Joint Effects of C-Reactive Protein and Glycated Hemoglobin in Predicting Future Cardiovascular Events of Patients With Advanced Atherosclerosis

Martin Schillinger, MD; Markus Exner, MD; Jasmin Amighi, MD; Wolfgang Mlekusch, MD; Schila Sabeti, MD; Helmut Rumpold, MD; Oswald Wagner, MD; Erich Minar, MD

Background—C-reactive protein (CRP) and glycohemoglobin (HbA1c) are established risk factors for the development of cardiovascular disease. We investigated the joint effects of these parameters on cardiovascular outcome of patients with advanced atherosclerosis.

Methods and Results—We studied 454 patients with advanced atherosclerosis (median age, 69 years; 264 male). Cardiovascular risk profile, high-sensitivity CRP (hs-CRP), and HbA1c were obtained at baseline, and patients were followed for a median of 21 months (interquartile range, 13 to 26) for the occurrence of major adverse cardiovascular events (MACE) (myocardial infarction, percutaneous coronary interventions, coronary artery bypass graft, carotid revascularization, stroke, and death). We observed 166 MACE in 128 patients (28%). Cumulative event-free survival rates at 6, 12, and 24 months were 91%, 85%, and 73%, respectively. Adjusted hazard ratios for the occurrence of MACE according to increasing quartiles of hs-CRP and HbA1c were 1.35 (P = 0.31), 1.90 (P = 0.026) and 2.13 (P = 0.007), and 1.40 (P = 0.26), 1.81 (P = 0.059), and 2.36 (P = 0.023), respectively, compared with the lowest quartiles. Considering both parameters jointly, we found that patients with hs-CRP >0.44 mg/dL and HbA1c >6.2% (upper quartiles) were at highest risk for MACE, with each parameter adding to the prognostic information of the other.

Conclusions—Inflammation, indicated by hs-CRP, and hyperglycemia, indicated by HbA1c, jointly contribute to the cardiovascular risk of patients with advanced atherosclerosis. Patients with both hs-CRP and HbA1c in the upper quartiles (≥0.44 mg/dL and ≥6.2%, respectively) are at particularly high risk for poor cardiovascular outcome.

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Key Words: arteriosclerosis • complications • inflammation • diabetes mellitus

Predicting future cardiovascular events in high-risk patients with advanced atherosclerosis remains challenging, because traditional risk factors account for only a part of the individual’s susceptibility for an adverse outcome. Among the panel of new biologic markers indicative of impending cardiovascular events, compelling evidence suggests that high-sensitivity C-reactive protein (hs-CRP) represents a powerful cardiovascular risk predictor, with a predictive value exceeding that of LDL cholesterol. CRP, a marker of inflammation, identifies a different high-risk group than the traditional parameters of the metabolic syndrome and provides additive information on the cardiovascular risk. However, as yet, observations mainly refer to cardiovascular risk prediction in initially healthy subjects or patients with subclinical atherosclerosis.

Elevated glycohemoglobin A1 (HbA1c) is an established predictor for developing atherosclerosis beyond the risk associated with diagnosed diabetes. Accumulating data indicate that insights gained from the link between inflammation and hyperglycemia can yield predictive and prognostic information of considerable clinical utility. In this context, chronic inflammation recently emerged as a new pathophysiologic determinant for diabetes mellitus. Elevated CRP indicates an increased risk for the development of diabetes in healthy subjects, and patients with manifest diabetes mellitus exhibit increased levels of CRP. Both enhanced inflammation and hyperglycemia contribute to the development and progression of atherosclerosis and are frequently found in patients with clinically advanced disease. Given the interrelation between inflammation, hyperglycemia, and atherosclerotic disease, we speculated that CRP and HbA1c jointly contribute to the cardiovascular risk of patients with clinically advanced atherosclerotic disease.

Methods

Study Design

We prospectively enrolled in a cohort study all consecutive patients with advanced peripheral artery disease (PAD) who were admitted to...
the Angiology Department of a tertiary care university hospital from March 1, 2000 to March 1, 2001. Patients with symptomatic PAD in terms of intermittent claudication or critical limb ischemia as well as patients with asymptomatic PAD and a history of surgical or endovascular lower limb revascularization were eligible for the study. The study was approved by the local ethics committee, and all patients gave their written informed consent.

