Prognostic Relevance of Early Serial C-Reactive Protein Measurements After First Ischemic Stroke

Kerstin Winbeck, MD; Holger Poppert, MD; Thorleif Etgen, MD; Bastian Conrad, MD; Dirk Sander, MD

Background and Purpose—Recent studies described an association between elevated levels of C-reactive protein (CRP) and outcome after ischemic stroke. We investigated the impact of early serial CRP measurements in hyperacute ischemic stroke on long-term outcome.

Methods—One hundred twenty-seven consecutive patients without thrombolysis with a first ischemic stroke no more than 12 hours after symptom onset were examined. Serial CRP measurements were done at admission (CRP 1), within 24 hours (CRP 2), and within 48 hours (CRP 3) after symptom onset. In addition to several cerebrovascular risk factors, the 1-year outcome and the lesion volumes of initial diffusion-weighted images were determined.

Results—The CRP concentration increased significantly during the first 48 hours after symptom onset (CRP 1, 0.86 mg/dL [95% CI, 0.69 to 1.02]; CRP 2, 1.22 mg/dL [95% CI, 0.88 to 1.55]; CRP 3, 1.75 mg/dL [95% CI, 1.25 to 2.25]; P=0.003). Multiple logistic regression analysis identified Barthel Index score at admission and CRP 2 and 3 as independent predictors of an unfavorable outcome. Kaplan-Meier analysis revealed a significantly higher rate of end point events (adjusted odds ratio, 3.9 [95% CI, 1.4 to 10.7]; P=0.008) only in patients with elevated CRP 2 concentrations.

Conclusions—The CRP level measured within 12 hours after symptom onset of an acute ischemic stroke is not independently related to long-term prognosis. In contrast, a CRP increase between 12 and 24 hours after symptom onset predicts an unfavorable outcome and is associated with an increased incidence of cerebrovascular and cardiovascular events. (Stroke. 2002;33:2459-2464.)

Key Words: C-reactive protein ■ magnetic resonance imaging, diffusion-weighted ■ outcome ■ stroke

Elevated levels of C-reactive protein (CRP) are present among patients at risk for further first-ever myocardial infarction and stroke.1–6 Recently, it was shown that elevated CRP levels independently predict the risk of future stroke and transient ischemic attack in the elderly.7 After acute stroke, a single CRP concentration measured within 72 hours was found to be an independent predictor of survival in a subgroup analysis from a prospective observational study.8 In this study survival in patients with CRP levels >1.01 mg/dL was significantly worse.8 Di Napoli et al9 studied the 1-year prognostic influence of CRP measured within 24 hours after ischemic stroke and described an association between increased levels of CRP and unfavorable outcome. A recent investigation analyzed the association between CRP measured within 24 hours after ischemic stroke, after 48 to 72 hours, and at hospital discharge and long-term outcome.10 The authors found that the CRP concentration at discharge was better related to outcome than earlier measurements. One possible shortcoming of the previous studies performed was that a reactive active phase response due to an accompanying infection could not be ruled out if blood samples were taken within 24 hours after symptom onset. Additionally, the changes in CRP during the hyperacute phase after stroke were not analyzed in detail until now. Therefore, the aim of our study was to evaluate the impact of early serial CRP measurements during the hyperacute phase of ischemic stroke on functional outcome.

