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# Effects of postconditioning in ST-elevation myocardial infarction:

## Assessment of myocardium at risk and infarct size

by

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*To my wonderful family,*
*Linda, Filippa, Elvira and Alexander*

# Contents

# ABSTRACT

**Background**
Myocardial infarction remains a major health problem, despite recent improvements in detection and treatment. Infarct size is a major determinant of future mortality and morbidity. Management strategies aimed at limiting infarct size, beyond what is achieved with early revascularization in combination with platelet stabilization, could be of great prognostic importance. Although opening of the infarct-related artery is mostly beneficial, it also initiates harmful processes that contribute to the final infarct size, so called reperfusion injury. When performing studies aiming at myocardial protection, it is important to have methods that accurately quantify ischemic but viable myocardium and the final infarct size. Postconditioning, a method that consists of cycles of brief reperfusion and ischemia during early stages of revascularization, seems to limit reperfusion injury. Further knowledge is important for understanding how efficient this technique is and if the protection leads to long-lasting benefits.

**Methods and results**
*Study I* investigated if postconditioning in addition to primary percutaneous coronary intervention (PCI) would limit infarct size and improve left ventricular ejection fraction (LVEF), compared with standard PCI. This was determined with cardiovascular magnetic resonance (CMR) after one week in 76 patients with ST-elevation myocardial infarction (STEMI). There was no difference in infarct size and LVEF within the total study population. Postconditioning did, however, have a beneficial effect on final infarct size and LVEF in patients with the largest volumes of myocardium at risk (MaR).
*Study II* investigated if the results from study I were consistent during long-term follow-up in 68 patients. In order to quantify infarct size and LVEF, the patients were re-examined with CMR at three and 12 months. There was no difference between patients who were postconditioned and those who underwent ordinary PCI in the complete study group. Postconditioned patients in the upper quartile of MaR did, however, still have less myocardial damage and improved LVEF after one year.
*Study III* compared MaR estimated with a new modified contrast-enhanced CMR sequence one week after admission with the reference standard method, myocardial perfusion single-photon emission computed tomography (SPECT), in 16 patients with STEMI. There was a good correlation between the two methods.
*Study IV* investigated 21 patients with STEMI one week after revascularization with CMR. T2-weighted (edema) images were compared with contrast-enhanced CMR sequence for the assessment of MaR. A strong agreement was found between the two methods.

**Conclusions**
Postconditioning did not decrease infarct size or improve LVEF one week or 12 months after the procedure in all patients with first time STEMI subjected to this method. Patients with large MaR seemed, however, to have a consistent benefit over time in the form of smaller infarct sizes and improved LVEF. There is a strong agreement between the newly developed contrast-enhanced CMR sequence compared with both reference standard SPECT and T2-weighted edema images. The implication is that the new technique can be used for quantification of MaR and final infarct size in patients with STEMI, through a single investigation performed several days after the event.

# SAMMANFATTNING

**Bakgrund**
Hjärtinfarkt är ett kvarstående hälsoproblem trots betydelsefulla förbättringar i handläggningen i form av tidig revaskularisering. Hjärtinfarktens storlek har stor betydelse för patientens framtida prognos. Strategier som kan minska hjärtinfarktens storlek är därför viktiga tillägg till tidig revaskularisering. Även om ett återställt kranskärlsblodflöde huvudsakligen är av godo initierar det också processer som leder till hjärtcellsdöd, så kallad ”reperfusionsskada”. Vid studier av möjligheten att reducera sådana skador är det av största vikt att förfoga över metoder som tillförlitligt kan mäta skillnaden mellan det hotade myokardområdet (riskarean) och den slutgiltiga hjärtmuskelskadan. S.k. postkonditionering, upprepade cykler av återskapat kranskärlsblodflöde med mellanliggande avstängning av flödet, är en metod som tycks minska reperfusionsskadan. Ytterligare studier behövs emellertid för att slutgiltigt värdera effekten av denna åtgärd.

