Coronary vasomotor response is related to the angiographic extent of coronary sclerosis in patients with stable angina pectoris

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ABSTRACT

Disturbed vasomotor function in coronary arteries has clinical importance in early stages of coronary artery disease (CAD), as it may contribute to the potential risk for an ischaemic coronary event. In the present study, we have investigated the relationship between coronary vasomotor function and the extent of CAD. The response to acetylcholine and nitrate infusion was assessed by quantitative coronary angiography. The extent of CAD was categorized into two groups: minor CAD (normal coronary arteries and vessel wall irregularities) and significant CAD (one-, two- and three-vessel disease). A total of 277 patients with stable angina pectoris, referred for a first diagnostic coronary angiography, were eligible for analysis (mean age 57 years, 61% male). The response to nitrate was significantly impaired in patients with significant CAD (P < 0.001). On the other hand, the response to acetylcholine was not different between the two groups (P = 0.12); however, a trend between the response to acetylcholine and the extent of CAD was observed in patients without a previous infarction (P = 0.07), which was a significant interaction variable. Furthermore, a significant relationship between coronary vasomotor response and the number of cardiovascular risk factors was observed (P < 0.05). In conclusion, in a heterogeneous group of patients, coronary vasomotor function measured by nitrate infusion was more strongly associated with the extent of CAD and the number of risk factors than the response to acetylcholine. These data suggest that, in patients with advanced atherosclerosis or multiple risk factors, the vasomotor dysfunction is not solely restricted to the endothelium.

INTRODUCTION

Historically, the ideas about the pathophysiological mechanism of myocardial ischaemia have varied between static or dynamic occlusions of coronary arteries. At present, the most plausible cause appears to be a combination of both explanations [1]. To evaluate the dynamic component, coronary vasomotor testing has been introduced. After the pioneering years of coronary angiography, the application of spasm provocation protocols using ergotalkaloids during standard coronary angiography were described by several high-volume centres [2–5]. At present, intracoronary acetylcholine infusion in combination with quantitative coronary angiography (QCA) is the most commonly used method to assess coronary vasomotor function.

Key words: acetylcholine, atherosclerosis, coronary artery disease, endothelium, myocardial infarction, nitroglycerin.

Abbreviations: ACE, angiotensin-converting enzyme; CAD, coronary artery disease; LAD, left anterior descending coronary artery; MI, myocardial infarction; QCA, quantitative coronary angiography.

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angiography (QCA) has become a generally accepted method to assess vasomotor function. ‘Endothelial dysfunction’ is thought to be present when acetylcholine induces vasoconstriction [6]. Endothelial dysfunction is considered to be the first step in the cascade of atherosclerosis [5] and has been shown to be related to traditional cardiovascular risk factors [7–10] and to predict future coronary events [11–13].

Ludmer and co-workers [14] reported for the first time in 1986 an association between the presence of atherosclerosis and an impaired response to acetylcholine. However, their study population consisted of 14 patients and four female control subjects. These results are partly confirmed by several groups in patients with early signs of atherosclerosis [15,16]. No study has yet validated the data of Ludmer et al. [14] in a patient population with more advanced atherosclerosis.

In contrast with the response to acetylcholine, the response to nitroglycerin, which is an endothelium-independent smooth-muscle-dependent vasodilator, has always been considered to remain within the normal ranges during the process of atherogenesis. However, Schächinger and co-workers [12] found that the response to nitrate has a strong prognostic value in patients with mild coronary artery disease (CAD), which suggests that the endothelium is not solely involved. No data are available regarding the extent of CAD and the response to nitroglycerin. We hypothesized that the response to nitroglycerin, in parallel with the response to acetylcholine, is related to the extent of CAD. Therefore the objective of the present study was to investigate further the key features of coronary vasomotor function and the extent of CAD.

**METHODS**

**Study population**

Patients between 18 and 80 years of age with angina pectoris, referred for their first diagnostic coronary angiography since November 1996, were considered for enrolment in the Intervention Cardiology Risk Stratification (ICaRiS) study. Patients with unstable angina, recent (<3 months) myocardial infarction (MI), valvular heart disease requiring surgical intervention, clinical evidence of heart failure, a history of previous coronary intervention or any serious disease that may interfere with the follow-up were excluded. Excluded from acetylcholine infusion were patients with significant left main coronary artery narrowing, severe angiographic abnormalities with ischaemic electrocardiographic changes and/or progressive angina pectoris during diagnostic catheterization.

Written informed consent was obtained from all patients before the study, and the Institutional Review Board of the University Hospital of Groningen approved the study protocol. The study was consistent with the principles outlined in the Declaration of Helsinki.