Subjects and Methods
From a series of 176 consecutive patients admitted to the Stroke Unit of the Department of Neurology of the Technical University of Munich with a proven first-ever stroke no more than 12 hours after symptom onset, 127 patients without thrombolysis were included in this prospective follow-up study. Potential patients were excluded from the study if they had a history of recent infection as outpatients (n=11), surgery or trauma in the previous month (n=12), obvious signs of acquired in-hospital infection before onset of the index stroke (n=8), or an initial CRP level >10 mg/dL (n=10). Previous infections were monitored by medical history, chest x-ray, examination of the urine with the use of a nitrite dipstick test and microscopic examination, and a complete physical examination. Eight patients were lost during follow-up. Cerebral infarction was defined as a focal neurological deficit of sudden onset that persisted >24 hours in surviving patients. All patients received a brain CT and/or MRI...
indicating the presence of infarction and the absence of hemorrhage, as well as a full neurological examination including screening with the Barthel Index and modified Rankin Scale, duplex sonography of the carotid arteries, transcranial Doppler sonography, 12-lead ECG, transthoracic and/or transesophageal echocardiography, and standardized blood tests. Several laboratory parameters and cardiovascular risk factors such as body mass index, pack-years of smoking, hypercholesterolemia (treatment with cholesterol-lowering drugs or a total cholesterol level ≥6.2 mmol/L), incidence of arterial hypertension (treatment with antihypertensive medication or documented blood pressure ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic), diabetes mellitus (treatment with anti-diabetic drugs or diagnosis of diabetes during hospital stay), ischemic heart disease (IHD) (documented by previous myocardial infarction or angina pectoris, bypass surgery, or >50% angiographic stenosis of ≥1 major coronary artery), carotid artery stenosis, and carotid plaques were determined. Depending on the etiology of the ischemic stroke, the patients received aspirin, clopidogrel, or an antiocoagulation therapy. To evaluate second stroke complication, eg, pneumonia or urinary tract infections, the patients received a daily physical examination and a continuous or repeated (every 4 hours) temperature measurement. Depending on these findings, a second chest x-ray and additional examination of the urine, including urine culture, was performed. Twenty-one percent of the patients developed fever (defined as rectal temperature >38.0°C) during the first 96 hours after stroke onset and were therefore treated with either mezlocillin 2 g and sulbactam 1 g in case of pneumonia or trimethoprim 160 mg and sulfamethoxazole 860 mg in case of a urinary tract infection. If necessary, treatment was then modified according to the antibiogram.

CRP Measurements
In all patients the first CRP concentration (CRP 1) was measured immediately after admission. The first CRP concentration was determined in serum specimen with the use of a Dimension RXL clinical chemistry analyzer with CRP Flex reagent cartridges. The assay range was 0.2 to 12 mg/dL. A concentration ≥0.5 mg/dL was defined as pathologically increased according to the reference values of our laboratory. For the second and third CRP measurements, the highly sensitive CRP concentration was determined with the use of a Tina-quant CRP (latex) highly sensitive assay (Roche). The measuring range is from 0.01 to 2 mg/dL. When the CRP concentration of the sample was above the measuring range, a manual dilution with CRP-free serum was performed. Intra-assay and inter-assay imprecisions were evaluated according to the guidelines of the European Committee for Clinical Laboratory Standards. Intra-assay imprecisions were evaluated for a sample being analyzed repeatedly in a single run. The coefficient of variation was 1.34%, with a mean CRP of 0.05 mg/dL. For inter-assay imprecisions, a sample was reanalyzed several times in different runs. The coefficient of variation for the interassay imprecision was 5.7%. More highly concentrated control samples showed lower interassay coefficient of variation values in the range of 1% to 3%. The determination limit was found at 0.004 mg/dL. A CRP increase was defined as a CRP elevation ≥0.5 mg/dL or an increase of ≥0.1 mg/dL between two measurements. An intermethod comparison demonstrated a high correlation between both methods. The comparison was performed with the use of the nonparametric regression analysis of Passing and Bablok. The correlation coefficient was 0.9965 for a measuring range of 0.0 to 18 mg/dL.

The second CRP measurement (CRP 2) was done within 24 hours after symptom onset, and the third CRP (CRP 3) was evaluated 24 hours after the second measurement.

Image Analysis
Diffusion-weighted imaging (DWI) and conventional T1- (repetition time [TR]/echo time [TE] 654/14 ms) and T2-weighted images (TR/TE 330/132 ms) were performed with a 1.5-T MRI (Magnetom Symphony, Siemens Medical Systems) in 125 patients. In 43 patients early DWI scans could be performed within 12 hours after symptom onset. Single-shot trace-weighted DWI with the use of multislice echo-planar imaging was obtained with the following measuring parameters: TR 4006 ms, TE 83 ms, slice thickness 6 mm, gap 0.1 mm, 128×128 matrix, field of view 240×240 mm, 3 b values = 0 to 1000 s/mm2. The infarct volume was determined morphometrically from DWI data with the use of a computer-supported image analysis system (Sigma Scan Pro, SPSS) as described previously.

Outcome Measurements
The Barthel Index and the modified Rankin Scale were used to assess functional disability and were evaluated at admission and after follow-up. A Barthel Index score ≥85 was defined as favorable and functionally independent. Additionally, the patients were divided according to the modified Rankin Scale score into the following categories: independent in terms of day-to-day activities and with a good outcome (score 0 to 2) and dependent or dead (score 3 to 6). We used the same end point criteria (combination of death due to any cause and any new nonfatal vascular event such as recurrent stroke, unstable angina, or myocardial infarction) as recently described by Di Napoli et al. The follow-up examinations were performed with the use of a standardized questionnaire with a face-to-face examination. Outcome events were evaluated by a direct contact with the patients, the patients’ families, and/or the physicians. Copies of hospital records, autopsy records, and death certificates were available.

