The use of venous myocardial contrast echocardiography in clinical evaluation of acute myocardial infarction

PhD thesis

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List of abbreviations

A	= examination at admission
AMI	= acute myocardial infarction
CK	= creatine kinase
DSE	= dobutamine stress echocardiography
FU	= follow-up
IRA	= infarct related artery
LV	= left ventricle
LAD	= left anterior descending coronary artery
LCX	= left circumflex coronary artery
MRI	= magnetic resonance imaging
PCI	= percutaneous coronary intervention
RCA	= right coronary artery
SPECT	= single photon emission computer tomography
TTC	= triphenyl tetrazoliumchloride
VI	= videointensity
VMCE	= venous myocardial contrast echocardiography
WMS	= wall motion score
99mTc sestamibi	= technetium-99$_m$ hexakis 2methoxyisobutyl-2metylpropyl-isonitrile

Summary

The aim of mechanical reperfusion in patients with acute myocardial infarction is to restore blood flow through the infarct related epicardial artery. The success of reperfusion, however, is indicated by perfusion of myocardial capillaries rather than simply patency of the infarct related artery. Venous myocardial contrast echocardiography can be used to assess myocardial capillary perfusion. The aim of our venous myocardial contrast echocardiographic study was:

- **1.** to examine the amount of viable myocardium early after successful mechanical reperfusion and to predict the subsequent functional recovery and contractile reserve.
- **2.** to determine the time course of microvascular and contractile improvement of risk area after successful reperfusion in patients with acute myocardial infarction
- **3.** to evaluate quantitatively risk area, infarct size and myocardial salvage.

1. Methods: VMCE (3ml Optison in 27ml NaCl, 200ml/h, intermittent harmonic imaging, 4- and 2-chamber views (six segments each), off-line digital image processing) was performed in 60 patients (male n=51, median age 58 years) with first AMI (IRA: LAD n=29, RCA n=25, LCX n=6; CK max. 825 U/l) three hours after primary PCI. Videointensity (VI) of akinetic segments were normalized to VI of the brightest normokinetic segment of the same view. Repeat coronary angiography and echocardiographic evaluation of contractile function (semiquantitative wall motion score (WMS): 1=normokinesia, 2=hypokinesia, 3=akinesia, 4=dyskinesia) at rest and during dobutamine stress (DSE) (5-40μg/kg/min) were performed 14 days after reperfusion. Recovery of contractile function of a formerly dys- or akinetic segment was defined as an improvement to at least hypokinesia at rest. Contractile reserve was defined as improvement to at least hypokinesia during dobutamine.

1. Results: Resting contractile function of 102/211 initially akinetic segments improved to day 14; another 33/211 segments improved during DSE at day 14. Receiver operator curve analysis identified a cut-off ratio of >50% for normalized VI as optimal VMCE criterion to predict subsequent functional recovery or contractile reseve: χ^2=76.2 (degree of freedom=1, p<0.001) and diagnostic accuracy 82% (sensitivity 83%, specificity 81%).

In the functional recovery group, relative videointensity was significantly different between segments with recovery to normokinesia versus hypokinesia (88% [77%,100%] vs. 74% [54%,99%], respectively, p<0.001. In segments without functional recovery,

relative videointensity was significantly higher in those with contractile reserve compared to segments without contractile reserve (61% [48%,76%] vs. 31% [22%,46%], respectively, p<0.001. Relative videointensity early after reperfusion and contractile function at 2 week follow-up were significantly correlated (rho=-0.67; p<0.0001).

2. Methods: VMCE was performed (as previously described) in 49 patients (male n=42, median age 57 years) with first AMI (IRA: LAD n=20, RCA n=21, LCX n=8; CK max. 837 U/l) before (A) and 3 hour (FU1), 14 days (FU2) and 6 months (FU3) after primary PCI. Echocardiographic evaluation of contractile function (semiquantitative wall motion score) at rest was performed simultaneously with VMCE, in the case of akinesia at 6 months' follow up DSE was performed to evaluate contractile reserve. A segment with initial wall motion abnormality was regarded as viable when improved at least to hypokinesia at rest or during DSE at FU3. Repeat coronary angiography was performed at FU2 and FU3. A risk area segment was regarded at any time as "reflow" segment, when the relative videointensity (VI) of the segment was higher than 50%. The VI of a "no-reflow" segment was equal or less than 50%. In "persistent reflow" group the VI was higher than 50% both at FU1 and at FU 3. In the "late reflow" group the VI was higher than 50% only at the FU3. In the "persistent no-reflow" group the VI was equal or less than 50% both at FU1 and FU3.

2. Results: Resting contractile function improved continuously in the risk area segments (n=188) from FU1 to FU3 (WMS FU1: 2.84±0.39; FU2: 2.26±0.87; FU3: 2.02±0.9; p<0.05). The WMS of „persistent reflow" segments (n=101) improved also continuously (FU1: 2.74±0.46; FU2: 1.77±0.79; FU3: 1.47±0.64, p<0.01). In the „late reflow" group WMS changed only between FU1 and FU3 (2.86±0.36, 2.07±0.81, respectively, p<0.01). No change was detected at all in the „persistent no-reflow" group. In the risk area segments (n=200) the VI increased between A and FU1 (40.4% (± 26) vs. 59.8% (±30.4) p<0.0001), but no further significant improvement was found. The microvascular perfusion in the viable segments (n=134) recovered continuously from A through FU1 and FU2 to FU3 (47.7%±26, 73.2%±25, 80.9%±24, 86.8%±17, respectively, p<0.05), while in the non-viable segments (n=66) did not change from FU1 to FU3.

3. Methods: VMCE was performed (as previously described) in 119 patients (male n=98, median age 59 years) with AMI (IRA: LAD n=55, RCA n=46, LCX n=18; CK max. 608 U/l) before and 14 days after reperfusion therapy. Tc-99m sestamibi (pre-reperfusion injection, post-reperfusion imaging) SPECT imaging was performed simultaneously with VMCE. Planimetry of myocardial contrast and sestamibi uptake defect size was performed in 4- and 2-

chamber views on VMCE and on polar maps (sestamibi activity threshold of 50% of myocardial maximum) on SPECT, respectively. Risk area (initial defect size), infarct size (follow-up defect size) and myocardial salvage (initial defect size minus defect size at follow-up) was described as a percentage of total LV.

3. Results: The correlation was significant between both methods to determine the risk area, the infarct size and the amount of myocardial salvage ($r=0.82$, $p<0.0001$; $r=0.8$, $p<0.0001$; $r=0.57$, $p<0.0001$, respectively).

	VMCE	Sestamibi	
Risk area (% of LV)	21% [14%-36%]	25% [14%-51%]	$p<0.05$
Infarct size (% of LV)	10% [0%-19%]	13% [5%-29%]	$p<0.005$
Salvage (% of LV)	9% [3%-17%]	10% [3%-22%]	ns.

Main findings

I. This is the first study in patients with first acute myocardial infarction to show, that early after successful mechanical reperfusion, the magnitude of myocardial signal of akinetic myocardium on venous contrast echocardiography is correlated with the degree of myocardial contractile function after two weeks.

II. Recovery of contractile function or presence of contractile reserve can be predicted with high diagnostic accuracy, with venous myocardial contrast echocardiography performed immediately after successful primary percutaneous coronary intervention. This is the first study to define a signal threshold for the prediction of myocardial contractile function on venous contrast echocardiography early after mechanical reperfusion of acute myocardial infarction.

III. Looking at the whole risk area microvascular recovery occurred during the first three hours after reperfusion and no further significant recovery was found. Recovery of the systolic function is a prolonged process.

IV. This is the first contrast echocardiographic study observed the slight but significant and continuous improvement of microvascular perfusion in the viable segments during the first six months after reperfusion therapy. The delayed microvascular healing was observed mostly in viable segments but in territories with a higher degree of transmurality than in the "early" and "persistent reflow" group.

V. In our study was first time described that in patients with acute myocardial infarction both the risk area and the infarct size measured by VMCE showed a close correlation with 99mTc sestamibi SPECT results.

VI. Risk area and infarct size are significant smaller measured by VMCE than by SPECT imaging, however in the amount of salvage no difference was found.

List of publications

Full papers

1. **Andrássy P**, Zielinska M, Busch R, Schömig A, Firschke C. Myocardial blood volume and the amount of viable myocardium early after mechanical reperfusion of acute myocardial infarction. *Heart.* 2002;87:350-355
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6. **Andrássy P**, Zámolyi K. Acute coronary syndrome and diabetes mellitus. *Diab. Hung.* 2004; in press.
7. **Andrássy P.** A szív ultrahang vizsgálatának leggyakoribb leletei. *Praxis* 9;(2);31-41
8. **Andrássy P.** Az akut miokardiális infarktus kezelése. *Hippocrates*/II/3/180

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Quotable abstracts

1. **Andrássy P**, Zielinska M, Firschke C. Serial evaluation of myocardial microvasculature before and after successful reperfusion in patients with acute myocardial infarction using venous echocardigraphy. *Circulation* 2001 Vol.104 No.17, (Suppl.II) pp.590.
2. **Andrássy P**, Zielinska M, Firschke C.Time course of improvement of microvascular perfusion and contractile function after successful reperfusion of acute myocardial infarction. *Eur Heart J.* 2002 Vol.23. (Abstr.suppl.) pp.397.
3. **Andrássy P**, Schricke U., Körner I, Firschke C. Quantitative evaluation of risk area and infarct size with venous contrast echocardiography compared to SPECT

imaging in patients with acute coronary syndrome. *Circulation.* 2000 Vol. 102. No.18. (Suppl.II.) pp.455.

