Department of Cardiology - Faculty of Medicine
Albert Szent-Györgyi Clinical Center
University of Szeged
Szeged, Hungary

CLINICAL VALUE OF PROCEDURES IN INVASIVE CARDIOLOGY FOR THE ASSESSMENT AND RELIEF OF MYOCARDIAL ISCHEMIA

Imre Ungi MD

PhD thesis

2009

List of publications related to the thesis

Full papers

I. Rodriguez O, Picano E, Fedele S, Morelos M, Marzilli M, *Ungi I*. Non-invasive prediction of angiographic progression of coronary artery disease by dipyridamole-stress echocardiography. Coron Artery Dis 2001; 12(3): 197-204. (IF: 1,098)

II. Nemes A, Forster T, *Ungi I*, Nagy V, Vass A, Pálinkás A, Varga A, Csanády M. The coronary flow velocity reserve measured by stress transoesophageal echocardiography evaluates the success of coronary interventions--results of a 5-year follow-up. Scand Cardiovasc J 2005; 39(5): 286-292. (IF:0,757)

III. *Ungi I*, Ungi T, Ruzsa Z, Nagy E, Zimmermann Z, Csont T, Ferdinandy P. Hypercholesterolemia attenuates the anti-ischemic effect of preconditioning during coronary angioplasty. Chest 2005; 128(3): 1623-1628. (IF: 4,008)

IV. *Ungi I*, Pálinkás A, Nemes A, Ungi T, Thury A, Sepp R, Horvath T, Forster T, Végh Á. Myocardial protection with enalaprilat in patients unresponsive to ischemic preconditioning during percutaneous coronary intervention. Can J Physiol Pharmacol 2008; 86(12): 827-834. (IF: 1,763)

Abstracts

1. Gliozheni E, Fedele S, *Ungi I*, Marraccini P, Marzilli M, Picano E: Angiographically assessed coronary collateral circulation increases vulnerability to myocardial ischemia during vasodilator stress testing. Z Kardiologie 1997; 4:178.
2. *I Ungi*, A Genovesi, R Testa, R Sicari, M Marzilli, E Picano: Non-invasive prediction of angiographic progression of coronary artery disease by dipyridamole stress echocardiography. Eur Heart J 1997; (S):P1244. (IF: 8,917)
3. *Ungi I*, Marraccini P, Gliozheni E, Fedele S, Djukic G, Marzilli M. Intracoronariás adenosin hatása a koszorúér-véráramlásra emberben. Cardiologia Hungarica 1997; 26(S3):87.
4. Kósa I, *Ungi I*, Thury A, Babai L, Csanády M, Végh Á: A collateralis keringés szerepe a PTCA során fellépő ischaemia megelőzésében. Cardiologia Hungarica 1999; 28(S2):8.
5. Nemes A, *Ungi I*, Forster T, Litvai E, Pálinkás A, Thury A, Csanády M: A coronaria áramlási rezerv értéke percutan transluminalis coronaria angioplastica során. Cardiologia Hungarica 1999; 28, (S2):36.
6. Zimmermann Zs, *Ungi I*, Szécsi J: Számítógépes program fejlesztése a coronaria áramlási rezerv videodenzitometriás meghatározása céljából. Cardiologia Hungarica 1999; 28(S2):46.
7. *Ungi I*, Végh Á, Thury A, Horváth T, Csanády M: PTCA során alkalmazott enalapril fokozza az ismételt koszorúér occlusiok protektív hatását. Cardiologia Hungarica, 2000.
8. Zimmermann Zs, *Ungi* I, Csanády M: A coronaria flow rezerv megítélése videodenzitometriával. Cardiologia Hungarica, 2000.
9. Nemes A, Palinkas A, Forster T, Varga A, Thury A, *Ungi I*, Csanady M: The effect of aortic valve replacement on coronary flow reserve in patients with significant aortic stenosis and normal coronary angiogram. Eur Heart J, 2002 23, (Suppl.):250. (IF: 8,917)

10. *Ungi I.*, Vegh A, Ruzsa Z, Zimmermann Zs, Rudas L, Csanady M: The protective effect of enalaprilat during percutaneous coronary intervention: the potential role of bradykinin. Am J Cardiol 2002; 111H. (IF: 3,905)
11. *Ungi I*, Nagy E, Ruzsa Z, Zimmermann Zs, Ungi T, Csont T, Ferdinandy P. Ischemic preconditioning induced by coronary angioplasty in patients: effect of hyperlipidemia. J Mol Cell Cardiol 2004;37:179. (IF: 5,054)

Abbreviations

ACE:	angiotensin converting enzyme
APV:	average peak diastolic flow velocity
ATP:	adenosine triphosphate
CABG:	coronary artery bypass graft
CAD:	coronary artery disease
CFR:	coronary flow reserve
DET:	dipyridamole-stress echocardiography
DNA:	deoxyribonucleic acid
ECG:	electrocardiogram
LAD:	left anterior descending
LV:	left ventricle
LVEF:	left ventricular ejection fraction
NYHA:	New York Heart Association
PCI:	percutaneous coronary intervention
RM-ANOVA:	repeated measures analysis of variance
ROC:	receiver-operating characteristics
STEE:	stress transesophageal echocardiography
TEE:	transesophageal echocardiography
WMSI:	wall-motion-score index

Table of Contents

1 Introduction

In the past decades, there is a growing body of understanding about the pathophysiology of CAD. The dynamic nature of this disease is defined by the atherosclerotic plaque, endothelial function and an interaction of cellular elements with the vessel wall. The severity and consequences of myocardial ischemia is influenced by several anatomical, pathophysiological, and pharmacological factors. All of these significantly modify the clinical picture of CAD, and the long-term outcome of the disease. This diversity of the clinical appearance is determined among the others by (1) variability of progression of coronary artery atherosclerosis, (2) different functional significance of apparently similar coronary stenoses, (3) influence of specific metabolic and pathophysiologic conditions of the heart on adaptation to myocardial ischemia, and (4) pharmacological modification of the ischemic response.

Currently, despite of the rapidly developing non-invasive diagnostic methods, the evaluation of coronary anatomy and function, the severity and extent of coronary stenosis is still performed mainly during cardiac catheterization. In patients with symptomatic and even asymptomatic ischemic heart disease the progression of CAD assessed by angiography, appears to be neither linear nor predictable (1–6). Coronary angiography is still the most accepted standard means for assessing the progression of atherosclerotic plaque formation (7,8), however, due to its invasive nature, repeated cardiac catheterizations can not be a routine method for the follow-up of progression of CAD. A non-invasive recognition of progression of CAD by stress testing would obviously be valuable. Encouraging data on limited populations of patients have been obtained with serial perfusion imaging by dipyridamole-positron-emission tomography (9,10) and exercise thallium (11–13) scanning. Theoretically, pharmacological DET might be an optimal candidate for non-invasive prediction of progression of CAD, since the timing, extent and severity of the abnormalities of wall motion induced are reasonably well related to the extent and severity of the underlying CAD (14–18).

While DET seems to be an optimal synoptic diagnostic method of the global progression of CAD, it might be inferior to other non-invasive methods targeting a specific vessel segment in post-PCI cases of possible restenosis. In this situation the suspected vessel of the coronary

stenosis is well known from the interventional history. If this segment can be visualized, and even the flow reserve capacity can be measured with a non-invasive method, with this diagnostic strategy a somewhat more exact information could be obtained on the restenosis process, and let to make decision on the indication of repeated cardiac catheterization. In a normal situation, coronary blood flow elevates approximately 4- to 6-fold to meet the increasing myocardial metabolic demands. This effect is mediated by vasodilation of the arteriolar bed, which reduces vascular resistance thereby increasing coronary flow. CFR represents the capacity of the coronary circulation to dilate following an increase in myocardial metabolic demands, and can be expressed by the difference between the hyperemic flow and the resting flow curves. In 1974 Gould and Lipscomb proposed the relationship between the anatomic condition and the behavior of coronary hyperemic flow, whereby an inverse curvilinear relationship exists between the narrowing of the lumen of the coronary artery and hyperemic capacity, up to a complete annulment or absence of CFR for stenosis >90%. (19) Coronary flow reserve is affected by micro- and macrovascular resistance, extravascular compressive forces (pathological LV hypertrophy), hypertension, metabolic factors. Several echocardiographic methods are suitable for the evaluation of CFR, including contrast echocardiography (20) and direct Doppler measurement during transthoracic or transesophageal measurements (21,22) The original protocol of transesophageal Doppler CFR measurement in the LAD coronary artery was described by Iliceto et al. (22). Theoretically, this method can be used for a semi-invasive follow-up for functional evaluation and prediction of long-term success of PCI. Sytematic use of STEE for follow-up of post-PCI patients can help us selecting subjects with significant restenosis and high-risk ischemia and referring them to a repeated invasive study. By this way, DET can alleviate the risk caused by the variable nature and dynamics of restenosis. The diverse clinical appearance of CAD is resulted not only from the variable nature of atherosclerosis progression and/or restenosis process, but also from the individual differences in response of myocardium to ischemia. This diversity is, at least in part, due to the preconditioned state of the myocardium to ischemia. Ischemic preconditioning was originally defined as an endogenous adaptive response to brief periods of ischemic stress that protects the myocardium against the severe consequences of a subsequent more prolonged ischemic insult. Since the first description of this phenomenon by Murry et al. (23) in anesthetized dogs, the effects of preconditioning have been extensively examined in various animal studies (24-26). There is now good clinical evidence that repeated

brief ischemic episodes can also be protective in the human myocardium (27). One of the models of ischemic preconditioning most frequently applied in humans is PCI, where, consecutive brief periods of balloon inflation provide an opportunity to perform and assess the effects of preconditioning.

Although preconditioning provides a remarkable cardioprotection, its effectiveness is attenuated in some animal models of diseases, including hyperlipidemia, diabetes, nitrate tolerance, heart failure, and aging (28,29). The loss of classic preconditioning was subsequently confirmed in hearts of cholesterol-fed rats in which no signs of atherosclerosis were observed (30). Other animal studies also confirm that hyperlipidemia, independently from the development of coronary atherosclerosis, attenuates the cardioprotective effect of preconditioning (28,29,31,32). However, little is known about the effects of hypercholesterolemia on preconditioning in humans.

Other data suggest that not only short ischemic episodes, but additionally certain pharmacological agents, such as adenosine, nitroglycerine or nicorandil, can also precondition the myocardium, or at least potentiate the protective effects of preconditioning (33-35). This phenomenon is termed ‘pharmacological preconditioning’ (36). There is strong evidence, mainly from experimental studies, that the protection associated with preconditioning involves the early release of bradykinin and the subsequent activation of bradykinin B2 receptors (26,37,38). Such a protective role for ischemia-induced bradykinin release has been confirmed in patients. Elevated kinin levels have been found in the arterial and coronary sinus blood of patients undergoing cardiopulmonary bypass surgery, suggesting that kinins can be generated rapidly even after short periods of ischemia and that the levels reached can sometimes be considerable (39). The protective effect of intracoronary infusions of bradykinin, administered prior to angioplasty, has been described in patients with severe coronary artery disease (40). Leesar et al. recently reported that intracoronary enalaprilat administered before PCI in patients with stable angina mimics the protective effect of ischemic preconditioning (41). These results suggest that locally administered ACE inhibitors have beneficial effects against myocardial ischemia in both acute coronary syndromes and chronic conditions of ischemic heart disease.

Overall, the aim of thesis was to determine the clinical value of different procedures in invasive cardiology for the accurate and case-specific assessment and mechanical or

parmacological relief of myocardial ischemia in the variable environment of CAD progression and ischemia sensitivity.

2 Primary goals

1. To test the role of non-invasive functional tests (DET, TEE-CFR) in the appropriate timing of coronary angiography follow-up in patients with known ischemic heart disease and in post-PCI patients. The difficulty of this question is the great variability and the unpredictable nature of atherosclerosis progression. This makes difficult to plan the regular invasive follow-up of patients with borderline coronary artery stenoses and/or diffuse atherosclerotic disease. Two series of clinical investigations were performed to work out a less invasive follow-up strategy in patients with CAD.

a) Analysis of the results of repeated dipyridamole-stress echocardiography to predict the angiographic progression of coronary artery disease.

We hypothesized that changes resulting from repeated dipyridamole-echocardiography tests (DET) mirror non-invasively the changes in coronary-artery atherosclerosis assessed independently by coronary angiography. In a retrospective analysis of prospectively acquired data, 60 in-hospital patients with stable angina pectoris were examined. These patients underwent both DET and coronary angiography within one week during two separate admissions to hospital separated by 45 ± 31 (range 3–144) months.

b) Repeated semi-invasive coronary flow velocity reserve measurements by transoesophageal echocardiography for prediction of restenosis following PCI.

We presumed that reduced CFR measured semi-invasively during TEE as a functional characteristic of LAD stenosis and microvascular integrity may have a prognostic value to predict the necessity of invasive procedures in patients who underwent PCI previously.

2. Demonstration of the variable ischemic responses of the myocardium by beat-to-beat analysis of intracoronary ECG. With this human experimental model of ischemic and pharmacologic preconditioning we intended to prove that

a) the adaptive response of the myocardium to ischemia is attenuated by different pathologic states, eg. hypercholesterolemia;

In both hypercholesterolemic and normocholesterolemic patients consecutive balloon inflations during PCI were performed and the time course of intracoronary ST-segment shifts during repeated balloon inflations and deflations were assumed.

b) pharmacologic stimuli – e.g. ACE inhibitor pretreatment – may have a potential for improving the protective effect of ischemic preconditioning.

In order to evaluate this, intracoronary infusion of enalaprilat prior to the second balloon inflation was administered in patients who do not respond with protection to the initial balloon inflation during PCI.

3 Methods

3.1 Study populations and protocols

3.1.1 Non-invasive prediction of angiographic progression of coronary artery disease by dipyridamole-stress echocardiography

From the Institute of Clinical Physiology National Research Council stress-echocardiography data bank (1983–1998), we initially selected in a retrospective manner a series of 156 patients for whom DET and diagnostic coronary angiography had been repeated in our unit over a period of 15 years. For all patients, the referring cardiologist required the angiographic evaluation to be repeated. All of the 156 patients satisfied the main criteria for inclusion in our study, having been subjected to DET and subsequent coronary angiography, within 1 week of each other, during two distinct periods in hospital. From this initial cohort of patients, 96 were excluded for one of the following reasons: at least one of the two coronary angiography films was of inadequate quality or incomplete for quantitative evaluation (n = 8); intercurrent coronary artery bypass surgery had been performed (n = 21); intercurrent PCI had been performed between the two DET (n = 47), or readmission for angiographic reassessment after a recent PCI had occurred (n = 16); and less than two months had elapsed between the two periods in hospital (n = 4). A history of previous PCI was not a criterion for exclusion from our study. The remaining 60 patients were enrolled in the study.

During the follow-up, all patients were administered anti-anginal medication prescribed by the treating physician, which consisted of combinations of antiplatelet agents, calcium antagonists, nitrates and beta-receptor blocking agents. The reasons for re-hospitalization were changes in symptomatic status with increase in Canadian Cardiovascular Society Scale angina (54) by > 1 grade (n = 49 patients); need for angiographic reassessment because of revascularization, due to a symptomatic status that, although remaining unchanged, had become unacceptable for the patient's lifestyle, compliance with or tolerance of the drug regimen being too poor, or choice of elective angioplasty after a period on a waiting list (n = 5); need for re-evaluation after intercurrent myocardial infarction (n = 4); and need to assess asymptomatic patients with positive results of exercise-stress tests (n = 2). Echocardiograms and coronary angiograms were recorded and interpreted independently by separate reviewers blinded to the results of the other tests.

3.1.2 The coronary flow velocity reserve measured by stress transoesophageal echocardiography evaluates the success of coronary interventions

The study population comprised 31 patients (13 women and 18 men) with significant proximal LAD stenosis; they were enrolled between October 1997 and August 2000. None of the patients had previous PCI (Table 4). After the first coronary angiography, all of them underwent LAD-PCI including bare-metal stent implantation within 2 weeks. In consequence of their clinical signs, 9 patients required rePCI, while a CABG operation was performed in 2 additional cases within 6 months (group 1). The reason for reintervention was primary failed procedure in 3 cases and restenosis in 8 cases. In all patients, the main symptom was the reappearance of angina pectoris. In group 1, the eventual symptom free interval was 75±20 days. The clinical status of the remaining 20 cases improved during the follow-up and they remained stable and angina-free within six months (group 2). Patients underwent STEE on average 8±13 days before PCI and on average 5±4 weeks after it. For the patients with a stable clinical status, a third control STEE examination was also performed, 33±20 weeks after the successful PCI. During the follow-up, a total of 54 STEE measurements were performed for CFR assessment in this patient population. All patients were controlled and undergone telephone consultation according to their medical history and clinical signs 58±10 months after PCI. The study complied fully with the Declaration of Helsinki. The written

study protocol was approved in advance by the locally appointed ethics committee, and informed consent was obtained from each patient prior to the procedure.