Statistical Analysis
All values were given as mean and 95% CI or median and percentiles. Independent t tests, the Fisher exact test, and the Wilcoxon test were used to test univariate differences between 2 groups. The distribution of the CRP was positively skewed, and therefore the data were log transformed. The difference between the CRP concentration at admission, CRP 2, and CRP 3 was analyzed with a 1-way ANOVA. If the overall test was significant, post hoc tests (Bonferroni) were performed. A multiple logistic regression analysis was done to evaluate the impact of age; Barthel Index score at admission; incidence of hypertension, IHD, diabetes mellitus, and hypercholesterolemia; and the different time-dependent CRP concentrations on dependence (Barthel Index score <85). In a second analysis, after adjustment for the incidence of fever after stroke, the occurrence of an end point was used as an independent variable. Kaplan-Meier survival curves were estimated for the groups of patients with CRP levels above and below the mean initial CRP concentration (0.86 mg/dL). Hazard ratios were calculated with the Cox proportional hazards regression model. A calculated difference of P < 0.05 was considered significant.

Results
Patient Characteristics
One hundred twenty-seven patients (74 men and 53 women; mean age, 65 years [95% CI, 63 to 68 years]) were included in this study. Seventy percent had a history of hypertension, 27% had an IHD, 42% had a history of hypercholesterolemia, and 29% had diabetes mellitus. The median initial Barthel Index score was 55 (interquartile range, 20 to 90), and the median Rankin Scale score was 4 with an interquartile range of 2 to 5 at admission. After 1 year of follow-up, 62% of the patients were functionally independent (Barthel Index score ≥85).

CRP Changes
The first determination immediately after admission was performed within 12 hours after symptom onset (mean, 5.0 hours [95% CI, 4.5 to 5.6]) (Table 1). In 17 patients (13%) the CRP concentration decreased and in 49% the CRP increased within 24 hours after symptom onset. The first CRP value was not significantly correlated with the development of
TABLE 1. Mean CRP Values and Time Between Symptom Onset and CRP Measurements and Values

<table>
<thead>
<tr>
<th>Concentration, mg/dL</th>
<th>Time After Symptom Onset, h</th>
<th>Mean Time From First Measurement, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP 1</td>
<td>0.86 (0.69 to 1.02)</td>
<td>5.0 (4.5 to 5.6)</td>
</tr>
<tr>
<td>CRP 2</td>
<td>1.22 (0.88 to 1.55)</td>
<td>19.6 (18.8 to 20.5)</td>
</tr>
<tr>
<td>CRP 3</td>
<td>1.75 (1.25 to 2.25)</td>
<td>43.1 (42.1 to 44.1)</td>
</tr>
</tbody>
</table>

Values in parentheses are 95% CIs. ANOVA showed an overall significant (P=0.003) difference between the 3 CRP values. Bonferroni post hoc testing revealed only a significant difference between CRP 1 and CRP 3 (P<0.01).

TABLE 2. Demographic Data, Cardiovascular Risk Factors, and Baseline Laboratory Parameters of Patients With CRP 2 Values <0.86 or ≥0.86 mg/dL

