Endothelial Function Predicts Future Development of Coronary Artery Disease
A Study of Women With Chest Pain and Normal Coronary Angiograms

Raffaele Bugiardini, MD; Olivia Manfrini, MD; Carmine Pizzi, MD; Fiorella Fontana, MD; Gianluigi Morgagni, MD

Background—The prognosis for women with chest pain and angiographically normal coronary arteries is believed to be totally benign. Previous studies, however, did not account for the delay of a decade or so in the development of coronary artery disease that women may experience.

Methods and Results—This study assessed long-term follow-up of 42 women with de novo angina, evidence of reversible myocardial perfusion defects on SPECT, and normal coronary angiograms. At recruitment, all women underwent endothelial function testing (intracoronary acetylcholine) during catheterization. Patients were followed up for >10 years. Angiography was repeated at the end of the follow-up in 37 patients. At recruitment, 22 patients developed diffuse vasoconstriction during acetylcholine in the absence of identifiable focal coronary spasm (acetylcholine-positive group). The remaining 20 patients showed vasodilation (acetylcholine-negative group). At the end of follow-up, in the acetylcholine-positive group, 1 patient developed cardiac death, 13 still complained of chest pain, and 8 had remission of symptoms. In the acetylcholine-negative group, all patients showed complete resolution of chest pain beginning 6 to 36 months after baseline assessment. Angiography showed development of coronary artery disease in the 13 symptomatic patients in the acetylcholine-positive group.

Conclusions—In women with angiographically normal-appearing coronary arteries, persistence of chest pain over the years often relates to development of coronary artery disease. Endothelial dysfunction in a setting of normal coronary arteries is a sign of future development of atherosclerosis. (Circulation. 2004;109:2518-2523.)

Key Words: acetylcholine • angina • endothelium • prognosis • women

The prognosis of middle-aged woman presenting with chest pain but found to have angiographically normal coronary arteries is assumed to be benign.1–3 Previous studies, however, did not account for the delay of a decade or so in the development of coronary artery disease (CAD) that women may experience. Nevertheless, these reports have contributed to a tendency to disregard these chest pain symptoms in women, and perhaps even more importantly, traditional preventive measures for coronary atherosclerosis.

Demonstrating loss of acetylcholine-induced vasodilation can prove endothelial dysfunction.4 Selective loss of endothelium-dependent vasodilatation in response to acetylcholine has been observed in angiographically atherosclerotic5,6 and in normal-appearing coronary arteries.7–9 Experimental and clinical findings suggest that endothelial dysfunction may be an early marker of atherosclerosis in human coronary arteries4 and could be associated with the prognosis of the disease.5–7

The aim of the present study was 2-fold: (1) to assess the long-term follow-up (>10 years) of women presenting with anginalike chest pain and myocardial reversible perfusion defects but found to have normal coronary angiograms and (2) to evaluate prospectively the relationship between prognosis and response of coronary arteries to intracoronary administration of acetylcholine as assessed at patient enrollment in the study.

Methods

Patients and Study Protocol
We studied 42 women (mean age, 51.6±8.8 years) having de novo angina without any angiographic lumen stenosis or irregularities in the coronary arteries. The population-based sample used for this report included women 38 to 69 years old at the time of enrollment from 1986 to 1990. Prospective 10-year follow-up was planned when these women were enrolled. Inclusion criteria were chest pain, normal angiograms, ECG ischemia during exercise stress test (ST-segment depression >0.1 mV), and myocardial reversible perfusion defects as assessed by SPECT. Patients with hypercholesterolemia (serum LDL cholesterol >160 mg/dL), hypertriglyceridemia (serum triglyceride >200 mg/dL), diabetes mellitus (fasting plasma glucose >126 mg/dL), valvular heart disease, and cardiomyopathy were not enrolled in the study.
Recruitment occurred after baseline angiography. All women underwent intracoronary acetylcholine testing during cardiac catheterization. Cardiac risk factors were assessed at baseline and monitored throughout the entire study period. Persons who smoked during the previous 12 months were classified as smokers. Height and weight were measured, and body mass index was calculated. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg. Family history was considered positive for CAD if at least 1 relative had a coronary event before 60 years of age.

