Aspects of endothelial function testing in coronary vascular disease
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CHAPTER 6

Vasomotor testing in symptomatic coronary artery disease: the relation with a previous myocardial infarction

submitted for publication

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CHAPTER 6

ABSTRACT

**Background** Disturbed endothelium dependent vasodilatation in coronary arteries, i.e. endothelial dysfunction, is considered to be the first step in the development of atherosclerosis and may contribute to the potential risk for a future ischemic coronary event. On the other hand, it is unknown how the presence of a previous coronary event, such as an old myocardial infarction, influences the endothelial function. The objective of this study was to investigate the relation between endothelial function and the extent of coronary artery disease (CAD) in the presence and absence of a previous myocardial infarction.

**Methods** In this cross-sectional study, stable angina pectoris patients referred for a first diagnostic coronary angiography, were included. Endothelium dependent vasodilatation in response to intracoronary acetylcholine infusion was assessed by quantitative coronary angiography.

**Results** A total of 286 patients were included (mean age 57 year (±11), 181 males (62%)). Fifty-five patients (19%) had a more than 3 months old myocardial infarction. In the absence of a previous myocardial infarction, the impairment of endothelium dependent vasodilatation was related to the extent of CAD. In contrast, in the presence of an old myocardial infarction, endothelium dependent vasodilatation was more impaired in case of minor CAD than in patients with severe CAD.

**Conclusions** Endothelial dysfunction is related to the extent of CAD in patients without a previous infarction and inversely related in patients with a previous infarction. These findings support the clinical importance of coronary endothelial vasomotor testing after myocardial infarction, especially in patients with minor coronary artery disease.
INTRODUCTION

Patients presenting with chest pain are often referred to the cardiologist for evaluation of potential underlying coronary artery disease (CAD). The typical presentation of angina pectoris is a strangling retrosternal pain that radiates down the inner aspect of the left arm, lower jaw, neck or teeth, and can be provoked by exercise or cold exposure. In addition to the clinical presentation, diagnostic tests such as exercise testing, or stress myocardial-imaging techniques can be used to substantiate the probability of CAD. The final diagnosis however has to be determined by coronary angiography. More than 40 years following the introduction of coronary angiography this diagnostic modality remains the golden standard for the detection of CAD.

Historically, the ideas about the pathophysiologic mechanism of myocardial ischemia varied between static or dynamic occlusions of coronary arteries. At present, the most plausible cause appears to be a combination of both explanations (1). After the pioneering years of coronary angiography the application of spasm provocation protocols using ergotalkaloids during standard coronary angiography was described by several high volume centres (2-5). Currently, intracoronary acetylcholine infusion in combination with quantitative coronary angiography has become a generally accepted method (6). Subtle changes in endothelium dependent vasodilatation, or 'endothelial function' can now be assessed. 'Endothelial dysfunction' is present when acetylcholine induces vasoconstriction (6). Endothelial dysfunction has been shown to be related to risk factors such as smoking, hypertension, and hypercholesterolemia (7-10) and also with the degree of atherosclerosis (11). Endothelial dysfunction is considered to be the first step in the development of atherosclerosis (5;12) and may contribute to the potential risk for a future ischemic coronary event. On the other hand, it is unknown how the presence of a previous coronary event, such as an old myocardial infarction, influences the endothelial function.

The objective of this study was to investigate the relation between endothelial function and the extent of coronary artery disease in the presence and absence of a previous myocardial infarction. The relation between the extent of coronary artery disease and degree of endothelial dysfunction was assessed in patients suspected of CAD during their first diagnostic coronary angiography. The study group consisted of a heterogeneous group of patients including patients with and without an old myocardial infarction.
METHODS

Study Population. Patients between 18 and 80 years 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. Excluded were patients with unstable angina, recent (< 3 months) myocardial infarction, valvular heart disease requiring surgical intervention, clinical evidence of heart failure, a history of previous coronary intervention (PTCA or CABG) or any serious disease that may interfere with the follow-up. Excluded from acetylcholine infusion were patients with significant left main coronary artery narrowing, severe angiographic abnormalities with ischemic electrocardiographic changes and / or progressive angina pectoris during diagnostic catheterisation.