<table>
<thead>
<tr>
<th>CRP 2 ≥0.86 mg/dL</th>
<th>CRP 2 &lt;0.86 mg/dL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>58</td>
<td>69</td>
</tr>
<tr>
<td>Age, y (95% CI)</td>
<td>66.2 (62.5 to 66.3)</td>
<td>64.9 (61.4 to 68.5)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>32/26</td>
<td>42/27</td>
</tr>
<tr>
<td>Hypertension, No.</td>
<td>44 (76%)</td>
<td>44 (64%)</td>
</tr>
<tr>
<td>IHD, No.</td>
<td>23 (40%)</td>
<td>12 (17%)</td>
</tr>
<tr>
<td>Hypercholesterolemia, No.</td>
<td>26 (45%)</td>
<td>28 (41%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>20 (34%)</td>
<td>17 (25%)</td>
</tr>
<tr>
<td>MRI deficit, mL (95% CI) (n=43)</td>
<td>36.0 (9.9 to 62.2)</td>
<td>21.9 (−0.9 to 44.6)</td>
</tr>
<tr>
<td>Body mass index (95% CI)</td>
<td>26.0 (24.7 to 27.3)</td>
<td>26.1 (25.0 to 27.3)</td>
</tr>
<tr>
<td>Pack-years of smoking (95% CI)</td>
<td>22.2 (14.1 to 30.4)</td>
<td>18.0 (13.2 to 22.9)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L (95% CI)</td>
<td>5.3 (4.9 to 5.7)</td>
<td>5.4 (5.1 to 5.7)</td>
</tr>
<tr>
<td>Time until CRP measurement, h (95% CI)</td>
<td>18.9 (17.7 to 20.2)</td>
<td>20.2 (18.9 to 21.4)</td>
</tr>
<tr>
<td>Treatment with antithrombotic drugs (aspirin or clopidogrel), No.</td>
<td>44 (76%)</td>
<td>55 (79%)</td>
</tr>
<tr>
<td>Treatment with intravenous heparin, No.</td>
<td>14 (24%)</td>
<td>14 (21%)</td>
</tr>
<tr>
<td>Incidence of fever within 96 h after stroke, No.</td>
<td>21 (36%)</td>
<td>6 (9%)</td>
</tr>
<tr>
<td>Barthel Index, median (interquartile range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>50 (10 to 83.8)</td>
<td>62.5 (25 to 95)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>80 (35 to 100)</td>
<td>92.5 (65 to 100)</td>
</tr>
<tr>
<td>Independent (Barthel Index ≥85)</td>
<td>30 (52%)</td>
<td>47 (68%)</td>
</tr>
<tr>
<td>End point event</td>
<td>15 (26%)</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>Death</td>
<td>8 (14%)</td>
<td>2 (3%)</td>
</tr>
</tbody>
</table>

MRI Measurements

In 43 patients it was possible to perform a DWI scan within 12 hours after symptom onset. The mean lesion volume was 33.4 mL (95% CI, 13.8 to 53.1). The infarct volume was larger in patients with an unfavorable outcome (69.1 mL [95% CI, 30.2 to 108] versus 4.9 mL [95% CI, 1.1 to 8.8]; P<0.003), indicating a correlation between the size of infarction on early DWI and 1-year outcome (P=0.005). In addition, an increased CRP level was significantly associated with a larger initial MRI lesion volume only for the third CRP measurement (51.9 mL [95% CI, 17.2 to 86.7] versus 13.6 mL [95% CI, 0 to 27.6]; P=0.042). Neither for the first nor for the second CRP measurement was the lesion volume significantly associated with elevated CRP ≥0.86 mg/dL (44.2 mL [95% CI, 11.7 to 76.8] versus 18.4 mL [95% CI, 4.0 to 32.8] [P=0.14] and 41.9 mL [95% CI, 11.2 to 71.8] versus 25.0 mL [95% CI, 0 to 52.9] [P=0.39]).

Outcome and End Point Analysis

During follow-up, 24 patients (19%; 17 men and 7 women) had a primary end point. Ten patients died (7 because of vascular death and 3 because of nonvascular death). Fourteen patients experienced a new vascular event (transient ischemic attack, n=3; recurrent stroke, n=9; unstable angina, n=2). Patients with increased CRP 2 levels had a significantly lower

known cardiovascular risk factors (pack-years of smoking, diabetes mellitus, hypertension, hypercholesterolemia, body mass index). Moreover, the use of antithrombotic and anti-coagulation medication was similar in both groups.
follow-up Barthel Index score (Figure 1). Accordingly, the incidence of dependency (Barthel Index score <85) after 1 year tended to be increased in the group of patients with CRP 2 levels ≥0.86 mg/dL (48% versus 32%; P = NS). Death (14% versus 3%; P = 0.025) was significantly more often observed in patients with CRP 2 levels ≥0.86 mg/dL. There was no significant difference between the patients who had or who did not have a primary end point regarding several parameters, such as age, body mass index, volume of MRI lesion, Barthel Index and modified Rankin Scale scores at admission, sex, and incidence of hypertension, IHD, diabetes mellitus, and hypercholesterolemia. The Cox proportional hazards regression analysis showed a significantly higher rate of end point events in patients with a CRP concentration ≥0.86 mg/dL, within 24 hours (CRP 2) after symptom onset, even after correction for other risk factors (age, initial Barthel Index score, and incidence of hypertension, IHD, diabetes mellitus, hypercholesterolemia, and fever after stroke), whereas the CRP 1 and CRP 3 were not significantly related to end point events (Figure 2).