**Metoder och resultat**
*Studie I* undersökte om postkonditionering i samband med perkutan koronarintervention (s.k. ballongsprängning; PCI) jämfört med enbart PCI utan postkonditionering minskar hjärtinfarktstorleken och ökar vänsterkammarens funktion mätt med magnetkamerateknik (MR) efter en vecka hos 76 patienter med förstagångsinfarkter. En sådan effekt kunde inte verifieras i den totala studiepopulationen. Dock utvecklade postkonditioneringspatienter med stora riskareor mindre hjärtinfarkter och hade en bättre hjärtmuskelfunktion jämfört med motsvarande kontrollindivider.
*Studie II* studerade de långsiktiga effekterna av postkonditionering. Efter tre och 12 månader återundersöktes 68 patienter från Studie I med MR. Liksom i den första studien förelåg ingen skillnad mellan grupperna vad avser infarktstorlek eller vänsterkammarfunktion, men postkonditionerade patienter med stora riskareor hade fortfarande mindre hjärtmuskelskador och bättre vänsterkammarfunktion jämfört med sina motsvarande kontrollpersoner.
*Studie III*: Riskarean hos 16 patienter med hjärtinfarkt bestämdes med en ny modifierad, kontrastförstärkt MR-sekvens en vecka efter insjuknandet och jämfördes med riskarean uppmätt med hjärtscintigrafi som referensmetod. Överensstämmelsen mellan de två metoderna var god.
*Studie IV*: Tjugoen patienter med hjärtinfarkt genomgick MR-undersökning av hjärtat en vecka efter ballongsprängning. De då erhållna T2-viktade ödembilder jämfördes med motsvarande fynd med den modifierade kontrastförstärkta MR-sekvensen. Även här förelåg en god överensstämmelse.

**Slutsats**
Postkonditionering hade, i studiepopulationen som helhet, ingen effekt på hjärtinfarktstorlek eller vänsterkammarfunktion, varken en vecka eller 12 månader efter en förstagångsinfarkt. Patienter med stora riskareor tycks emellertid ha nytta av postkonditionering, som hos dessa minskade hjärtmuskelskadan och förbättrade vänsterkammarfunktionen på ett bestående sätt. Det förelåg en god överensstämmelse mellan den nyutvecklade, kontrastförstärkta MR-sekvensen jämfört med både referensmetoden hjärtscintigrafi och T2-viktade ödembilder vad avser fastställande av det hotade myokardområdet. Fortsättningsvis kan den nya sekvensen användas i framtida infarktstudier som behöver mäta både riskarea och infarktstorlek samt utföras med endast en undersökning flera dagar efter det akuta insjuknandet.

# List of Original Papers

This thesis is based on the following original studies which will be referred to by their Roman numerals.

**I.** **Sörensson P**, Saleh N, Bouvier F, Böhm F, Settergren M, Caidahl K, Tornvall P, Arheden H, Rydén L, Pernow J.
Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction.
*Heart*. 2010;96:1710-1715

**II.** **Sörensson P**, Rydén L, Saleh N, Tornvall P, Arheden H, Pernow J.
Preserved benefits of postconditioning on infarct size in patients with large myocardial areas at risk - One year follow-up of patients with ST-elevation myocardial infarction.
Manuscript

**III.** **Sörensson P**, Heiberg E, Saleh N, Bouvier F, Caidahl K, Tornvall P, Rydén L, Pernow J, Arheden H.
Assessment of myocardium at risk with contrast enhanced steady-state free precession cine cardiovascular magnetic resonance compared to single-photon emission computed tomography.
*J Cardiovasc Magn Reson*. 2010;12:25-32

**IV.** Ubachs J, **Sörensson P**, Engblom H, Carlsson M, Jovinge S, Pernow J, Arheden H.
Myocardium at risk by magnetic resonance imaging: head-to-head comparison of T2-weighted imaging and contrast enhanced steady state free precession.
Manuscript