Data Collection. Baseline screening included history of coronary risk factors, physical examination, 12-lead electrocardiogram (ECG) and fasting whole blood collection for serum lipid profile and blood glucose. A standard diagnostic catheterisation procedure with concomitant intracoronary acetylcholine and nitro-glycerine infusion was performed. The response to both stimuli was measured by automatic contour detection technique (quantitative coronary angiography).

Definitions. Hypercholesterolemia was defined as a fasting serum cholesterol value > 6.5 mmol/l or a history of hypercholesterolemia for > 3 months that led to the initiation of lipid lowering therapy by the primary physician. Family history of coronary artery disease was defined as evidence of the disease in a parent or sibling before 60 years of age at the time of diagnosis. Hypertension was defined as a systolic blood pressure > 160 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. The smoking status was divided into 2 categories: no cigarette smoking for > 3 months or currently a cigarette smoker. Patients had a history of a myocardial infarction when pathological Q-waves > 0.04 s in duration were present in two adjacent leads on the 12-leads ECG or had a history of hospitalisation with ST-segment elevation > 0.1 mV measured 80 ms after the J-point in two adjacent leads on the 12-leads ECG, eventually supported by biological markers of myocardial necrosis. Diabetes was defined as high blood glucose levels requiring glucose lowering therapy. The coronary angiogram was evaluated by two independent angiography experts and categorized in two groups: Minor CAD (up to single vessel disease), and severe CAD (2- and 3-vessel disease). The coronary vasomotor responses were tested uniformly in the
proximal part of the left anterior descending coronary artery (LAD). The response was the change in diameter in response to maximal concentration acetylcholine and nitro-glycerine respectively, expressed as the percentage of the mean baseline diameter. A negative response represents a vasoconstriction.

**Coronary angiography.** Before the coronary angiogram, vasoactive agents were discontinued for at least 3 days (24 hours if recurrent angina was expected). Considered as vasoactive agents were long acting nitrates, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin II receptor blockers (adrenergic blockers were allowed). Smokers did not smoke for at least 4 hours before examination. Using a standard percutaneous femoral approach, a 6F diagnostic catheter was advanced into the left main coronary artery. Intracoronary nitro-glycerine was not given before the diagnostic catheterisation procedure.

**Vasomotor function test.** After completion of the diagnostic CAG, the diagnostic 6 French Judkins catheter was left in the left main coronary artery. For accurate vasomotor response measurement, the subsequent angiographic recordings were made with 25 frames per second and care was taken to have an adequate part of the catheter visible for calibration. A baseline coronary angiogram was done, visualizing the proximal left anterior descending artery (LAD). Then acetylcholine-chloride (concentration 0.16 μg/ml; Clinalfa AG, Läufelfingen, Switzerland) was infused through the catheter for at least 3 minutes. To achieve a coronary blood concentration of $10^{-8}$ mol/L acetylcholine, infusion rate was 82 ml/hour (with the assumption that the blood flow in the left main coronary artery was 120 ml/minute). At the end of the infusion, the acetylcholine in the catheter was removed carefully and an angiographic recording of the endothelium-dependent vasodilative response to acetylcholine was made. This procedure was repeated using $10^{-7}$ mol/L and $10^{-6}$ mol/L acetylcholine concentrations, respectively. Finally, the endothelium-independent dilative response was recorded one minute after an intracoronary bolus of 0.5-mg nitro-glycerine.

**Quantitative coronary angiography.** Quantitative Coronary Angiography (QCA) was performed by a previously described and validated automatic contour detection technique (CMS, Medis Co., Nuenen, the Netherlands) (13). End-diastolic frames of the non-stenotic proximal segment of the LAD were selected for QCA. User interaction was limited to the definition of the start and end points of the coronary segment to be analysed. Easily identifiable side branches were used as anatomic landmarks to allow the analysis of the same segments in successive
angiograms. The length of the analysed segment was always within a range of 10% from baseline segment length. In case of overlap with other branches, the independent analyst manually edited automatic contour detection. Mean segment diameter of the proximal LAD was determined in millimetres.