Patients were followed up at regular intervals for at least 10 years. SPECT and coronary angiography were repeated at the end of follow-up and during follow-up if clinically indicated. Investigators who examined the angiograms at follow-up were blinded to clinical outcome and SPECT and acetylcholine findings. Written informed consent was obtained from all patients.

Chest Pain
Location, radiation, intensity, and character of the pain; what caused and relieved the pain; time relationships; and the pattern of recurrence of pain were measured with self-made questionnaires based on textbook sources.10 Intensity was graded as follows: 1=discom- fort, 2=mild pain, 3=moderate pain, and 4=severe pain. Physicians were asked to classify patients’ symptoms as typical or atypical of angina on the basis of the above information. Patients were required to specify the number of anginal episodes per week.

Acetylcholine Testing
Cardiac catheterization was performed after an overnight fast. All drugs except sublingual nitroglycerin were withdrawn 48 hours before cardiac catheterization. Patients were premedicated with diazepam (5-mg oral dose). Five minutes after routine angiography, patients underwent acetylcholine testing. Graded doses of acetylcholine (0.182, 1.82, and 18.2 μg/mL (10⁻³, 10⁻², and 10⁻¹ mol/L) were given in the left main coronary artery over 3 minutes (infusion rate, 1 mL/min) at 10-minute intervals. Angiograms were taken 1 minute after each dose. Acetylcholine was upgraded if angina or ECG ischemia did not occur. At the end of the study, an intracoronary bolus of isosorbide dinitrate (1 to 2 mg) was administered in the left coronary artery of all patients.

Angiographic Measurements
Measurements of coronary diameters were performed by previously validated techniques.12 Two investigators examined the angiograms. Two orthogonal views of the left coronary artery (frontal and left lateral or 30° right anterior oblique and 60° left anterior oblique) were chosen for quantitative analysis before and after drug administration. All proximal, mid, and distal segments (4 to 6 mm) of the left anterior descending and left circumflex coronary arteries free of vessel overlap and side branches were chosen for analysis. Selection allowed the analysis of 3 to 6 coronary segments per patient. The selected end-diastolic frames were projected (Tagarno 55CX) into a 52×67-cm screen with an ×5-fold magnification. The borders of the selected coronary artery segments were then traced by hand. Data were digitized by a computer (IBM PC-AT 0386). The software used the catheter as a scaling device to determine absolute diameter in millimeters. Segments were averaged to give the reference coronary artery luminal diameter. The response of coronary arteries to acetylcholine and nitroglycerin was expressed as percentage change in the diameter compared with baseline angiograms.

SPECT
A semiquantitative scoring method was used to analyze thallium-201 or technetium-99 sestamibi uptake in 20 myocardial segments in each patient.13 In each segment, a 4-point scoring system was used to describe radionuclide uptake (0=normal, 1=equivocal, 2=moderate, 3=severe reduction in activity). A segment with a score ≥2 was considered to have a defect. A difference in score between stress and resting uptake in at least 1 segment was classified as reversible perfusion defect.

End Point and Follow-Up
Patients were discharged on calcium channel blockers. When angina was refractory, β-blockers were recommended as substitutive medication.

End points of the study were cardiac events (myocardial infarction, cardiac death, and hospital readmission for unstable angina), persistence of symptoms, and development of CAD at the 10-year follow-up.

Cardiology research staff collected clinical event data at regular intervals (6 to 12 months). Follow-up assessments were carried out through patient visit for 37 patients. The remainder were sent a postal follow-up questionnaire every 6 months that asked about current symptoms and treatment, satisfaction with medical services, and causes of the symptoms. All patients completed the symptom inventory.