**Patient Data**
At admission, patient’s demographic data, clinical characteristics, and current medication were recorded by 2 independent observers. Data were evaluated for interobserver agreement at the day of patient’s discharge. In case of discrepancies, the patient was reevaluated by both investigators in consensus. Efforts to detect undiagnosed diabetes at admission were routine measurement of overnight fasting blood glucose and HbA1c levels. In case of pathologic findings (fasting glucose >110 mg/dL or HbA1c >6.0%), an oral glucose tolerance test was applied. During the hospital stay, repetitive blood pressure measurements were applied 2 to 4 times daily to detect undiagnosed hypertensive patients.

**Laboratory Parameters**
We used a high-sensitivity assay for measurement of serum hs-CRP (N Latex CRP Mono, DADE Behring) with a lower detection level of 0.03 mg/dL and a coefficient of variation of 4.6%. HbA1c was measured by high-pressure liquid chromatography (HPLC) separation of hemoglobin fractions with a reference value of 4.0% to 6.0% and a coefficient of variation of 1.8% on a Hi Auto A1c HA-8140 (KDK).

**Study End Point**
The study end point was the occurrence of major adverse cardiovascular events (MACE), a composite of myocardial infarction (MI), percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), stroke, carotid revascularization (carotid stenting or carotid endarterectomy), and death.

**Follow-Up Procedure**
Patients were clinically reevaluated routinely at 3, 6, and 12 months after hospital discharge and then annually at the outpatient ward of our department until December 2002. A follow-up questionnaire was then sent to each patient during December 2002 reevaluating the occurrence of MACE. Information from the follow-up questionnaire was validated by reviewing the original hospital discharge reports of corresponding readmissions attributable to MACE. If the follow-up questionnaire was not returned, personal telephone contact to the patients, their relatives, or the treating physicians was established. Additional information was obtained by reviewing the hospital discharge reports of any other readmission during the follow-up period. The performance of PCI, CABG, carotid stenting, and carotid endarterectomy 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.

**Definitions**
Diabetes mellitus was defined according to the criteria of the American Diabetes Association by pathologic oral glucose tolerance tests and was assumed to be present in patients with a history of diabetes taking antidiabetic medication. Arterial hypertension was defined according to the International Society of Hypertension classification, was evaluated by treadmill exercise testing, dobutamine echocardiography, myocardial scintigraphy, and coronary angiography in selected cases. MI was defined according to the consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. Stroke was defined as a neurological deficit that persisted longer than 24 hours, evaluated by a neurologist according to the modified Rankin stroke scale. Mandatory cranial computed tomography or, if available, MRI was used for confirmation of the diagnosis.

**Statistical Analysis**
Data are given as the median and the interquartile range (IQR, range from the 25th to the 75th percentile) or as counts and percentages. We used chi² tests, Mann-Whitney U tests, and the Spearman rank correlation for univariate analyses, as adequate. Event-free survival rates until the first cardiovascular adverse event are presented as Kaplan-Meier curves and compared by means of the log-rank test. A multivariate Cox proportional hazards model was applied to assess the joint effects of CRP and HbA1c on event-free survival, giving hazard ratios (HRs) and 95% CIs. Baseline variables were entered as possible predictor variables into the model to adjust for confounding effects if they were imbalanced between patients with and without MACE, indicated by a P<0.2, or had a clinical or biological association with CRP or HbA1c levels. We tested for interactions between baseline variables by multiplicative interaction terms using log likelihood ratio tests. A 2-sided P<0.05 was considered statistically significant. Calculations were performed with SPSS for Windows (Version 10.0, SPSS Inc.).

**Results**
We studied 454 of 467 patients (97%) who were admitted with advanced PAD during the study period. Thirteen patients (3%) had to be excluded because of missing follow-up data. These 13 patients with missing data closely resembled patients with complete data with respect to baseline demographic and clinical characteristics, without significant differences (data not shown). Follow-up data were derived from follow-up examinations in 412 patients (91%); in the remaining 42 patients (9%), follow-up was based on information from the follow-up questionnaire (n=42), personal phone calls (n=36), and review of hospital discharge letters from other institutions (n=22).

The median age of the 454 patients who were eligible for the final analysis was 69 years (IQR, 59 to 76), and 264 patients were male (58%). Diabetes mellitus was diagnosed in 181 patients (40%). Of these, 138 (78%) had known diabetes at the time of admission; in 43 patients (24%), diabetes was newly diagnosed. The median HbA1c level of patients with diabetes was 7.3% (IQR, 6.4 to 8.2), and 72 patients (40%) had an advanced diabetic nephropathy, as indicated by either an elevated serum creatinine (>1.2 mg/dL) or macroalbuminuria. At discharge, oral antidiabetic medication was administered in 122 patients (67%); 59 patients (33%) received insulin therapy.