3.1.3 The effect of hypercholesterolemia on anti-ischemic effect of preconditioning during coronary angioplasty

The study protocol was approved by the Ethics Committee of the University of Szeged. A written informed consent was obtained from all patients enrolled in the study. The investigations were carried out in single-vessel coronary disease patients elected for PCI (Table 7). Exclusion criteria were as follows: (1) angina pectoris or other signs of myocardial ischemia 1 week before intervention; (2) Lown 3–4 ventricular arrhythmia before the intervention; (3) left ventricular dysfunction (<35% ejection fraction or greater than New York Heart Association functional circulatory stage II); (4) treatment with adenosine 5-triphosphate–sensitive potassium channel inhibitors; (5) history of myocardial infarction or baseline ST-segment abnormalities; (6) serum electrolytes out of the physiologic range; (7) serum creatinine level <150 μmol/L; (8) hypothyreosis; and (9) angiographically visible collateral vessels interfering with the treated coronary artery. Based on the exclusion criteria, 41 patients were included in our study. A further 11 patients were excluded from the study due to unsuccessful registration of intracoronary ECG, such as lack of ST-segment elevation during the first occlusion in 5 patients, occurrence of arrhythmias that made the beat-to-beat evaluation of ST segments impossible in 4 patients, and poor quality of recordings in 2 patients. The remaining 30 patients were classified into normocholesterolemic (3.5 to 5.3 mmol/L, n = 15) and hypercholesterolemic (5.5 to 7.8 mmol/L, n = 15) groups. Our boundary cholesterol value (5.3 mmol/L) is associated with borderline high risk of ischemic heart disease according to various recommendations including the National Cholesterol Education Program. Time to normalization of the ST segment during reperfusion could be assessed onlyin 13 patients in each group due to various technical problems unrelated to the end points ofthe study.

3.1.4 Myocardial protection with enalaprilat in patients unresponsive to ischemic preconditioning during percutaneous coronary intervention

Patients referred for elective PCI with stable angina and single-vessel coronary artery disease were selected for the study. All of them had a reduction >70 % and <90 % in the vessel

diameter, as assessed by digital caliper method (Siemens Hicor system). The criteria for the exclusion of patients from the study: (i) angina pectoris or other signs of myocardial ischemia within 2 days before the intervention, (ii) Lown 2-4 ventricular arrhythmia before or during the intervention, (iii) a decreased LVEF and/or symptoms of left heart failure (<35% LVEF or greater than NYHA II functional circulatory stage), (iv) treatment with ATP-sensitive potassium channel inhibitors, (v) a history of myocardial infarction in the territory of the dilated vessel, (vi) angiographically visible collateral vessels interfering with the treated coronary artery, (vii) a bundle branch block or baseline ST-segment abnormalities, (viii) serum electrolytes out of the physiological range, (ix) serum creatinine >176 μmol L-1. This was a single-center, investigator-blinded, randomized study. The 20 patients included in the investigation were randomly allocated to either the control group or the enalaprilat group, in such a way that each group contained 10 patients. All 20 patients were treated with aspirin (100 mg) and clopidogrel (75 mg) daily for at least 2 days before the commencement of PCI. Long-acting nitrates were stopped 2 days, and any ACE inhibitors 5 days before angioplasty. Other antianginal medication was not discontinued before the study. The study was performed in accordance with the 1983 revision of the Declaration of Helsinki, and adhered to local guidelines for good clinical practice. Permission was obtained in advance from the Local Research Ethics Committee, all 20 patients provided their written, informed consent before participation in the study.

3.2 Diagnostic and interventional methods applied in the studies

3.2.1 Coronary angiography

Diagnostic coronary arteriography was carried out with the use of standardized projections. Several views of each coronary artery were obtained, including craniocaudal views. All coronary arteriograms were reviewed and analysed visually by two independent observers. When there was disagreement between the two observers, a third observer reviewed the angiogram and his judgement was binding. The stenosis was evaluated from multiplane projections and was considered significant in the event of a lumen area reduction > 75%. Description of the coronary anatomy is presented in Figure 1. For the study to evaluate prediction of angiographic progression of coronary artery disease, the following extra measurements were performed. For any given stenosis and both angiograms, end-diastolic

frames were selected and the diameter narrowings were evaluated also by an automatic edge-detection system (Mipron II, Kontron, Berlin, Germany).

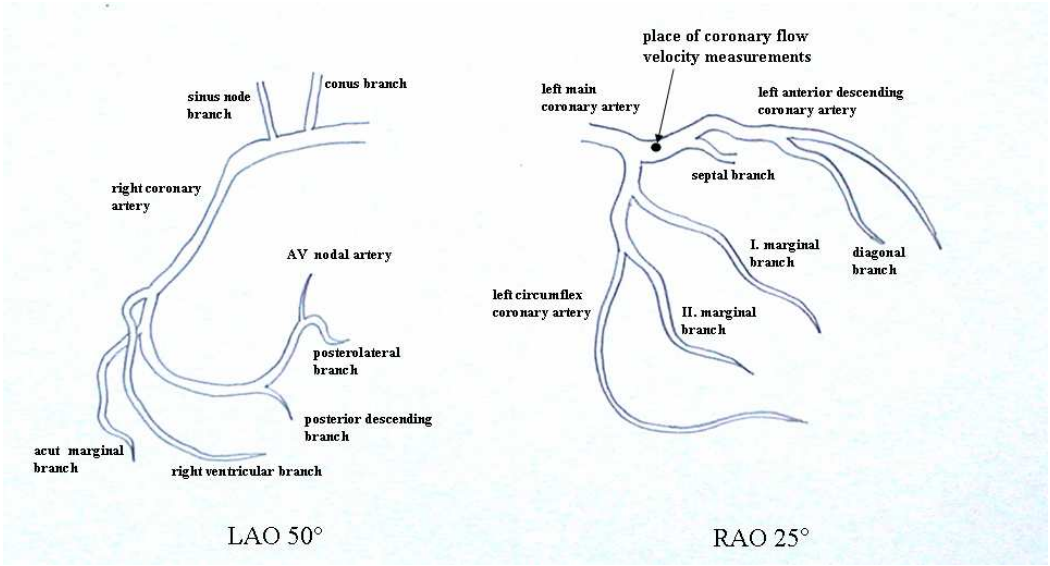

Figure 1 Schematic representation of the right (left side) and left (right side) coronary arteries according to the 15-segment AHA coronary artery model. Each coronary segment was analyzed in multiple projections for obstructive lesions. In this figure right coronary artery is depicted in left anterior oblique (LAO) projection, left coronary artery system in right anterior oblique (RAO) projection.projections for obstructive lesions. In this figure right coronary artery is depicted in left anterior oblique (LAO) projection, left coronary artery system in right anterior oblique (RAO) projection.

Matching segments and narrowings in these two coronary angiograms were carefully selected by use of identical projections. The intra-observer and inter-observer variabilities of the method had previously been found to be 7 and 6%, respectively, in our laboratory (42). On the basis of a-priori criteria, patients were defined as angiographic progressors if any progression of stenosis to occlusion was detected visually and if there was any stenosis > 30% for which > 20% progression of stenosis was measured by quantitative coronary angiography (43-45). From the raw data a quantitative measure of the extent of CAD was also evaluated by means of the Duke scoring system. The Coronary Artery Disease Prognostic Index (Duke score) considers the number of the diseased vessels (one, two or three as well as left-main-artery disease) and also the involvement of the proximal segment and the severity of stenosis (i.e. > 95% narrowing of diameter). The range of the prognostic weight is a 0–100-grade scale (46).

3.2.2 PCI protocol

A percutaneous femoral or transradial approach was used for PCI. After placement of the guiding catheter into the target vessel, a baseline coronary angiography was performed, and a bolus of 100 IU kg-1 heparin was administered intravenously. Nonionic contrast medium (Ioversol 350, Mallinckrodt Inc., St. Louis, MO) was used in all patients. Intracoronary nitrate administration was avoided during the study; when it was required due to a clinically relevant coronary artery spasm, the patient was excluded from the study. The coronary artery lesion was crossed with a 0.014-in guidewire. PCI was performed with semicompliant balloon dilatation catheters ranging in diameter from 2.5 to 3.5 mm. Balloon sizes were determined by measuring the distal reference segment of the coronary artery adjacent to the stenosis (digital caliper method; Siemens Hicor, Erlangen, Germany). After the balloon had been positioned in the lesion, the patients underwent repeated balloon inflations, with periods of reperfusion, during which the balloon was deflated and withdrawn proximal to the lesion with the guidewire remaining across the lesion. In the preconditioning studies the completeness of the coronary occlusion was assessed by a short injection of contrast material.

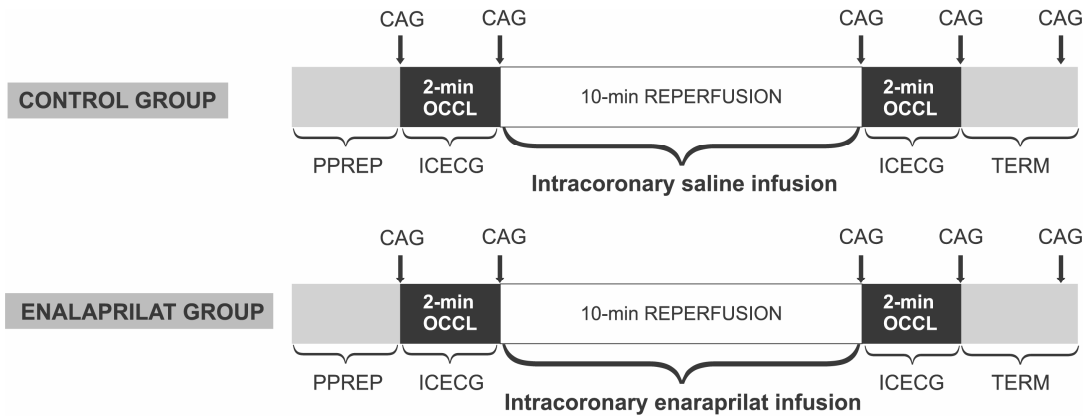

Figure 2 The study design to evaluate the protective effect of enalaprilate in patients unresponsive to ischemic preconditioning during PCI. (Abbreviations: PPREP: preparation of the patient; CAG: coronary angiography; ICECG: intracoronary electrocardiogram; OCCL: coronary artery occlusion by balloon inflation.)

The study design to evaluate the protective effect of enalaprilate in patients unresponsive to ischemic preconditioning during PCI is outlined in Figure 2. A continuous intracoronary

infusion of saline (control group; n = 10) or enalaprilat (enalaprilat group; n = 10) was started immediately after deflation of the first balloon occlusion using the guiding catheter. The enalaprilat (Enap; Krka Pharmaceutical Co., Novo Mesto, Slovenia) infusion was freshly prepared just before the commencement of the study. The content of the original vial was diluted in normal saline to a concentration of 50 µg ml-1, which was infused at a rate of 50 µg min-1 over 10 min. This dose and rate of enalaprilat infusion have been found to result in coronary ACE inhibition (47) without producing arterial hypotension (48). The control group received an equivalent volume of physiological saline. After termination of the infusion the balloon was again led into the lesion segment and the second 2-min occlusion was performed. One min after each occlusion, coronary angiography was commenced in order to assess the result of the dilation. After cessation of the second inflation, the study protocol was terminated, and decisions regarding further inflations or other interventional procedures were made on an individual basis.

3.2.3 Quantitative assessment of myocardial ischemia during PCI

In the studies to evaluate the preconditioning and pharmacological protection myocardial ischemia was assessed by measurement of the intracoronary ST-segment elevation. Lead C1 of the ECG monitoring system was connected to the coronary guidewire. Intracoronary ECG, and intraaortic blood-pressure signals were recorded online with Wsmon Application Version 3.3 software throughout the procedure. Every beat was evaluated by an independent physician in a blinded arrangement. The ST-segment elevation was defined as the difference between voltage values measured 80 ms after the J point and that in the PQ segment. The total ischemic burden was characterized by measuring the mean ST-segment deviation (mV) and the peak ST-segment elevation (mV). The dynamics of the evolution of ischemia was characterized with the time to reach 0.5 mV ST-segment elevation. The aortic pressure curve during the procedure was obtained from the guiding catheter, and was recorded by the hemodynamic monitoring system (Siemens Cathcor).

3.2.4 Transthoracic and stress transoesophageal echocardiography

Transthoracic echocardiography was performed with commercially available imaging systems at rest. The left ventricular internal dimensions were measured by 2-dimensional directed M-mode echocardiography. The ejection fraction was calculated by the method of Teichholz et

al. (50). TEE was performed with a Toshiba Powervision 8000 echocardiography equipment using multiplane transoesophageal transducer. TEE-derived CFR measurements were carried out according to the standard protocol proposed by Iliceto et al. (22). In all patients, the aortic root and the proximal portion of the LAD were visualized in the transversal plane. The biphasic coronary flow waveform in the LAD was recorded by pulsed Doppler. Flow measurements were made under baseline conditions and after the administration of 0.56 mg/kg dipyridamole during 4 minutes. The peak velocities were measured at maximal vasodilation at 6 minutes. In each case, five consecutive cycles were measured and averaged.

The CFR was calculated as the ratio of the APV during hyperemia to the resting APV (Figures 3 and 4). Blood pressure was continuously monitored throughout the stress.

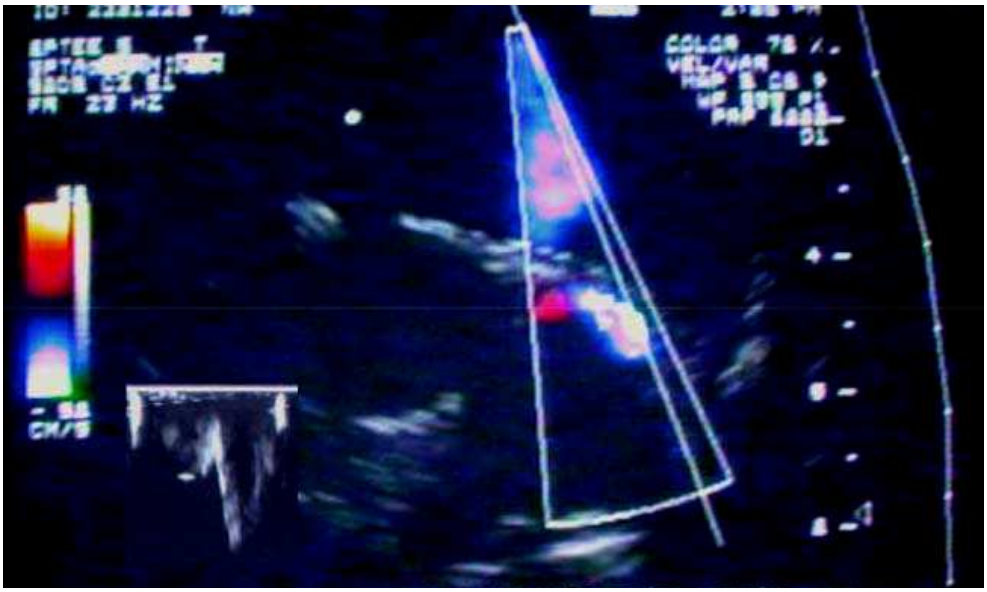

Figure 3 Typical biphasic coronary waveform recorded by pulsed Doppler in the left anterior descending coronary artery and the place of measurement in transversal plane during transesophageal echocardiography

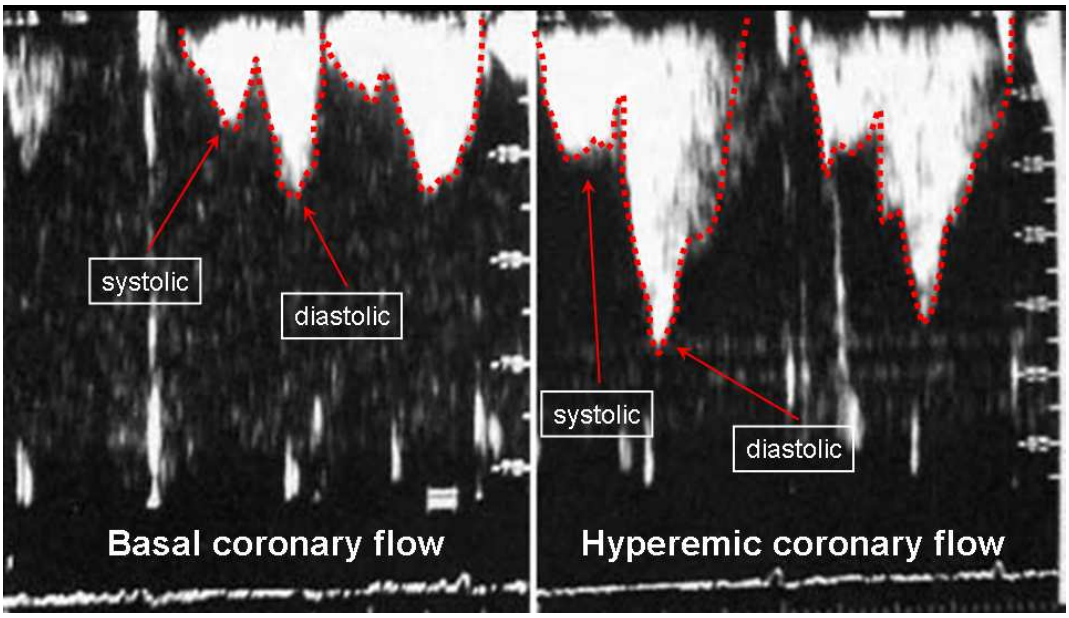

Figure 4 CFR measurement before PCI of LAD. Typical biphasic coronary waveform: lower systolic and higher diastolic flow. In this case, 40 cm/s diastolic coronary flow velocity could be measured at resting condition, which increased to 54 cm/s during the maximal vasodilation. The calculated coronary flow velocity reserve (CFR) is 54/40=1.35.