**Statistical analysis.** Baseline characteristics are presented as mean (standard deviation), and number of valid observations by the subgroups of patients based on their coronary artery disease status assessed by coronary angiography. Categorical data are presented per group percentage. For the normally distributed continuous variables differences between disease status subgroups were evaluated by the T-test, for skewed distributed continuous variables (p-value Shapiro-Wilk test for normality <0.05), the Kruskal-Wallis test was used. For qualitative parameters (categorical or ordered), frequency counts and percentages of each category were calculated by disease status. Differences between status subgroups were evaluated by the use of the Fisher exact test or the Chi-square test. A p-value < 0.05 was considered statistically significant. SAS version 6.12 (Cary, N.C) was used for all statistical analyses.

**Ethical considerations.** 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. All studies were performed in line with institutional guidelines. The Netherlands Heart Foundation that partly funded this study did not have any influence on study conduct, analysis nor interpretation and presentation of the results.

**RESULTS**

A total number of 312 patients undergoing a first diagnostic angiogram for the suspicion of coronary artery disease were included. Of these, 13 patients did not undergo acetylcholine infusion because of left main or severe three-vessel disease. In addition, 13 patients were excluded because of a recent myocardial infarction (between 11 days and 3 months before the diagnostic angiogram). These patients were excluded from further analysis. The remaining 286 patients had a mean age of 57 years (± 11), and 181 (62%) of them were male (table 1).
29.4% of the patients had normal smooth coronary arteries, 20.6% had vascular wall irregularities (<50% luminal narrowing in all coronary arteries), 21.3% had 1-vessel disease, 19.6% had 2-vessel disease and 9.1% had 3-vessel disease. A substantial number of patients had normal coronary arteries. A positive exercise test was present in 31% of these patients, a positive nuclear test in 27% and 90.5% had angina pectoris according the CCS classification. Patients with more severe CAD were significantly older than patients with minor CAD. Furthermore, more males were present in the group with severe CAD and more patients had hypercholesterolemia and a previous myocardial infarction in comparison to the group with minor CAD (table 1).

As shown in figure 1, the mean ± standard deviation epicardial luminal area change after infusion of the maximum dose of acetylcholine was \(-4.65 \pm 11.73\%\) in the group with minor CAD versus \(-5.85 \pm 10.30\%\) in the group with severe CAD \((p = 0.26)\). In contrast, the endothelium independent vasodilatation, induced by intracoronary nitro-glycerine, was significantly different between the two groups. The mean ± standard deviation nitrate response was 10.72 ± 9.06% in patients with minor CAD and 5.55 ± 7.72% in patients with severe CAD \((p<0.001)\). Due to technical failure, nine values of the vasomotor response of the LAD were missing during the procedure.
Multivariate analysis on all relevant variables including second order interactions revealed a previous myocardial infarction as only significant interaction variable. Based on this analysis, data were further analysed in strata by the presence or absence of a previous myocardial infarction. A total of 55 patients (19%) had a history of a more than 3 months old myocardial infarction, 23 (11%) in the minor CAD group, and 32 (39%) in the group with severe CAD. The median (range) interval between the documented MI and the diagnostic coronary angiography was 14.1 (5.0-70.0) months.

The groups with and without infarction were comparable regarding age and common risk factors except for sex (73% male in the myocardial infarction group versus 58% in the group without previous infarction). The number of patients using angiotensin converting inhibitors and statins was significantly higher in the group with a previous myocardial infarction. The response to acetylcholine, divided by the presence of a previous myocardial infarction, is shown in figure 2.

Figure 1: The response to intracoronary acetylcholine and nitro-glycerine in patients with minor and severe CAD.
Vasomotor testing in patients with a previous myocardial infarction

In the group without a previous infarction, the mean ± standard deviation response to acetylcholine infusion was more negative when the coronary artery disease was more severe, -3.96 ± 11.29 % in the minor CAD group versus −7.22 ± 11.16 % in the more severe group (p = 0.052). In contrast, in patients with a previous myocardial infarction, there was an inverse relation between acetylcholine response and the extent of coronary artery disease, the mean ± standard deviation endothelial vasodilatation was −9.95 ± 13.89 % in the patients with minor CAD versus −3.70 ± 8.52 % in patients with more severe CAD (p = 0.049).