**C-Reactive Protein and Glycated Hemoglobin**
Median levels of hs-CRP and HbA1c at admission were 0.43 mg/dL (IQR, 0.19 to 0.90) and 6.2% (IQR, 5.8 to 7.1), respectively. Hs-CRP and HbA1c were significantly but weakly correlated (r=0.17, P<0.001), indicating that 1 parameter contributed by approximately 3% to the variation of the other parameter.

**Risk Factors for MACE**
During the median follow-up period of 21 months (IQR, 13 to 26), 166 MACE occurred in 128 of 454 patients (28%).
including 17 MIs (4%), 43 PCIs (10%), 6 CAGBs (1%), 15 carotid artery stenting procedures (3%), 7 carotid endarterectomies (2%), 21 strokes (5%), and 57 deaths (13%). Of 57 case fatalities, 53 patients (92%) died of cardiovascular causes and 4 patients died of malignancies. Cumulative event-free survival rates (freedom from MACE) at 6, 12, 18, and 24 months were 91%, 85%, 80%, and 73%, respectively.

Demographic data and clinical characteristics comparing 128 patients with MACE and 326 patients without MACE are given in Table 1. The frequencies of diabetes mellitus, arterial hypertension, and hyperlipidemia refer to the hospital discharge diagnosis, including patients with previously known and newly diagnosed disease. Diabetes mellitus, antidiabetic medication, coronary artery disease, history of MI, and history of stroke were clinical variables associated with adverse cardiovascular outcome (Table 1). Higher HbA1c and hs-CRP values were laboratory parameters indicative of an increased cardiovascular risk. A gradually increased risk for MACE with increasing quartiles of both parameters was observed (Figure 1). Analyzing the associations between hs-CRP, other traditional cardiovascular risk factors, and MACE, we found that hs-CRP was associated with MACE independently of smoking, hyperlipidemia, and arterial hypertension (Figure 2).

### Table 1. Demographic Data and Clinical Characteristics of 454 Patients With Advanced Atherosclerosis Comparing Patients With and Without MACE

<table>
<thead>
<tr>
<th>Event-Free Survival</th>
<th>MACE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=326, 72%</td>
<td>n=128, 28%</td>
<td></td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>69 (58 to 76)</td>
<td>69 (60 to 76)</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td>189 (58%)</td>
<td>75 (59%)</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>25.7 (23.4 to 28.3)</td>
<td>26.0 (23.1 to 28.6)</td>
</tr>
<tr>
<td><strong>Arterial hypertension</strong></td>
<td>236 (72%)</td>
<td>101 (79%)</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td>120 (37%)</td>
<td>61 (48%)</td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td>6.2 (5.7 to 6.8)</td>
<td>6.5 (5.9 to 7.9)</td>
</tr>
<tr>
<td><strong>Medication at hospital discharge</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral antidiabetics</td>
<td>85 (26%)</td>
<td>37 (29%)</td>
</tr>
<tr>
<td>Insulin</td>
<td>35 (11%)</td>
<td>24 (19%)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>46 (14%)</td>
<td>26 (20%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>146 (45%)</td>
<td>53 (41%)</td>
</tr>
<tr>
<td><strong>Hyperlipidemia</strong></td>
<td>257 (79%)</td>
<td>98 (77%)</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>213 (183 to 244)</td>
<td>202 (179 to 253)</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mg/dL</strong></td>
<td>125 (101 to 157)</td>
<td>124 (94 to 143)</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mg/dL</strong></td>
<td>48 (41 to 58)</td>
<td>46 (39 to 56)</td>
</tr>
<tr>
<td><strong>Critical limb ischemia</strong></td>
<td>72 (22%)</td>
<td>33 (26%)</td>
</tr>
<tr>
<td><strong>Ankle brachial index</strong></td>
<td>0.58 (0.43 to 0.73)</td>
<td>0.56 (0.37 to 0.70)</td>
</tr>
<tr>
<td><strong>Coronary artery disease, CCS (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>81 (25)</td>
<td>32 (25)</td>
</tr>
<tr>
<td>II</td>
<td>52 (16)</td>
<td>38 (30)</td>
</tr>
<tr>
<td>III</td>
<td>6 (2)</td>
<td>4 (3)</td>
</tr>
<tr>
<td><strong>History of myocardial infarction</strong></td>
<td>65 (20)</td>
<td>46 (36)</td>
</tr>
<tr>
<td><strong>History of stroke</strong></td>
<td>27 (8)</td>
<td>22 (17)</td>
</tr>
<tr>
<td><strong>Statin therapy at hospital discharge</strong></td>
<td>163 (50)</td>
<td>72 (56)</td>
</tr>
<tr>
<td><strong>hs-CRP, mg/dL</strong></td>
<td>0.35 (0.17 to 0.82)</td>
<td>0.61 (0.24 to 1.37)</td>
</tr>
</tbody>
</table>

