Prognostic Value of Abnormal Vasoreactivity of Epicardial Coronary Arteries to Sympathetic Stimulation in Patients With Normal Coronary Angiograms

Thomas H. Schindler, Burkhard Hornig, Peter T. Buser, Manfred Olschewski, Nobuhisa Magosaki, Matthias Pfisterer, Egbert U. Nitsche, Ulrich Solzbach, Hanjörg Just

Objective—We aimed to evaluate prospectively whether patients with normal coronary angiogram but abnormal epicardial vasoreactivity to cold pressor test (CPT) are at increased risk for cardiovascular events.

Methods and Results—Vasoreactivity in response to CPT and dilation of epicardial coronary arteries to intracoronary application of nitroglycerin were assessed quantitatively (percent change of luminal area, ΔDLA%) in 130 patients with normal coronary angiograms. Cardiovascular events (cardiovascular death, acute coronary syndrome, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary bypass grafting, ischemic stroke, or peripheral revascularization) were assessed as clinical outcome parameters over a mean follow-up period of 45±9 months. Based on their vascular responses to CPT, patients were assigned into the following 3 groups: group 1, patients with normal vasodilator response (ΔDLA >0%; n=37); group 2, patients with moderate vasoconstrictor response (ΔDLA between 0% and −15%; n=42); and group 3, patients with severe vasoconstrictor response (ΔDLA ≤ −15%; n=51). Although patients from groups 2 and 3 had significantly increased vasoconstrictor response to CPT (group 2, ΔDLA =6±3% and group 3, ΔDLA =−24±6% versus group 1, ΔDLA =11±9%; P=0.0001), they showed normal endothelial-independent epicardial vasodilation to intracoronary application of nitroglycerin similar to patients from group 1 (ΔDLA =39±16% and 34±14% versus 41±14%; P=NS, respectively). During follow-up, none of the patients from group 1 developed cardiac events. However, 7 cardiovascular events occurred in group 2 and 30 occurred in group 3 in 4 and 22 patients, respectively (P<0.0001, univariate by log-rank test). After adjustment for known risk factors for coronary artery disease, impaired epicardial coronary vasoreactivity to CPT remained significantly associated with the risk of developing cardiovascular events (P=0.040, multivariate by Cox regression model).

Conclusions—In patients with normal coronary angiogram, abnormal vasoreactivity of epicardial coronary arteries in response to sympathetic stimulation is associated with the risk of developing cardiovascular events. (Arterioscler Thromb Vasc Biol. 2003;23:000–000.)

Key Words: coronary disease □ C-reactive protein □ endothelium □ free radicals □ prognosis

Atherosclerotic heart disease is the most prevalent cause of morbidity and mortality in the Western society.1 It has been realized that the vascular endothelium plays an active and pivotal role in regulating aspects of the integrity and metabolism of the vascular wall, such as vascular structure and permeability, vasomotor tone, and hemoostasis.2 Experimental and clinical studies indicate that the functional integrity of the endothelium implicates antiatherosclerotic and anti thrombotic effects.2,3 Indeed, impaired endothelial-dependent vasomotion has been implicated in the development and progression of atherosclerosis.3–5 Clinically, coronary endothelial function can be assessed specifically with the muscarinic, endothelial receptor–mediated vasomotor response to intracoronary infusion of acetylcholine.2 Moreover, coronary vasoreactivity may be determined by cold pressor test (CPT), which has been shown to induce a complex sympathetic stimulation with a mixed, adrenergic receptor activation of the endothelium and smooth muscle cells of the vascular wall, leading to a vasoconstrictor and vasodilatory response, whereby the vasodilatory response is thought to be mediated through β-adrenoceptor with direct stimulation of nitric oxide synthesis and through a flow-dependent release of endothelial-derived nitric oxide.6–8 In this regard, coronary vasomotion in response to sympathetic activation may serve as an additional tool to probe the functional integrity of the vascular wall.6,9–10 Abnormal
endothelial-dependent vasoreactivity of epicardial coronary arteries has been shown to precede and accompany the development and progression of atherosclerosis with substantial diagnostic and prognostic implications.2,11-15

Hence, we aimed to evaluate prospectively whether patients with normal coronary angiogram but abnormal epicardial vasoreactivity to CPT are at increased risk for cardiovascular events.