3.2.4.1 DET protocol

Two-dimensional echocardiographic and 12-lead electrocardiographic monitoring were performed in combination with infusion of dipyridamole at an initial rate of 0.56 mg/kg over 4 min. The maximal cumulative dose achieved was 0.84 mg/kg over 10 min. In cases of negative results of tests, 40–70 mg aminophylline was administered over 1 min during the 15th minute. Two-dimensional echocardiograms were continuously obtained during and up to 5 min after administration of dipyridamole. Blood pressure and the electrocardiogram were recorded each minute. Echocardiographic diagnostic end point was the development of obvious echocardiographic positivity. The test was stopped in the absence of diagnostic end points, if sub-optimal results [intolerable symptoms and limiting asymptomatic side effects, including hypotension (relative or absolute: > 30 mmHg decrease in blood pressure] were obtained.

Regional wall motion was assessed according to the recommendations of the American Society of Echocardiography with a 16-segment model of the left ventricle (53). Left ventricular segments were assigned to pre-determined coronary vascular territories according

to generally accepted pre-defined criteria (53). In all studies, segmental wall motion was semiquantitatively graded as 1, normal; 2, hypokinetic, marked reduction of endocardial motion and thickening; 3, akinetic, virtual absence of inward motion and thickening; and 4, dyskinetic, paradoxical wall motion away from the centre of the left ventricle in systole. The wall-motion-score index was derived by dividing the sum of scores for individual segments by the number of segments for which data were interpretable. Positivity of a result of a test was defined as the occurrence of at least one of these conditions: new dys-synergy in a region with normal resting function (i.e. normokinesis becoming hypokinetic, akinetic or dyskinetic); and worsening of a resting dys-synergy (i.e. hypokinesia becoming akinesia or dyskinesia). Inadequately visualized segments were not scored. For patients with dysfunction at rest, positivity of a result of a test was defined as 'remote' (or 'heterozonal') ischemia when the development of asynergy was not directly adjacent to the area of infarction and was assumed to be related to another vascular region (53).

Echocardiographic criteria for progressors and non-progressors. According to DET criteria, progressors were defined according to the satisfaction of at least one of two criteria: (1) a negative result of DET in initial testing and a positive result of DET in second testing; (2) positive results both of initial and of second tests, with the latter having a peak WMSI 0.12 greater than that in the former. Non-progressors were defined according to the satisfaction of at least one of three criteria: (1) negative results of DET both in initial and in second tests; (2) a positive result of initial test and a negative result of second test; (3) positive results both of initial and of second tests, with the latter having a peak WMSI 0.12 less than that of the former. The cut-off point of peak WMSI variations was chosen post hoc on the basis of ROC analysis providing the optimal sensitivity and specificity in DET results for identification of angiographic progressors.

3.3 Statistical analysis

Statistical analysis was performed by using analysis of variance or the two-tailed Student's t test for paired data as appropriate. The unpaired Student t test was applied for between group comparisons. For dichotomous variables, Fisher's exact test was used. Data are reported as means□standard deviation; 95% confidence intervals are also given. $P < 0.05$ value was considered statistically significant. Correlation of [kappa] indices was used when appropriate.

The sensitivity, specificity and positive and negative predictive value were calculating using standard formulae. A SPSS 7.0 package (Statistical Package for the Social Sciences (SPSS) Inc., Chicago, Illinois, USA) running on a personal computer was applied for the statistical analysis. In the enalaprilate study, the statistical analysis was performed with MedCalc for Windows Version 8.2.0.3 (MedCalc Software, Mariakerke, Belgium). In DET study, ROC analysis was performed to identify the stress-echocardiographic parameter providing the best accuracy. To establish the predictioning power of the CFR early after PCI, ROC curve was constructed and the area under curve was reported. For the preconditioning studies, ECG recordings were analyzed using software (WinDaq Version 1.78; Dataq Instruments; Akron, OH). The number of heart beats in each ischemic period were separated into 15-s intervals, and the ST-segment elevation values of each cardiac cycle in the 15-s periods were averaged to get an eight-point curve. The effects of repeated occlusions were analyzed by RM-ANOVA with Greenhouse-Geisser adjustment if needed (SPSS version 11.0; SPSS; Chicago, IL); p values were corrected according to Bonferroni for repeated measurements. Effect of repeated occlusions on time to normalization of the ST segment in the normocholesterolemic group was also analyzed by RM-ANOVA. Confidence intervals in pairwise comparisons were adjusted to multiplications according to the Sidak formula.

4 Results

4.1 Non-invasive prediction of angiographic progression of coronary artery disease by dipyridamole-stress echocardiography

Clinical history. The clinical characteristics of the patients at entry to our study are reported in Table 1.

Age (years)	59 ± 9
Sex	
Male	54 (90)
Female	6 (10)
Risk factors	
Hypertension	23 (38)
Smoking	21 (35)
Diabetes	8 (13)
Family history of disease	15 (25)
Hyperlipidaemia	20 (33)
Obesity	30 (50)
Previous myocardial infarction	31 (52)
Previous PTCA	15 (25)
Therapy at time of testing	
None	44 (74)
Some (β-blockers, calcium antagonists and nitrates)	11 (18)
Variable	5 (8)

Values are expressed as numbers (percentages) and mean ± SD.
PTCA, percutaneous transluminal coronary angioplasty.

Table 1. Baseline clinical characteritics of 60 patients involved in the study

During our second assessment 53 patients were identified clinically as 'progressors' (i.e. with worsening of Canadian Class Angina by > 1 grade or with intercurrent myocardial infarction). Resting echocardiography. By resting echocardiography, 27 patients were identified as 'progressors' (i.e. patients with worsenings of > 0.12 in baseline regional WMSI). Coronary angiography. The extents of CAD detected by first and second evaluations are reported in Table 2. The number of diseased vessels was higher at the time of the second evaluation (Table 2). Angiographic progressors amounted to 44 of 60 patients (73%) and angiographic non-progressors to 16 of 60 patients (27%). For progressors, the Duke score was significantly increased with respect to that in the first angiography (from 37±17 to 51±15, $p<0.001$); for non-progressors the Duke score did not change significantly (from 26±20 to 24±19, n.s.).

	First evaluation	Second evaluation
Normal	7	5
One-vessel disease	21	13
Two-vessel disease	23	25
Three-vessel disease	9	16
Left-main-artery disease	0	1
Diseased vessels	1.5 ± 0.8	1.9 ± 0.9***

Values are numbers of patients and means ± SD. *** $P < 0.001$, versus first evaluation.

Table 2. Data obtained from coronary angiography

Dipyridamole-stress echocardiography. Resting WMSI was 1.06 ± 0.12 at first and 1.10 ± 0.22 (NS) at second testing. Results of DET were positive for 30 of 60 patients at first and 46 of 60 patients at second testing (50 versus 77%, P < 0.001). According to previously specified criteria, 40 of 60 patients were 'progressors' in terms of stress-echocardiographic criteria. For 20 patients we found negative results of DET at first testing and positive results of DET at second testing; for an additional 26 patients we found positive results both at initial and at second testing, with the latter having a peak WMSI 0.12 greater than that of the former (i.e. a more severe dysfunction at peak stress).

Comparison of data from coronary angiography and clinical, resting and stress echocardiography. Taking the coronary-angiographic evolution as the reference standard, the agreement ([kappa]) for each of the three levels of assessment (medical history, resting echocardiography and stress echocardiography) was computed (Table 3; identifying 'progressors' according to the previously defined criteria). The coefficient of agreement ([kappa]) with coronary angiography was poor for resting echocardiography ([kappa] = 0.144), moderately high for clinical variables ([kappa] = 0.266), and substantial for stress echocardiography ([kappa] = 0.764). The concordance of stress-echocardiographic data with respect to coronary-angiography progressors was found for 39 of 44 cases (89%). The remaining 20 patients were 'non-progressors' in terms of stress-echocardiographic criteria. An example of an angiographic stress-echocardiographic 'non-progressor' is shown in Figure 5. Different groups of patients could be identified according to the variation in DET response between the first and second test. An excellent rate of concordance was achieved with a

positive DET response becoming negative (four of four, 100%) and with a negative DET response becoming positive (18 of 19, 95%). The concordance was 100% (six of six for non-progressors and 20 of 20 for progressors) for patients for whom the peak variations in WMSI and DET were chosen post hoc on the basis of ROC analysis.

		Result of DET		Coronary angiography
		Positive	Negative	
Coronary Angiography				
Positive		39	5	44
Negative		1	15	16
DET	Total cases	40	20	60
κ=0.764				
		Clinical variables		Coronary angiography
		Positive	Negative	
Coronary angiography				
Positive	41		3	44
Negative		12	4	16
Clinical variables	Total cases	53	7	60
κ=0.266				
		Results of resting echocardiography		Coronary angiography
		Positive	Negative	
Coronary angiography				
Positive	22		22	44
Negative		5	11	16
Resting echocardiography	Total cases	27	33	60
κ=0.144				

DET, dipyridamole-echocardiography test.

Table 3 Indices of kappa correlations of results of dipyridamole-stress-echocardiography testing, clinical variables and resting echocardiography with respect to data from coronary angiography

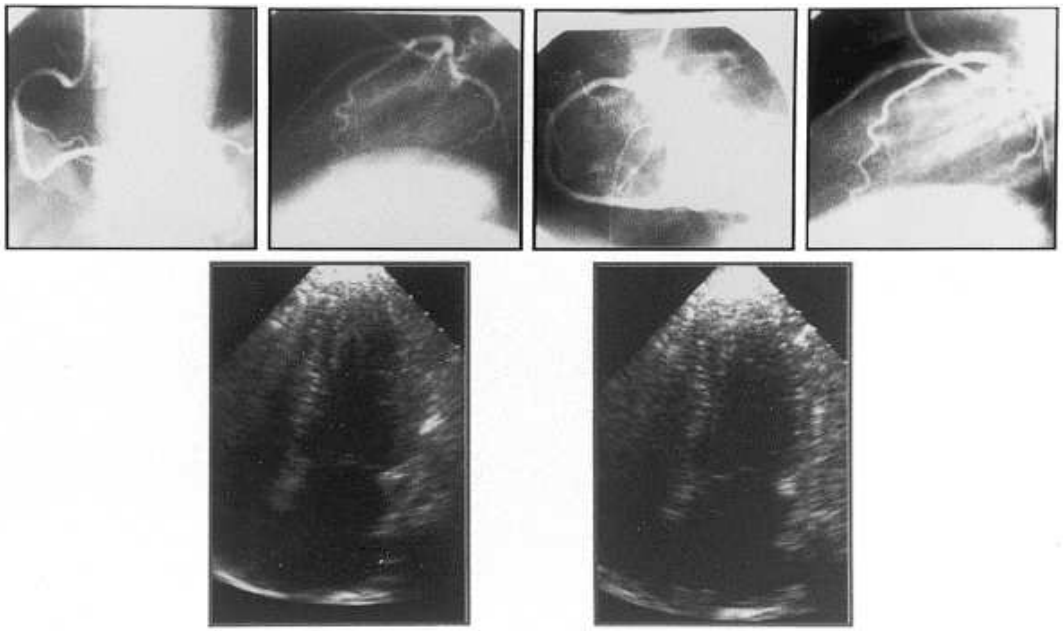

Figure 5. An example of a 'non-progressor', identified by concordance of angiographic (upper panels) and stress-echocardiographic (lower panels) criteria. Normal coronary-angiographic findings at the time of the initial evaluation (left-hand upper panel). . Stress-echocardiographic finding: the end-systolic frame at peak stress exhibits a normal thickening of septal, apical and lateral walls (left-hand lower panel). Six years later: There was no change in the coronary-angiographic findings at the time of the second evaluation (right-hand upper panel). The stress-echocardiographic finding shows the end-systolic frame at peak stress exhibiting a normal echocardiographic systolic thickness (right-hand lower panel).

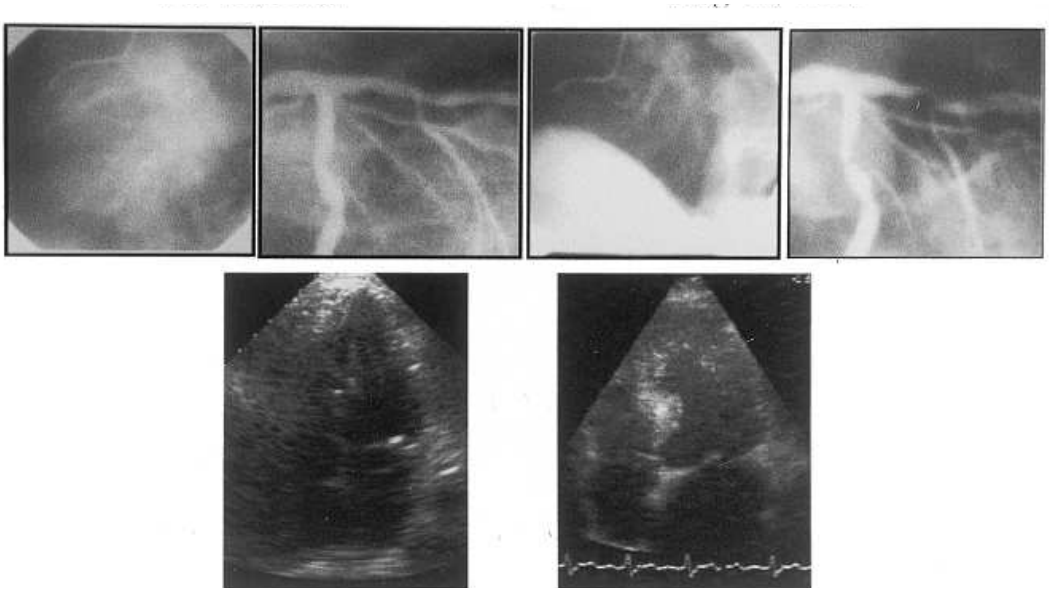

Figure 6. An example of a 'progressor', identified by concordance of angiographic (upper panels) and stress-echocardiographic (lower panels) criteria. Coronary angiography at the time of the initial evaluation (left-hand upper panel): occluded right coronary artery and patent left anterior descending coronary artery. The stress-echocardiographic finding (left-hand lower panel): the end-systolic frame at peak stress exhibits a normal thickening of septal, apical and lateral walls. Five years later: Coronary-angiographic findings (right-hand upper panel): tight stenosis of left anterior descending coronary artery, which was absent from the first evaluation. The stress-echocardiographic finding (right-hand lower panel) shows the end-systolic frame at peak stress exhibiting an obvious akinesia of medium anterior and septo-apical wall as well as of latero-apical wall. The new abnormality of regional wall motion detected by repeated testing is in agreement with the angiographic progression of disease.

For 13 of the 20 'progressors', the echocardiographic pattern of repeated testing was also characterized by a 'remote' type of positivity, correctly identifying instances of multivessel disease with angiographic progression in a vessel different from the ischemia-producing one at initial testing. An example of a 'progressor' pattern is shown in Figure 6.

Sensitivity of the 'progressor-pattern' of stress-echocardiography test for predicting progression of CAD was 87%, specificity was 93%, positive predictive value was 96%, negative predictive value was 77% and accuracy was 90%.

4.2 The coronary flow velocity reserve measured by stress transoesophageal echocardiography evaluates the success of coronary interventions

Demographic and clinical data on the patients and echocardiographic parameters are presented in Table 4.