**Definitions**

The following traditional cardiovascular risk factors were predefined. Male gender and an age above 60 years were defined as risk factors. The smoking status was divided into two categories: no cigarette smoking for >3 months or currently a cigarette smoker. Hypercholesterolaemia was defined as a fasting serum cholesterol value >6.5 mmol/l or a history of hypercholesterolaemia for >3 months that led to the initiation of lipid-lowering therapy by the primary physician. Hypertension was defined as a systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg (measured twice), or a history of high blood pressure that led to the initiation of antihypertensive therapy by the primary physician. Diabetes was defined as high blood glucose levels requiring glucose-lowering therapy. Family history of CAD was defined as evidence of the disease in a parent or sibling before 60 years of age at the time of diagnosis. Patients had a history of a MI when pathological Q-waves >0.04 s in duration were present in two adjacent leads on the 12-lead ECG or had a history of hospitalization with ST-segment elevation >0.1 mV measured 80 ms after the J-point in two adjacent leads on the 12-lead ECG, eventually supported by biological markers of myocardial necrosis. A stenosis was defined as >50% luminal narrowing in a coronary artery. The severity of CAD was categorized in two groups: minor CAD (normal coronary arteries and vessel wall irregularities) and significant CAD (one-, two- and three-vessel disease). To investigate the association between vasomotor response and cardiovascular risk factors, we divided the number of traditional risk factors into three groups: no risk factors, 1–3 risk factors and 4–7 risk factors. The vasomotor response was the change in diameter in response to a maximal concentration of acetylcholine and nitroglycerin respectively, expressed as the percentage of the mean baseline diameter. A negative response represents vasoconstriction.

**Coronary angiography**

Before the coronary angiogram, vasoactive agents were discontinued for at least 3 days (24 h if recurrent angina was expected). The vasoactive agents used were long-acting nitrates, calcium channel blockers, angiotensin-converting-enzyme (ACE)-inhibitors and angiotensin II-receptor blockers (adrenergic blockers were allowed). Smokers did not smoke for at least 4 h before examination. Using a standard percutaneous femoral approach, a 6 French Judkins diagnostic catheter (Cordis, Roden, The Netherlands) was advanced into the left main coronary artery. Intracoronary nitroglycerin was not given before the diagnostic catheterization procedure.
Vasomotor function test
After completion of the diagnostic coronary angiography, the diagnostic 6 French Judkins catheter was left in the left main coronary artery. For accurate vasomotor response measurements, the subsequent angiographic recordings were made with 25 frames/s, and care was taken to have an adequate part of the catheter visible for calibration. A baseline coronary angiogram was done to visualize the proximal left anterior descending artery (LAD). Acetylcholine chloride (concentration 0.16 μg/ml; Clinalfa AG, Läufelfingen, Switzerland) was then infused through the catheter for at least 3 min. This gave an infusion of $120 \times 10^{-8}$ mmol/min, resulting in a final concentration in the coronary blood of $1 \times 10^{-6}$ mol/l (with the assumption that the blood flow in the left main coronary artery was 120 ml/min). This procedure was repeated using $10^{-7}$ mol/l and $10^{-6}$ mol/l acetylcholine chloride. Finally, the response to nitrate was recorded 1 min after an intracoronary bolus of 0.5 mg of nitroglycerin. The response to both stimuli was measured by automatic contour detection technique (QCA) in the non-stenotic proximal segment of the LAD. QCA was performed by a validated automatic contour detection technique (CMS; Medis Co., Nuenen, The Netherlands) as described previously [17]. Mean segment diameter of the proximal LAD was determined in millimetres.

Statistical analysis
Values are presented as means ± S.D. and the number of valid observations by the subgroups of patients based on their CAD status assessed by coronary angiography. Categorical data are presented as per group percentages. Differences between disease status subgroups were evaluated by Student’s t test for the normally distributed continuous variables, or with the Wilcoxon two-sample test if data were skewed. For categorical data, the Fisher’s exact test or the $\chi^2$ test was used.

The relationship between coronary vasomotor response and extent of CAD, as well as other known factors associated with the coronary vasomotor response were evaluated using one way analysis of variance. In addition, multivariate analysis of variance was performed allowing the evaluation of potential interactions influencing the relationship between coronary vasomotor response and extent of CAD. $P < 0.05$ was considered statistically significant. SAS version 6.12 (Cary, NC, U.S.A.) was used for all statistical analyses.

