C-Reactive Protein Predicts Future Cardiovascular Events in Patients With Carotid Stenosis

Oliver Schlager, MD; Markus Exner, MD; Wolfgang Mlekusch, MD; Schila Sabeti, MD; Jasmin Amighi, MD; Petra Dick, MD; Oswald Wagner, MD; Renate Koppensteiner, MD; Erich Minar, MD; Martin Schillinger, MD

Background and Purpose—Atherosclerosis is a systemic inflammatory disease. We demonstrated previously that high-sensitivity C-reactive protein (hs-CRP) is associated with short-term progression of carotid atherosclerosis. We now investigated whether baseline levels of hs-CRP predict midterm clinical outcome in these patients.

Methods—We prospectively studied 1065 of 1268 consecutive patients who were initially asymptomatic with respect to carotid artery disease and were investigated with serial carotid ultrasound examinations at baseline and after a 6- to 9-month interval. Patients were followed-up clinically for the occurrence of cardiovascular events, a composite of myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, and death.

Results—We recorded progression of carotid stenosis in 93 patients (9%) after 6 to 9 months, and 381 cardiovascular events in 337 patients (27%) during a median of 3 years of clinical follow-up (interquartile range, 2.5 to 3.5 years). The hs-CRP levels were significantly elevated in patients with progressive carotid stenosis ($P<0.001$), and hs-CRP was significantly associated with the occurrence of a first future cardiovascular event ($P<0.001$). Adjusted hazard ratios for a first cardiovascular event for increasing quintiles of hs-CRP were 1.41 (95% confidence interval, 0.92 to 2.17), 1.76 (95% confidence interval, 1.17 to 2.66), 2.22 (95% confidence interval, 1.48 to 3.32), and 2.41 (95% confidence interval, 1.61 to 3.60) as compared with the lowest quintile, respectively. This association was independent of traditional cardiovascular risk factors and the baseline degree of carotid stenosis.

Conclusion—Inflammation was associated with morphological and clinical progression of atherosclerotic disease. Patients with elevated levels of hs-CRP exhibit an increased risk for adverse cardiovascular outcome attributable to clinical adverse events of progressive atherosclerotic disease. (Stroke. 2007;38:1263-1268.)

Key Words: asymptomatic carotid stenosis ■ carotid artery ■ outcome ■ prognosis

Atherosclerosis is a systemic inflammatory disease.\textsuperscript{1-4} Inflammation is a key feature of plaque instability and triggers progression of atherosclerosis.\textsuperscript{5-7} Patients with progressive or unstable plaques are at high risk for future clinical complications.\textsuperscript{5-7} With the rapidly evolving scope of preventive therapies, there is a growing interest in cardiovascular risk stratification. Several prognostic biomarkers have been evaluated to noninvasively identify patients with unstable plaques who are at high risk for incident clinical events. Among the panel of potentially useful parameters, high-sensitivity C-reactive protein (hs-CRP) has been unequivocally demonstrated to predict disease progression and clinical adverse events in the coronary, cerebrovascular, and peripheral circulation in apparently healthy subjects as well as in patients with prevalent atherosclerosis.\textsuperscript{8-18} Nevertheless, an association of hs-CRP with morphological and clinical progression of atherosclerosis has not been demonstrated in a large clinical study as yet.

We have demonstrated previously in the prospective IC-ARAS protocol that inflammation measured by hs-CRP is associated with short-term morphological progression of atherosclerosis in the carotid arteries.\textsuperscript{19-21} We now investigated whether baseline levels of hs-CRP predict midterm clinical outcome in these patients.

Methods

Study Design

We prospectively enrolled all consecutive patients who underwent duplex ultrasound investigations of the extracranial carotid arteries from March 2002 until March 2003 at our institution who were neurologically asymptomatic in the Inflammation in Carotid Arteries Risk for Atherosclerosis Study (ICARAS).\textsuperscript{19-21}

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria have been reported previously.\textsuperscript{19} Briefly, we included patients who were initially asymptomatic with respect to carotid artery disease, defined as the absence of transient...
ischemic attacks, amaurosis fugax, or stroke in patients’ recent history. Patients with a history of transient ischemic attacks or stroke were only eligible if the event occurred at least 12 months before inclusion and no residual or recurrent symptoms were identified by a neurologist.