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5. Firschke C, **Andrássy P**, Schricke U, Paliga J, Schwaiger M, Schömig A. Spatial extent and magnitude of myocardial "no/low reflow" by contrast echocardiography after reperfusion in patients with acute coronary syndrome represent segmental and transmural extent of infarct. *J Am Coll Cardiol.* 2001 Vol.37. No.2.(Suppl.A) pp.396.
6. Firschke C, **Andrássy P**, Zielinska M,. Schömig A. Serial evaluation of myocardial microvasculature in patients with acute myocardial infarction before and after successful mechanical reperfusion using venous contrast echocardiography. *J Am Coll Cardiol.* 2001 Vol.37. No.2.(Suppl.A) pp.452.
7. Firschke C, **Andrássy P**, Zielinska M, Schricke U, A. Schömig, M. Schwaiger. Comparison of myocardial perfusion defect size between venous contrast echocardiography and sestamibi SPECT in patients with acute myocardial infarction before and after mechanical reperfusion. *Eur Heart J.* 2001 Vol.22. (Abstr.suppl.) pp.616.
8. Firschke C, Zielinska M, Schömig A, **Andrássy P**. Venous contrast echocardiography for assessment of myocardial perfusion in patients with non ST-segment elevation acute coronary syndrome. *Eur.Heart J.* 2001 Vol.22. (Abstr.suppl.) pp.469.
9. Firschke C, **Andrássy P**, Schricke U, Paliga J, Blasini R, Schwaiger M, Schömig A. Spatial extent and magnitude of myocardial "no/low reflow" by contrast echocardiography after reperfusion in patients with acute coronary syndrome represent segmental and transmural extent of infarct. *Eur.Heart J.* 2000 Vol.21. (Abstr.suppl.) pp.116
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11. Firschke C, Odaka K, **Andrássy P**, Schricke U, Schömig A, Schwaiger M. Venous myocardial contrast echocardiography and SPECT imaging in patients with

coronary disease: quantitative comparison of signals at rest and during stress. *Eur Heart J.* 2000 Vol.21. (Abstr.suppl.) pp.336.

12. Szabóki F, **Andrássy P**, Heltai K, Palásti I, Zámolyi K. Quantitative and semiquantitative evaluation of low-dose Dipyridamole Dobutamine stress echocardiography. *Eur.Heart J.* 1999. Vol.20, (Abstr.suppl.) pp.617.
13. **Andrássy P**, März I, Szabóki F. Resting Echocardiographic Parameters In Coronary Artery Disease. *Echocardiography* Vol.14. No.:6,Part 2, Nov.1997 pp.S 107
14. **Andrássy P**, Szabóki F, Balogh I. Long axis M-mode parameters for the evaluation of myocardial viability, a quantitative method. *Echocardiography* Vol.15. No.:8,Part 2, Nov.1998 pp.S 34
15. **Andrássy P**, Paliga J, Lange S, Blasini R, Firschke C. Kontrastechokardiographische Erkennung vitalen Myokards unmittelbar nach Reperfusion im akutem Myokardinfarkt. *Kardiologie* (89) Suppl.5, 2000,pp.44
16. Firschke C, **Andrássy P**, Lange S, Schricke U, Blasini R. Definition eines kontrastechokardiographischen Kriteriums für normale und reduzierte Myokardperfusion unter Ruhebedinungen. *Kardiologie* (89) Suppl.5, 2000,pp.44.
17. Firschke C, **Andrássy P**, Lange S, Schricke U, Blasini R. Kontrastechokardiographischen Beurteilung des "Myokardial Salvage"nach Reperfusionstherapie im akuten Myokardinfarkt. *Kardiologie* (89) Suppl.5, 2000,pp.44.
18. **Andrássy P**, Paliga J, Lange S, Firschke C. Evaluation of myocardial viability by venous myocardial contrast echocardiography immediately after reperfusion therapy in patients with acute myocardial infarction.*Cardiologia Hungarica* (Suppl.2000/3) pp.50.
19. Firschke C, **Andrássy P**, Lange S, Martinoff S, Schwaiger M, Schömig A. Venous myocardial contrast echocardiography can guide clinical decision making in the cardiac catheterization laboratory. *Cardiologia Hungarica* (Suppl.2000/3) pp.50.

1. Introduction

The aim of mechanical reperfusion in patients with acute myocardial infarction is to restore blood flow through the infarct related artery to limit myocellular necrosis and ultimately preserve myocardial contractile function, which is the most powerful predictor of survival after acute myocardial infarction (1). The success of reperfusion, however, is indicated by perfusion of myocardial capillaries rather than simply patency of the infarct related artery (2,3). Myocardial contrast echocardiography can be used to assess myocardial capillary perfusion (4,5). The signal from the myocardium following an intravenous injection of microbubbles has been shown to be a corollary of the blood volume of myocardial capillaries and thus functional capillary integrity (6).

Identification of viable myocardium after reperfusion of acute myocardial infarction through contrast enhancement after intracoronary injection of microbubbles was, however, limited in most studies by low specificity for the binary prediction of recovery of resting contractile function (7-9). We expected that the more physiologic intramyocardial distribution of the contrast agent after venous administration would result in a precise image of differential degrees of myocardial microvascular damage. We, therefore hypothesized that measuring the transmural contrast signal from the myocardium will correlate with the extent of myocardial viability and functional recovery after mechanical reperfusion of acute myocardial infarction. Since endocardial necrosis may not be associated with recovery of resting contractile function (10), we assessed myocardial viability by measuring contractile function during dobutamine in patients who did not exhibit functional recovery. Furthermore, because reactive hyperemia may mask the degree of capillary damage immediately after reperfusion (11), we made our contrast intensity measurements several hours following reperfusion when the hyperemic response had abated. Later, two weeks and six months after the reperfusion the contrast and two-dimensional echocardiography were repeated to examine the long-term changes in microvasculature and to describe its relation to contractile function of risk area.

We also measured quantitatively strong prognostic factors, such as risk area of myocardial infarction and the subsequent size of infarction with contrast echocardiography. We compared this data with the gold standard method of infarct size and risk area measurement: 99mTc sestamibi SPECT images (12).

The aim of our venous myocardial contrast echocardiographic study was:

- 1. to examine the amount of viable myocardium early after successful mechanical reperfusion and to predict the functional recovery and contractile reserve.
- 2. to determine the long term time course of microvascular and contractile improvement of risk area after successful reperfusion in patients with acute myocardial infarction
- 3. to evaluate quantitatively risk area, infarct size and myocardial salvage.

2. Myocardial blood volume and amount of viable myocardium early after successful mechanical reperfusion, prediction of functional recovery and contractile reserve

2.1. Methods

2.1.1. Patient Population

This prospective study included patients with first acute myocardial infarction who presented to hospital within 24 hours after onset of chest pain. Acute myocardial infarction was diagnosed in the presence of two of the following criteria: (1) typical anginal chest pain lasting $\geq$ 20 minutes, (2) presumed new ST-elevation of $\geq$ 0.1mV in two or more contiguous limb ECG leads or $\geq$ 0.2 mV in two or more contiguous precordial leads, (3) an increased creatine kinase to two ore more times the upper reference limit with a concomitant rise in creatine kinase MB-isoenzyme. Exclusion criteria were inability to give informed consent, cardiogenic shock and inadequate echocardiographic window for assessment of wall thickening in any segment of the apical four- and two-chamber views. All patients underwent successful mechanical reperfusion, which was defined as the presence of TIMI (thrombolysis in myocardial infarction) grade 3 flow immediately and two weeks after the intervention.

2.1.2. Study Protocol

Two-dimensional and venous myocardial contrast echocardiography were performed approximately three hours after successful mechanical reperfusion (median (25^{th}, 75^{th} percentile) 190 minutes (160 minutes, 242 minutes)). At follow-up after two weeks coronary angiography and two-dimensional echocardiography were repeated. Patients with akinetic segments at follow-up underwent dobutamine stress echocardiography. The study protocol was approved by Institutional Review Board of German Heart Center, Munich, and patients gave written informed consent.

2.1.3. Venous myocardial contrast echocardiography

Three milliliters of Optison, which consists of microbubbles with a mean size of 3.9 μm and a concentration of 0.8×10^9/ml (Mallinckrodt Medical Imaging, Dublin, Ireland) was

diluted with 27 ml 0.9% sodium chloride and infused intravenously at 200ml/h. A volumetric pump (Perfusor fm, Braun Melsungen, Melsungen, Germany) was used for intravenous infusion of the solution at 200ml/h and adequate mixing of the solution was maintained by careful shaking of the pump. A phased array echocardiographic system (Agilent Sonos 5500) was employed. Harmonic (mean transmit frequency 1.8 MHz, mean receive frequency 3.6 MHz) and intermittent imaging (every seventh cardiac cycle, EKG-triggered to endsystole) were applied (13,14). The maximum power output and dynamic range of the system (60 dB) were used and the most linear postprocessing mode (prostprocessing A) selected. A dual-focus was placed in the upper and lower third of the imaging sector to avoid beam overlap in the nearfield of the transducer. Gain was set to keep myocardial tissue signal low with myocardial contours barely seen before contrast infusion. Machine settings were adjusted before each study and kept constant throughout. Four- and two-chamber views (each view was divided into six segments) were stored on 1.25 cm videotape using an S-VHS recorder (Panasonic Model AG-7350, Matsushita Electrical Co.). Contrast images were off-line digitally processed as previously described (6). Mean videointensity was measured in each of the six segments in the apical four and two chamber views. To account for differences in echogenity between patients, the relative videointensity (VI) of a risk area segment was calculated as the ratio between the videointensity of the risk area segment and the brightest normokinetic segment of the same view. This parameter has been recently clinically validated with quantitative radionuclide single photon emission computed tomography data (15). Variability of relative videointensity of normokinetic segments early after reperfusion was 9% [1%, 13%]. Intraobserver and interobserver variability of relative videointensity of segments were 5% [1.5%,8.5%] and 7.5% [1.5%,15%], respectively. Quantitative venous myocardial contrast echocardiography was performed by experienced investigators blinded to all clinical, echocardiographic, and angiographic data.