Statistical Analysis
Baseline demographic and biochemistry analytical information is presented as mean±SD for continuous variables and as absolute number (percentage) for categorical variables. Differences between baseline characteristics of the 2 groups were assessed by use of Student’s t test for continuous variables and χ² test for categorical variables. All statistical tests were performed with SPSS-Win 10.1 (statistical package for social science, SPSS Inc). Values of P<0.05 were considered significant.

Results
None of the 42 enrolled patients had a family history of CAD. Thirty-seven women were postmenopausal as a result of bilateral oophorectomy in 2 patients and aging in the others. None of the women were receiving estrogen therapy.

Vasoresponses to Acetylcholine
The response of coronary arteries to acetylcholine was homogeneous in individual patients but heterogeneous among different patients, varying from dilation to constriction.

At the peak acetylcholine dose (10⁻⁴ mol/L), 22 patients developed diffuse coronary vasoconstriction (acetylcholine-positive group). The mean coronary diameter decreased by 22.6±13.7% compared with baseline. Most of these patients (17 of 22) experienced chest pain and ST-segment depression. Relief of chest pain and ECG changes were spontaneously obtained a few minutes after discontinuation of acetylcholine infusion.

 Vasodilatation was the response to 10⁻⁴ mol/L acetylcholine in the remaining 20 patients with a mean diameter change of 15.6±11.5% (acetylcholine-negative group). In these patients, acetylcholine did not generate chest pain or ECG changes.

The Figure shows the percentage of lumen changes related to dose of acetylcholine injection. There was no significant intrapatient difference in percentage diameter changes between left anterior descending and left circumflex coronary arteries. Isosorbide dinitrate dilated coronary artery diameter (23.6±10.8%) in the overall study population. No relationship was found between the response to acetylcholine and isosorbide dinitrate.

Clinical characteristics of patients are summarized in Tables 1 and 2. A considerable number of women in both groups had low HDL cholesterol. Yet, there was no significant difference between the 2 groups regarding age, body mass index, and traditional risk factors.
Chest Pain Characteristics
In both groups, the location of pain was most frequently represented in regions favoring an ischemic origin: substernal areas, across midthorax and arms, shoulders, and neck (82% and 72% in acetylcholine-positive and acetylcholine-negative groups, respectively). Patients in the acetylcholine-negative group rated pain as more intense ($P < 0.02$) and long-lasting ($P < 0.01$) and used more

TABLE 1. Clinical Characteristic of Patients in Acetylcholine-Positive Group

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<th>HDL-C, mg/dL</th>
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HTN indicates hypertension; BMI, body mass index; C, cholesterol; TG, triglycerides; ACh, acetylcholine; and NTG, nitroglycerin.
emotional words to describe their pain. Symptoms were referred similarly during follow-up.

A cardiologist labeled the pain reported by women as “typical” in 52% of acetylcholine-negative group and 64% acetylcholine-positive group.

**Patient Outcome**

Patients were followed up for an average of 10.3 years (range, 10 to 11 years). Angiography was repeated at the end of follow-up in 37 patients who gave consent.

In the acetylcholine-positive group, 1 patient (5%) experienced acute myocardial infarction and cardiac death as a result of coronary dissection 43 weeks after enrollment, 13 patients (59%) still complained of chest pain at the end of follow-up, and 8 (36%) patients had remission of symptoms 24 to 36 months after enrollment. SPECT was repeated in all survivors. It showed reversible perfusion abnormalities in 15 patients (13 symptomatic, 2 asymptomatic). Perfusion abnormalities were in the same myocardial segments that showed reduced uptake at enrollment. The severity of uptake reduction in each myocardial region was not significantly changed from baseline (3.4±0.5 versus 3.6±0.5). Angiography was repeated in 17 patients. The 13 symptomatic patients showed variable degrees of coronary lumen stenosis (10 in the left and 3 in the right coronary system). Mean percentage of lumen narrowing was 32.4±23.7%. Smooth coronary arteries were found in 4 asymptomatic patients.