### Study End Point

The primary clinical study end point was the occurrence of a first cardiovascular event, a composite of myocardial infarction, percutaneous coronary interventions, coronary artery bypass graft, stroke, and death during the follow-up period. As a secondary objective we investigated the association between hs-CRP and the occurrence of the triple-end point myocardial infarction, stroke, and death.

### Clinical Data

After patient identification at the ultrasound laboratory, medical history, and data from physical examination were recorded. We screened for current infectious or inflammatory diseases by evaluating patients’ clinical history and current symptoms. Clinical suspicion for infectious or inflammatory diseases prompted further specific investigations according to clinical judgment. Completeness and accuracy of all data were ascertained by 2 independent observers.

### Color-Coded Duplex Sonography and Grading of Internal Carotid Artery Stenosis

Duplex examinations at baseline and during follow-up were performed on an Acuson 128 XP10 with a 7.5-MHZ linear array probe (Acuson) by experienced technical assistants who were supervised by 2 of the authors. Two independent investigators determined progression of carotid atherosclerosis based on the baseline and follow-up duplex investigations. All duplex operators were blinded with respect to patients’ clinical data and laboratory findings. Duplex grading of the carotid stenosis was performed as described previously. The accuracy of our classification of the degree of stenosis with respect to angiography was assessed previously in our duplex laboratory in an independent cohort including 1006 carotid arteries. Using angiography as the gold standard, positive predictive values and negative predictive values ranged from 70% to 98%, and interobserver agreement was calculated by 2 independent observers.

### Laboratory Data

For determination of hs-CRP values, a high-sensitivity assay (N Latex CRP Mono; DADE Behring) with a detection level of 0.03 mg/dL and a coefficient of variation of 4.6% was used. Serum amyloid A (SAA) was measured by N Latex SAA (DADE Behring) with a detection level of 3.8 mg/L and a coefficient of variation of 6.4%.

### Surveillance Protocol

Patients were scheduled for a follow-up visit 6 to 9 months after the initial presentation for clinical re-examination, neurological history with standard questionnaires, duplex examination, and blood sampling. Patients with clinical suspicion of neurological events during the follow-up time were further evaluated by a neurologist and mandatory cranial computed tomography.

Thereafter, patients were clinically re-investigated every 6 months at the outpatient ward of our department until December 2005. A follow-up questionnaire was then sent to each patient during January 2006, re-evaluating the occurrence of cardiovascular events. Information from the follow-up questionnaire was validated by reviewing the original hospital discharge reports of corresponding readmissions because of cardiovascular events. If the follow-up questionnaire was not returned, personal telephone contact to the patients, their relatives, or to the treating physicians was established. Further information was obtained by reviewing the hospital discharge reports of any other re-admission during the follow-up period. The performance of percutaneous coronary interventions or coronary bypass graft was validated by review of the original procedure protocols. Outcome was assessed by 2 independent observers who were blinded with respect to patients’ baseline clinical and laboratory data.

### Statistical Methods

Continuous data are presented as the median and the interquartile range (range from the 25th to the 75th percentile), or the total range. Discrete data are given as counts and percentages. We used χ² tests, Mann–Whitney U tests, exact tests, and Spearman correlation coefficients for univariate analyses, as appropriate. Time-dependent variables were analyzed using the Kaplan–Meier method and compared by the log rank test. Multivariable Cox proportional hazards models were applied to assess the association between hs-CRP levels and the occurrence of a first cardiovascular event. Baseline variables that were imbalanced between patients with and without progressive carotid stenosis were entered into the models to adjust for potentially confounding effects. Results of the Cox models are presented as the hazards ratio and the 95% CIs. Additionally, we analyzed the association between hs-CRP and cardiovascular events in predefined strata according to patients’ cardiovascular risk profile at baseline. A 2-sided P<0.05 was considered as statistically significant. Calculations were performed with Stata (release 8.0; Stata) and SPSS for Windows (version 10.0; SPSS Inc).