	Group 1	Group 2
No. of patients	11	20
Age (years)	60±12	58±9
Male gender (%)	5 (45)	13 (65)
Hypertension (%)	9 (82)	13 (65)
Diabetes mellitus (%)	4 (36)	5 (25)
Hyperlipidemia (%)	8 (73)	10 (50)
Previous MI (%)	5 (45)	10 (50)
Smoking (%)	6 (55)	8(40)
BMI (kg/m^2)	29.1±4.4	28.5±5.2
prePCI area stenosis (%)	82±5	81±6
Transthoracic echocardiography		
EDD (mm)	54.3±7.1	51.0±5.5
IVS (mm)	11.0±2.8	10.5±1.3
PW (mm)	11.0±2.9	10.2±1.1
EF (%)	58.8±6.4	59.7±12.9
LVMI (g/m^2)	151.8±5.2	132.0±31.7

Table 4. Demographic and clinical data of the patients and echocardiographic parameters. (p>0.05)

There was no significant difference in the prevalence of risk factors between the groups. The medications applied in patient groups before LAD-PCI were also found to be similar (Table 5). After the PCI, the residual coronary area stenosis evaluated by coronary angiography was 48±12% in group 1 and 25±5% in group 2.

Dipyridamole stress transoesophageal echocardiography. No major side-effect of the infusion of dipyridamole or severe discomfort from the STEE examination was reported. The resting and maximal hyperemic diastolic coronary flow velocities before and after LAD-PCI are

presented in Table 6. The CFR in group 2 increased continuously during the follow-up, whereas that in group 1 remained unchanged.

Result of the statistical analysis in predicting coronary reintervention. The CFR early after PCI (ROC area, 78%, $p < 0.04$) was found to exhibit good prognostic value in predicting patients with reintervention (Figure 7).

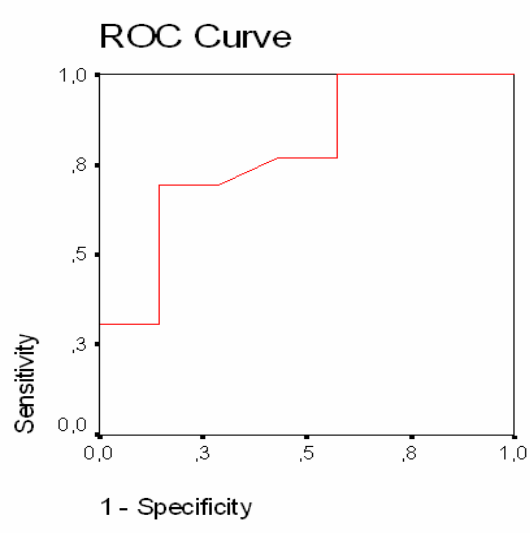

Figure 7 CFR early after PCI (ROC area, 78%, p < 0.04) was found to exhibit good prognostic value in predicting patients with reintervention.

The CFR < 1.87 found to have optimal accuracy selected as cut off value with 69% sensitivity and 86% specificity.

Results of the 5-year follow-up study. The success rate of the 5-year follow-up was 97% (30 of 31). The missing patient was from group 2. From patients who required rePCI or CABG (group 1), two patients died: one male patient from colon tumor with pulmonary metastasis 46 months after the PCI (non-cardiac death) and one female patient from acute anterior myocardial infarction 29 months after the PCI (cardiac death). From group 2, only one woman had a myocardial infarction 30 months after the PCI, no other coronary events were found in this patient group. New coronary interventions were not performed within the 5-year follow-up in group 2 and after the rePCI or CABG in group 1; all living patients are clinically stable now.

	Group 1	Group 2
No. of patients (n)	11	20
B-Blockers (%)	8 (73)	15 (75)
ACE inhibitors (%)	8 (73)	16 (80)
Nitrates (%)	8 (73)	16 (80)
Calcium-antagonists (%)	2 (18)	4 (20)
Lipid-lowering therapy (%)	5 (45)	8 (40)
Antidiabetics (%)	1 (9)	2 (10)
Anti-platelet therapy (%)	11 (100)	20 (100)

Table 5 The most important medications applied before PCI. Values are expressed as number (%) unless otherwise indicated. p=ns

Group	Velocity	Before PCI	5±4 weeks after PCI	33±20 weeks after PCI
1	Drest	70.3±15.4	66.3±29.8	-
	Dmax	115.5±29.2	105.5±41.1	-
	CFR	1.66±0.37	1.68±0.42	-
2	Drest	52.1±20.5	59.8±18.0	51.5±17.3
	Dmax	86.1±36.6	120.8±27.7	107.4±28.9
	CFR	1.73±0.45	2.09±0.46*	2.14±0.28*

Table 6 Diastolic coronary flow velocities and CFR before and after LAD-PCI (expressed in cm/s) *p<0.05 in comparison with the before LAD-PCI (groups 1 and 2) and 5±4 weeks after LAD-PCI (group 1). Abbreviations. CFR: coronary flow velocity reserve, Dmax: diastolic velocity measured at the peak of stress, Drest: diastolic velocity measured at rest, LAD: left anterior descending coronary artery, PCI: percutaneous coronary intervention

4.3 The effect of hypercholesterolemia on anti-ischemic effect of preconditioning during coronary angioplasty

With the exception of the fasting blood cholesterol values, there was no difference between the demographic, clinical and angiographic parameters of the two groups (Table 7).

Features	Normocholesterolemic group (n=15)	Hypercholesterolemic group (n=15)
Age, yr	59 ±2	61 ±2
Male/female gender	9/6	8/7
Hypertension	11	12
Smoking	4	2
Diabetes mellitus	1	2
Target vessel		
LAD	9	11
CX	2	2
RCA	4	2
Previous PCI	1	3
Left ventricular EF, %	59 ±2.5	53 ±1.8
Triglyceride, mg/dL	55.5 ±10.2	80.8 ±8.7
Cholesterol, mg/dL	166.8 ±17.0	234.7 ±9.8 *
HDL cholesterol, mg/dL	43.8 ±3.0	46.8 ±2.3
Statin treatment	11	9
Data are presented as mean ±SEM or No. * p<0.05 vs. Normocholesterolemic group.		

Table 7 Clinical feaures of the patients

Time course of ST-segment elevation during ischemia. In the normocholesterolemic group, ST-segment elevation showed a continuous rise during the 2-min occlusions (Figure 8). Repeated occlusions resulted in lower ST-segment elevations, which reached a statistically significant level in the last 30-s periods of the occlusions, showing the anti-ischemic effect of preconditioning. In the hypercholesterolemic group, a rapid elevation of the ST segment was developed in the initial 30 s of the first occlusion, which was not observed in the subsequent two occlusions. However, from 45 to 120 s of the occlusions, there was no difference between ST-segment elevations. This shows that in hypercholesterolemic patients, preconditioning only slowed down the rapid onset of ischemia seen at the initial 30 s of the first occlusion but

did not protect against the evolution of ST-segment elevation observed by the end of the occlusions.

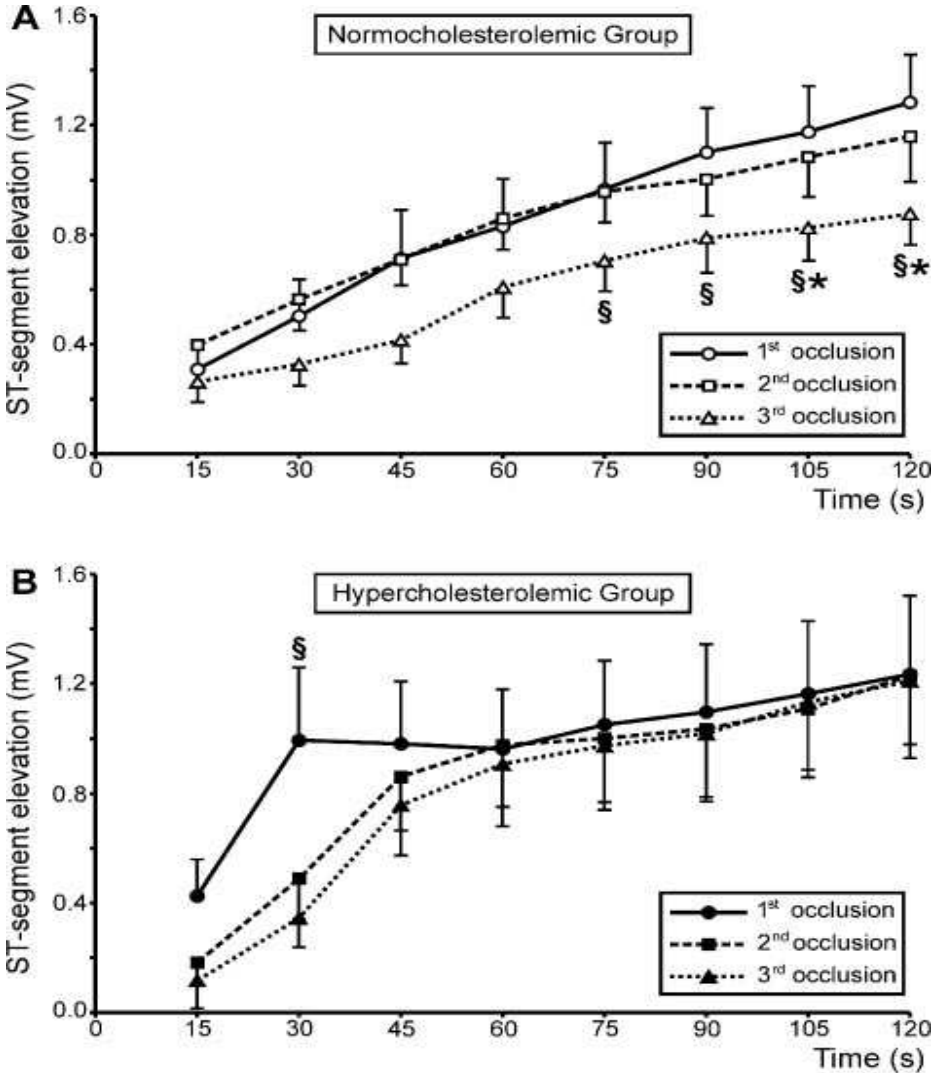

Figure 8 ST-segment elevations during balloon inflation periods in normocholesterolemic (top, A) and hypercholesterolemic (bottom, B) patients. Values are mean ± SEM of 15-s periods (n=15 in both groups). *p <0.05 (RM-ANOVA, Bonferroni correction for eight repeats); §p <0.05 vs first occlusion (RM-ANOVA).

Time to normalization of the ST segment during reperfusion. We determined the time to normalization of intracoronary ST segment during the reperfusion periods on balloon deflations as an indicator of the recovery of the heart from ischemia. In normocholesterolemic

patients, we observed a significant decrease in time to normalization of the ST segment during repeated reperfusions, showing the preconditioning effect (Figure 9). In the hypercholesterolemic group, time to normalization of the ST-segment after all the three ischemic periods was significantly prolonged as compared to the normocholesterolemic group, and repeated occlusion/reperfusion periods did not decrease time to normalization of ST segment. This shows the lack of the protective effect of preconditioning in hypercholesterolemia.

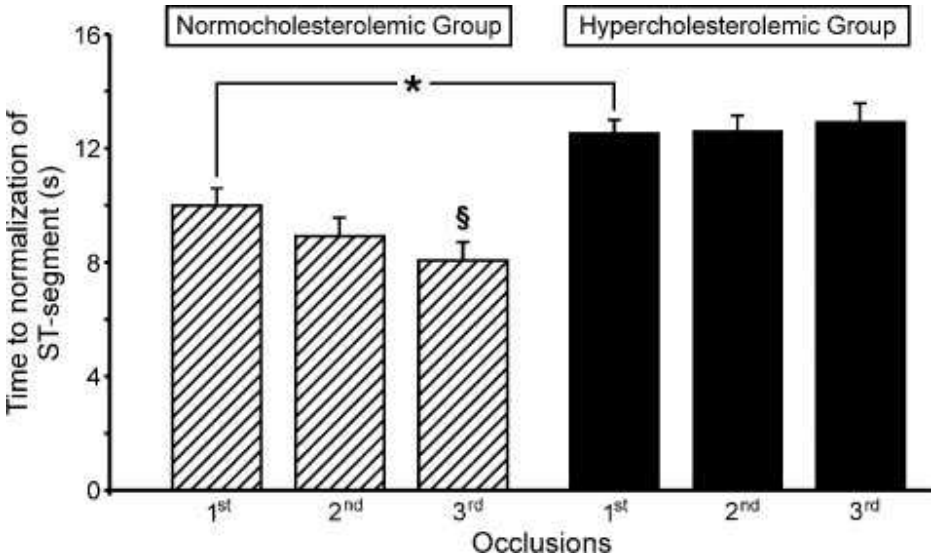

Figure 9 Time to normalization of the ST segment following balloon deflations after coronary occlusions in normocholesterolemic and hypercholesterolemic patients. Values are mean± SEM (n=13 in both groups). *p <0.05 vs normocholesterolemic group (independent-samples t test; Bonferroni correction, p = p x 3). §p <0.05 vs first occlusion (pairwise comparisons based on estimated marginal means).

4.4 Myocardial protection with enalaprilat in patients unresponsive to ischemic preconditioning during percutaneous coronary intervention

Clinical data on the study group. Ten patients in the control group and 10 in the enalaprilat group satisfied the criteria for inclusion in the study, and yielded technically adequate intracoronary ECG recordings associated with complete resolution of the ischemia between the balloon inflations. The angioplasty procedure was successfully completed in all subjects; no adverse cardiovascular event was detected in any of the patients. Characteristic data on the

study subjects are listed in Table 8. There were no significant differences between the groups as regards their demographic and clinical parameters. In both groups, the systolic and diastolic aortic blood pressures remained unchanged following the release of the first balloon inflation.

Features	Control group (n=10)	Enalaprilat group (n=10)
Age, yr	52 ±6	58 ±7
Male/female gender	6/4	5/5
Hypercholesterolemia	4	5
Previous infarction (remote)	2	3
Anginal status (CCS classification)	2.4 ±0.5	2.2 ±0.5
Date of last angina (days before PCI)	6.3 ±2.4	7.6 ±3.6
LVEF, %	57.2 ±6.8	51.4 ±6.5
Hypertension	5	6
Diabetes mellitus	4	3
Target vessel		
LAD	4	4
CX	3	4
RCA	3	2
Data are presented as mean ±SEM or No.		

Table 8 Demographic and clinical data on the patients. There were no significant differences between the groups in any of the examined parameters. Values are means±SEM.

The severity of ischemia following consecutive balloon inflations. The beat-to-beat analysis of the ST-segment elevations during consecutive balloon inflations in one control and one enalaprilat-treated patient is illustrated in Figure 10A and B.

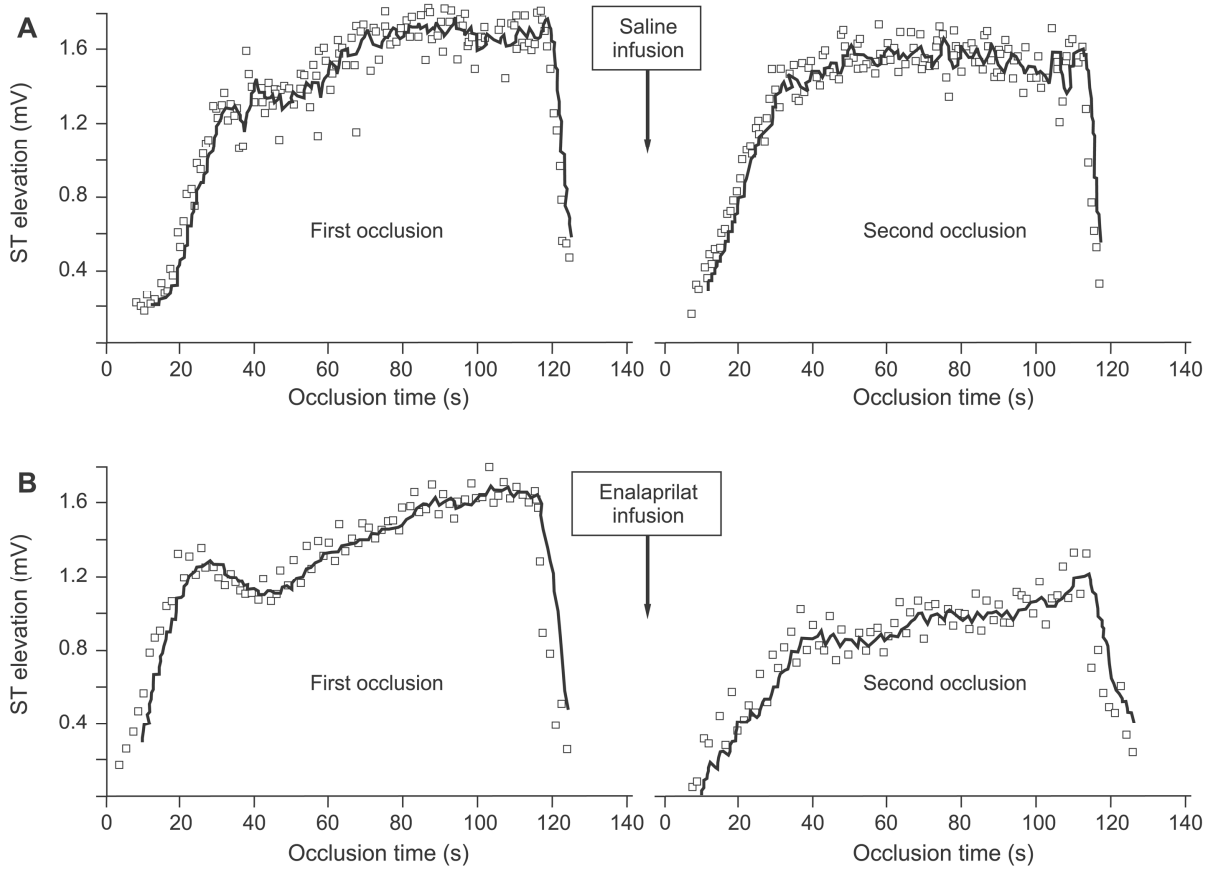

Figure 10 A representative figure illustrating beat-to-beat analysis of the ST-segment elevation (mV) during coronary occlusions in one control (A) and one enalaprilat-treated (B) patient. The diagrams of patient B demonstrate a marked difference in the evolution of ischemia as measured by the ST elevation on the intracoronary ECG. At the first occlusion there was a steeper ascent and higher plateau of the ST elevation, while after enalaprilat infusion the evolution of ischemia was much slower. Both the peak and the mean ST elevation were less during the second than during the first occlusion.