Methods

Patient Population and Study Protocol

Between January 1996 and October 2000, 130 patients (91 men and 39 women; mean age, 59±7 years) referred for diagnostic cardiac catheterization and with normal coronary angiograms as defined by the absence of angiographic evidence for coronary artery disease were studied prospectively. Each patient was screened by complete history, physical examination, and laboratory analyses. Excluded were patients with a history of acute coronary syndrome including myocardial infarction, hypertrophic obstructive and nonobstructive cardiomyopathy, congestive heart failure, malignant hypertension, valvular heart disease, or significant endocrine, hepatic, renal, or inflammatory disease. Vasoactive medications, including calcium channel blockers, ACE inhibitors, long-acting nitrates, and β-blockers, were discontinued at least 24 hours before angiographic evaluation. Risk factors assessed at the time of assessment of endothelial function included hypertension, hypercholesterolemia, smoking, and family history. Hypertension was defined as a well-established history of chronically elevated blood pressure. Smoking was defined as a history of smoking for ≥2 pack-years.

Patient recruitment was performed by a team of cardiologists, who selected patients with normal coronary angiogram and established history of chronic systemic hypertension. Hypertension was defined as systolic blood pressure ≥140/90 mm Hg or treatment with dietary modification, β-blockers, diuretics, oral anti-diabetic (such as metformin, sulfonylurea, or α-glucosidase inhibitors), insulin therapy, or nitroglycerin, which might influence both vasodilator function and disease progression, were documented. Medical therapy with ACE inhibitors, lipid lowering, calcium-channel blockers, and, in addition, β-blockers, diuretics, oral anti-diabetic (such as metformin, sulfonylurea, or α-glucosidase inhibitors), insulin therapy, or nitroglycerin, which might influence both vasodilator function and disease progression, were documented.

Assessment of Epicardial Vascular Function

Selective coronary angiography was performed using a biplane, isocentric multidirectional digital angiographic system (Siemens BICOR-HICOR). End-diastolic images of coronary arteries were evaluated quantitatively from biplane views at baseline, after CPT with immersion of the left hand into ice water, and after nitroglycerin application, as described previously.17 In all patients, quantitative measurements were performed on biplane images in a 4-mm-long, nonbranching vessel segment of the proximal part of the left anterior descending coronary artery corresponding to vessel segment 12 according to the ACC/AHA guidelines.18 The accuracy, reproducibility, and interobserver and intraobserver variability of these measurements have been described previously.6,9,17 In each patient, quantitative angiographic evaluation at baseline and during the CPT established vasoreactivity of epicardial coronary arteries.17 Normal vasoreactivity was defined as vasodilation (percent change of luminal area, ΔDLA >0%), whereas an absent vasodilation or vasoconstriction during CPT was regarded as abnormal vasoreactivity (ΔDLA ≤0%).6 The maximal endothelium-independent vasodilator response of epicardial coronary arteries was tested by injection of 0.2 mg nitroglycerin intracoronally.

The decision to divide patients into different groups was based on the previously described association between changes in coronary blood flow to acetylcholine and scintigraphic myocardial perfusion defects.19,20 To assess the incidence of perfusion defects among patients with abnormal vasoreactivity, we applied subsequent criteria to group abnormal epicardial vasoreactivity to CPT. When defined as ΔDLA ≤−15% in response to CPT in this selected study population, 35 of 45 patients had exercise-induced perfusion defects, compared with 0 of 33 patients with ΔDLA between 0% and −15% and with 0 of 27 patients with ΔDLA >0%. In view of the previous investigation,19 patients were divided into the following 3 groups: group 1, normal epicardial vasoreactivity (ΔDLA >0%; n=37); group 2, moderate epicardial vasoconstriction (ΔDLA between 0% and −15%; n=42); and group 3; severe epicardial vasoconstriction (ΔDLA ≤−15%; n=51).