### Results

#### Patient Characteristics and Progressive Disease

We enrolled 1363 eligible patients in the study. Of these, 95 patients (7%) had to be excluded because of missing duplex ultrasound follow-up data after the initial 6- to 9-month period (28 deaths; 67 refused the repeated duplex ultrasound investigation) and another 203 patients (15%) who were lost to clinical follow-up, leaving 1065 patients for the final analysis. Clinical baseline characteristics (age, sex, frequency of atherothrombotic risk factors, cardiovascular comorbidities, and baseline degree of stenosis) of the 298 patients with missing follow-up data were not significantly different compared with the study sample (data not shown).

Median age of the study population was 69 years (interquartile range, 61 to 76) and 668 patients (63%) were male. In 17% of the patients a history of stroke was found without residual or recurrent symptoms; in these patients, the time interval between previous stroke and inclusion in the study was a median of 5.2 years (range, 2.1 to 12.4). During the initial follow-up period of a median of 7.5 months (range, 6 to 9), progression of carotid lesions was found with duplex ultrasound in 93 of 1065 patients (8.7%). Patients with progressive disease were older, exhibited more frequently traditional cardiovascular risk factors, had higher levels of hs-CRP at baseline, and had a more extensive degree of atherosclerosis at baseline reflected by the history of stroke and symptomatic peripheral artery disease (Table). Baseline degree of stenosis was strongly associated with progressive disease; patients with a degree of stenosis ≥50% had 14% risk for disease progression as compared with 5% in patients with a degree of stenosis <50% (P<0.001).
Follow-Up for Cardiovascular Events

We recorded 381 cardiovascular events in 337 patients (27%) during a clinical follow-up period of a median of 3 years until occurrence of a first event (interquartile range, 2.5 to 3.5 years), including 42 myocardial infarctions (3.9%), 79 percutaneous coronary interventions (7.4%), 47 coronary bypass grafts (4.4%), 56 strokes (5.3%), and 157 deaths (14.7%). Cumulative event-free survival rates at 1, 2, and 3 years were 89% (95% CI, 0.87 to 0.91), 82% (95% CI, 0.80 to 0.84), and 73% (95% CI, 0.70 to 0.76).

Inflammation and Cardiovascular Events

Patients with higher baseline levels of hs-CRP had a significantly increased risk for cardiovascular events (P<0.001).

Being aware of several potential confounding factors, we calculated the adjusted risk for adverse cardiovascular outcome by multivariable Cox proportional hazards analysis, adjusting for age (years), sex (male/female), smoking (in categories), arterial hypertension, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, history of diabetes mellitus, family history of atherosclerosis, peripheral artery disease (Fontaine I, II, III), coronary artery disease (CCS I, II, III), history of myocardial infarction, history of stroke, baseline degree of carotid stenosis (right/left), baseline serum creatinine, statin treatment, and hs-CRP. By multivariable analysis, hs-CRP was significantly associated with cardiovascular events (P<0.001; Figure 1); adjusted hazard ratios for increasing quintiles of hs-CRP as compared with the lowest quintile were 1.41 (95% CI, 0.92 to 2.17), 1.76 (95% CI, 1.17 to 2.71), 2.17 (95% CI, 1.38 to 3.39), and 2.71 (95% CI, 1.71 to 4.30).
to 2.66), 2.22 (95% CI, 1.48 to 3.32), and 2.41 (95% CI, 1.61 to 3.60), respectively. Consistent with the overall effect, we observed significant associations between increasing quintiles of hs-CRP and cardiovascular events within the pre-defined strata according to patients’ baseline cardiovascular risk profile (Figure 2).

With respect to the occurrence of the secondary clinical end point, the composite of myocardial infarction, stroke, and death, adjusted hazard ratios for increasing quintiles of hs-CRP and cardiovascular events within the predefined strata according to patients’ baseline cardiovascular risk profile (Figure 2).