We assessed the severity of ischemia by measurement of the intracoronary ECG, expressed in terms of various parameters, such as the peak ST-segment elevation (Figure 11), the time required to reach a 0.5 mV elevation (Figure 12), and the total ischemic burden, the latter being evaluated by calculating the mean ST-segment elevation over the entire 2-min occlusion period (Figure 13).

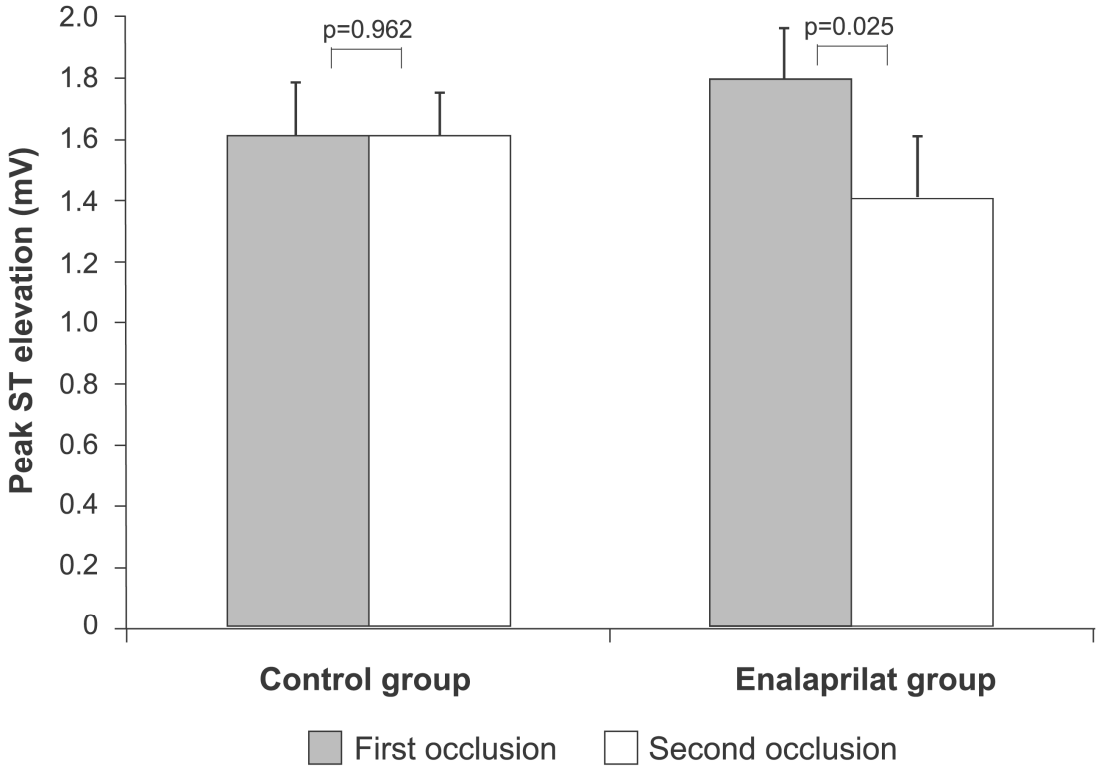

Figure 11 Changes in peak ST-segment elevation (mV) during the first and the second occlusion in the control and enalaprilat-treated patients. As compared with the controls, where the peak ST-segment elevation was similar during the consecutive balloon inflations, in the patients given enalaprilat the peak ST-segment elevation was markedly reduced during the second inflation. Values are means±SEM. *p<0.05 vs first occlusion.

In the control patients subjected to consecutive balloon inflations without drug treatment, both the magnitude of the peak ST-segment elevation (Peak-ST: 1.61±0.17 vs. 1.61±0.16 mV; Figure 11) and the time course of ischemia development (time to reach 0.5 mV ST-elevation: 16±4 vs. 22±7 s; Figure 12) were almost identical during the first and second balloon inflations. In contrast, in the patients infused with enalaprilat the peak ST-segment elevation was significantly less (1.41±0.19 vs. 1.80±0.18 mV, $p<0.05$; Figure 11), and the time to reach the 0.5 mV elevation was significantly longer (18±4 vs. 30±4 s, $p<0.01$; Figure 12) during the second than during the first occlusion.

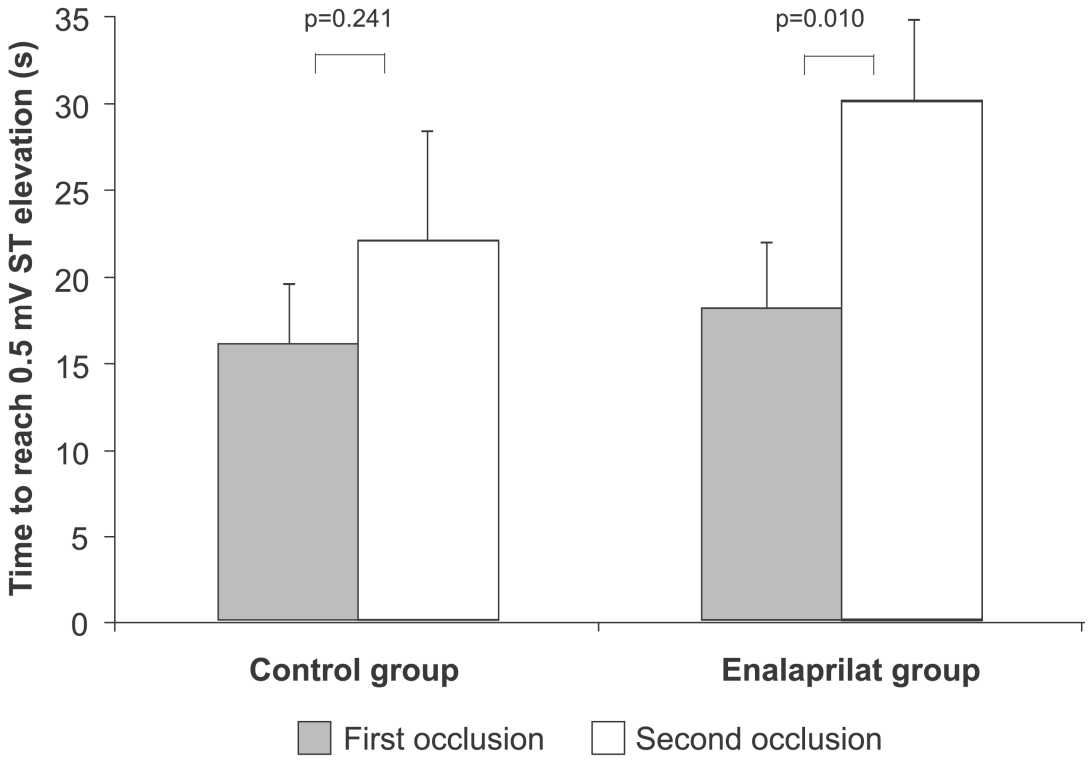

Figure 12 Time to reach 0.5 mV ST elevation during the first and second occlusions in the control and enalaprilat groups. We characterized the evolution of ischemia by the time necessary to reach a predefined (0.5 mV) ST elevation. Although the length of this period in both groups was somewhat longer during the second occlusion, a significant increase in this time period was found only in the enalaprilat group. Values are means±SEM.

The total ischemic burdens during the first and second balloon inflations were very similar in the control group (mean-ST: 1.03±0.12 vs. 1.02±0.11 mV; Figure 13) whereas in the enalaprilat group the burden was significantly lower during the second occlusion (1.04±0.11 vs. 0.85±0.14 mV; $p<0.01$; Figure 13).

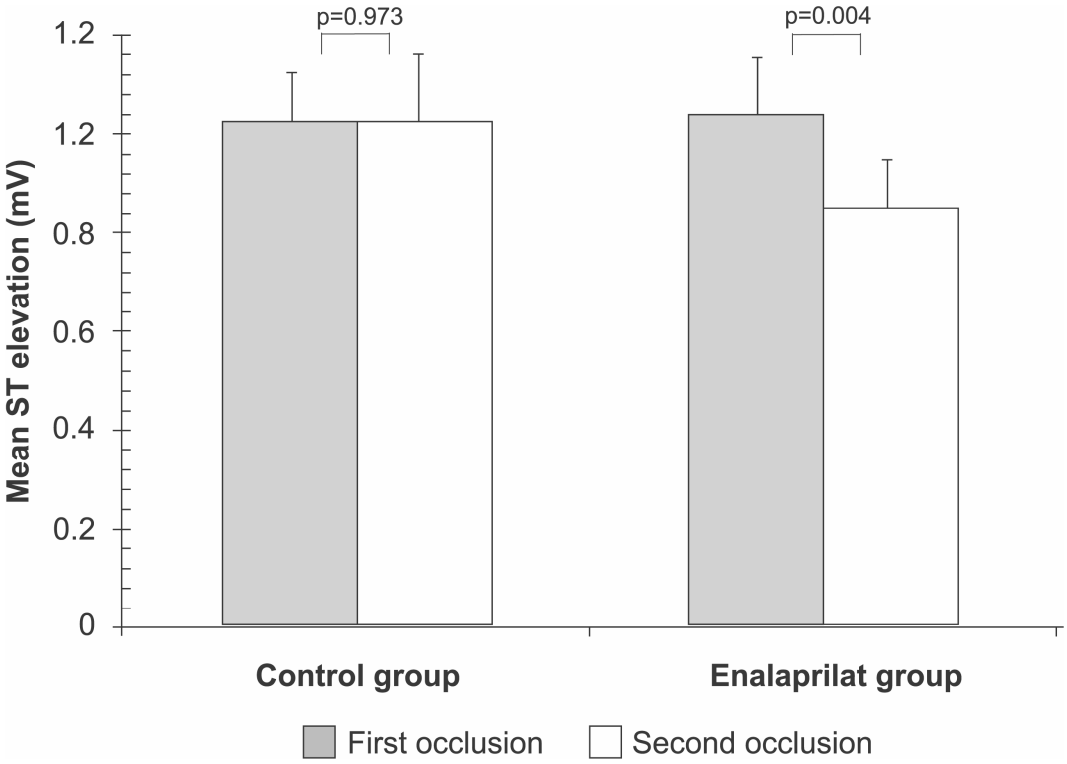

Figure 13 Mean ST elevations during the first and second occlusions in the control and enalaprilat groups. In the control group, the mean ST elevations were similar when saline was applied between the first and second balloon inflations. In contrast, the infusion of enalaprilat significantly decreased the mean ST elevation from the first to the second occlusion. Values are means±SEM.

5 Discussion

These studies were primarily focused to examine the extremely great variability of myocardial ischemia and its clinical appearance. This is manifested in the non-linear progression of coronary atherosclerosis, and in the limited predictability of post-PCI restenosis, as well. Another principal manifestation of this variability is the diverse adaptation capability of the myocardium to ischemia.

5.1 Non-invasive quantification of myocardial ischemia to improve the timing of repeated cardiac catheterization.

One of the primary goals of the thesis was to test the reliability of different non-invasive diagnostic methods for the quantification of myocardial ischemia in this variable environment. The clinical relevance of these diagnostic tests is that theoretically they facilitate to plan the optimal timing of repeated catheterization in patients with proved ischemic heart disease or already treated by PCI.

The first two studies proved that both DET and STEE can correctly predict the progression of native coronary artery disease or the restenosis process. Both of these non-invasive tests can be of great benefit for the invasive cardiologist to correctly assess the indication of repeated cardiac catheterization.

5.1.1 Non-invasive prediction of angiographic progression of coronary artery disease by dipyridamole-stress echocardiography

Serial assessment of repeat dipyridamole-stress-echocardiography results allows one to separate angiographic progressors and non-progressors efficiently, by taking into account the presence, extent and severity of stress-induced abnormalities of wall motion. The results of the present study are consistent with those of several studies showing that the extent and severity of induced dysfunction observed during stress echocardiography somewhat mirrors the extent and severity of the underlying CAD (51,55). Also the ischemia-free duration under stress might be a measure of the severity, being shorter for patients with more advanced forms of single-vessel disease, in terms of anatomic and functional criteria (16,56,57). However, the ischemia-free duration under stress is related to the severity of disease in the ischemia-related vessel, rather than to the extent of the atherosclerotic disease. This could account for the briefer duration of the time to ischemia, in comparison with the peak WMSI, in our study's model (16). The unique feature of the present study is that coronary-angiography and stress-echocardiography results were not only assessed horizontally, at one point in time, comparing different patients, but also longitudinally, at two different times, using each patient as his or her own control in order to assess individual variations in anatomic angiographic progression and results of functional stress testing.

The rationale of our study is along the line pioneered by Gould et al. (10), who were the first to propose an imaging stress test to monitor non-invasively the angiographic progression of the disease. They used positron-emission-tomography-perfusion imaging, which is technologically demanding and characterized by high cost and low availability. Exercise thallium scintigraphy has also been proposed as a means for assessing evolution of angiographically assessed CAD. For 18 patients subjected to a regimen of intensive physical exercise and a low-fat diet, Schuler et al. found that myocardial perfusion in individual patients improved markedly despite there being no change in or a worsening of angiographically assessed coronary disease (58). In fact, perfusion imaging detects impairment of coronary flow (59), whereas abnormalities of wall motion are consistently induced only by impairment of coronary flow reserve due to CAD (57,60), not impairment of flow reserve due to microvascular disease in angiographically normal coronary arteries (61). Therefore, perfusion imaging might be more suitable for detecting microvascular endothelial dysfunction and healing (9,10), whereas results of functional stress testing may be more tightly linked to impairment of coronary flow reserve due to coronary-artery stenosis.

As a functional stress-imaging test, we employed pharmacological dipyridamole-stress-echocardiography, which has obvious advantages in terms of cost and logistics, and has been recognized to be of proven diagnostic and prognostic value for the assessment of patients with CAD in the recent guidelines of American College Cardiology/American Heart Association (62) and American Society of Echocardiography (63). This study has a few limitations. We employed a quantitative assessment of coronary arteriograms with a widely adopted semiquantitative scoring method. Angiography provides a mere luminogram, depicting coronary anatomy from a planar two-dimensional silhouette of the lumen. Confounding factors include tortuosity of vessels, overlap of structures and the effects of shape of lumen (64). Quantitative coronary arteriography, which we employed in the present study whenever feasible, is more reproducible, but not necessarily more accurate, than simple visual assessment. In addition, results of several studies show that there is a marked disparity between the apparent severity of the lesions and their pathophysiological effects, the latter being more tightly related to stress-echocardiographic results than are the former. All these factors could have introduced sources of errors into the validity of our assumption of angiographic assessment of CAD as a standard against which to assess the capability of this non-invasive test to predict progression of CAD. Ideally, intravascular ultrasound

measurements would have been much more appropriate references. Nevertheless, angiography is currently the standard method for diagnosing disease; it has been validated widely with regard to its prognostic and therapeutic implications; and it was used in this study under especially favourable conditions, with each patient acting as his or her own control.

