatory markers on survival, and hence a history of infection around the time of stroke was not recorded; however, studies indicate that recent infections are a risk factor for stroke.\(^6\) In addition, as previously described, treatment on discharge was not obtained and we only analyzed total mortality in this study because of recognized inconsistencies in reporting on death certificates.

In conclusion, our data suggest that the relationship between CRP and poststroke mortality may in part reflect inflammation-induced endothelial cell dysfunction and platelet activation.

### Sources of Funding

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### Disclosures

None.

### References

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