Although endothelium dependent vasodilatation was investigated in the left anterior descending coronary artery, the presence of an old anterior wall myocardial infarction had no effect on the inverse relation between endothelium dependent vasodilatation and extent of coronary artery disease. The use of angiotensin converting enzyme inhibitors and statins was significantly related to a smaller vasoconstrictive response. In the group without previous myocardial infarction, no significant interaction variables were found. In the group with an old myocardial infarction the severity of the coronary artery disease was a significant interaction variable.
DISCUSSION

In the present study, endothelium dependent and endothelium independent vasodilatation was investigated in a heterogeneous group of patients with the clinical suspicion of symptomatic CAD. We demonstrated that the endothelium independent vasodilatation, induced by nitro-glycerine infusion, is significantly impaired in patients with more severe CAD. Furthermore, our results confirmed that endothelial dysfunction was related to the extent of CAD. However, this was only found in the group of patients without a previous myocardial infarction. Interestingly, in the group with a previous infarction (more than 3 months before endothelial testing), an inverse relation between endothelial function and extent of coronary artery disease was present.

The impaired dilative response to exogenous nitric oxide in patients with severe CAD, suggests that the vascular dysfunction in high-risk patients is not only present in the endothelium, but might also be located in the smooth muscle cells. One explanation for this finding could be the increased stiffness of the vessel wall in patients with severe atherosclerosis, which could cause the impaired endothelium independent vasodilatation. Another, more likely, explanation for this impaired nitrate response is a selective defect of the smooth muscle cell function, in particular of the soluble guanylate cyclase / cyclic guanosine monophosphate signalling system (14).

The coronary endothelium plays an important role in maintenance of myocardial perfusion and hence myocardial function. Nitric oxide produced by the endothelium is essential in vasodilatation, and inhibits vascular smooth muscle cell proliferation, monocyte adhesion and platelet aggregation. For these reasons, the observed reduction in endothelium dependent vasodilatation after myocardial infarction might have important implications in terms of risk stratification.

In most studies on coronary endothelial function, it has been mainly investigated in small or selected groups of patients. Few large scale studies in heterogeneous patient groups have been performed (2,5,15,16). Bertrand et al. (2) were the first to describe the effect of a previous myocardial infarction on ergonovine-induced spasm, a form of 'endothelial dysfunction'. They evaluated intracoronary ergonovine in eight different groups of patients. When patients had an old myocardial infarction, they found that a recent (< 6 weeks previously) infarction coincided with a high incidence of coronary spasm. Spasm was provoked by ergonovine in 20% of these patients. A myocardial infarction longer than 6 weeks previously was not related to such a high incidence of spasm (6.2%). Sueda et al. (16) studied, amongst others, a group of patients with a recent myocardial infarction (4 weeks) and a group with an older infarction (> 1 month old;
range 2-36, mean10.6±7.4 months). In both groups comparable incidences of acetylcholine induced spasm were found (37.5 and 37.8). This study was performed in a Japanese population. In these two studies, no indication about the relation between post-myocardial infarction, endothelial dysfunction and the extent of coronary artery disease was given. Our results indicate that endothelial dysfunction persists after myocardial infarction for a longer period than has been described thus far. This is especially true in patients without significant coronary artery stenosis. These results suggest that in patients with an old myocardial infarction and minor coronary artery disease extra attention should be given to the presence of endothelial function as a possible cause of the previous infarction, and potentially future events. This observation is also underscored by studies on the prognostic significance of endothelial function in patients with mild coronary artery disease (17;18). Furthermore, it is known that myocardial infarction develops predominantly in coronary vessels with mild or moderate stenosis, and especially in coronary vessels known to have endothelial dysfunction. In fact, endothelial dysfunction was found to be the most important predictor for future myocardial infarction (5). Also in our study group a causal role of endothelial dysfunction with respect to the previous infarction can not be excluded. Angiotensin converting inhibitors as well as statins are known to improve coronary endothelial function (19;20). Although all vasoactive drugs were stopped prior to the study a longer-term benefit cannot be excluded. At present the use of both drugs after a myocardial infarction is widely accepted. The results of our study provide a mechanistic rationale for the implementation of daily secondary preventive care.

In conclusion, our observation supports the clinical importance of coronary endothelial vasomotor testing after myocardial infarction, especially in patients with minor coronary artery disease.

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REFERENCES


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