Long-Term Follow-Up

After a minimum of 12 months (range, 24 to 54; average, 45±9 months), all patients were contacted for end point assessment. Cardiovascular death, myocardial infarction, acute coronary syndrome, percutaneous transluminal coronary angioplasty, coronary bypass grafting, ischemic stroke, and peripheral revascularization were counted as clinical end points based on a questionnaire that was sent to patients and primary physicians. All information regarding potential cardiovascular events was validated by documentation, including the analysis of repeated coronary angiograms, hospital discharge letters, or hospital chart reviews. Death from any cause was documented. Cardiovascular death was defined as death attributable to myocardial or cerebral infarction or documented sudden cardiac death. Acute coronary syndrome was defined as hospitalization because of unstable angina pectoris of Braunwald classification IIB or IIIB. Myocardial infarction was defined as an elevation of creatine kinase levels ≥2 times the upper limit of normal or new ST elevations (≥0.1 mV) in ≥2 leads. PTCA was counted when performed in a newly developed (de novo) stenosis (luminal narrowing >50%) that was estimated as hemodynamically significant or when performed in acute coronary syndromes attributable to plaque rupture without hemodynamically significant coronary lesions during the follow-up. Ischemic stroke was defined as clinical evidence of stroke without intracranial hemorrhage on brain imaging studies. Medical therapy with ACE inhibitors, lipid lowering, calcium-channel blockers, and, in addition, β-blockers, diuretics, oral anti-diabetic (such as metformin, sulfonylurea, or α-glucosidase inhibitors), insulin therapy, or nitroglycerin, which might influence both vasodilator function and disease progression, were documented.
Statistical Analysis

Data of baseline characteristics, responses to CPT and NTG, and cardiovascular events during follow-up are expressed as mean ± SD or n (%). Comparison between groups was performed by the Wilcoxon or the Kruskal-Wallis test for quantitative variables and by the Fisher’s exact test for qualitative data. Cumulative event rates for groups 1 through 3 were estimated by the method of Kaplan-Meier and compared by means of univariate and multivariate Cox models.

For univariate comparisons of groups with no event, the log-rank test was used. The variables included in the multivariate model were age, sex, arterial hypertension, serum cholesterol levels, smoking, diabetes mellitus, and positive family history for coronary artery disease. Medical therapy, myocardial ischemia, as well as CRP serum levels were not entered into the multivariate model, because co-medication was highly correlated with its indications (coronary risk factors) whereas the latter were highly correlated with abnormal epicardial vasoreactivity in response to CPT. Results are presented as relative risks with corresponding 95% confidence intervals and probability values from Wald’s test. Statistical significance was assumed if the null hypothesis could be rejected at the $P \leq 0.05$ level.

Results

Clinical Characteristics at Baseline

The clinical characteristics of the study population are given in Table 1. One third of patients were female. Groups 2 and 3 had somewhat more coronary risk factors compared with group 1. In group 1, the lipid profile was within normal range. However, in groups 2 and 3, elevated total cholesterol and LDL levels were significantly higher ($P \leq 0.05$). There were no significant differences in total cholesterol, LDL, and HDL levels between groups 2 and 3 ($P = NS$). In all groups, glucose levels were within normal range but significantly higher in group 3 compared with the other groups ($P \leq 0.05$). Moreover, in group 3, CRP serum levels were significantly more elevated than in groups 1 and 2 ($P \leq 0.05$). However, the body mass index did not differ significantly between the groups ($P = NS$). In regard to the hemodynamics, CPT induced a similar significant increase in systolic and diastolic blood pressure.