Consistent with hs-CRP, SAA also showed a significant association with adverse clinical outcome ($P < 0.001$). Adjusted hazard ratios for cardiovascular events for increasing quintiles of SAA as compared with the lowest quintile were 0.96 (95% CI, 0.62 to 1.48), 1.52 (95% CI, 1.06 to 2.17), 1.60 (95% CI, 1.11 to 2.37), and 1.70 (95% CI, 1.19 to 2.40), respectively.

**Discussion**

We found that inflammation measured by acute phase parameters was associated with morphological and clinical progression of atherosclerosis. Elevated baseline levels of hs-CRP seem to reflect activity of atherosclerotic disease and indicate an increased risk for systemic progression of atherosclerosis. Determination of hs-CRP levels in patients with high cardiovascular risk, as included in the present study, may add to conventional risk stratification strategies as the observed effect was independent of traditional cardiovascular risk factors.

Accumulating epidemiological data have evolved confirming that elevation of acute phase parameters like hs-CRP and SAA herald clinically relevant atherothrombotic events.\(^{5-18,25}\) The hs-CRP exerts pro-atherogenic effects and promotes endothelial cell activation, adhesion molecule expression, and resultant endothelial dysfunction.\(^{26-29}\) However, large population-based cohort studies failed to demonstrate an independent association between hs-CRP and early stages of atherosclerosis measured by carotid intima-media thickness.\(^{10}\) In contrast, particularly in patients with advanced stages of the disease, hs-CRP proved to be a robust predictor of adverse clinical outcome.\(^{18}\) Putting these findings together it seems reasonable to speculate that hs-CRP merely acts as a marker of advanced and progressive disease rather than as a relevant underlying pathophysiological substrate in early stages of atherosclerosis development.

In the present study, hs-CRP predicted morphological progression of carotid disease and clinical progression of atherosclerosis in the coronary and cerebrovascular vessel area. Elevated levels of inflammatory biomarkers likely uncover the presence of a multiple vulnerable lesions, thus identifying a "vulnerable patient" with several coexisting high-risk lesions in different arterial segments.\(^{5,7}\) Rothwell et al\(^{30}\) demonstrated a systemic predisposition to irregularity and rupture of atherosclerotic plaque independently of traditional vascular risk factors in a large series of patients with carotid stenosis. Elevation of hs-CRP and SAA indicates enhanced inflammation and may help to identify these patients who generally are at a high-risk for morphological and clinical progression of atherosclerotic disease. The concept of identifying vulnerable patients who are susceptible to cardiovascular adverse events early seems appealing, and measurement of inflammatory biomarkers may be a potent adjunctive tool for this purpose. In this context it seems particularly interesting that hs-CRP was predictive for cardiovascular events in all investigated subgroups, independently of traditional risk factors and baseline extent of the disease, suggesting clinical utility of this biomarker adjunctive to conventional clinical risk factors.
Limitations
Several limitations of the study have to be acknowledged. Several medications may influence levels of acute phase parameters, progression of atherosclerosis, and occurrence of clinical adverse events. In particular, statins and platelet inhibitors certainly play a relevant role in this context. Although we adjusted statistically for the use of statins at baseline, we are unable to exclude residual confounding by changes of the medication during the course of the study.

Conclusion
Inflammation was associated with morphological and clinical progression of atherosclerotic disease. Patients with elevated levels of hs-CRP exhibit an increased risk for adverse cardiovascular outcome due to clinical adverse events of progressive atherosclerotic disease.

Disclosures
None.
References

C-Reactive Protein Predicts Future Cardiovascular Events in Patients With Carotid Stenosis
Oliver Schlager, Markus Exner, Wolfgang Mlekusch, Schila Sabeti, Jasmin Amighi, Petra Dick, Oswald Wagner, Renate Koppensteiner, Erich Minar and Martin Schillinger

*Stroke*. 2007;38:1263-1268; originally published online February 22, 2007;
doi: 10.1161/01.STR.0000259890.18354.d2

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/38/4/1263