2.1.4. Two- dimensional and dobutamine stress echocardiography

Tissue harmonic imaging (2.1/4.2 MHz) was used for assessment of wall thickening at rest and during dobutamine stress echocardiography. Dobutamine stress echocardiography was performed with incremental doses of intravenous dobutamine (5, 10, 20, 30 and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$) at 3-minute intervals (16). Single systoles obtained at rest and before each dobutamine increment from the apical four- and two-chamber views were stored digitally for review using a quad screen format. The entire study was also continuously recorded on S-VHS videotape. Wall thickening assessment at rest and during dobutamine stress was

performed by two separate experienced readers blinded to clinical, angiographic and contrast echo data. In cases of disagreement, wall motion score was resolved by consensus of opinion. Each of the same segments used for videointensity measurements was assigned a score (1=normokinesia, 2=hypokinesia, 3=akinesia, 4=dyskinesia). Recovery of contractile function of a formerly dys- or akinetic segment was defined as an improvement to at least hypokinesia at rest. Contractile reserve was defined as improvement to at least hypokinesia during dobutamine. Wall motion score index was defined as mean score of the 12 segments of a patient.

2.1.5. Statistical analysis

Nominal variables were expressed as counts and percentages. Differences between groups were assessed with use of a two-sided chi-square test. Continuous variables were presented as median (with the 25^{th} and 75^{th} percentiles) and, as appropriate, Kruskal-Wallis and Mann-Whitney tests were applied for comparison between groups. Alpha–correction using the method of Marcus et al. (17) was employed. Spearman correlation coefficient (r) was given. Sensitivity and specificity of venous contrast echocardiography for prediction of functional status at two week follow-up was calculated for each relative videointensity value. Areas under the resulting receiver operator characteristic curves were compared with Wilcoxon signed rank test. The Youden-index (sensitivity+specificity-100%) was derived to assess optimal cut-off value. Differences between groups were considered significant at $p<0.05$. SPSS 10.0 (Spss Inc, Chicago, Illinois, USA) and Statview was used as the statistical software package.

2.2. Results

2.2.1.Patient characteristics

Seventy-four patients were enrolled in the study. In six patients, image analysis could not be performed due to inadequate assessability of wall thickening at baseline. Follow-up could not be performed in six patients (one died, three underwent emergency coronary artery bypass grafting, and two refused to participate). The infarct related artery did not show TIMI 3 flow at follow-up in two patients. Demographic, clinical and angiographic characteristics of the 60 patients who were eligible for image analysis and who completed the study protocol are listed in Table 1. In seven patients, dobutamine stress echocardiography was not performed at two-week follow-up for clinical reasons (congestive heart failure in two, sustained ventricular tachycardia in one, atrial flutter in one, chest pain in one, by patient request in two).

Table 1 Patient characteristics

Women, n (%)	9 (15)
Age, median y [25th, 75th percentiles]	58 [51, 63]
Mechanical reperfusion	
Stent, n (%)	46 (77)
Percutneous transluminal angioplasty, n (%)	14 (23)
Time from onset of symptoms to reperfusion, median h [25th, 75th percentiles]	8 [3, 17]
Peak CK, median IU [25th, 75th percentiles]	825 [426, 1410]
Infarct related artery, n (%)	
Left anterior descending artery	29 (48)
Right coronary artery	25 (42)
Left circumflex coronary artery	6 (10)
Multivessel disease, n (%)	36 (60)
Q wave infarction, n (%)	49 (82)
Arterial hypertension, n (%)	34 (57)
Hypercholesterolaemia, n (%)	39 (65)
Diabetes mellitus, n (%)	10 (17)
Smoking, n (%)	39 (65)
β Blocker medication at follow up, n (%)	57 (95)

2.2.2. Relation between relative videointensity early after reperfusion and differential levels of contractile function at follow-up

The vast majority of myocardial segments (211 of 720) in the territory of the infarct related artery were akinetic at the time of post-reperfusion echocardiography. Sequential changes in contractile function of initially akinetic segments at rest and during dobutamine at the two-week follow up are summarized in Figure 1.

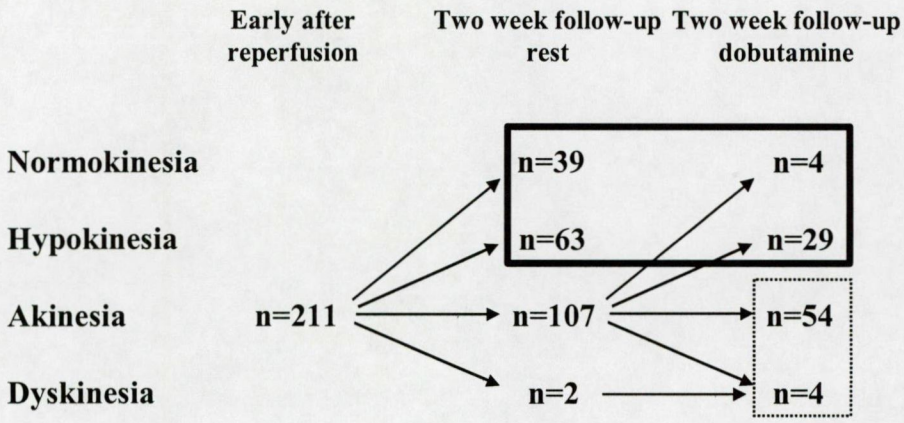


Figure 1 *Segmental wall thickening early after reperfusion and after two weeks at rest and during dobutamine stress. In seven patients (18 akinetic segments at follow-up), dobutamine was not infused for clinical reasons. Thick frame: either recovery of contractile function or presence of contractile reserve; thin frame: neither recovery of contractile function nor presence of contractile reserve.*

We specifically looked at the relation between relative videointensity of initially akinetic segments and contractile function after two weeks. Relative videointensity was higher (83% [66%, 99%]) in segments with functional recovery (normokinesia, n=39; hypokinesia, n=63) than in those that remained akinetic or dyskinetic (relative videointensity 40% [28%, 57%], $p<0.0001$). In the functional recovery group, relative videointensity was significantly different between segments with recovery to normokinesia versus hypokinesia (88% [77%, 100%] vs. 74% [54%,99%], respectively, $p<0.001$, Figure 2). In segments without functional recovery, relative videointensity was significantly higher in those with contractile reserve (n=33) compared to segments without contractile reserve (n=58); (61% [48%, 76%] vs. 31% [22%, 46%], respectively, $p< 0.001$, Figure 2). Relative videointensity of segments with functional recovery to hypokinesia was significantly higher than of segments without functional recovery but presence of contractile reserve ($p<0.001$). Relative videointensity early after reperfusion and contractile function at 2-week follow-up were significantly correlated (rho=-0.67; Figure 2).

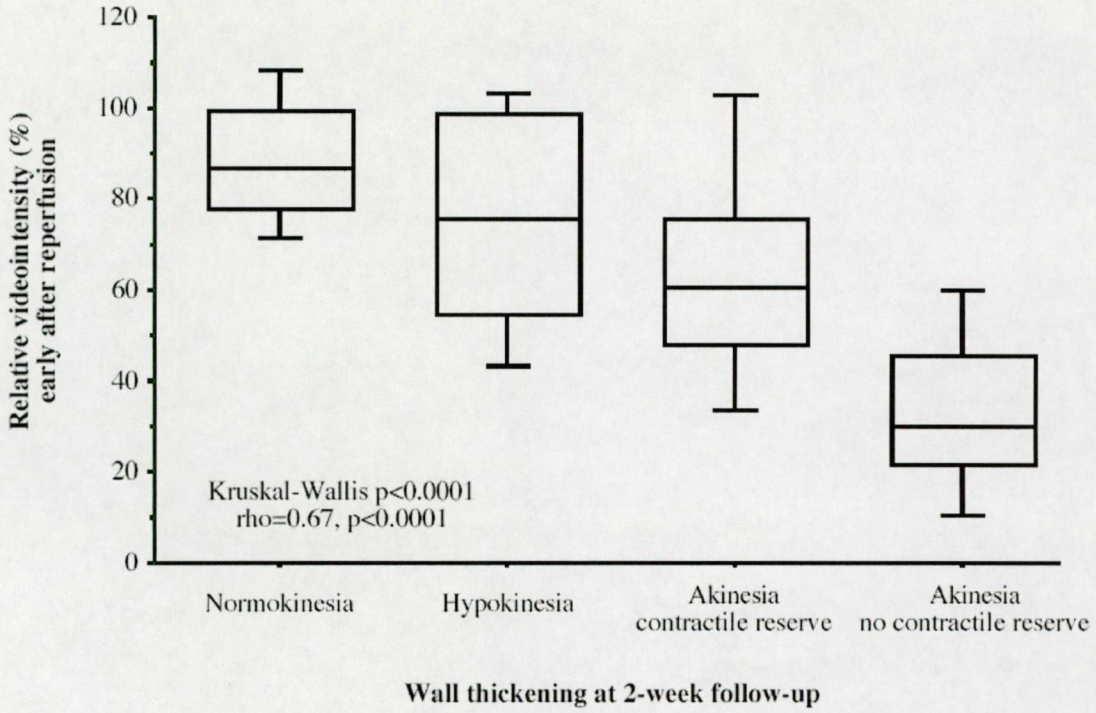


Figure 2 *Relative videointensity of akinetic segments early after reperfusion is significantly different between different functional groups as observed at two weeks' follow- up.*

2.2.3. Prediction of segmental contractile function with contrast echocardiography

Figure 3 depicts examples of differential relative videointensities within akinetic myocardium of patients early after successful mechanical reperfusion of first acute myocardial infarction. Figure 4A (curve A) depicts the receiver operator characteristic curve for contrast echocardiographic prediction of myocardial segments with recovery of resting contractile function or presence of contractile reserve at 2-week follow-up. Curve B depicts the receiver operator characteristic curve for prediction of recovery of resting contractile function alone. The curves differ only slightly albeit significantly ($p<0.001$). Youden-Index derived from curve A is depicted in Figure 4B and plateaus between values of 42% and 62%. In light of intra- and interobserver variabilities of relative myocardial videointensity of 5% and 7.5%, respectively, a cut-off value of 50% relative videointensity for prediction of recovery of contractile function or presence of contractile reserve by contrast echocardiography was determined. The power of this criterion for early discrimination between segments with and without contractility at rest or during dobutamine at 2-week follow-up is characterized by a $\chi^2=76.2$ (degree of freedom=1, $p<0.001$) and diagnostic accuracy was 82% (sensitivity 83%, specificity 81%).