In the acetylcholine-negative group, all patients showed complete resolution of chest pain beginning 6 to 36 months after initial assessment. SPECT was repeated in all patients but did not show reversible perfusion abnormalities. Angiography was performed in all patients and confirmed normal-appearing coronary arteries.

Vasoconstriction during acetylcholine as assessed at enrollment was strongly associated with the occurrence of CAD (P<0.001).

At the end of follow-up, cardiovascular risk factors did not differ significantly between groups. The number of patients receiving calcium channel blockers decreased from 42 to 33. Nine patients shifted to β-blockers (5 in the acetylcholine-positive group, 4 in the acetylcholine-negative group). Six patients combined the above therapy with ACE inhibitors (4 in the acetylcholine-positive group, 2 in the acetylcholine-negative group), and 5 patients combined it with statins (3 in the acetylcholine-positive group, 2 in the acetylcholine-negative group). None of these women received estrogen therapy.

Follow-up appointments avoided readmission to hospital for further episodes of chest pain in both groups. Patients were continuously reassured and were not greatly concerned by their symptoms in view of the normal angiograms. They were satisfied by the research assessment, which provided an opportunity for better evaluation of the causes and prognosis of their chest pain.

**Discussion**

Our aim was to evaluate prospectively whether women with chest pain, normal coronary angiograms, but abnormal epi-
Cardiac vasoreactivity to an acetylcholine test were at increased risk for future development of atherosclerosis. Results of the present study show that most patients with vasoconstriction developed CAD after 10 years. No data like these have been published before; all previous data relate to events only.5–7 Our finding is powerful proof for the hypothesis that endothelial dysfunction in a setting of normal coronary arteries is a sign of future development of atherosclerosis.

CAD in Women

CAD is the leading cause of death in women in the Western world. This issue assumes particular importance in light of the troubling recent trends in the health profile of US women.14,15 Several mechanisms may worsen outcomes. Genetic, hormonal, or inflammatory factors may cause unstable atherosclerotic plaques or hypercoagulable states in women with CAD.16 Recent evidence has suggested that sex disparities in treatment of myocardial infarction have diminished, at least among elderly patients.17 Coronary atherosclerosis, however, is still considered a predominantly male disease, which results in underestimation of its presence and severity in women. CAD is often diagnosed later in women than in men when they are suffering from more severe prognosis.18 Late diagnosis may also result from less sensitivity of standard diagnostic methods to detect CAD in women.18 Interpretation of early signs of atherosclerosis may constitute a turning point in the management and prevention of CAD in women.

Prognosis in Women With Chest Pain and Normal Angiograms

Chest pain is the most common symptom of coronary atherosclerosis prompting subjects to seek attention from physicians. Chest pain may be associated with normal-appearing coronary arteries at angiography. This finding is 5 times more common in women than men.19 The presence of exercise-induced ST-segment depression and/or reversible radionuclide perfusion defects in most patients with normal angiograms led to the assumption that the pain is likely to be ischemic in origin and to the diagnosis of microvascular angina or syndrome X.20,21 Diagnosis of syndrome X could be misleading for physicians. They believe that the prognosis is benign, and they often think that further treatment and follow-up are not necessary even in those patients who have chest pain and limitation of activities persisting over the years. Clinical decision making is often supported by the presence of an atypical symptom profile. The present study demonstrates that the prognosis of these women is not as benign as initially suggested.1–3 After 10 years of follow-up, one third of the patients showed variable degrees of coronary atherosclerosis, with 1 patient experiencing fatal myocardial infarction. Follow-up appointments avoided readmission to hospital for further episodes of chest pain. Results also indicated that the quality of symptoms reported by patients was not helpful in predicting development of atherosclerosis. Patients commonly reported pain in the substernal areas regardless of their future outcome. Symptoms were referred to similarly during follow-up. Conversely, chest pain persistence over the years is more interesting. In the present study, chest pain was associated with the development of coronary atherosclerosis. Although chest pain in women with normal angiograms is very subjective,20 routine follow-up of these patients seems necessary.