Stress echocardiography is based on the subjective assessment of regional wall motion. Although there can be substantial inter-centre variability in stress-echocardiographic readings even between experienced observers, the agreement is substantially higher when a-priori criteria for reading data are developed by a pool of accredited readers with extensive experience of joint reading and deliberately ignoring minor forms of questionable hypokinesia (63,64). In our experience, this is the best approach for obtaining reliable and consistent results with stress-echocardiographic reading (65-68).

Finally, the criteria for selection of patients should be considered. The design of our study was observational and the analysis of the data bank was retrospective. However, the data were prospectively acquired and entered in the data bank at the time of execution of our study, by observers who remained unaware of the results of the other tests.

5.1.2 The coronary flow velocity reserve measured by stress transoesophageal echocardiography evaluates the success of coronary interventions

This is the first investigation of the potential usefulness of the STEE-derived CFR in the evaluation of the long-term success of LAD-PCI. The CFR of patients with successful LAD-PCI improved continuously (became >2) and these cases did not require further invasive procedures during a 5-year follow-up period (group 2). Patients whose condition required rePCI or CABG within half a year, CFR remained unchanged and two patients died during this follow-up period (group 1). Transoesophageal echocardiography can be used for a direct evaluation of the coronary flow dynamics. Previous studies have examined coronary flow velocity patterns during PCI (69,70). There is a good correlation between CFR assessed by Doppler guide-wire and that determined by TEE (71). The CFR is reduced in patients with significant LAD disease, in case of proximal location or more severe stenosis, further impairment can be observed (22,71-77). Paraskevaidis et al. performed several studies with stress TEE as CFR measurement for the evaluation of the success of LAD-PCI (72-74). Paraskevaidis et al. suggested that TEE can be utilized to evaluate the functional status of

LAD-PCI. When the CFR is >2, there is less residual coronary artery stenosis, as detected by intravascular ultrasound (72). The CFR is significantly higher early after successful than after unsuccessful LAD-PCI, as assessed by STEE. The TEE–CFR may serve as a noninvasive index of early postangioplasty restenosis (73). They verified that CFR after PCI of proximal LAD can be evaluated serially by TEE. CFR of LAD in patients without restenosis is increased 3 months after PCI and remains stable thereafter (75). In contrast, Tsutsui et al. found that periprocedural CFR conferred no predictive value for subsequent intrastent restenosis (78).

The possibility now exist for the measurement of the CFR by transthoracic echocardiography (79-81). Pizzuto et al. evaluated the flow changes and the CFR in the LAD before and after stenting, as measured by transthoracic Doppler echocardiography during adenosine infusion. They found that the CFR may identify patients with ≥90% stenosis; and it normalizes early after stenting, even in patients with ≥90% stenosis (79). A CFR<2 had 91% sensitivity, 95% specificity, and 96% positive and 97% negative predictive values to detect significant stenosis in patients with LAD stents (80). Non-invasive CFR assessment by contrast-enhanced transthoracic echocardiography is an accurate method for monitoring significant restenosis in the LAD when following up patients submitted to elective PCI (81).

Serruys et al. demonstrated in the DEBATE study (Doppler Endpoints Balloon Angioplasty Trial Europe), that using Doppler guidewire the measurement of distal CFR after PCI, in combination with diameter stenosis (%), have a predictive value, albeit modest for the short- and long-term outcomes after PCI (82). A low postprocedural CFR was associated with a worse perprocedural outcome, but there was no significant difference at late follow-up as concluded from the DEBATE II study (83). According to the DESTINI study (Doppler Endpoint STenting INternational Investigation), when balloon angioplasty is guided by online quantitative angiography and Doppler-derived coronary flow reserve, with provisional stenting reserved for suboptimal results, early and late clinical outcomes are comparable to those achieved by elective stenting of all patients (84).

The present study verified the prognostic role of TEE-CFR in the long-term functional evaluation of the success of PCI. Regarding to its semi-invasivity, the clinical usefulness of this method is questionable in the future, the stress transthoracic echocardiography can indicate an alternative way. However, it can be stated that more studies are needed to clarify

the prognostic role of stress transthoracic echocardiographically-detected CFR in the long-term evaluation of PCI. One of the main limitation is that the population sample was relatively small. It was not our aim to evaluate the flow velocity changes during the follow-up. The evaluation of successful coronary angioplasty was symptom-limited; control coronary angiography was performed only in hospitalized patients (group 1). This approach measures blood flow velocities, and not the blood flow itself. The measurement of coronary blood flow requires an evaluation of the luminal cross-sectional area. Further, there is an angle between the ultrasound beam and the vessel direction, as a result of which blood flow velocities measured by this approach can be lower than the actual values. However, both the numerator and the denominator in the formula for the CFR are measured at the same angle, and thus their ratio is not appreciably influenced by the angle or the vessel direction.

5.2 Limitation of ischemic preconditioning by hypercholesterolemia and pharmacological enhancement of myocardial ischemic adaptation

The object of the second part of our studies was to examine the source of variability in ischemia sensitivity in angiographically proved coronary artery disease. It has already been published both in animal and human experiments that different pathologic states and aging, as well can influence the adaptation capability of the myocardium. The target of these pathologic states was hypercholesterolemia in the third part of our investigations, because this is one of the most common metabolic disturbances as a risk factor of ischemic heart disease. Our results proved that in patients with hypercholesterolemia ischemic preconditioning is limited compared to those with normal cholesterol blood level. These data can serve as explanation of the clinical observation that the outcome of acute myocardial infarction in patients with elevated cholesterol level can be worse compared to normocholesterolemic subjects. This observation is a partial explanation of the interindividual variability of preconditioning, however it must be considered clinically important, because this raise attention to the fact that hyperlipidemia is a “double” risk factor; not only that of coronary atherosclerosis, but for the severity of myocardial ischemia, as well.

This variability of adaptive response to ischemia of myocardium emphasizes the importance of therapeutic methods improving the efficacy of ischemic preconditioning. That is why we

completed another study on the pharmacologic potentiation of ischemic preconditioining in patients, who poorly responded short ischemic epizodes with ischemic adaptation.

The last phase of our research investigated the therapeutic aspects of the diversity in ischemic preconditioning. It is well-known that the adaptive response of the myocardium to brief periods of ischemia is extremely variable. We attempted to decrease this variability by pharmacological potentiation of the ischemic preconditioning in patients with reduced adaptive response to short coronary occlusions. Intracoronary infusion of enalaprilat was selected for this purpose by previous animal and human experimental results, which demonstrated the role bradykinin B2 receptors.

The results of this study proved that the myocardium of patients with limited ischemic preconditioning can still be protected by combination of the short ischemic episodes with intracoronary enalarilate infusion.

5.2.1 The effect of hypercholesterolemia on anti-ischemic effect of preconditioning during coronary angioplasty

We investigated if hypercholesterolemia attenuates ischemic preconditioning in 30 patients. We have shown here for the first time in the literature that in hypercholesterolemic patients, there is a rapid elevation of ST segment during the initial 30 s of the first balloon inflation, a phenomenon not seen in normocholesterolemic patients. Our results further show that in hypercholesterolemic patients, the repeated occlusions although abolished the initial rapid elevation of ST segment but did not attenuate ST-segment elevation when measured at 45 to 120 s, and failed to decrease the time to normalization of ST segment after balloon deflations when compared to normocholesterolemic patients. Our results provide clear-cut evidence that hypercholesterolemia enhances the evolution of myocardial ischemia on coronary occlusion and significantly inhibits the antiischemic effect of preconditioning in humans. A high-cholesterol diet is regarded as an important factor in the development of ischemic heart disease. There is a linear relationship between elevation of serum total cholesterol concentration and the incidence of myocardial infarction; furthermore, the heart of hyperlipidemic/atherosclerotic patients is hardly capable of adapting to physical exercise or other kinds of stress (86,87). This has been attributed solely to the development of atherosclerosis, and the possibility of the deterioration of endogenous adaptive mechanisms

against myocardial ischemia, ie, preconditioning, has not been considered in hypercholesterolemic patients. Although the majority of animal studies (28,29,88) show that experimental hypercholesterolemia interferes with the cardioprotective effect of preconditioning, little is known about the effect of hypercholesterolemia on human preconditioning.

Our present study provided solid evidences showing that hypercholesterolemia inhibits the cardioprotective effect of acute preconditioning in man. We have assessed the time course of the evolution of myocardial ischemia and that of the recovery from ischemia by a beat-to-beat analysis of the intracoronary ST-segment shifts induced by balloon inflations and deflation during PCI and observed that in hypercholesterolemic patients, there is a rapid increase in ST-segment elevation at the beginning (0 to 30 s) of the first balloon inflation; however, this is not seen in the second and third occlusions. This phenomenon could be considered as a preconditioning effect; however, if one looks at ST-segment elevation at 45 to 120 s of the occlusions, no preconditioning effect can be observed in hypercholesterolemic patients. This shows that a week preconditioning effect can be observed in hypercholesterolemic patients, but it only slightly delays the evolution of ischemia and does not alleviate the severity of ischemia. Our results support that of Kyriakides et al (88) who reported that ST-segment elevation on the surface ECG measured at 120 s of balloon inflations was not attenuated during repeated occlusions in hypercholesterolemic patients. Furthermore, we have found that the time to normalization of the ST segment was decreased after repeated coronary occlusions/reperfusion periods in normocholesterolemic patients, showing the beneficial effect of preconditioning. However, in hypercholesterolemic patients, time to normalization of the ST segment was significantly longer even after the first occlusion, and repeated occlusion/reperfusion periods did not decrease this parameter. These results clearly show that hypercholesterolemia aggravates ischemia/reperfusion injury and impairs the anti-ischemic effect of preconditioning in humans.

The mechanism by which hypercholesterolemia may influence the severity of myocardial ischemia and the effects of preconditioning in humans is not known; however, several mechanisms have been suggested in animal studies (28,29), such as deterioration of myocardial nitric oxide metabolism (30), increased formation of reactive oxygen species such as super- oxide and peroxynitrite (89), disruption of the mevalonate pathway (90), attenuation

of heat shock response (91), and the accumulation of cholesterol in the sarcolemmal and mitochondrial membranes (92,93). However, it seems that hypercholesterolemia induces very complex changes in cellular mechanisms of the myocardium, as our study (94) using DNA microarray assay of 3,200 genes showed that hypercholesterolemia leads to significant alterations in the expression of 51 genes (4% of the examined genes) in the rat heart. There appears a controversy in some studies10–12 on the effectiveness of preconditioning induced by PCI in humans. We assume that the reasons for the controversy among others might be the following: (1) that hypercholesterolemic and normocholesterolemic patients have not been separated in these studies, and (2) that ST-segment elevation in myocardial ischemia was evaluated by different ways. Study protocols usually include an arbitrarily selected time point to assess ischemia during balloon inflations. Dupouy et al11 failed to show preconditioning induced by 2-min balloon inflations when measured intracoronary ST-segment elevation at 90 s of balloon inflations in 13 patients. Billinger et al10 failed to observe the anti-ischemic effect of pharmacologic preconditioning induced by intracoronary adenosine before angioplasty when measuring intracoronary ST-segment elevation at 60 s of balloon inflations in 30 patients. Laskey and Beach12 studied preconditioning in 382 patients by the assessment of the maximal ST-segment elevations on either surface or intracoronary ECG during two 90-s balloon occlusions, and they could elicit preconditioning only in 80% of their patients. In these studies, the reason for the lack of preconditioning was not shown. Our present findings, ie, the differences in the time course of ST-segment elevation between normocholesterolemic and hypercholesterolemic patients and the limitation of preconditioning in hyper-cholesterolemic patients, show the necessity to distinguish between normocholesterolemic and hypercholesterolemic patients and emphasize the importance of a beat-to-beat analysis of ST-segment elevation du ring the entire periods of balloon inflations and deflations in human preconditioning studies. It should be noted here that similarly to hypercholesterolemic patients, in patients with other diseases such as diabetes, heart failure, and aging, the time course of ischemia during PCI may also be altered that can be assessed only by a beat-to-beat analysis of ST-segment elevation.

5.2.2 Myocardial protection with enalaprilat in patients unresponsive to ischemic preconditioning during percutaneous coronary intervention

The finding of this study supports our previous observation (95) that in patients with severe coronary artery disease who undergo elective PCI, a single brief (2-min) period of balloon inflation is not sufficient to give rise to significant improvements in the intracoronary ST-segment changes that occur during a subsequent, similar period of balloon inflation. We have now demonstrated for the first time that the administration of enalaprilat to such patients during PCI does induce protection, as revealed by significant reductions in the mean and peak ST-segment elevation and the onset of ischemic changes during the second coronary artery occlusion. From the present study it is difficult to decide whether this protection is due to the action of enalaprilat alone or whether it results from the combined effects of the previous occlusion and the enalaprilat treatment. Whatever the precise roles of the preceding ischemia and enalaprilat in this protection, the primary aim of the study was to examine whether patients who seem to be unresponsive to a single balloon inflation and who do not exhibit a preconditioning-like effect during the subsequent ischemia, would benefit from acute drug administration during PCI. We consider, that this timing of the drug administration could be of particular importance in everyday clinical practice, since, a variable degree of myocardial ischemia is already present in a substantial proportion of patients with acute coronary syndromes who are referred for emergency PCI. In this patient subset, persistence of ischemic symptoms demonstrate considerably jeopardized state of the myocardium and, limited preconditioning effect of the sequential short ischemic episodes. Our results served indirect information that in these high-risk patients – particularly in those with slow flow and no-reflow phenomenon - the administration of intracoronary enalaprilat can still be cardioprotective. Patients with non-ST-elevation myocardial infarction may also have special interest in this respect, since in these subjects there are short repeated ischemic attacks without considerable preconditioning effect. The potential benefit of intracoronary enalaprilat in this clinically relevant population may reproduce the same mechanism, what we demonstrated now in elective patients.

There is an ongoing debate as to whether a short (90 to 120-s) period of balloon inflation in patients with various degrees of coronary artery disease, and with comorbidities such as diabetes, atherosclerosis, hypercholesterolemia, hypertension, etc., is able to produce protection similar to ischemic preconditioning (96,97). Dupouy et al. (98), using consecutive

balloon inflations for a duration of 2 min, found no significant differences between the occlusions as concerns modification of the intracoronary ECG or the left ventricular systolic function. They concluded that such a brief period of ischemia is insufficient to produce protection against the consequences of a subsequent ischemic event. There is, of course, a possibility to increase the duration of the occlusion but prolongation of the balloon inflation over a certain period may raise ethical concerns. Johansen et al. (99) reported that, on increase of the duration of the occlusion from 181 s to 307 s, the risk of irreversible myocardial injury was enhanced, as assessed from a 10-fold increase in the blood troponin T levels. Matsubara et al. (100) examined the relationship between the duration of balloon inflation and its effectiveness during PCI, and demonstrated that only 180-s, but not shorter occlusions were effective in inducing a substantial reduction in the ST-segment alterations during the subsequent balloon occlusion. In an investigation of the effects of ischemic preconditioning in 382 patients, Laskey and Beach (101) showed that some of the patients exhibited a preconditioning-like protection following two 90-s balloon occlusions, while the others remained resistant to these stimuli.

Other studies (41,102), involving the use of 120-s balloon inflations revealed marked ST-segment reductions during repeated occlusions. The reasons for the potential success or failure of preconditioning in patients are not fully understood, but the severity of the coronary artery disease, age and gender differences, demographic features, the existence of comorbidities, etc, appear to be the most important factors, which certainly determine the ability of the human myocardium to respond with protection or with resistance to a brief period of occlusion.