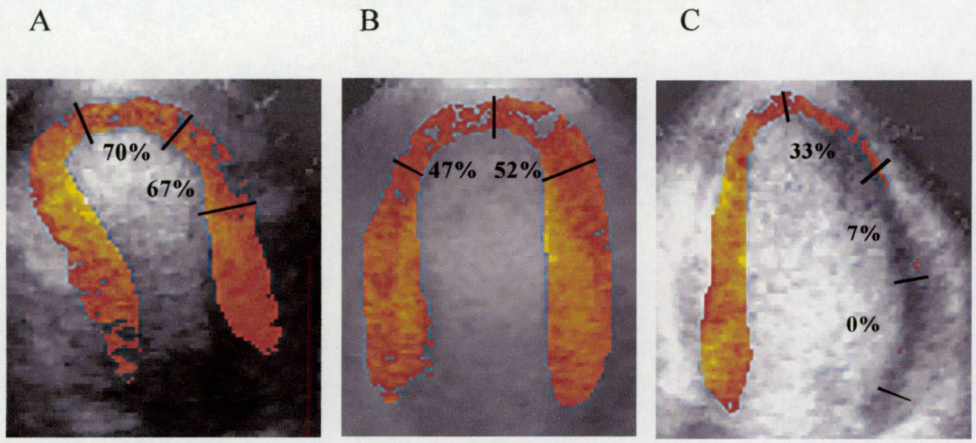


Figure 3 *Relative videointensity values of akinetic segments of 3 patients in the two chamber view early after reperfusion: (A) with functional recovery at rest at two weeks follow-up, (B) without functional recovery at rest but presence of contractile reserve at two weeks follow-up and (C) with neither functional recovery at rest nor presence of contractile reserve at two weeks follow-up.*

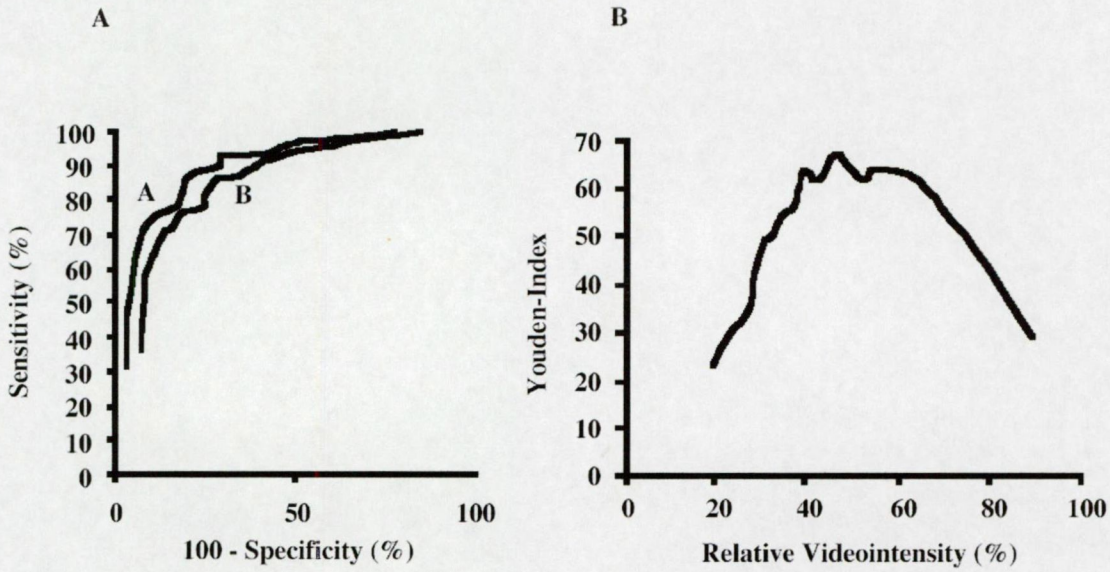


Figure 4 *Receiver operator characteristic curves for identification of functional recovery or presence of contractile reserve (curve A) or functional recovery alone (curve B) by contrast echocardiography. (B) The Youden index (sensitivity + specificity-100) of different relative videointensities for the prediction of functional recovery or presence of contractile reserve at two weeks' follow-up.*

2.2.4. Prediction of recovery of global left ventricular function with contrast echocardiography

We then compared the wall motion score index of two groups of patients early after reperfusion and at the two week follow-up: ≥ 50% (group 1, n=23) and < 50% (group 2, n=37) of initially akinetic segments are contrast positive (relative videointensity ≥ 50%). Clinical and angiographic characteristics were not different between group 1 and 2 except for the presence of non-Q wave AMI (n=9 in group 1 vs. n=0 in group 2, p<0.01). Early after reperfusion, wall motion score index was not different between group 1 and group 2 (1.5 [1.33,1.90] vs. 1.75 [1.31,1.96], respectively). At follow-up, wall motion score index was significantly lower in group 1 compared to group 2 (1.25 [1.08,1.5] vs. 1.5 [1.25,1.83], respectively, p=0.002). Improvement of wall motion score index between early post-reperfusion and follow-up was significant only in group 1 (p<0.0001, Figure 5).

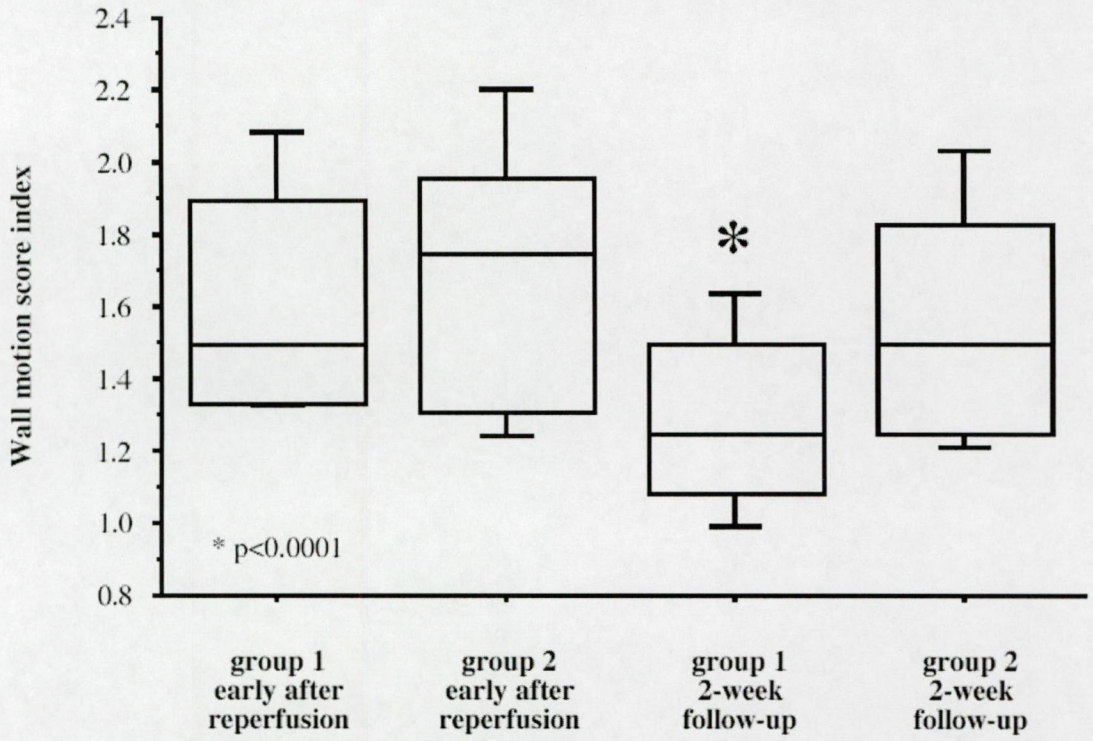


Figure 5 *Wall motion score index early after reperfusion and at follow up in patients with ≥50% (group 1) and <50% (group 2) contrast positive (relative videointensity ≥50%) segments of initially akinetic myocardium.*

3. Time course of microvascular and contractile improvement of risk area after successful reperfusion in patients with acute myocardial infarction

3.1. Methods

3.1.1. Patient population

The same patients were enrolled in this long term follow-up study, than in the previous one, but all patients had to present TIMI grade 3 IRA flow not only immediately and two weeks after primary PCI but at the six month follow up as well.

3.1.2. Study Protocol

Two-dimensional and venous myocardial contrast echocardiography was performed approximately three hours after successful mechanical reperfusion (FU 1) (median [25th, 75th percentile] 180 minutes [150 minutes, 240 minutes]). At follow-up after two weeks (FU 2) and six months (FU 3) coronary angiography and both echocardiographic studies were repeated. Dobutamine stress echocardiography was performed at the six month follow-up (FU 3). This protocol was also approved by Institutional Review Board of German Heart Center, Munich, and patients gave written informed consent.

3.1.3. Venous myocardial contrast echocardiography

The contrast echocardiography was performed and on the same segmental basis quantitatively evaluated as previously described. A risk area segment was regarded at any time as "reflow" segment, when the relative videointensity (VI) of the segment was higher than 50%, The VI of a "no-reflow" segment was equal or less than 50%. The risk area segments were divided into three groups: In "persistent reflow" group the VI was higher than 50% both at FU1 and at FU3. In the "late reflow" group the VI was higher than 50% only at the FU3. In the "persistent no-reflow" group the VI was equal or less than 50% both at FU1 and FU3.

3.1.4. Two dimensional and dobutamine stress echocardiography

Only the definition of myocardial viability was different compared to the previous study, because of the long-term follow-up. An initial risk area segment (segment with wall motion abnormality at admission) was regarded as viable when it was hypo- or normokinetic at rest or improved at least to hypokinesia during the dobutamine stress echocardoigraphy at the sixth month follow-up (FU3).

3.1.5. Statistical analysis

Nominal variables were expressed as counts and percentages. Continuous variables were presented as mean ± standard deviation (SD) instead of time parameters, which were presented as median (interquartile range) and, as appropriate, Kruskall-Wallis and Mann-

Whitney tests were used for comparison between groups. Differences between groups were considered significant at $p<0.05$.