### TABLE 1. Baseline Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>37</td>
<td>42</td>
<td>51</td>
</tr>
<tr>
<td>Age, y</td>
<td>58±6</td>
<td>61±6</td>
<td>58±8</td>
</tr>
<tr>
<td>Sex, female</td>
<td>14 (38)</td>
<td>11 (26)</td>
<td>14 (27)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±3</td>
<td>24±3</td>
<td>25±3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (8)</td>
<td>16 (38)</td>
<td>20 (39)*</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>7 (19)</td>
<td>21 (50)</td>
<td>35 (69)*</td>
</tr>
<tr>
<td>Smoking</td>
<td>4 (11)</td>
<td>15 (36)</td>
<td>33 (65)*</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0 (0)</td>
<td>5 (12)</td>
<td>8 (16)*</td>
</tr>
<tr>
<td>Positive family history of coronary artery disease</td>
<td>3 (3)</td>
<td>13 (31)</td>
<td>6 (12)*</td>
</tr>
<tr>
<td>Lipid status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol levels, mg/dL</td>
<td>189±54</td>
<td>223±40</td>
<td>238±40*</td>
</tr>
<tr>
<td>Serum LDL level, mg/dL</td>
<td>114±40</td>
<td>147±20</td>
<td>151±23*</td>
</tr>
<tr>
<td>Serum HDL level, mg/dL</td>
<td>52±11</td>
<td>49±11</td>
<td>47±13*</td>
</tr>
<tr>
<td>Triglyceride level</td>
<td>124±39</td>
<td>142±58</td>
<td>144±69</td>
</tr>
<tr>
<td>Glucose level, mg/dL</td>
<td>89±6</td>
<td>96±14</td>
<td>100±15*</td>
</tr>
<tr>
<td>CRP levels, mg/dL</td>
<td>0.63±0.68</td>
<td>1.09±0.69</td>
<td>2.64±0.92*</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP at rest</td>
<td>131±16</td>
<td>131±15</td>
<td>133±18</td>
</tr>
<tr>
<td>DBP at rest</td>
<td>78±8</td>
<td>75±6</td>
<td>75±10</td>
</tr>
<tr>
<td>SBP during CPT</td>
<td>144±19</td>
<td>147±17</td>
<td>145±19</td>
</tr>
<tr>
<td>DBP during CPT</td>
<td>77±7</td>
<td>77±8</td>
<td>78±9</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%).

* $P \leq 0.05$ between groups by Kruskal-Wallis test.

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

### Statistical Analysis

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### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>CPT</th>
<th>NTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5.24±1.34</td>
<td>5.81±1.39*</td>
<td>7.38±1.95</td>
</tr>
<tr>
<td>Group 2</td>
<td>5.73±2.14</td>
<td>5.35±1.87*</td>
<td>8.12±2.59</td>
</tr>
<tr>
<td>Group 3</td>
<td>5.68±1.68</td>
<td>4.27±1.17*</td>
<td>7.59±2.04</td>
</tr>
</tbody>
</table>

$\Delta$CPT: * $P = 0.0001$ and $\Delta$NTG: $P = 0.15$ (NS) between groups by Kruskal Wallis Test.
pressure in the different study groups \((P \leq 0.05)\), indicating a distinct sympathetic activation during CPT (Table 1).

**Coronary Vasoreactivity**

Table 2 depicts the mean changes of luminal area of all analyzed coronary segments at baseline, during CPT, and after i.c. nitroglycerin application in each study group. (The corresponding percent change of luminal area to CPT and nitroglycerin is shown in Figure 1.) The baseline values of mean luminal area did not differ significantly between the study groups \((P = 0.38)\). Sympathetic activation by cold pressor testing led to a significant increase of percent change of luminal area of epicardial arteries of 11\(\pm\)9\% (group 1), whereas group 2 demonstrated a decrease in luminal area of 2\(\pm\)6\% and group 3 a decrease in luminal area of 24\(\pm\)6\%.

The group comparison of the CPT-induced increase in mean luminal area in group 1 was significant compared with the decrease in luminal area in groups 2 and 3 \((P \leq 0.0001)\). Endothelium-independent vasodilation of epicardial coronary arteries induced by nitroglycerin was not significantly different between the different study groups (group 1, 41\(\pm\)14\% versus group 2, 39\(\pm\)16\% versus group 3, 34\(\pm\)14\%; \(P = NS\)).

**Correlation Between Responses of Epicardial Artery to CPT and CRP Plasma Levels**

The regression analysis between the change of luminal area of epicardial artery to CPT and CRP serum levels showed a significant inverse correlation in group 1 with normal epicardial vasoreactivity as well as combined for both groups 2 and 3 with abnormal epicardial vasoreactivity \((r = -0.427, P = 0.008\) and \(r = -0.784, P = 0.0001)\) (\(n = 37\) and \(n = 92\), respectively). However, the regression analysis in the 2 study groups of abnormal epicardial vasoreactivity revealed a significant correlation between epicardial vascular response to CPT and elevated CRP levels in group 3 \((r = -0.755, P = 0.0001)\) (\(n = 51\)) but not in group 2 \((r = -0.174, P = 0.270)\) (\(n = 42\)).