Regarding the incidence of a favorable outcome after 1 year of follow-up (Barthel Index score ≥85), the multiple logistic regression analysis revealed only the initial Barthel Index score, CRP 2, and CRP 3 as independent predictors. The adjusted odds ratio for being independent 1 year after a first ischemic stroke according to the serial CRP concentrations is given in Table 3. Only the CRP 2 and CRP 3 were independent predictors of an unfavorable outcome even after correction for the other risk factors (age, Barthel Index score at admission, and incidence of IHD, hypercholesterolemia, diabetes mellitus, and hypertension) with the use of a Cox regression model. To exclude the possibility that the CRP 1 levels were not predictive for outcome because we did not use the sensitive assay for this value, we analyzed separately the subgroup of patients with initial CRP values ≥0.5 mg/dL (n = 55) and observed the same results.

Discussion

The aim of our study was to evaluate for the first time, to the best of our knowledge, the relationship between serial CRP measurements during the hyperacute phase of ischemic stroke and long-term outcome. Several other studies described an association between increased CRP levels measured within 24 to 72 hours after ischemic stroke and outcome.8–10 One might argue that in these studies at least a part of the CRP increase was due to in-hospital acquired inflammatory pro-
In conclusion, using serial CRP measurements during the hyperacute phase of stroke, we observed that increased CRP levels within 12 to 24 hours after stroke onset represent a strong predictor of further cardiovascular or cerebrovascular events as well as unfavorable outcome, whereas increased CRP concentrations measured at admission within 12 hours after stroke onset, as well as 24 to 48 hours after stroke onset, were not correlated with end point events. We postulate that the CRP determination between 12 and 24 hours after symptom onset and not the initial or later CRP measurements is the best parameter to identify patients with a high risk for further vascular events and unfavorable outcome.

**CRP measured within 4±2 days after symptom onset and infarct size.**

Several explanations might be given for the different prognostic impact on long-term outcome of CRP during the acute stages of ischemic stroke. It is conceivable that an increased CRP concentration after stroke onset was due to inflammation related to the pathophysiology of ischemic stroke and might reflect the extent of the ischemic area, as described by Dirnagl et al.25 Experimental data demonstrate that the acute phase response to brain ischemia occurred within 2 hours.26 However, limited data are present regarding the time course of acute phase responses in humans. Montaner et al27 described a peak level of interleukin-6 after 24 hours of symptom onset. Recently, Di Napoli28 observed an increase of CRP within 3 hours after stroke compared with the prestroke value. The peak level of CRP was reached within 36 to 48 hours after stroke onset. In our study only the second CRP concentration measured within 12 to 24 hours after stroke was predictive of new vascular events during follow-up. This may suggest that the inflammatory system reacts more intensely in some patients and may explain the individual differences in inflammatory response to brain ischemia and the independence in regard to DWI lesion volumes in the acute phase of stroke. Serial CRP measurements, particularly the second CRP concentration measured between 12 and 24 hours after symptom onset, may help to identify those patients who are predisposed to an intensive activation of the inflammatory system due to even small infarcts. Because inflammation is known to play a causal role not only in vascular damage but also in coagulation, host defense against infections, and cancer,29 the occurrence of increased individual activation of the inflammatory process in these patients might explain the increased risk for future cardiovascular and cerebrovascular events. On the basis of our results, we hypothesize that the measurement of CRP levels between 12 and 24 hours after stroke onset seems to be the best marker to estimate these individual reactions of the inflammatory system. Interestingly, the third CRP measurement was not related to end point events but was related to functional outcome and DWI lesion volume. Compared with the second CRP measurement, this value is the best predictor (odds ratio, 3.5) of 1-year functional outcome. We hypothesize that a CRP increase after 24 to 48 hours reflects mainly secondary complications of stroke and hospitalization rather than the individual inflammatory response to brain ischemia and therefore is closely related to functional outcome but not to long-term events.

**TABLE 3. Adjusted Odds Ratio of Unfavorable Functional Outcome (Barthel Index<85) After 1 Year of Follow-Up in Relation to Serial CRP Concentrations in 127 Patients With a First Ischemic Stroke**

<table>
<thead>
<tr>
<th>CRP 1</th>
<th>CRP 2</th>
<th>CRP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratio</td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.5–2.4</td>
<td>1.3–4.8</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Adjusted for age, Barthel Index at admission, and incidence of hypertension, IHD, diabetes mellitus, and hypercholesterolemia.
Acknowledgment
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References
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