3.2. Results

3.2.1. Patient characteristics

Between July 1999 and August 2000 sixty-eight patients were included in the study. In six patients poor acoustic window prevented to quantify contrast images. Thirteen patients were excluded because of death (n=2), history of reinfarction (n=2) or inadequate infarct related artery perfusion (TIMI flow <3) at any control coronary angiography during the follow-up period (n=9). Table 2 lists the demographic, clinical and angiographic characteristics of the remaining 49 patients.

Table 2 Patient characteristics

Age, median years [25th, 75th percentiles]	57 [51, 63]
Women, n (%)	7 (14)
Mechanical reperfusion	
Stent, n (%)	37 (76)
Percutaneous transluminal coronary angioplasty, n (%)	12 (24)
Time from onset of symptoms to reperfusion , median h [25th, 75th percentiles]	7 [3, 15]
Peak creatine kinase level, mean±SD	837±663
Infarct related artery	
Left anterior descending coronary artery, n (%)	20 (41)
Right coronary artery, n (%)	21 (43)
Left circumflex coronary artery, n (%)	8 (16)
Multivessel disease, n (%)	36 (73)
Q wave infarction, n (%)	37 (76)
Arterial hypertension, n (%)	30 (61)
Hypercholesterolaemia, n (%)	33 (67)
Diabetes mellitus, n (%)	11 (22)
Smoking, n (%)	35 (71)
ß Blocker medication at follow up n (%)	47 (96)

3.2.2. Time course of systolic recovery

Resting contractile function was abnormal initially in 200 (akinesia n=174, hypokinesia n=26) from the total of 488 segments. The wall motion score values of risk area segments did not change during the first three hours (2.87±0.34 vs. 2.84±0.39; p=NS). Sixty eight percent of systolic functional recovery had happened during the first two weeks after reperfusion therapy of MI (between FU1 and FU2) and a further 28% improvement was found between the FU2 and FU3 (WMS= 2.84 ± 0.39; 2.26 ± 0.87; 2.02 ± 0.9; respectively, $p<0.05$) (Figure 6).

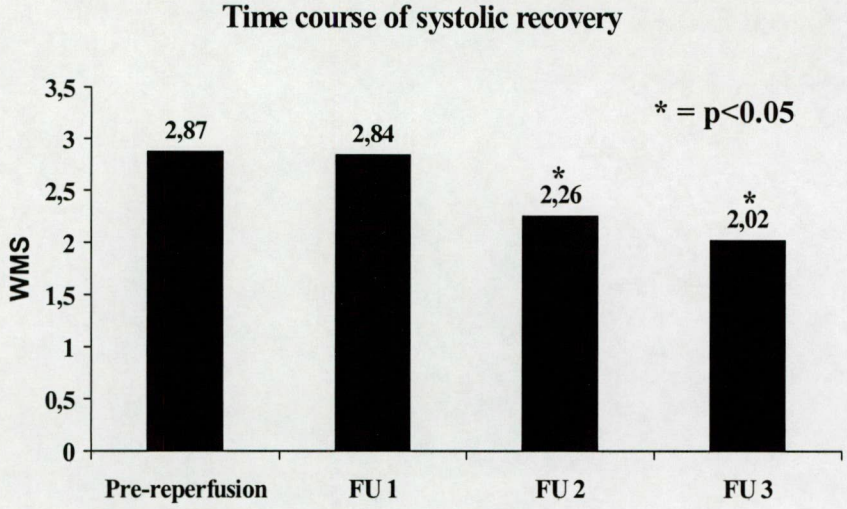


Figure 6 *Wall motion score (WMS) of risk area segments at pre-reperfusion, three hours (FU1), two weeks (FU2) and six months (FU3) after reperfusion.*

3.2.3. Time course of systolic recovery in different "microvascular healing" groups

The FU1 echocardiography was not performed in four patients (12 risk area segments), therefore 188 risk area segments were examined both at FU1 and FU3. Hundred one segments had higher than 50% VI both at FU1 and at FU3 („persistent reflow"). Twenty-eight segments improved from no-reflow to reflow until the FU3 („delayed reflow") and 49 segments remained in the no-reflow range untill FU3 („persistent no-reflow") (Figure 7). The wall motion score values of „persistent reflow" segments showed a continuous improvement during the first six months (FU1-FU2-FU3) after reperfusion of AMI (2.74 ± 0.46, 1.77 ± 0.79, 1.47 ± 0.64, respectively, $p<0.01$). In the „delayed reflow" group the systolic function improved significantly only between FU1 and FU3 (2.86 ± 0.36, 2.07 ± 0.81, respectively, $p<0.01$). In the „persistent no-reflow" group did not change the contractile function at all until the six-month follow up. The WMS of „persistent reflow", „delayed reflow" and „persistent no-reflow" segments was significantly different at FU3 (1,47 ± 0.64, 2,07 ± 0.81, 3.02 ± 0.25, respectively, $p<0.05$) (Figure 8). In the „persistent reflow" group 93 segments improved at least to hypokinesia at rest and further 5 segments had contractile reserve (97% viability), in the „delayed reflow" group 18 segments were at least hypokinetic at rest and further 6 had contractile reserve (86% viability), 2% of „persistent no-reflow" segments were viable.

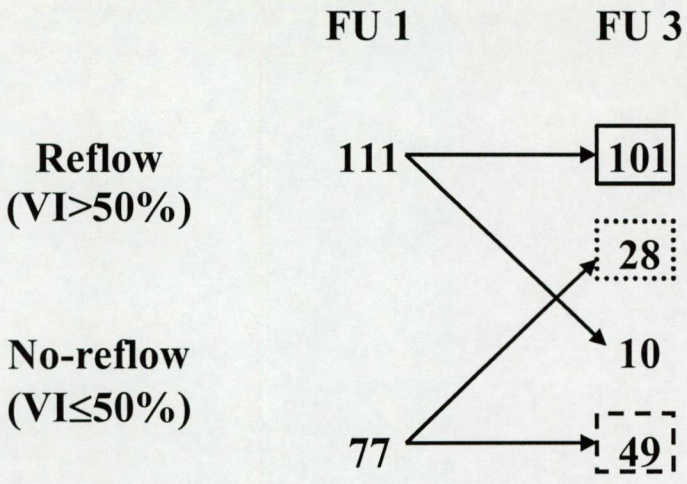


***Figure** 7 Changes of microvascular status of risk area segments three hours (FU1) and six months (FU3) after reperfusion. „Persistent reflow" segments are in solid frame „Late reflow" segments are in dotted frame and dashed frame contains the „persistent no-reflow" segments. (VI: relative videointensity)*

3.2.4. Time course of microvascular recovery in the risk area

The VI of risk area segments improved significantly during the first three hours after reperfusion therapy (40.4% ± 26 vs. 59.8% ± 30.4, $p<0.0001$). During the follow up period (FU1, FU2, FU3) we did not find any further significant improvement of microvascular obstruction in the risk area segments (59.8% ± 30.4, 64.5% ± 32, 68.2% ± 31.9), respectively, p=NS).

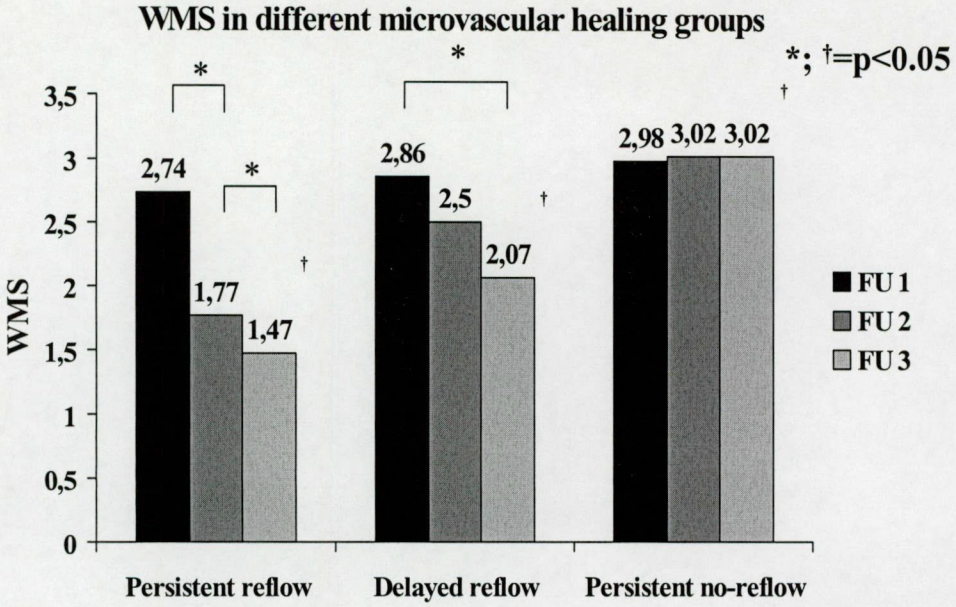


Figure 8 *Wall motion score (WMS) values in three different microvascular healing groups, three hours (FU1), two weeks (FU2) and six months (FU3) after reperfusion.*

3.2.5. Time course of microvascular recovery in the viable and non-viable regions of risk area

From the 200 segments with initial contractile abnormality 122 was normo-(n=77) or hypokinetic (n=45) at FU3, and further 12 segments had contractile reserve (viable group). Sixty-six segments remained akinetic without contractile reserve (non-viable group). The pre-reperfusion VI of the subsequent viable risk area segments were higher than of the non-viable segments (47.7% ± 26 vs. 25% ± 18, respectively; $p<0.01$) and the microvascular obstruction of these viable segments showed a continuous improvement at FU1, FU2 and FU3 (73.2% ± 25, 80.9% ± 24, 86.8% ± 17, respectively, $p<0.05$). There was no change at all in the microvascular perfusion of the non-viable segments at the post-reperfusion follow up studies (33.2% ± 20, 31.3% ± 20, 30.2% ± 19, respectively, p=NS) (Figure 9).