**Clinical Events During Follow-Up**

The mean duration of follow-up was 45\(\pm\)9 months. During follow-up, a total of 26 of 130 (20\%) patients experienced at least 1 cardiovascular event. Eighteen patients experienced 1 event, 5 patients had 2 events, and 3 had 3 events. During the follow-up, none of the patients in group 1 with normal vasodilation of epicardial arteries to CPT had

<table>
<thead>
<tr>
<th>TABLE 3. Cardiovascular Events During Long-Term Follow-Up (n=130)</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Sudden death</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
</tr>
<tr>
<td>Myocardial infarction (nonfatal)</td>
</tr>
<tr>
<td>Coronary angioplasty</td>
</tr>
<tr>
<td>Coronary bypass surgery</td>
</tr>
<tr>
<td>Peripheral arterial bypass surgery</td>
</tr>
<tr>
<td>Ischemic stroke</td>
</tr>
<tr>
<td>Patients with at least one cardiovascular event, n (%)</td>
</tr>
</tbody>
</table>
cardiovascular events. However, in patients with moderate vasoconstrictor response (group 2), 4 patients experienced cardiovascular events. In group 3 with severe vasoconstrictor response, 22 patients developed cardiovascular events.

The cumulative proportion of patients with cardiovascular events according to the vascular responses to CPT is shown in Figure 2 for groups 1 through 3. Overall, patients with abnormal vasoreactivity of epicardial vessels revealed a significantly higher incidence of cardiovascular events compared with patients with normal vasodilator response ($P < 0.0001$). Of note, the incidence of cardiovascular events increased with increasing vasoconstrictor responses to CPT in groups 2 and 3 compared with group 1 ($P < 0.0001$, respectively).

Determinants of Prognosis

On univariate analysis, abnormal vasoreactivity attributable to CPT ($P < 0.0001$), myocardial ischemia ($P < 0.0001$), CRP serum levels ($P < 0.0001$), arterial hypertension ($P < 0.001$), hypercholesterolemia ($P = 0.003$), glucose level ($P < 0.0005$), smoking ($P = 0.004$), diabetes ($P = 0.008$), and age ($P = 0.012$) were significantly associated with the occurrence of cardiovascular events during follow-up. A positive family history of coronary artery disease ($P = 0.263$), body mass index ($P = 0.797$), and sex ($P = 0.713$) were not associated with a poor outcome. In accordance with earlier results, we observed a strong correlation between abnormal vasoreactivity to CPT and myocardial ischemia and CRP serum levels, respectively. Therefore, the latter were excluded as candidates for the additional multivariate analysis. Furthermore, in patients of group 2 and 3, neither lipid-lowering therapy nor ACE-inhibitor therapy were associated with improved long-term outcome by Cox models.

According to the multivariate analysis, as shown in Table 4, the independent predictors of a poor outcome were abnormal vasoreactivity to CPT, hypercholesterolemia, hypertension, and age. Diabetes mellitus, a positive family history for coronary artery disease, smoking, and sex did not have a significant effect in the multivariate analysis.

Discussion

The present study is first to demonstrate that patients with normal coronary angiograms but moderate to severe abnormal vasoreactivity of epicardial coronary arteries in response to CPT are at increased risk for cardiovascular events. These observations are in keeping with the results of recent studies

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Kaplan-Meier analyses demonstrating the proportion of patients without cardiovascular events during the long-term follow-up in patients with normal (group 1, solid line), moderate (group 2, dotted line), and severe (group 3, dotted line) abnormal epicardial vasoreactivity to CPT. *$P < 0.0001$ for 3-group log-rank test.

**TABLE 4. Multivariate Cox Regression Analysis (n=130)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal epicardial vasoreactivity</td>
<td>0.95 (0.90–0.99)</td>
<td>0.040</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>5.13 (1.86–14.17)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>3.16 (1.07–9.35)</td>
<td>0.038</td>
</tr>
<tr>
<td>Sex</td>
<td>0.94 (0.37–2.42)</td>
<td>0.896 (NS)</td>
</tr>
<tr>
<td>Smoking</td>
<td>3.13 (0.89–10.98)</td>
<td>0.076</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.46 (0.38–5.67)</td>
<td>0.581 (NS)</td>
</tr>
<tr>
<td>Age</td>
<td>0.92 (0.87–0.98)</td>
<td>0.011</td>
</tr>
<tr>
<td>Positive family history of coronary artery disease</td>
<td>0.83 (0.23–2.97)</td>
<td>0.776 (NS)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval.
in patients with no apparent CAD and in patients with mild or more advanced stages of coronary atherosclerosis \cite{12,13,15,22} but extend the latter findings to abnormal epicardial vasoreactivity attributable to sympathetic activation in patients with normal coronary angiograms.