There is considerable evidence that, in experimental animals, various subthreshold preconditioning stimuli, which are alone insufficient to elicit protection, will result in significant cardioprotection if they are applied together (103). Furthermore, endogenous substances, such as bradykinin (37), adenosine (104) or nitric oxide (105) that are released in the early phase of myocardial ischemia, either from the cardiac myocytes or from the coronary vascular endothelium, have been shown to play a trigger role in the cardioprotective effects of preconditioning. It is likely that under conditions where an endothelial dysfunction is evident (hypertension, atherosclerosis, hypercholesterolemia, diabetes, etc.) the ability to generate such endogenous myocardial protective substances may be impaired. This would

lead to the attenuation of a major pathway for protection and may explain, at least in part, why short coronary artery occlusions in these patients fail to produce a preconditioning-like effect. Thus, it might be expected that drugs which are able to replace the loss of these substances due to acute or chronic injury (e.g. nitric oxide donors), or drugs that enhance the production of these substances (e.g. inhibition of the kinin metabolism with ACE and neutral endopeptidase inhibitors with a subsequent increase in nitric oxide production) would be cardioprotective. Since our studies consistently we have consistently indicated that patients subjected to two balloon inflations for a duration of 120 s, did not exhibit a significant improvement in their ST-segment parameters during the second balloon occlusion, the question was raised of whether another, albeit different stimulus could elicit protection. Thus, we selected an ACE inhibitor that is known to induce preconditioning-like protection (38,106-108). The dose of enalaprilat was selected from the studies of Mohri et al. (47). and Rundqvist et al. (48) who reported that 50 µg min-1 enalaprilat does inhibit coronary ACE, but does not cause arterial hypertension. We then observed the infusion of enalaprilat between the two balloon inflations resulted in a marked reduction in the development and also in the magnitude of the ST-segment elevation that occurred during the second occlusion. This result indicated that, in contrast with the controls, enalaprilat, perhaps together with the preceding ischemic event, serves as an adequate stimulus to induce protection. Leesar et al. (41) recently reported that a higher dose of enalaprilat, given prior to the first balloon inflation, was cardioprotective, mimicking the effect of the first preconditioning occlusion. We should note that there are substantial differences between their and our studies. First, the objectives of the two studies were not the same since the patient populations differed substantially. As we have pointed out above, they worked with patients who responded with protection to a 120-s coronary artery occlusion, whereas our patients were not responsive to this stimulus per se. Secondly, the total dose of enalaprilat that induced cardioprotection in the study of Leesar et al. (41) was 0.75 mg (50µg min-1 over 15 min). We have now demonstrated that a lower dose (0.5 mg) is sufficient to evoke protection, even though our patients proved unresponsive to the ischemic stimulus. The most relevant difference was that Leesar et al. infused a high dose of enalaprilat before the ischemic stimuli, while we administered the drug only after the ineffective first brief coronary occlusion. We consider that our experimental set-up provides a more realistic model as regards clinical practice, when we meet patients who have undergone recent anginal attacks. The study by Leesar et al. furnished good evidence of the preventive

effect of enalaprilat against subsequent ischemia. In contrast, our model has clearly shown that, in the event of ongoing ischemia which is not sufficient to precondition the myocardium, enalaprilat administration can still be effective. It is worthy of note that the objective of our study was not the myocardial protection from potentially harmful consequences of the first balloon inflation, since the length of coronary occlusion is usually shorter in the everyday clinical practice, and on the other hand, the duration of a 2-min balloon occlusion has no risk of irreversible myocardial damage. Instead, our experimental setup served as a model of patients with acute coronary syndrome, treated with PCI, and suffering from preceding short ischemic attacks.

The present study has some limitations. First, as we have mentioned above, in the absence of an enalaprilat control group, it is difficult to define whether the protection in patients treated with enalaprilat is a consequence of the action of enalaprilat alone or whether the preceding occlusion also contributes to this effect. The limited number of patients selected for this study did not allow us to perform dose-finding examinations for enalaprilat. Secondly, the absence of collateral circulation was assessed only visually on coronary angiograms; the enhancement of potential recruitable collateral channels was not examined directly. Moreover, the effect of enalaprilat on the coronary blood flow was not evaluated in this study. However, there is convincing evidence that collateral circulation is unlikely to be a significant factor in the cardioprotective effect of preconditioning (109), and that in anesthetized dogs bradykinin protects against ischemia and reperfusion-induced arrhythmias in a dose (25 ng kg-1 min-1) that has no effect on the coronary circulation (37). Thirdly, the assessment of ischemia during repetitive coronary occlusions was based on beat-to-beat analysis of the ST-segment elevation on intracoronary ECG. Although this parameter can be regarded as a surrogate endpoint, this is a well-accepted and highly sensitive method for the quantitative evaluation of myocardial ischemia (95,110), and the ST-segment shift accurately reflects the presence of ischemic preconditioning (111). An additional limitation of our study is that although we could not detect any systemic hemodynamic effect of this dose of enalaprilat, this case can not be excluded in patients with acute myocardial infarction. This potential side effect must be evaluated in future studies, and may limit the spectrum of clinical scenery, in which this cardioprotective measure can be applied.

6 Conclusions (new observations)

1. Spontaneous evolution of angiographically assessed coronary artery disease can usefully be monitored non-invasively through recording variations in serial dipyridamole-stress echocardiography. This technique is highly feasible, widely available, of low cost, employing non-ionizing energy and with a total time for imaging and analysis of < 20 min. This application can further contribute to the expanding use of stress echocardiography in modern cardiological practice.

2. CFR is significantly higher early after successful than after unsuccessful PCI as assessed by STEE. The CFR of patients with successful LAD-PCI became >2 and suffered no major clinical events during a 5-year follow-up. In contrast, in those a priori had a low CFR and did not exhibit an improvement after PCI, further invasive procedures and major events did occur during this period.

3. Hypercholesterolemia attenuates the anti-ischemic effect of preconditioning, accelerates the evolution of myocardial ischemia, and delays the recovery from ischemia on reperfusion in humans. These findings further emphasize the importance of serum cholesterol as a predictive risk factor for the incidence and severity of myocardial ischemic events, and call for the development of new cardioprotective drugs that reverse the increased susceptibility of hearts to ischemic stress and recapture the cardioprotective effect of preconditioning in hypercholesterolemic patients.

4. There is a population of patients with severe coronary artery disease and assigned to elective PCI in whom a brief, 120-s period of coronary occlusion fails to produce protection against the consequences of a subsequent, similar period of ischemia. We have now further shown that, in these patients who seem to be unresponsive to this initial preconditioning ischemia, the administration of intracoronary enalaprilat during the procedure can still elicit adequate protection. It is our view that the combination of preconditioning stimuli which are different in nature (i.e. ischemic and pharmacological) may well provide protection even in patients who do not respond with protection to either of the stimuli separately.

7 References

1. Waters D , Craven TE , Lesperance J . Prognostic significance of progression of coronary atherosclerosis. Circulation 87: 1993; 1067–1075.
2. Chen L , Chester MR , Redwood S , Huang J , Leatham E , Kaski JC . Angiographic stenosis progression events in patients with 'stabilized' unstable angina. Circulation 91: 1995; 2319–2324.
3. Chen L , Chester MR , Crook R , Kaski JC . Differential progression of complex culprit stenoses in patients with stable and unstable angina pectoris. J Am Coll Cardiol 12: 1988; 56–62.
4. Azen SP , Mack WJ , Cashin-Hemphill L , Labree L , Shircore AM , Selzer RH et al. Progression of coronary artery disease predicts clinical coronary events. Circulation 93: 1996; 34–41.
5. Buchwald H , Matts JP , Fitch LL , Campos CT , Sanmarco ME , Amplatz K et al. Changes in sequential coronary arteriograms and subsequent coronary events: program on the Surgical Control of the Hyperlipidemias (POSCH) group. JAMA 268: 1992; 1429–1433.
6. Mulcahy D , Husain S , Zalos G , Rehman A , Andrews NP , Schenke WH et al. Ischemia during ambulatory monitoring as a prognostic indicator in patients with stable coronary artery disease. JAMA 4: 1997; 318–324.
7. Roussouw JE . Lipid-lowering interventions in angiographic trials. Am J Cardiol 76(suppl): 1995; 86C–92C.
8. Kaski JC , Chester MR , Chen L , Katritsis D . Rapid angiographic progression of coronary artery disease in patients with angina pectoris. Circulation 92: 1995; 2058–2065.
9. Gould KL , Ornish D , Scherwitz L , Brown S , Edens RP , Hess MJ et al. Changes in myocardial perfusion abnormalities by positron emission tomography after long-term, intense risk factor modification. JAMA 274: 1995; 894–901.
10. Gould KL , Martucci JP , Goldberg DI , Hess MJ , Edens RP , Latifi R et al. Short-term cholesterol lowering decreases size and severity of perfusion abnormalities by positron emission tomography after dipyridamole in patients with coronary artery disease. A potential noninvasive marker of healing coronary endothelium. Circulation 89: 1994; 1530–1538.
11. Schuler G , Hambrecht R , Schlierf G , Grunze M , Methfessel S , Hauer K et al. Myocardial perfusion and regression of coronary artery disease in patients on a regimen of intensive physical exercise and low fat diet. J Am Coll Cardiol 19: 1992; 34–42.
12. Eichstadt HW , Eskotter H , Hoffmann I , Amthauer HW , Weidinger G . Improvement of myocardial perfusion by short-term fluvastatin therapy in coronary artery disease. Am J Cardiol 76: 1995; 122A–125A.
13. Czernin J , Barnard RJ , Sun KT , Krivokapich J , Nitzsche E , Dorsey D et al. Effect of short-term cardiovascular conditioning and low-fat diet on myocardial blood flow and flow reserve. Circulation 92: 1995; 197–204.
14. Picano E , Lattanzi F , Masini M , Distante A , L'Abbate A. High dose dipyridamole echocardiography test in effort angina pectoris. J Am Coll Cardiol 1986; 8: 848–854.
15. Picano E , Lattanzi F . Dipyridamole echocardiography. A new diagnostic window on coronary artery disease. Circulation 83(suppl. III): 1991; III19–III26.
16. Picano E , Parodi O , Lattanzi F , Sambuceti G , Andrade MJ , Marzullo P et al. Assessment of anatomic and physiologic severity of single vessel coronary artery lesions by dipyridamole echocardiography: comparison with positron emission tomography and quantitative arteriography. Circulation 89: 1994; 753–761.
17. Picano E , Lattanzi F , Masini M , Distante A , L'Abbate A . Different degrees of ischemic threshold stratified by dipyridamole echocardiography test. Am J Cardiol 59: 1987; 71–73.

18. Picano E , Severi S , Michelassi C , Lattanzi F , Masini M , Orsini E et al. Prognostic importance of dypiridamole echocardiography test in coronary artery disease. Circulation 80: 1989; 450–457.
19. Gould KL, Lipscomb K: Effects of coronary stenoses on coronary flow reserve and resistance. Am J Cardiol 1974; 34: 48–55.
20. Vogel R, Indermuhle A, Reinhardt J, Meier P, Siegrist PT, et al.: The quantification of absolute myocardial perfusion in humans by contrast echocardiography: algorithm and validation. J Am CollCardiol 2005; 45: 754–762.
21. Nemes A, Forster T, Gruber N, Csanady M. Coronary flow velocity reserve and indices describing aortic distensibility in patients after coronary angiography. Int J Cardiol 2004; 96: 29–33.
22. Iliceto S, Marangelli V, Memmola C, Rizzon P. Tranesophageal Doppler echocardiography evaluation of coronary blood flow velocity in baseline conditions and during dipyridamole-induced coronary vasodilation. Circulation 1991; 83: 61-69.
23. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74: 1124-1136.
24. Liu GS, Thornton J, VanWinkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1-adenosine receptors in the rabbit heart. Circulation. 1991; 84: 350-356.
25. Schott RJ., Rohmann S, Braun ER, Schaper W. Ischemic preconditioning reduces infarct size in swine myocardium. Circ Res. 1990; 66: 1133-1144.
26. Goto M, Liu Y, Yang XM, Ardell JL, Cohen MV, Downey JM. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. Circ Res 1995; 77: 611-621.
27. Tomai F, Crea F, Chiariello L, Gioffre PA. Ischemic preconditioning in humans: models, mediators, and clinical relevance. Circulation. 1999; 100: 559-563.
28. Ferdinandy P, Szilvassy Z, Baxter GF. Adaptation to myocardial stress in disease states: is preconditioning a healthy heart phenomenon? Trends Pharmacol Sci 1998; 19: 223–229.
29. Ferdinandy P. Myocardial ischemia/reperfusion injury and preconditioning: effects of hypercholesterolaemia/hyperlipidaemia. Br J Pharmacol 2003; 138: 283–285.
30. Ferdinandy P, Szilvassy Z, Horvath LI, et al. Loss of pacing induced preconditioning in rat hearts: role of nitric oxide and cholesterol-enriched diet. J Mol Cell Cardiol 1997; 29: 3321–3333.
31. Kocic I, Konstanski Z, Kaminski M, et al. Experimental hyperlipidemia prevents the protective effect of ischemic preconditioning on the contractility and responsiveness to phenylephrine of rat-isolated stunned papillary muscle. Gen Pharmacol 1999; 33: 213–219.
32. Ueda Y, Kitakaze M, Komamura K, et al. Pravastatin restored the infarct size-limiting effect of ischemic preconditioning blunted by hypercholesterolemia in the rabbit model of myocardial infarction. J Am Coll Cardiol 1999; 34: 2120–2125.
33. Leesar MA, Stoddard MF, Ahmed M, Broadbent J., Bolli R. Preconditioning of human myocardium with adenosine during coronary angioplasty. Circulation 1997; 95: 2500-2507.
34. Leesar MA, Stoddard MF, Dawn B, Jasti VG, Masden R, Bolli R. Delayed preconditioning-mimetic action of nitroglycerin in patients undergoing coronary angioplasty. Circulation 2001; 103: 2935-2941
35. Yasuda T, Hashimura K, Matsumura Y, Kato Y, Ueda T, Mori I, et al. Nicorandil, a hybrid between nitrate and ATP-sensitive potassium channel opener, preconditions human heart to ischemia during percutaneous transluminal coronary angioplasty. Jpn Circ J 2000; 65: 526-530.
36. Yellon DM, Baxter GF. Protecting the ischemic and reperfused myocardium in acute myocardial infarction: distant dream or near reality? Heart 2000; 83: 381-387.
37. Végh Á, Szekeres L, Parratt JR. Local intracoronary infusions of bradykinin profoundly reduce the severity of ischemia-induced arrhythmias in anaesthetized dogs. Br J Pharmacol 1991; 104: 294-295.

38. Miki T., Miura T, Ura N, Ogawa T, Suzuki K, Shimamoto K, Iimura O. Captopril potentiates the myocardial infarct size-limiting effect of ischemic preconditioning through bradykinin B2 receptor activation.J Am Coll Cardiol 1996; 28: 1616-1622.
39. Ahmad M, Zeitlin IJ., Parratt JR, Valen G, Takeshima S, Vaage J. Changes in bradykinin levels in the blood of patients undergoing cardiopulmonary bypass. J Physiol 1996; 494: 116P-117P.
40. Leesar MA, Stoddard MF, Manchikalapudi S, Bolli R. Bradykinin-induced preconditioning in patients undergoing coronary angioplasty. J Am Coll Cardiol 1999; 34: 639-650
41. Leesar MA, Jneid H, Tang XL, Bolli R. Pretreatment with intracoronary enalaprilat protects human myocardium during percutaneous coronary angioplasty. J Am Coll Cardiol 2007; 49: 1607-1610.
42. Picano E, Parodi O, Lattanzi F, Sambuceti G, Andrade MJ, Marzullo P, et al. Assessment of anatomic and physiologic severity of single vessel coronary artery lesions by dipyridamole echocardiography: comparison with positron emission tomography and quantitative arteriography. Circulation 1994; 89: 753–761.
43. Kaski JC, Chester MR, Chen L, Katritsis D. Rapid angiographic progression of coronary artery disease in patients with angina pectoris. irculation 1995; 92: 2058–2065.
44. Ambrose JA, Winters SL, Arora RR, Eng A, Riccio A, Gorlin R, et al. Angiographic evolution of coronary artery morfology in unstable angina. J Am Coll Cardiol 1986; 7: 472–478.
45. Lichtlen P, Nikutta P, Jost S, Deckers J, Wiese B, Raffenbeul W. Anatomical progression of coronary artery disease in humans as seen by prospective, repeated, quantitated coronary angiography: relation to clinical events and risk factors. The INTACT Study Group. Circulation 1992; 86: 828–838.
46. Mark DB, Nelson CL, Califf RM, Harrell FE, Lee KL, Jones RH, et al. Continuing evolution of therapy for coronary artery disease. Initial results from the era of coronary angioplasty. Circulation 1994; 89:2015–2025.
47. Mohri M, Tagawa H, Egashira K, Takeshita A. Intracoronary enalaprilat improves metabolic coronary vasodilation in patients with idiopathic dilated cardiomyopathy. J Cardiovasc Pharmacol 2000; 35: 249-255.
48. Rundqvist B, Eisenhofer G, Emanuelsson H, Albertsson P, Friberg P. Intracoronary blockade of angiotensin-converting enzyme in humans: Interaction with cardiac sympathetic neurotransmission? Acta Physiologica Scandinavica 1997; 161: 15-22.
49. Smith SC Jr, Dove JT, Jacobs AK, et al. ACC/AHA guidelines of percutaneous coronary interventions (revision of the 1993 PTCA guidelines): executive summary; a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (committee to revise the 1993 guidelines for percutaneous transluminal coronary angioplasty). J Am Coll Cardiol 2001; 37: 2215–2239.
50. Teichholz LE, Cohen MV, Sonnenblick EH, Gorlin R. Study of the left ventricular geometry and function by B-scan ultrasonography in patients with and without asynergy. N Eng J Med 1974; 291: 1220-1226
51. Picano E , Ostojic M , Sicari R , Baroni M , Cortigiani L , Pingitore A . on behalf of the EPIC (Echo Persantin International Cooperative) study group. Dipyridamole stress echocardiography: state of the art 1996. Eur Heart J 1997; 18(suppl A): D16–D23.
52. Picano E , Pingitore A , Conti U , Kozakova M , Boem A , Cabani E et al. Enhanced sensitivity for detection of coronary artery disease by addition of atropine to dipyridamole echocardiography. Eur Heart J 1993; 14: 1216–1222.
53. American Society of Echocardiography Committee on standards, subcommittee on quantitation of two dimensional echocardiograms: Schiller NB, Shah PM, Crawford M, De Maria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. J Am Soc Echocardiogr 1989; 2: 358–367.
54. Campau L . Grading of angina pectoris [letter]. Circulation 1976; 52: 522–523.