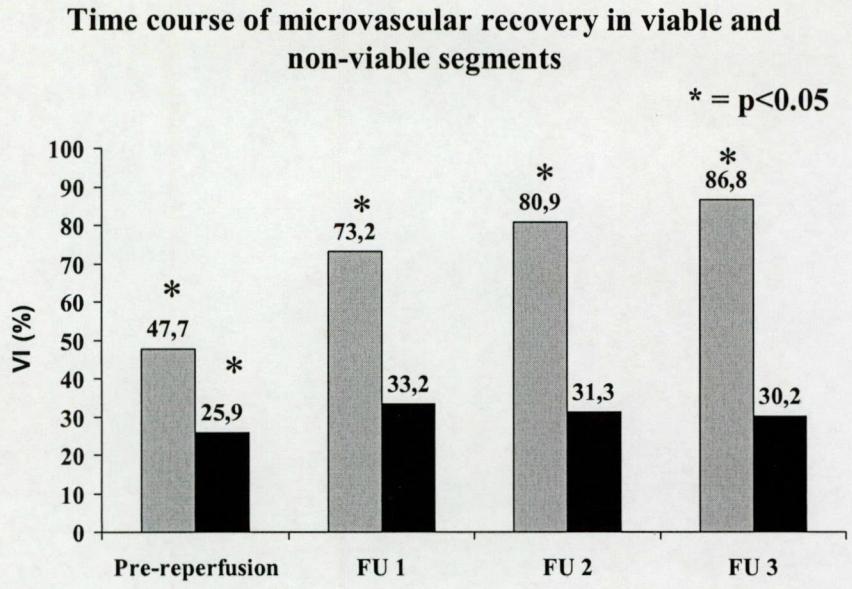


Figure 9 *Gray bars represents the relative videointesity (VI) values of viable segments (n=134), the black bars represents the VI values of non-viable segments (n=66) at pre-reperfusion, three hours (FU1), two weeks (FU2) and six months (FU3) after reperfusion.*

4. Quantitative evaluation of risk area, infarct size and the myocardial salvage

4.1. Methods

4.1.1. Patient population

In this study consecutive AMI patients were enrolled according to the previously described diagnostic criteria. Exclusion criteria was inability to give informed consent. All patients underwent thrombolysis or mechanical reperfusion.

4.1.2. Study protocol

Before the initiation of primary percutaneous coronary intervention or thrombolysis (accelerated tissue plasminogen activator therapy) patients received an intravenous injection of 27 mCi (1000MBq) of technetium Tc 99m sestamibi. Single-photon-emission computed tomography was performed within six to eight hours after the injection of the radionuclide. VMCE was performed at the same time of Tc 99m sestamibi injection. A follow-up VMCE and scintigraphic study was scheduled to perform approximately two weeks after treatment.

The study protocol was approved by Institutional Review Board of German Heart Center Munich, and patients gave written informed consent.

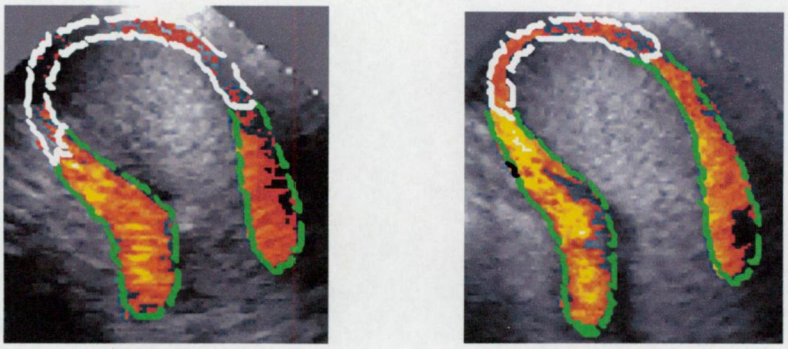
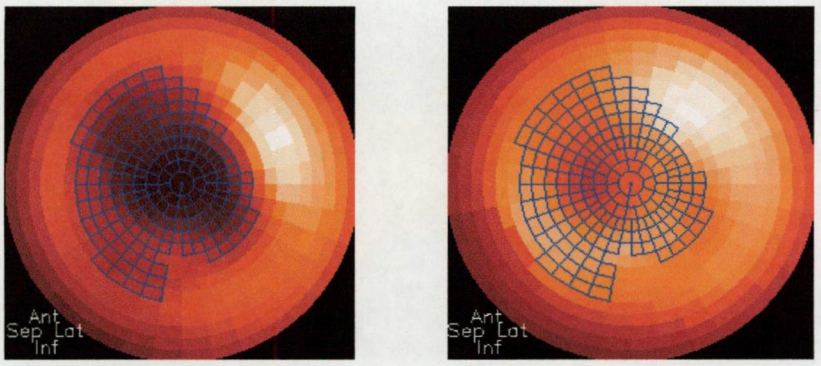


Figure 10 *Quantitative planimetry of a four-chamber view venous myocardial contrast echocardiography (VMCE) image before and ten days after reperfusion therapy of anterior AMI.*

4.1.3. Venous myocardial contrast echocardiography

The VMCE was performed on the same way as previously described. Planimetry of myocardial contrast defect size was performed in four and two chamber views on VMCE. The initial defect size, the defect size at follow up and the myocardial salvage (initial defect size minus defect size at follow-up) was expressed as a fraction of total left ventricular myocardium (Figure 10). Quantitative venous myocardial contrast echocardiography was performed by experienced investigators blinded to all clinical, echocardiographic and angiographic data. Values for intra- and interobserver variability of VMCE defect size were derived from analysis of 30 randomly selected records. Intra- and interobserver variability were 3% [1%, 4%] and 3% [2%, 4%], respectively, based on the absolute difference, and 18%[10%, 26%] and 21% [11%, 26%], respectively, based on relative difference.

Quantitative Analysis of SPECT



Pre-interventional
42%

Post-interventional
0%

Figure 11 *Quantitative planimetry of polar map Sestamibi single photon emission computer tomography SPECT images before and ten days after reperfusion therapy of anterior AMI.*

4.1.4. Radionuclide studies

The percentage of the left ventricle (LV) that was compromised by the initial defect; the size of the infarct (as a percentage of the LV) at the time of follow-up scintigraphy; the degree of myocardial salvage (as a percentage of the LV) was determined (Figure 11). The performance of the radionuclide studies and the quantitative analysis of the initial and follow-up polar map SPECT images were carried out according to the previously described method (18). Each polar map was adjusted for its own maximal value. The size of the defect was calculated with the use of 50 percent threshold, which was derived from studies that used a phantom, according to previously described method (19,20).

4.1.5. Statistical analysis

The previously described statistical methods were applied.

4.2. Results

4.2.1. Patient characteristics

From the 132 patients, enrolled in the study 13 were excluded because of image analysis could not be performed due to inadequate VMCE quality. Demographic, clinical and angiographic characteristics of the 119 who were eligible for image analysis are listed in

Table 3. Initial VMCE was performed in 118 follow-up in 117 patients. Initial SPECT analysis was performed in 112, follow-up in 117 patients.

Table 3 Patient characteristics

Age, median years [25th, 75th percentiles]	59 [50, 68]
Women, n (%)	21 (18)
Type of reperfusion	
Stent, n (%)	76 (64)
Percutaneous transluminal coronary angioplasty, n (%)	31 (26)
Thrombolysis, n (%)	12 (10)
Time from onset of symptoms to reperfusion , median h [25th, 75th percentiles]	8 [3, 18]
Peak creatine kinase level, [25th, 75th percentiles]	608 [338, 1230]
Infarct related artery	
Left anterior descending coronary artery, n (%)	55 (46)
Right coronary artery, n (%)	46 (39)
Left circumflex coronary artery, n (%)	18 (15)
Multivessel disease, n (%)	80 (67)
Q wave infarction, n (%)	92 (77)
Arterial hypertension, n (%)	72 (61)
Hypercholesterolaemia, n (%)	85 (71)
Diabetes mellitus, n (%)	20 (17)
Smoking, n (%)	74 (62)
ß Blocker medication at follow up n (%)	104 (87)

4.2.2. Defect sizes

The initial defect size measured by VMCE and sestamibi imaging was 21% [14%-36%] and 25% [14%-51%] ($p<0.05$), respectively. The correlation was significant between both methods ($r=0.82$, $p<0.0001$) (Figure 12). The residual defect size was also significantly different but VMCE and sestamibi imaging showed a fair correlation 10% [0%-19%] v. 14% [5%-29%] ($p<0.005$), respectively, ($r=0.8$, $p<0.0001$) (Figure 13). In the size of the salvaged myocardium no significant difference was found between VMCE and sestamibi imaging 9% [3%-17%] vs. 10% [3%-22%], ($r=0.57$, $p<0.0001$) (Table 4).

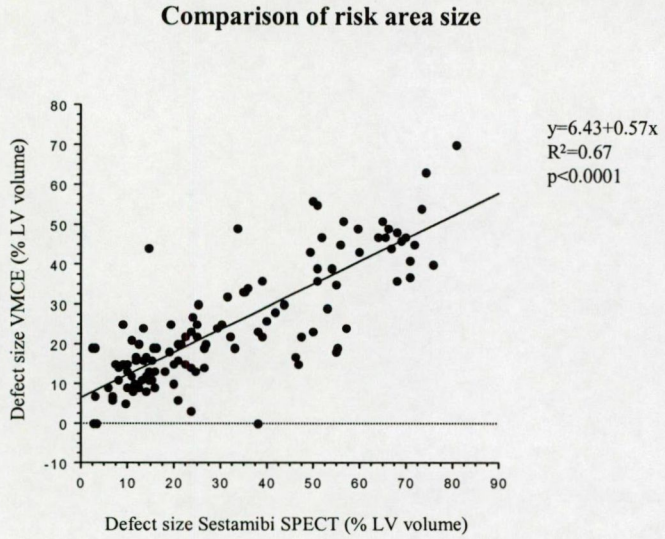


Figure 12 *Simple regression analysis of initial defect size measured by Sestamibi single photon emission computer tomography (SPECT) and venous myocardial contrast echocardiography (VMCE).*

Table 4 *Defect size by venous myocardial contrast echocardiography (VMCE) and Sestamibi SPECT (LV: left ventricle, SPECT: single photon emission computer tomography)*

	VMCE	Sestamibi	
Risk area (% of LV)	21% [14%-36%]	25% [14%-51%]	p<0.05
Infarct size (% of LV)	10% [0%-19%]	13% [5%-29%]	p<0.005
Salvage (% of LV)	9% [3%-17%]	10% [3%-22%]	ns.