Data are given as counts and percentages or median and IQR (range from the 25th to the 75th percentile). CCS indicates Canadian Cardiovascular Society class. MACE includes myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, any stroke, carotid revascularization, and death. HbA1c indicates glycated hemoglobin.

* Hospital discharge diagnosis, including patients with previously known and newly diagnosed disease.

hs-CRP, HbA1c, and MACE

Adjusted HRs for the occurrence of MACE according to increasing quartiles of hs-CRP and HbA1c were 1.35, 1.90, and 2.13, and 1.40, 1.81, and 2.36, respectively, compared with the lowest quartiles (Table 2). The final model adjusted for age (quartiles), sex, smoking, arterial hypertension, LDL cholesterol (quartiles), history of MI, history of stroke, critical limb ischemia, statin therapy, and antidiabetic medication (none/oral antidiabetics/insulin). The variable Canadian Cardiovascular Society classification of coronary artery disease was not included in the final model because of collinearity with history of MI.
We then tested for interaction between hs-CRP, HbA1c, and MACE. The frequencies of MACE according to patients’ hs-CRP and HbA1c levels are given in Figure 3. Because the main effects of hs-CRP and HbA1c were examined with the use of indicator variables, interactions between these variables were considered by adding an interaction term of the products among these indicator variables (hs-CRP [in quartiles]/HbA1c [in quartiles]) to the multivariate model. Adding this term to the fully adjusted model, we found a significant change of the model fit as indicated by a log likelihood ratio test ($\chi^2$, 7.82; df, 2; $P = 0.02$). The patient population was therefore divided into 3 groups considering the patient’s hs-CRP and HbA1c levels jointly. Group I (n=135, 30%) consisted of patients with both hs-CRP and HbA1c in the lower 2 quartiles (hs-CRP $\leq$ 0.44 mg/dL, HbA1c $\leq$ 6.2%), group II (n=194, 43%) consisted of patients with one of the parameters in the upper quartiles (either hs-CRP $>$ 0.44 mg/dL or HbA1c $>$ 6.2%) and the other parameter in the lower quartiles, and group III (n=125, 27%) consisted of patients with both parameters in the upper quartiles (hs-CRP $>$ 0.44 mg/dL, HbA1c $>$ 6.2%). Adjusted HRs for the occurrence of MACE compared with group I were 1.34 (95% CI, 0.83 to 2.16; $P = 0.24$) for group II and 2.92 (95% CI, 1.75 to 4.46; $P < 0.001$) for group III, indicating that patients with both parameters in the upper quartiles had a substantially increased risk for poor cardiovascular outcome.

Discussion

We found that higher levels of hs-CRP and glycohemoglobin jointly increased the risk for cardiovascular adverse events in patients with advanced atherosclerotic disease. Patients with PAD and both hs-CRP and HbA1c in the upper quartiles (>0.44 mg/dL and >6.2%, respectively) were at particularly high risk for complications of atherosclerotic comorbidities.

The present study focused on high-risk patients with advanced atherosclerotic disease. Patients therefore exhibited considerably higher baseline hs-CRP and HbA1c values compared with healthy subjects1–3,15,16 and high cardiovascular event rates of 20% to 27% within 12 to 24 months. Nevertheless, similarly to the observations in healthy subjects or in patients with subclinical atherosclerosis,1–11 CRP also predicted cardiovascular events in these patients with clinically advanced disease.