# List of Abbreviations

| | |
|---|---|
| ACEi/ARB | angiotensin converting enzyme inhibitor/ angiotensin receptor blocker |
| ACS | acute coronary syndrome |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| CABG | coronary artery bypass grafting |
| CK | creatine kinase |
| CKMB | creatine kinase isoenzyme MB |
| CMR | cardiac magnetic resonance |
| DTPA | diethylenetriamine penta-acetic acid |
| ECG | electrocardiography |
| Erk1/2 | extracellular regulated kinase |
| GP | glycoprotein |
| GSK3β | glycogen synthase kinase-3β |
| IS | infarct size |
| LAD | left anterior descending artery |
| LCx | left circumflex artery |
| LDL | low density lipoprotein |
| LGE | late gadolinium enhancement |
| LV | left ventricle |
| LVEF | left ventricular ejection fraction |
| MaR | myocardium at risk |
| MI | myocardial infarction |
| MPS | myocardial perfusion SPECT |
| MVO | microvascular obstruction |
| mPTP | mitochondrial permeability transition pore |
| NO | nitric oxide |
| NOS | NO synthase |
| PCI | percutaneous coronary intervention |
| PET | positron emission tomography |
| PKC/G | protein kinase C/G |
| PI3K | phosphatidylinositol-3-kinase |
| RCA | right coronary artery |
| RISK | reperfusion injury salvage kinases |
| ROI | region of interest |
| ROS | reactive oxygen species |
| SAFE | survivor activating factor enhancement |
| SNR | signal-to-noise ratio |
| SPECT | single-photon emission computed tomography |
| CE-SSFP | contrast-enhanced steady-state free precession |
| STEMI | ST-elevation myocardial infarction |
| STIR | short inversion time inversion recovery |
| $^{99m}$Tc | technetium |
| TE | echo time |
| TI | inversion time |
| TIMI | thrombolysis in myocardial infarction |
| TNFα | Tumor necrosis factor α |
| TR | repetition time |
| TTC | triphenyltetrazolium chloride |

# INTRODUCTION

## Acute myocardial infarction

Worldwide coronary heart disease remains a major health problem despite substantial improvements in detection and treatment.[1] In 2010 the SWEDHEART registry, comprising information on all Swedish hospital admissions for acute myocardial infarction (MI), recorded approximately 19,600 patients of whom 5,100 had ST-elevation myocardial infarctions (STEMI). The risk profile of patients with STEMI has changed during 1995-2010 with an increasing proportion of subjects with obesity, treated hypertension and hyperlipidema and also smokers. The proportion of patients with diabetes mellitus has remained around 20 % persistently. Reperfusion treatment, either by percutaneous coronary intervention (PCI) or thrombolysis, was performed in 70 % of the STEMI population during 1995-2004 and successively increased to 90 % in 2010, above all in the form of primary PCI. During the period 1995-2010, there has been an increase in the short- and long-term survival of patients with STEMI regardless of sex and age. This is most likely the result of improved reperfusion treatment, more aggressive anti-platelet therapy, increased use of betablockers, statins and angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers.[2] Still, many STEMI patients develop large areas of myocardial necrosis and infarct size is a major determinant of subsequent mortality and morbidity.[3, 4] Accordingly, early instituted therapeutic strategies aimed at limiting infarct size should be of great prognostic importance as a supplement to current management focused on primary revascularization in combination with platelet stabilization.[5] The development of such treatment modalities is critically dependent on methods that accurately determine the size of the ischemic myocardium at risk (MaR) and final infarct size. Moreover, these methods must be feasible to use in connection to the acute revascularization procedure, usually a PCI.