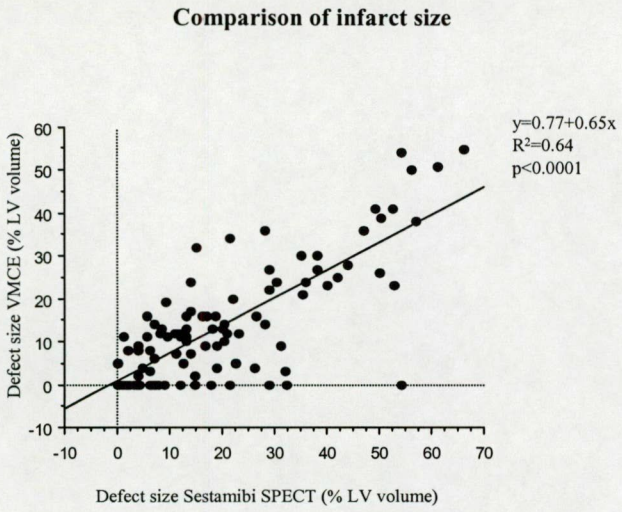


Figure 13 *Simple regression analysis of infarct sizes measured by Sestamibi single photon emission computer tomography (SPECT) and venous myocardial contrast echocardiography (VMCE).*

5. Discussion

5.1. Myocardial contrast signal and pathophysiology of contractile dysfunction early after reperfusion

The pathophysiology of myocardial contractile dysfunction after mechanical reperfusion in acute myocardial infarction is heterogeneous and may, besides myocardial stunning, include various amounts of myocardial necrosis or recurrent ischemia. Early after reperfusion, most of the previously ischemic myocardium is still akinetic and conventional echocardiographic measures of contractile function cannot be used to differentiate the underlying pathophysiology. Later, once stunning has abated, myocardium with only minor transmural extent of necrosis will show functional recovery. The presence of persistent akinesia, however, is not synonymous with transmural myocardial necrosis. Evaluation of myocardial contractile function at rest only allows evaluation of the inner (endocardial) third of the myocardium: if there is no or only minor necrosis the myocardium is normo- or hypokinetic, but subendocardial necrosis results in akinesia (10). Akinesia, therefore, occurs irrespective of the amount of vital tissue in the mid and epicardial portions of the myocardium. Although these portions contribute little to contraction at rest, they can be recruited to produce contractile reserve of akinetic

myocardium during dobutamine stimulation (21). Contractile reserve of akinetic myocardium is also clinically relevant because it has been shown to protect against ventricular dilatation and development of heart failure (22).

The four functional classes differentiated at two-week follow-up in the present study can therefore be regarded as functional corollary of differential degrees of transmural myocardial necrosis. Our data indicate that the magnitude of myocardial signal on venous contrast echocardiography gives an estimate of transmural extent of myocardial necrosis as soon as three hours after reperfusion. The present study extends the concept of myocardial "no-reflow" as an all or none phenomenon by quantitation of the continuum between myocardial "no-reflow" and "reflow" and the demonstration of its relevance by establishing a relation to the subsequent degree of contractile function. This result is in line with the findings of previous animal studies comparing the extent of myocardial contrast enhancement after venous contrast injection and myocardial necrosis using triphenyltetrazolium chloride staining (23, 24). With respect to the relevance of spatial extent of contrast enhancement for subsequent recovery of global left ventricular function, our findings confirm the results of Porter et al (25) and Lepper et al (26). These first 2 reports on the use of venous contrast echocardiography in patients after reperfusion of acute myocardial infarction used a semiquantitative score for the assessment of moycardial contrast enhancement in the risk area 2 days (25) and 24 hours (26) after reperfusion. Our results were subsequently also confirmed by Balcells at all (27) who performed contrast echocardiography at 3-5 days and four weeks after coronary stenting of AMI.

5.2. Prediction of functional recovery or presence of contractile reserve with venous contrast echocardiography early after reperfusion

This is the first study to define a signal threshold for the prediction of myocardial contractile function on venous contrast echocardiography early after mechanical reperfusion of acute myocardial infarction. Prediction of recovery of contractile function has been attempted by several investigators early after reperfusion therapy in acute myocardial infarction using semiquantitative assessment of myocardial contrast enhancement with intracoronary injection of microbubbles. Depending on the strictness of criteria for positive contrast effect, sensitivities for prediction of subsequent functional recovery were found between 66% (7) and 100% (8). Specificity, however, was only between 18% (9) and 60% (7). We found higher diagnostic accuracy of venous contrast echocardiography for prediction of recovery of contractile function or presence of contractile reserve after acute myocardial infarction. This finding cannot be solely

attributed to our consideration of contractile reserve in distinction to previous studies with intracoronary contrast injection because our receiver operator curves using functional recovery alone (Figure 4 A, curve B) or both variables (curve A) differ only slightly.
Several additional factors could contribute to the improved diagnostic accuracy. First, intracoronary bolus injection of sonicated radiopaque dye with a hand held syringe used in previous studies may force contrast material into the dependent microvasculature. Therefore, lack of opacification may be observed only in myocardium with the most seriously damaged microvessels whereas it may still be seen in areas with moderate microvascular damage. Venous infusion, in distinction, may result in more physiological distribution of microbubbles and provide a better representation of differential degrees of microvascular damage.

Secondly, the diagnostic accuracy for the prediction of functional recovery depends on the definition of the threshold for positive contrast effect. Quantification of the magnitude of contrast effect after poorly standardized intracoronary bolus injection is difficult since myocardial contrast signal, for the most time, is found on the upper flat part of microbubble concentration-videointensity relation curve (28). Therefore, only semiquantitative assessment of contrast effect has been employed in most intracoronary studies. Threshold-definition, however, is less accurate with a framework of only a few scores compared to the quantitative approach used in the present study.

Thirdly, myocardial contrast enhancement was evaluated 10-16 min after successful reperfusion in previous intracoronary studies while reactive hyperemia might have been present likely causing an overestimation of contrast effect within the infarct bed (29). Venous contrast echocardiography was performed later (190 min. after rerperfusion) in the present study and, relative videointensity was even slightly higher after two weeks in our subsequent observations. It is therefore unlikely that relative videointensity measurements were affected by reactive hyperemia.
Swinburn et al., recently reported a sensitivity of 62% of venous contrast echo score obtained approximately 5 days after acute myocardial infarction for binary prediction of recovery of resting contractile function of myocardial segments (30). Apart from methodological differences and the sole consideration of functional recovery at rest, however, undefined coronary anatomy at the time of contrast echocardiography and at follow up may have prevented unequivocal interpretation of the association between early contrast score and contractile function at follow-up in the study of Swinburn et al. Shimoni

and her colleagues found 88% sensitivity and 73% specificity for detection of hibernating myocardium with similar method to ours in their recently published paper (31).

5.3. Recovery of systolic function

Immediately (three hours) after optimal restoration of epicardial infarct related artery did not change the contractile function of the risk area segments. Most of the resting contractile recovery (68% of the total improvement) occurred in the first two weeks after coronary intervention, the rest of the systolic healing process (28%) was carried out until the 6th month follow-up. This means that restoration of global systolic dysfunction caused by myocardial stunning mainly accomplishes in the post-infarction two weeks. Our data is comparable with others. Affridi and colleagues (32) found 80% „early recovery" (1 week follow-up) and 20 % „late recovery" (within 6 weeks), although they examined revascularised hibernating myocardium. Ito and colleagues (33) examined also patients underwent successful primary PCI and in their study major functional improvement was achieved within 14 days of reflow. However in this study the longest follow up was only 24 days.

5.4. Recovery of microvascular function

The recovery of microvascualture of the whole risk area occurs during the first three hours after primary PCI, later a slight, non-significant improvement of microvascular obstruction was observed.

Some authors found early progressive deterioration (34,35,36) or no change in microvasculature (37) with an intermediate follow-up (2-9 days), but these observations were carried out on experimental animal models with different methods: MRI (34,35,37), microsphere tracer (36), PET (38). These changes occurred as early as 1 (36,38) or 6 hours (35) after reperfusion. The occlusion of the infarct related artery lasted 90 minutes (34,35,36), which is a significantly shorter duration of ischaemia, than in the real life infarct patients. In animals, substantial extension of tissue injury during reperfusion has not been observed after periods of ischaemia > 120 to 180 min (39). Our time interval from onset of pain to primary PCI was 7 hours (Table 2.). In another animal study with not only ligation of the coronary artery but with artificial thrombus creation in it microvascular obstruction decreased in the first hour after recanalisation (40).

In one study only 6% change was detected in the microvascular perfusion of RA between the first 4 days and 1 month, in another human study 56% of "early no-reflow" healed until the one month follow up (27,41). In our study 18% of early no-reflow RA segments improved until 1 month and 36 % (28 of 77) of early no-reflow RA segments belonged to

the delayed reflow group (improvement until the 6th month follow-up (FU3)) (Figure 7). Brochet and his colleagues found that 85% (41/48) of the „early no-reflow" segments improved during the first 9 days after reperfusion, but a semiquantitative scoring system was used in an intracoronary contrast echocardiographic study (7).

On the one hand the definition of no-reflow was different in these studies, for example in the study of Galiuto et al. 66% of patients was in the early no-reflow group which is extremely high (41). The incidence of „no-reflow" phenomenon depends on the definition. For example Ito and his colleagues determined no-reflow as follows: when the endocardial length of the area showing residual contrast defect exceeded a fourth of that of risk area, it was considered „no-reflow" (42). Thirty seven percent of their patients showed signs of „no-reflow" with this definition. On the other hand the FU1 was performed three hours after reperfusion in our study whereas the early follow-up had happened 3-5 days after reperfusion therapy in the paper of Balcells (27). We used a quantitative method for the definition of no-reflow contrary to the previously mentioned authors.