Although atherosclerosis was formerly considered a bland lipid storage disease, substantial advances in basic and clinical studies have illuminated the role of inflammation and the underlying cellular and molecular mechanisms that contribute to atherogenesis.24–27 In this context, accumulating epidemiological data evolved indicating that elevation of CRP heralds atherothrombotic events.1–11 However, CRP was described not merely as a marker of atherosclerosis risk but also to directly promote endothelial cell activation, adhesion molecule expression, and resultant dysfunction.28–31 Our data suggest that in the presence of hyperglycemia, CRP is an even more powerful predictor of cardiovascular outcome, or, assuming a causal relationship, in the presence of hyperglycemia...
cemia, CRP exerts particularly harmful effects on the course of atherosclerotic disease. The mechanisms through which hyperglycemia might potentiate proatherogenic effects of CRP remain to be determined. However, an interrelationship between CRP, hyperglycemia, and atherosclerosis already has been described in experimental and clinical observations.1,2 On the one hand, chronic inflammation and diabetes mellitus are risk factors for atherosclerosis and atherothrombotic events.24–27,32,33 On the other hand, chronic inflammation is thought to be involved in the pathogenesis of diabetes.16–20 Hyperglycemia in states of high CRP may serve to exaggerate the proatherogenic effects of CRP28,29 and thus uncover a severe atherosclerotic phenotype.

Considering hs-CRP and HbA1c jointly as prognostic parameters may help to more adequately identify and better treat highest-risk patients with atherosclerosis.34,35 and optimization of the glycemic control is deemed necessary on the long term.32,33

Limitations
Some limitations of the present study have to be acknowledged. We do not believe that selection bias plays a major role, because complete baseline and follow-up data were available in 97% of patients and patients with missing follow-up data were comparable to the remaining patients. Information bias is unlikely, because outcome assessors were blinded to baseline and laboratory data. Furthermore, our study is necessarily of an observational nature. Accordingly, our results may be explained by confounding. Therefore, we tried to control for baseline imbalances (Table 2) by multi-

**TABLE 2. Multivariate Cox Proportional Hazards Model Assessing the Association Between hs-CRP, Glycated Hemoglobin (HbA1c), and MACE in 454 Patients With Peripheral Artery Disease**

<table>
<thead>
<tr>
<th>Laboratory Range</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP quartile 1*</td>
<td>&lt;0.19 mg/dL</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>hs-CRP quartile 2</td>
<td>0.19 to 0.44 mg/dL</td>
<td>1.37</td>
<td>0.77 to 2.44</td>
</tr>
<tr>
<td>hs-CRP quartile 3</td>
<td>0.44 to 0.90 mg/dL</td>
<td>1.83</td>
<td>1.06 to 3.18</td>
</tr>
<tr>
<td>hs-CRP quartile 4</td>
<td>&gt;0.90 mg/dL</td>
<td>2.18</td>
<td>1.27 to 3.74</td>
</tr>
<tr>
<td>HbA1c quartile 1*</td>
<td>&lt;5.8%</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td>HbA1c quartile 2</td>
<td>5.8% to 6.2%</td>
<td>1.30</td>
<td>0.73 to 2.30</td>
</tr>
<tr>
<td>HbA1c quartile 3</td>
<td>6.2% to 7.1%</td>
<td>1.59</td>
<td>0.90 to 2.83</td>
</tr>
<tr>
<td>HbA1c quartile 4</td>
<td>&gt;7.1%</td>
<td>1.85</td>
<td>1.06 to 3.20</td>
</tr>
</tbody>
</table>

*Reference category.
†Adjusted for age (quartiles), sex, smoking, arterial hypertension, LDL cholesterol (quartiles), history of myocardial infarction, history of stroke, critical limb ischemia, statin therapy, and antidiabetic medication.

Figure 3. Joint effects of hs-CRP protein and HbA1c on MACE (MI, PCIs, CABG, any stroke, carotid revascularization, and death) in 454 patients with PAD. Bars indicate the frequency of cardiovascular events during a median follow-up time of 21 months (IQR, 13 to 26) according to the levels of hs-CRP and HbA1c (in quartiles).
variante modeling. The possibility of residual or undetected confounding is small but cannot be ruled out completely.

Conclusion
Inflammation, indicated by hs-CRP, and hyperglycemia, indicated by HbA1c, jointly contribute to the cardiovascular risk of patients with advanced atherosclerosis. Patients with hs-CRP and HbA1c in the upper quartiles (>0.44 mg/dL and >6.2%, respectively) are at particularly high risk for poor cardiovascular outcome.

References
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