RESULTS
A total number of 312 patients undergoing a first diagnostic angiogram for the suspicion of CAD were included. Owing to technical failure, nine values of the vasomotor response of the LAD were missing. Furthermore, 13 patients did not undergo acetylcholine infusion because of left main or severe three-vessel disease, and 13 patients were excluded because of a recent MI (between 11 days and 3 months before the diagnostic angiogram). These patients were excluded from further analysis. The remaining 277 patients had a mean age of 57 ± 12 years, and 169 (61 %) of them were male. In this group, 30.0 % of the patients had normal smooth coronary arteries, 21.3 % had vessel wall irregularities (< 50 % luminal narrowing in all coronary arteries), 20.9 % had one-vessel disease, 18.8 % had two-vessel disease and 9.0 % had three-vessel disease. Surprisingly, a substantial number of patients had normal coronary arteries. A positive exercise test was present in 26.5 % of these patients, a positive nuclear test in 25.3 % and 90.4 % had angina pectoris according to the Canadian Cardiovascular Society classification. A total of 21 % used ACE inhibitors, 65 % used aspirins, 65 % used β-blockers, 42 % used calcium antagonists, 16 % used diuretics, 27 % used long-acting nitrates and 31 % used lipid-lowering drugs. Patients with significant CAD were significantly older than patients with minor CAD. Furthermore, more males were present in the group with significant CAD, and more patients had a history of hypercholesterolaemia, hypertension, diabetes mellitus and a previous MI in comparison with the group with minor CAD (Table 1). The nitrate response was significantly different between the two groups (mean response to nitrate was $11.74 ± 9.69$ % in patients with minor CAD and $6.70 ± 7.42$ % in patients with significant CAD; $P < 0.001$). In contrast, the response to acetylcholine was not different between the two groups. The mean epicardial luminal area change after infusion of the maximum dose of acetylcholine was $-4.34 ± 12.16$ % in the group with minor CAD compared with $-5.66 ± 10.43$ % in the group with significant CAD ($P = 0.12$). Vasoconstriction to acetylcholine occurred in 186 (65 %) of the subjects. Interestingly, if vasoconstriction to acetylcholine was observed, a significant disturbed nitrate response was demonstrated. The mean response to nitrate was $13.36 ± 8.69$ % in patients with vasodilatation and $7.29 ± 8.49$ % in patients with vasoconstriction after infusion of acetylcholine ($P < 0.001$).

In order to evaluate the relationship between cardiovascular risk factors and the response to acetylcholine and nitrate, we investigated subgroups based on the number of traditional cardiovascular risk factors (Figure 1). A stepwise negative relationship was observed between the coronary vasomotor response and the number of risk factors ($P < 0.05$). It is important to notice that the vasodilatative response to acetylcholine in the group without cardiovascular risk factors were all women under 60 years of age.
Table 1  Baseline characteristics of the total study population and the population separated by the extent of CAD
Values shown are means ± S.D. or total number (percentage).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall (n = 277)</th>
<th>Minor (n = 142)</th>
<th>Significant (n = 135)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>169/108</td>
<td>74/68</td>
<td>95/40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 ± 12</td>
<td>53 ± 12</td>
<td>62 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>79 (29 %)</td>
<td>45 (32 %)</td>
<td>34 (25 %)</td>
<td>0.29</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>139 (50 %)</td>
<td>50 (41 %)</td>
<td>81 (60 %)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypertension</td>
<td>147 (45 %)</td>
<td>62 (44 %)</td>
<td>85 (63 %)</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>30 (11 %)</td>
<td>7 (5 %)</td>
<td>23 (17 %)</td>
<td>0.002</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.29 ± 1.30</td>
<td>5.11 ± 0.97</td>
<td>5.47 ± 1.55</td>
<td>0.12</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>123 (44 %)</td>
<td>62 (44 %)</td>
<td>61 (45 %)</td>
<td>0.81</td>
</tr>
<tr>
<td>Previous MI</td>
<td>53 (19 %)</td>
<td>8 (6 %)</td>
<td>45 (33 %)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 1  Median responses to acetylcholine and nitroglycerin compared with the number of traditional cardiovascular risk factors

In a multivariate analysis with acetylcholine response as the dependent variable, a significant interaction between the extent of CAD and a previous MI was observed (P < 0.001). Based on this result, data are described further in strata by the presence or absence of a previous MI. Of note, the multivariate analysis of the nitrate response did not show any significant interactions between the extent of CAD and traditional risk factors. A total of 53 patients (19 %) had a history of having an 'old' MI (an MI occurring more than 3 months before angiography), with eight (6 %) of these patients in the minor CAD group and 45 (33 %) in the group with significant CAD. The median interval between the documented MI and the diagnostic coronary angiography was 14.1 (range, 5.0–70.0) months.