5.5. Relation between microvascular and contractile improvement

This is the first study to observe a long-term (6 months) improvement of microvascular perfusion in the viable segments of risk area after reopening of infarct related artery. Others found some improvement only during a determined period of the first month interval (43,44). One group showed decrease in microvascular obstruction (between first and eighth day after primary percutaneous coronary intervention) only in myocardium with subsequent inotropic reserve, during an eigth day follow-up (45). Brochet and colleagues found a significant improvement of microcirculation in the whole risk area until the 9th day follow up (7). His semiquantiative assessment of microvascular healing was probably not so strictly and exactly determined than ours. It can be confirmed by the fact, that functional recovery or contractile reserve was found only in 36% of Brochet's „improved contrast score" group, whereas 86% of our „delayed reflow" segments were viable. One other group found immediate deterioration in „no-reflow", a slower (lasting hours) destruction in infarcted territories, and permanent viability in the „salvaged" area (46).

5.6. Infarct size estimation with different methods

In experimental studies the quantitation of risk area during coronary occlusion and the measurement of infarct size after reopening of infarct related artery with Technetium-99m Methoxisbutyl Isonitrile (99mTc-MIBI) correlated well with the direct radiotracer risk area and triphenyltetrazolium chloride (TTC) measurement results: $r = 0.94$, $r = 0.98$, respectively according to Sinusas and colleagues (47) and other authors (48,49). With

cardiac MRI (50) and myocardial contrast echocardiography (51,23) similar correlation was found between the post-reperfusion defect size and the infarct size determined by TTC.

According to our knowledge only in one human study was examined the correlation between direct pathologic quantification of the extent of scarred myocardium and the 99mTc-MIBI defect size (52). In this study despite a slight overestimation scintigraphic scar size correlated closely with pathological scar size ($r = 0.89$). Several data available on the comparisons of SPECT sestamibi defect size and multiple other parameters, which are strong predictors of mortality of myocardial infarction: ejection fraction($r = 0.81$-0.76) (20,53,54), end systolic volume (55), regional wall motion (20,53), cretine kinase release (56,57). Sestamibi infarct size is highly associated directly with the subsequent overall and cardiac mortality as well (58). Microvascular obstruction size measured by magnetic resonance imaging is also a predictor cardivascular events (cardiac death, reinfarction, congestive heart failure, stroke, or unstable angina requiring hospitalisation) (59).

5.7. Difference between VMCE and SPECT defect size

The number of clinical studies in comparison of SPECT and VMCE defects are limited the patient population in the formerly studies are not homogenous (the date of AMI is not clear in these studies, the VMCE data analysis is different, bolus contrast administration, shorter time interval for intermittent imaging, no background subtraction, without colour coding, no quantitative analysis) (60,61,62). The sensitivity of contrast echocardiography for detection of SPECT defect ranged from 18% (61) to 76% (60). In another intracoronary administered contrast agent study in patients underwent coronary angiography (not only post MI patients) in 78% of patients agreed the two techniques. A significant correlation ($p<0.001$) was observed between these techniques in assessing the size of perfusion defects with a relatively low r value ($r = 0.62$) (62). In these studies "more defect" were found with SPECT than with VMCE. These finding might be analogue with our smaller VMCE defect sizes compared to SPECT. In our study the correlation is much better between the two methods examined, either the risk area or the infarct size, $r = 0.82$ and $r = 0,8$, respectively. The better results might be explained with methodological differences (we used continuous contrast infusion, harmonic imaging, pulsing interval of 7 cardiac cycles), between our and the previously mentioned studies. Lindner and colleagues (15) found that the videointensity ratio between the abnormal and normal myocardium using continuous contrast infusion at a long pulsing interval (8 cardiac cycles) had correlated well with the activity ratio between these segments on SPECT ($r = 0.73$, $p<0.01$). The correlation with

bolus infusion and at shorter pulsing interval (1 and 5 cardiac cycles) is poorer: r=46; r=0.48, respectively.

However, there is a close correlation between scintigraphic and histological scar size therefore SPECT defect size was regarded as clinical gold standard, SPECT imaging slightly overestimates the scar size (52).

In the experimental studies of Kloner was proved that myocardial cell injury could exist without microvascular damage, but microvascular damage could not occur without myocardial cell injury (63). With VMCE the microvasculature is examined, but for 99mTc-MIBI uptake not only intact microvasculature, but also normal mithocondrial function is necessary (64). This is another explanation for the larger SPECT defect sizes, found in our study.

5.8. Study limitations

Due to anisotropy, heterogeneous beam profile and attenuation, contrast signal intensity of differential myocardial segments is subject to variability independent of the volume of myocardial microvasculature (9% [1%, 13%] in normokinetic segments in the present study), which might have prevented a closer correlation between relative myocardial videointensity early after reperfusion and the amount of viable myocardium.

Advantage of intermittent harmonic imaging, used in the present study, is high signal amplitude caused by microbubble disruption with high mechanical index imaging, but off line image processing is mandatory because the tissue component of the harmonic signal is considerable. Recent advances in this technique including multiple pulse transmission, reduction of myocardial tissue signal by modified transmit and receive frequencies, and colour coding of row image data may, however, obviate the need for time consuming off line processing in the future. Recently developed technologies offers alternative echocardiographic modalities (power pulse inversion, power modulation technique) for venous contrast echocardiography.

In our study myocardial blood volume was measured to characterise myocardial perfusion. The myocardial blood flow velocity can be also assessed by analysis of increasing pulsing intervals (6). This parameter is significantly better than the qualitative evaluation of contrast pictures for assessment of myocardial viability. However, blood flow velocity parameters can not be significantly more accurate predict the functional recovery than myocardial blood volume parameters, such as relative videointensity with a long pulsing interval (65).

Diagnostic accuracy found for prediction of contractile myocardium by venous myocardial contrast echocardiography in the present study may be too optimistic since the underlying contrast intensity cut-off value was determined in the same patient population. Although raw signal intensity data were not used in the present study, despite theoretical superiority, validity of videodensitometric data could be demonstrated in a head to head comparison with digital raw data (23).

Contractile response to dobutamine could have been attenuated by beta-blocker therapy, which was not discontinued in the present study. Therefore, although we used high doses of dobutamine, we may have incorrectly classified patients as having no contractile reserve.

Although planimetry is a quantitative method, only in SPECT polar map pictures were clearly defined perfusion defect size (territory of pixels with <50% of maximum contrast intensity). The borderline of the defects on the contrast echocardiographic images was determined and drawn around by the investigator without clear definition of contrast defect. In spite of this fact the intra- and interobserver variability was only 3%, based on the absolute difference.

5.9. Clinical implications and perspective

Our data imply that characterization of myocardial capillary perfusion by venous contrast echocardiography is a sensitive and meaningful marker for the evaluation of the success of reperfusion therapy in patients with acute myocardial infarction. Determination of TIMI myocardial perfusion grade (30), Doppler flow wire (26,66), nuclear myocardial perfusion imaging (43) and magnetic resonance imaging (67) have been used for assessment of myocardial microvasculature after acute myocardial infarction. Each of these techniques has its specific strengths and limitations but none of them has been implemented in routine diagnostic workup of patients with acute myocardial infarction because they are either invasive or not readily available in most hospitals. Venous myocardial contrast echocardiography used in the present study is a bedside technique and has the potential of wide availability. Furthermore, the application of pharmacological stress, so far needed for echocardiographic assessment of myocardial viability is not required.

Multiple end points, including global left ventricular function, early arterial patency, and clinical outcomes have been used in various randomized trials to measure the efficacy of reperfusion therapy in acute myocardial infarction. The most important clinical outcome is that of patient mortality. However, use of mortality, as an end point requires increasingly large sample sizes to test advances compared with existing therapy, which is already highly

effective. End points (such as infarct size, myocardial salvage) that are potential „predictors" for both early and late patient mortality are therefore attractive for several purposes: to conduct early pilot studies to demonstrate potential efficacy of a new treatment, to indicate a possible late mortality benefit for a new therapy, that may be equivalent to existing therapy with respect to early mortality. Infarct size can be measured shortly after reperfusion therapy, while ventricular systolic function changes (recovery of stunning, remodeling) for a long time after the therapy.

Based on the present data, it appears also reasonable to evaluate the role of venous contrast echocardiography for risk stratification after acute myocardial infarction in future studies.

Analysis of time-intensity curves based on signals from incremental imaging intervals or real-time registration of signals using low mechanical index imaging has the potential for evaluation of myocardial microvascular flow velocity and blood flow (6). This may provide further insight in the process of recovery of myocardial microvascular and contractile function after reperfusion of acute myocardial infarction.

6. Main findings

1. This is the first study in patients with first acute myocardial infarction to show, that early after successful mechanical reperfusion, the magnitude of myocardial signal of akinetic myocardium on venous contrast echocardiography is correlated with the degree of myocardial contractile function after two weeks.
2. Recovery of contractile function or presence of contractile reserve can be predicted with high diagnostic accuracy, with venous myocardial contrast echocardiography performed immediately after successful primary percutaneous coronary intervention. This is the first study to define a signal threshold for the prediction of myocardial contractile function on venous contrast echocardiography early after mechanical reperfusion of acute myocardial infarction.
3. Looking at the whole risk area microvascular recovery occurred during the first three hours after reperfusion and no further significant recovery was found. Recovery of the systolic function is a prolonged process.
4. This is the first contrast echocardiographic study observed the slight but significant and continuous improvement of microvascular perfusion in the viable segments during the first six months after reperfusion therapy. The delayed microvascular healing was observed mostly in viable segments but in territories with a higher degree of transmurality than in the "early" and "persistent reflow" group.
5. In our study was first time described that in patients with acute myocardial infarction both the risk area and the infarct size measured by VMCE showed a close correlation with the clinical gold standard, the 99mTc sestamibi SPECT results.
6. Risk area and infarct size are significant smaller measured by VMCE than by SPECT imaging, however in the amount of salvage no difference was found.

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