**Abnormal Epicardial Vasoreactivity and Prognosis**

On univariate analysis, apart from classical risk factors such as arterial hypertension, hypercholesterolemia, diabetes mellitus, and smoking, abnormal vasoreactivity of epicardial arteries in response to CPT was significantly associated with the occurrence of cardiovascular events. In addition, the multivariate analysis revealed that the increased risk of developing cardiovascular events in patients with normal coronary angiogram but with abnormal vasoreactivity of epicardial vessels to sympathetic activation was independent of the classical risk factors for CAD. Hence, with regard to the early development of atherosclerosis, this study provides evidence that abnormal vasoreactivity of epicardial arteries in response to CPT may be useful as an integrating index of the overall stress burden imposed by various risk factor states on the arterial wall. Noteworthy, in previous studies, \cite{12,22} even once atherosclerosis was present, abnormal endothelial-dependent vasoreactivity of coronary circulation remained an independent predictor of disease progression and cardiovascular events. The latter findings may also accord with recent observations \cite{13,11} that have shown a dissociation between endothelial vasomotor responses to acetylcholine and coronary atherosclerosis as assessed by intravascular ultrasound, supporting the contention that intracoronary ultrasound parameters alone may not predict cardiovascular events. Taken together, these studies clearly indicate that endothelial vasodilator dysfunction, implicating proatherosclerotic and prothrombotic effects, \cite{2,3} may provide an important mechanistic link between endothelial dysfunction and adverse cardiovascular outcomes. \cite{12,13,22}

The presented clinical study, however, cannot determine the exact mechanisms responsible for the association between impaired vasomotion of epicardial arteries to CPT and cardiovascular events. Abnormal epicardial vasoreactivity to sympathetic stimulation is certainly multifactorial in etiology but has been attributed in part to increased amounts of reactive oxygen species, which, apart from reducing the bioavailability of endothelial-derived nitric oxide associated with impaired endothelium-dependent vasodilator function, may lead to the induction of a whole array of inflammatory genes involved in the pathogenesis of atherosclerosis. \cite{15,23,25}

**Relation to C-Reactive Protein**

In line with previous results of forearm blood flow responses to acetylcholine-elevated C-reactive protein (CRP) serum levels, an acute-phase reactant produced in response to inflammatory cytokines \cite{23} correlated significantly with severe abnormal epicardial vasoreactivity to sympathetic stimulation. Moreover, in the actual study, elevated baseline levels of CRP were significantly associated with the occurrence of cardiovascular events, as reported previously. \cite{24,26,28} Thus, it is intriguing to speculate that elevated serum levels of CRP in this study population may act as a marker of systemic microinflammation that could reflect at least in part the presence of abnormal epicardial vasoreactivity to CPT, providing a link between systemic inflammation and cardiovascular events. \cite{29} In view of the latter findings, it has to be pointed out that although elevated CRP levels correlated significantly with abnormal epicardial vasoreactivity to CPT, the additional analysis of the single study groups revealed a significant correlation between elevated CRP levels and the group with severe abnormal epicardial vasoreactivity but not for the group with moderate abnormal vascular responses. These contrary findings may emphasize the complexity of the development of abnormal epicardial vasoreactivity with both genetic and environmental determinants, \cite{2,3} whereby in more advanced stages of abnormal epicardial vasoreactivity, markers of systemic microinflammation such as CRP may prevail. \cite{26,28} The association, however, of abnormal vasoreactivity of epicardial coronary arteries in response to CPT and clinical cardiovascular events suggests functional abnormalities of the vascular wall as a promising target of therapy. \cite{21,29,31} Ongoing multicenter, large-scale trials will have to determine whether an improvement in abnormal endothelial-dependent vasoreactivity of the coronary circulation, for example by statin or ACE inhibitor therapy, is indeed directly related to the well-established beneficial effects of these drugs on the prognosis in patients with atherosclerotic heart disease. \cite{32}