The response to acetylcholine, divided by the presence of a previous MI, is shown in Figure 2.

DISCUSSION

In the present study, coronary vasomotor function was investigated in a heterogeneous group of patients. The main finding of our study is that the nitrate response was significantly impaired in patients with significant CAD. On the other hand, the response to acetylcholine was not different between the two groups. However, the presence of an old MI was a significant interaction variable between the extent of atherosclerosis and the response to acetylcholine. In patients without a previous MI, a trend between the response to acetylcholine and the extent of CAD was observed. Interestingly, in the group with a previous MI (more than 3 months before vasomotor testing), an inverse relationship between the response to acetylcholine and the extent of CAD was observed. Moreover, we found an inverse relationship between
vasomotor function and the number of traditional cardiovascular risk factors.

The impaired vasodilator response to nitrate in patients with significant CAD and multiple risk factors suggests that vasomotor dysfunction in high-risk patients is not only present at the endothelial level, but might also be located in the smooth muscle cells. This observation is in line with the data of Schächinger et al. [12], who showed that an impaired nitrate response was more strongly related to the occurrence of cardiovascular events than the vasoconstrictive response to acetylcholine.

An explanation for this finding could be the limited distension of the vessel wall, due to fibrosis, or a change in baseline vasomotor tone in patients with severe atherosclerosis. Another, more likely, explanation for this impaired nitrate response is a specific defect in smooth muscle cell function and, in particular, of the soluble guanylate cyclase/cGMP signalling system [18]. Moreover, it has been shown [19] that certain cardiovascular risk factors increase the production of the endothelial superoxide anion, which may decrease the availability of nitroglycerin/nitric oxide to the smooth muscle cell.

The coronary endothelium plays an important role in the integrity and progression of myocardial perfusion and, hence, myocardial function. Nitric oxide produced by the endothelium is a prerequisite for vasodilatation and inhibits vascular smooth muscle cell proliferation, monocyte adhesion and platelet aggregation. For these reasons, the impaired response to acetylcholine observed after MI might have important implications for risk stratification.

In most studies, coronary vasomotor function has been mainly studied in small or selected groups of patients. On the other hand, few large scale studies in heterogeneous patient groups have been conducted [2,5,20,21]. Bertrand et al. [2] were the first to describe the effect of a previous MI on ergonovine-induced spasm, a form of 'vasomotor dysfunction'. They evaluated intracoronary ergonovine in eight different groups of patients. In the group of patients with an old MI, they found that an additional recent (<6 weeks previously) infarction coincided with a high incidence of coronary spasm. Spasm was provoked by ergonovine in 20% of these patients. An MI >6 weeks previously was not related to such a high incidence of spasm (6.2%). Sueda et al. [21] studied, in a Japanese population, amongst others, a group of patients with a recent MI (4 weeks) and a group with an older infarction (>1 month; 10.6 ± 7.4 months, range 2–36 months). In both groups, comparable incidences of acetylcholine-induced spasm were found (37.5 and 37.8). In these two studies [2,21], no consideration about the relationship between post-MI, vasomotor dysfunction and the extent of CAD was reported.

Our present results indicate that coronary vasomotor dysfunction is present in patients with a previous MI after a longer period than has been described so far. Interestingly, the response to acetylcholine is more disturbed in patients without significant CAD. This observation is also underlined by studies on the prognostic significance of endothelial function in patients with mild CAD [11–13]. Furthermore, it is known that MI develops predominantly in coronary vessels with mild or moderate stenosis and especially in coronary vessels known to vasoconstrict after infusion of acetylcholine. In fact, an impaired response to acetylcholine was found to be the most important predictor for future MI [5]. Considering the aforementioned arguments, the results of our present study may provide a mechanistic rationale for the implementation of aggressive secondary prevention in subjects with minor CAD and an old MI.

A limitation of this present study is the descriptive nature of the data. Therefore we cannot state any conclusion about the precise relationship in time between the impaired response to acetylcholine and the occurrence of a MI; consequently, the data shown are only hypothesis-generating. Longitudinal study designs are necessary to determine the underlying mechanism. In addition, the number of subjects (n = 8, 2.6%) with a previous MI and no significant CAD is in line with previous studies [22] but, due to this small number, no significance could be reached in our present study. ACE inhibitors as well as statins are known to improve coronary vasomotor function [23,24]. Although all vasoactive drugs were stopped prior to the study, a longer-term benefit cannot be excluded.

In conclusion, our present data suggest that, in patients with significant CAD or multiple cardiovascular risk factors, coronary vasomotor dysfunction is present and that this dysfunction is not solely restricted to the endothelial layer.

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