55. Marwick T , D'Hondt A , Baudhuin T , Willemart B , Wijns W , Detry JM et al. Optimal use of dobutamine stress for the detection and evaluation of coronary artery disease: combination with echocardiography, scintigraphy or both?. J Am Coll Cardiol 1993; 22: 159–167.
56. Baptista J , Arnese M , Roelandt JR , Fioretti P , Keane D , Escaned J et al. Quantitative coronary angiography in the estimation of the functional significance of a coronary stenosis. Correlations with dobutamine–atropine stress test. J Am Coll Cardiol 23: 1994; 1934–1940.
57. Sheikh KH , Bengtson JR , Helmy S , Juarez C , Burgess R , Bashore TM et al. Relation of quantitative coronary lesion measurements to the development of exercise induced
58. Schuler G , Hambrecht R , Schlierf G , Grunze M , Methfessel S , Hauer K et al. Myocardial perfusion and regression of coronary artery disease in patients on a regimen of intensive physical exercise and low fat diet. J Am Coll Cardiol 1992; 19: 34–42.
59. Gould KL , Ornish D , Scherwitz L , Brown S , Edens RP , Hess MJ et al. Changes in myocardial perfusion abnormalities by positron emission tomography after long-term, intense risk factor modification. JAMA 1995; 274: 894–901.
60. Picano E , Simonetti I , Masini M , Marzilli M , Lattanzi F , Distante A et al. Transient myocardial dysfunction during pharmacological vasodilation as an index of reduced coronary reserve: a coronary hemodynamic and echocardiographic study. J Am Coll Cardiol 1986; 8: 84–90.
61. Lucarini AR , Picano E , Lattanzi F , Camici P , Marini C , Salvetti A , L'Abbate A . Dipyridamole echocardiography stress testing in hypertensive patients. Targets and tools. Circulation 1991; 83(suppl III): III68–III72.
62. Cheitlin MS , Alpert JS , Armstrong WF , Aurigemma GP , Beller GA , Bierman FZ et al. ACC/AHA guidelines for the clinical application of echocardiography: executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Clinical Application of Echocardiography). J Am Coll Cardiol 1997; 29: 862–879.
63. Armstrong WF , Pellikka PA , Ryan T , Crouse L , Zoghbi WA . Stress echocardiography task force of the Nomenclature and Standards Committee of the American Society of Echocardiography. Stress echocardiography: recommendations for performance and interpretation of stress echocardiography. J Am Soc Echocardiogr 1998; 11: 97–104.
64. Topol EJ , Nissen SE . Our preoccupation with coronary luminology. The dissociation between clinical and angiographic findings in ischemic heart disease. Circulation 1995; 92: 2333–2342.
65. Hoffmann R , Lethen H , Marwick T , Arnese M , Fioretti P , Pingitore A et al. Analysis of inter-institutional observer agreement in the interpretation of dobutamine stress echocardiograms. J Am Coll Cardiol 1996; 27: 330–336.
66. Hoffmann R , Lethen H , Marwick T , Rambaldi R , Fioretti P , Pingitore A et al. Standardized guidelines for the interpretation of dobutamine echocardiography reduce inter-institutional variance in interpretation. Am J Cardiol 1998; 82: 1520–1524.
67. Picano E , Lattanzi F , Orlandini A , Marini C , L'Abbate A. Stress echocardiography and the human factor: the importance of being experts. J Am Coll Cardiol 1991; 17: 666–669.
68. Varga A , Picano E , Dodi C , Barbieri A , Pratali L , Gaddi O. Madness and method in stress echo reading. Eur Heart J 1999; 20: 1271–1275.
69. Isaaz K, Bruntz JF, Ethevenot G, Courtalon T, Aliot E. Noninvasive assessment of coronary flow dynamics before and after coronary angioplasty using transoesophageal Doppler. Am J Cardiol 1993; 72: 1238-1242
70. Segal J, Kern MJ, Scott NA, King SB 3rd, Doucette JW, Heuser RR, Ofili E, Siegel R. Alteration of phasic coronary artery flow velocity in humans during percutaneous coronary angioplasty. J Am Coll Cardiol 1992; 20: 276-286.
71. Gadallah S, Thaker KB, Kawanishi D, Mehra A, LAu S, Rahstian M, Chandraratna AN. Comparison of intracoronary Doppler guide wire and transesophageal echocardiography in measurement of flow velocity and coronary flow reserve in the left anterior descending coronary artery. Am Heart J 1998; 135: 38-42.

72. Paraskevaidis IA, Katritsis DG, Tsiapras DP, Kyriakides ZS, Korovesis ST, Kremastinos DTh. Coronary flow reseve assessed by transesophageal echocardiography identifies early restenosis of the left anterior descending coronary artery angioplasty. Am J Cardiol 1997; 79: 803-807
73. Paraskevaidis IA, Tsiapras DP, Kyriakides ZS, Kremastinos DTh. Transesophageal Doppler evaluation of left anterior descending coronary artery angioplasty. Am J Cardiol 1997; 80: 947-951
74. Paraskevaidis IA, Tsiapras D, Karavoilas GK, Kyriakides ZS, Kremastinos DTh. Serial evaluation of coronary flow reserve by transesophageal Doppler echocardiography after angioplasty of proximal left anterior descending coronary artery: a 6-months follow-up study. Coron Artery Dis 2001; 12: 45-52
75. Caiati C, Aragona P, Iliceto S, Rizzon P. Improved Doppler detection of proximal left anterior descending coronary artery stenosis after intravenous injection of a lung-crossing contrast agent: a transesophageal Doppler echocardiographic study. J Am Coll Cardiol 1996; 27: 1413-1421
76. Nemes A, Forster T, Pálinkás A, Vass A, Borthaiser A, Ungi I, Thury A, Litvai E, Nádaskay M, Csanády M. The value of coronary flow reserve in ischemic heart disease measured by dipyridamole stress transoesophageal echocardiography. Orv Hetil 2000; 141: 2327-2331
77. Hutschinson SJ, Soldo SJ, Gadallah S, Kawanishi DT, Chandraratna PA. Determination of coronary flow measurements by transesophageal echocardiography: Dependence of velocity reserve on the location of stenosis. Am Heart J 1997; 133: 44-52
78. Segal J, Kern MJ, Scott NA, King SB 3rd, Doucette JW, Heuser RR, Ofili E, Siegel R. Alteration of phasic coronary artery flow velocity in humans during percutaneous coronary angioplasty. J Am Coll Cardiol 1992; 20: 276-286
79. Pizzuto F, Voci P, Mariano E, Puddu P, Sardella G, Nigri A. Assessment of flow velocity reserve by transthoracic Doppler echocardiography and venous adenosine infusion before and after left anterior descending coronary artery stenting. J Am Coll Cardiol 2001; 38: 155-162
80. Pizzuto F, Voci P, Mariana E, Puddu P, Chiavari PA, Romeo F. Noninvasive coronary flow reserve assessed by transthoracic coronary Doppler ultrasound in patients with left anterior descending coronary artery stents. Am J Cardiol 2003; 91: 522-526
81. Ruscazio M, Montisci R, Colonna P, Caiati C, Chen L, Lai G, Cadeddu P, Pirisi R, Iliceto S. Detection of coronary restenosis after coronary angioplasty by contrast-enhanced transthoracic echocardiographic Doppler assessment of coronary flow velocity reserve. J Am Coll Cardiol 2002; 40: 896-903
82. Picano E , Lattanzi F . Dipyridamole echocardiography. A new diagnostic window on coronary artery disease. Circulation 1991; 83(suppl. III): III19–III26.
83. Serruys PW, di Mario C, Piek J, Schroeder E, Vrints C, Probst P, de Bruyne B, Hanet C, Fleck E, Haude M, Verna E, Voudris V, Geschwind M, Emanuelsson H, Mühlberger V, Danzi G, Peels HO, Ford AJ Jr, Boersma E. Prognostic value of intracoronary flow velocity and diameter stenosis in assessing the short- and long-term outcomes of coronary balloon angioplasty. The DEBATE Study (Doppler Endpoints Balloon Angioplasty Trial Europe). Circulation 1997; 96: 3369-3377
84. Albertal A, Voskuil M, Piek JJ, de Bruyne B, Van Langenhove G, Kay PI, Costa MA, Boersma E, Beijsterveldt T, Belardi JA, Serruys PW, Doppler Endpoints Balloon Angioplasty Trial Europe (DEBATE) II. Study Group. Coronary flow velocity reserve after percutaneous interventions is predictive of periprocedural outcome. Circulation 2002; 105: 1573-1578
85. Di Mario C, Moses JW, Anderson TJ, Bonan R, Muramatsu T, Jain AC, de Lezo JS, Cho SY, Kern M, Meredith IT, Cohen D, Moussa I, Colombo; on belhalf of the DESTINI Study Group (Doppler Endpoint STenting International Investigation). Randomized comparison of elective stent implantation and coronary balloon angioplasty guided by online quantitative angiography and intracoronary Doppler. Circulation 2000; 102: 2938-2944

86. Houterman S, Verschuren WM, Hofman A, et al. Serum cholesterol is a risk factor for myocardial infarction in elderly men and women: the Rotterdam Study. J Intern Med 1999; 246: 25–33.
87. Roberts WC. Preventing and arresting coronary atherosclerosis. Am Heart J 1995; 130: 580–600.
88. Kyriakides ZS, Psychari S, Iliodromitis EK, et al. Hyperlipidemia prevents the expected reduction of myocardial ischemia an repeated balloon inflations during angioplasty. Chest 2002; 121: 1211–1215.
89. Onody A, Csonka C, Giricz Z, et al. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. Cardiovasc Res 2003; 58: 663–670
90. Ferdinandy P, Csonka C, Csont T, et al. Rapid pacinginduced preconditioning is recaptured by farnesol treatment in hearts of cholesterol-fed rats: role of polyprenyl derivatives and nitric oxide. Mol Cell Biochem 1998; 186: 27–34
91. Csont T, Balogh G, Csonka C, et al. Hyperlipidemia induced by high cholesterol diet inhibits heat shock response in rat hearts. Biochem Biophys Res Commun 2002; 290: 1535–1538
92. Hexeberg S, Willumsen N, Rotevatn S, et al. Cholesterol induced lipid accumulation in myocardial cells of rats. Cardiovasc Res 1993; 27: 442–446.
93. Vigh L, Maresca B, Harwood JL. Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem Sci 1998; 23: 369–374.
94. Puskas LG, Nagy ZB, Giricz Z, et al. Cholesterol diet-induced hyperlipidemia influences gene expression pattern of rat hearts: a DNA microarray study. FEBS Lett 2004; 562: 99–104.
95. Ungi I, Ungi T, Ruzsa Z, Nagy E, Zimmermann Z, Csont T, Ferdinandy P. Hypercholesterolemia attenuates the anti-ischemic effect of preconditioning during coronary angioplasty. Chest 2005; 128: 1623-1628.
96. Billinger M, Fleisch M, Eberli FR, Garachemani A, Meier B, Seiler Ch. Is the development of myocardial tolerance to repeated ischemia in humans due to preconditioning or to collateral recruitment? J Am Coll Cardiol 1999; 33: 1027-1035.
97. Airaksinen KEJ, Huikuri HV. Antiarrhythmic effect of repeated coronary occlusion during balloon angioplasty. J Am Coll Cardiol 1997; 29: 1035-1038.
98. Dupouy P, Geschwind H, Pelle G, Aptecar E, Hittinger L, El Ghalid A, et al. Repeated coronary artery occlusions during routine balloon angioplasty do not induce myocardial preconditioning. in humans. J Am Coll Cardiol 1996; 27: 1374-1380.
99. Johansen O, Brekke M, Stromme JH, Valen V, Seljeflot I, Skjaeggestad O, et al. Myocardial damage during percutaneous transluminal coronary angioplasty as evidenced by troponin T measurements. Eur Heart J 1998; 19: 112-117.
100. Matsubara T, Minatoguchi Sh, Matsuo H, Hayakawa K, Segawa T, Matsuno Y, et al. Three minute, but not one minute, ischemia and nicorandil have a preconditioning effect in patients with coronary artery disease. J Am Coll Cardiol 2000; 35: 345-351.
101. Laskey WK, Beach D. Frequency and clinical significance of ischemic preconditioning during percutaneous coronary intervention. J Am Coll Cardiol 2003; 42: 998-1003.
102. Tomai F, Crea F, Gaspardone A, Versaci F, De Paulis R, Penta de Peppo A, et al. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K+ channel blocker. Circulation 1994; 90: 700-705.
103. Cohen MV, Liu Y, Downey JM. Activation of protein kinase C is critical to the protection of preconditioning. In: Wainwright, Ch.L. and Parratt, J.R. eds. Myocardial Preconditioning, Chapman & Hall RG Landes Co. 1996; 185-206.
104. Liu GS, Thornton J, VanWinkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1-adenosine receptors in the rabbit heart. Circulation 1991; 84: 350-356.
105. Bolli R, Manchikalapudi S, Tang XL, Takano H, Qiu Y, Guo Y, et al. The protective effect of late preconditioning against myocardial stunning in conscious rabbits is mediated by nitric

oxide synthase: evidence that nitric oxide acts both as a trigger and as a mediator of the late phase of ischemic preconditioning. Circ Res 1997; 81: 1094-1107.
106. Martorana PA, Kettenbach B, Breipohl G, Linz W, Scholkens BA. Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. Eur J Pharmacol 1990; 182: 395-396.
107. Nozawa Y, Miura T, Tsuchida A, Kita H, Fukuma T, Shimamoto K. Chronic treatment with an ACE inhibitor, temocapril, lowers the threshold for the infarct size-limiting effect of ischemic preconditioning. Cardiovasc Drugs Ther 1999; 13: 151-157.
108. Kurz T, Schafer U, Dendorfer A, Hartmann F, Raasch W, Tölg R, et al. Effects of intracoronary low-dose enalaprilat as an adjunct to primary percutaneous transluminal coronary angiography in acute myocardial infarction. Am J Cardiol 2001; 88: 1351-1357.
109. Cohen M, Rentrop KP. Limitation of myocardial ischemia by collateral circulation during sudden controlled coronary artery occlusion in human subjects: a prospective study. Circulation 1981;74: 469-476.
110. Friedman PL, Shook TL, Kirshenbaum JM, Selwyn AP, Ganz P. Value of the intracoronary electrogram to monitor myocardial ischemia during percutaneous transluminal coronary angioplasty. Circulation 1986; 74: 330-339.
111. Shattock MJ, Lawson CS, Hearse DJ, Downey JM. Electrophysiological characteristics of repetitive ischemic preconditioning in the pig heart. J Mol Cell Cardiol 1996; 28: 1339-1347

Acknowledgements

First of all, I wish to thank Prof. Ágnes Végh, for her inspiration, patience and persistence in guiding my work at the time of preconditioning investigations and also during the preparation of data for publication.

My special thanks to Prof. Péter Ferdinandy for his particularly invaluable help in designing and performing a part of the clinical investigations.

I am grateful to Prof. Eugenio Picano, Prof. Mario Marzilli and Dr. Paolo Marraccini for their education in interventional cardiology practice and scientific methods of clinical investigations in 1995-96 at the Istituto di Fisiologia Clinica, CNR, Pisa, Italy.

I would also like to express my thanks to Prof. Miklós Csanády, the former and Prof. Tamás Forster, the present Director of the Department of Cardiology for creating the conditions of my scientific development and for guiding my clinical work.

This paper would not have been possible without the powerful support and encouragement of Dr. Attila Nemes, Dr. Attila Pálinkás, Dr. Attila Thury and Dr. Tamás Ungi.

I additionally offer my thanks to my other former and present colleagues, assistants, nurses physicians, and students who played a determinant role in performing the examinations.

Finally, I wish to thank my parents for starting me in the medical carrier and my wife and children for their love, patience and tolerance during my work.

Photocopies of essential publications