Prognostic Value of Endothelial Function Testing

Attention has been given recently to the development of more effective strategies based on a better understanding of mechanisms underlying the early stages of atherosclerotic disease. Loss of endothelium-dependent vasodilation in response to acetylcholine may be regarded as a sign of endothelial dysfunction.4 Normal endothelial function plays a central role in vascular homeostasis, including inhibition of thrombus formation, inhibition of leukocyte adhesion, oxidative modification of LDL, and regulation of vascular smooth muscle proliferation.6 An impaired ability of the endothelium to release vasoactive substances may facilitate inflammation, platelet aggregation, and coronary vasoconstriction.22,23 Recent observations indicate that endothelial dysfunction may be able to predict future cardiovascular events even in patients with mild CAD.5,6 Interestingly, endothelial dysfunction may also predict cardiovascular events in patients with angiographically smooth coronary arteries.7 All of these studies clearly suggest that endothelial vasodilator dysfunction implicates proatherosclerotic and prothrombotic effects that may provide a link between dysfunction itself7 or the degree of dysfunction5 and adverse cardiovascular outcome. These studies, however, could not determine the mechanisms responsible for the association between impaired vasomotion of coronary arteries to acetylcholine and cardiovascular events.

The results of the present study indicate that intracoronary acetylcholine may predict an evolution toward CAD in some women found to have angiographically normal-appearing coronary arteries at the time of testing. Patients who showed diffused vasoconstriction in response to intracoronary infusion of acetylcholine developed CAD at the 10-year follow-up. Most of these patients had chest pain and ECG changes during testing.

One explanation for this finding may be the erroneous classification of coronary arteries as normal, which is due to the failure of angiography to detect atherosclerosis in the vessel wall. Angiographically normal-appearing coronary segments may harbor large subintimal atherosclerotic plaques. Alternative explanation is that abnormal vasoconstriction during acetylcholine testing may be a feature of a pronounced impairment of endothelium-dependent vasodilation, which can be seen as an early functional disorder preceding and promoting structural reversible vessel wall changes linked to atherosclerosis.4

Study Limitations

Some limitations should be considered. First, the study population is relatively small. These prospective data, however, are based on highly selected patients, which limits the enrollment of large numbers. Also, the statistical power for detecting a prognostic factor is determined chiefly by the number of outcome events, not by the total number of
patients. In the present study, 13 patients developed an outcome event, overt atherosclerosis. Larger studies are needed to confirm that the likelihood of no CAD is as low as it appears to be in the present study among women who are negative to acetylcholine.

Second, many studies showed an association between endothelial dysfunction and the presence of traditional risk factors for atherosclerosis, with hypercholesterolemia and diabetes being the most powerful causes. We tried to circumvent this problem. Inclusion criteria focused on normal routine lipid profile and fasting plasma glucose. Despite this precaution, a considerable number of women had low HDL cholesterol and hypertension and were smokers, all factors that may impair endothelium-dependent vasodilation. However, the 2 groups did not significantly differ with regard to any of these potential confounding factors.

Third, intravascular ultrasound was not performed at baseline assessment. Therefore, it is unknown whether any of the patients had atherosclerosis not identifiable by coronary angiography and whether this contributed to the response to acetylcholine and to the progression of atherosclerosis noted at follow-up in many women. Nevertheless, previous studies demonstrated that abnormal endothelium-dependent vasoreactivity of coronary circulation is an independent predictor of cardiovascular events, even when atherosclerosis is assessed by intravascular ultrasound.

Conclusions

We conclude that the heterogeneity of patients included in the broad definition of angina with normal angiograms, their chest pain characteristics at referral, and their varied outcomes support the need for a flexible approach to management and treatment. It is possible that endothelial function testing might define a subgroup of patients who will develop CAD and would benefit from aggressive medical or lifestyle interventions. Persistence of episodes of typical chest pain over the years in women who have showed an abnormal response to acetylcholine would require careful specialist attention and management.

References

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