**Endothelium-Independent Dysfunction and Prognosis**

Of note, nitroglycerin-induced vasodilation was not significantly reduced in the described study groups with abnormal vasoreactivity of epicardial coronary arteries, indicating preserved endothelium-independent vasodilator function. This is in contrast to recent findings, \cite{12} which showed that significantly impaired vasodilator capacity of epicardial vessels in response to exogenous nitric oxide was also an independent prognostic parameter for cardiovascular events, apart from the presence of endothelial dysfunction. Indeed, previous investigations have reported a significant inverse relationship between endothelium-independent vasodilation in response to exogenous nitroglycerin and endothelium-dependent vasodilation. \cite{12,33,34} However, the previous study \cite{12} to assess the prognostic significance of endothelial dysfunction of coronary circulation included patients with more advanced stages of atherosclerosis, indicating that impaired vasodilator function in patients with more advanced disease may not be confined to endothelium-mediated mechanisms but may also implicate impairment in smooth muscle cell vasodilator function.

**Myocardial Perfusion Defects and Abnormal Epicardial Vasoreactivity**

Noteworthy, in the present study, patients with normal coronary angiograms but abnormal coronary vasoreactivity and regional myocardial perfusion defects were at increased risk for cardiovascular events. This may be somewhat surprising, because patients with exertional angina pectoris, a positive response to exercise testing, and normal coronary angiogram, commonly referred to as the so-called syndrome.
X, have been shown to be associated with a benign long-term prognosis. Different inclusion criteria and patient selection may explain these differences. Patients described by previous studies did not undergo testing of coronary vasoreactivity to CPT or other more specific stimuli, such as acetylcholine. Thus, it is not known whether the syndrome X in these patients was related to endothelium-dependent microvascular dysfunction, reduced coronary flow reserve, or merely the results of an abnormal pain perception. The actual finding of an association between severe abnormal coronary vasoreactivity to CPT and exercise-induced regional myocardial perfusion defects is supported by previous findings. Thus, within a wide range of patients with so-called syndrome X, the assessment of abnormal vasoreactivity to CPT may indeed help identify a subset of patients at higher risk for future cardiovascular events and, therefore, should be subjected to additional investigations.

Limitations
There are several limitations to be considered in interpreting our data. First, the results presented were obtained from a sample of highly selected patients referred for coronary angiography to evaluate persistent chest pain. Hence, the proportion of patients with abnormal vasoreactivity of epicardial vessels in response to CPT associated with myocardial perfusion defects during exercise may be different among patients being examined for chest pain in other settings. Second, it can be observed that the 3 groups are significantly different with regard to some coronary risk factors. This fact may univariately explain some of the differences observed between groups in the rates of cardiovascular events. However, we were able to show in a multivariate analysis adjusting for the effect of other coronary risk factors that abnormal vasoreactivity to sympathetic stimulation remained significantly associated with the risk of developing cardiovascular events. Admittedly, the number of patients in our study seems to be relatively small and the probability value of 0.04 for abnormal epicardial vasoreactivity in the multivariate analysis may also be tending to borderline significance to predict cardiovascular events. Hence, although the actual study represents the largest patient cohort study to assess vasoreactivity of epicardial coronary arteries in response to CPT in patients with coronary risk factors and normal coronary angiograms with a sufficiently long-term follow-up, large-scale trials are needed to draw definite conclusions. Third, because distinct differences in second messenger systems of the individual stimuli to assess coronary vasomotion are involved, epicardial vascular responses to CPT cannot be regarded as an alternative approach for the specific determination of endothelial-dependent vasomotion to intracoronary infusion of acetylcholine but may serve as an additive test to provide information in regard to sympathetic activation. Fourth, because we did not perform intravascular ultrasound to assess the vascular wall structure in patients with normal coronary angiogram, diffuse atherosclerosis or eccentric plaque may have been present.  

Conclusions
In patients with normal coronary angiogram, abnormal vasoreactivity of epicardial coronary arteries in response to sympathetic stimulation is associated with the risk of developing cardiovascular events. Thus, epicardial coronary vasoreactivity to cold pressor test may add important diagnostic and prognostic information additional to that derived from angiographic and coronary risk factor assessment.

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