## Pathophysiology

All acute coronary syndromes (ACS) comprising unstable angina, non-STEMI and STEMI, share a common pathophysiological sequence: the disruption of an atherosclerotic plaque leading to platelet aggregation, thrombus formation and a nearly or completely occluded coronary artery. This causes myocardial ischemia due to insufficient blood supply in relation to the demands of the jeopardized myocardial area. If blood flow is not restored within an appropriate time period the ischemia will result in cellular necrosis, which starts in the subendocardial myocardium and progresses like a "wave-front" towards the epicardial region, eventually developing into a transmural myocardial infarct.[6, 7] The ischemic cardiomyocytes generate lactic acid from anaerobic metabolism, thereby reducing the intracellular pH which activates their sarcolemmal $Na^+$-$H^+$ channels exchanging $H^+$ at the expense of intracellular $Na^+$ accumulation. This is followed by an activation of the $Na^+$-$Ca^{2+}$ channel resulting in an increased intracellular $Ca^{2+}$ concentration and elevated levels of $Ca^{2+}$ in the mitochondria. At the same time, attempts to maintain the mitochondrial oxidative phosphorylation deplete adenosine triphosphate (ATP), which is broken down to adenosine diphosphate (ADP) and phosphate, resulting in high mitochondrial levels of phosphate. In the end, this will lead to

increased mitochondrial membrane permeability and the loss of viability (myocyte necrosis or apoptosis) and release of large molecules like creatine kinases and troponins into the blood.[8] Myocardial injury initiates cellular and extracellular processes in the reperfusion phase leading to cell death, inflammation and scar formation. Molecular, cellular and interstitial events, collectively termed LV remodeling, culminate in changes in the size, shape and function of the left ventricle (LV). The LV remodeling process thereby involves the entire LV: the injured myocardium, the surrounding border zone and the remote myocardium.

## Diagnosis and current treatment

When managing patients with STEMI, the goals are early diagnosis, the earliest possible institution of reperfusion therapy and optimal secondary prevention.[9] The diagnosis of an acute myocardial infarction is, according to internationally established criteria, based on typical symptoms in combination with ECG changes and the release of myocardial enzymes, in particular troponins.[10] The initial diagnosis of acute coronary occlusion can be made using several different techniques and methods. Classic symptoms like severe chest pain, referred pain in either arm or the jaw and/or acute onset of shortness of breath are still the best indications of coronary occlusion. Electrocardiography (ECG) in the acute setting has a fairly good sensitivity and specificity for acute myocardial ischemia (ST-elevation, and T-wave changes) and necrosis (Q-wave development). Repeated ECG should be obtained if possible. Cardiac biomarkers, preferable high-sensitivity troponin I or T, are highly sensitive for myocardial necrosis. With strong suspicion from symptoms and ECG, one should not wait to see elevated biomarkers before the initiation of reperfusion treatment. Additional evidence from cardiac imaging regarding loss of viability or newly developed regional wall motion abnormalites will support the diagnosis of myocardial injury, but wall motion abnormalities are not specific for STEMI and may be due to ongoing ischemia or an old infarction.[10]

For patients with a clinical presentation of STEMI less than 12 hours and with persistent ST-elevation or new onset left bundle-branch block, early mechanical or pharmacological reperfusion should be performed. If available within a short time, primary PCI – i.e. angioplasty and stenting without prior or concomitant fibrinolytic therapy – is considered the treatment of choice. Primary PCI decreases mortality and nonfatal re-infarctions and causes fewer haemorrhagic strokes compared with thrombolysis.[11, 12] The procedure requires transportation to hospitals equipped for acute PCI which may delay the onset of reperfusion. It is, however, always preferred if the PCI can be performed within two hours after the first medical contact.[12, 13] In patients presenting early with a large myocardium at risk (MaR), the delay should be shorter, and although not truly evidence-based a maximum of 90 min is recommended in such patients. Current guidelines stress the need for the shortest possible delay between the first medical contact and the onset of PCI, preferrably within 90 minutes or less in all patients.[9] In the absence of contraindications, the use of pre-hospital thrombolysis should always be considered if the time to admission or PCI is significantly delayed.

Activated platelets are critical to the mechanism following acute plaque rupture and thrombus formation. Specific and potent inhibition of platelet activation aims to protect the myocardium against ischemic events by maintaining the patency of the infarct-related coronary artery without significantly increasing the risk of major bleeding. Improved clinical outcomes have

been achieved by inhibiting platelet activation and aggregation.[5, 14-19] Such therapy includes antithrombotic treatment with: a bolus dose of aspirin (320-500 mg), which inhibits the release of thromboxane A2 by the thrombocyte; a bolus dose of clopidogrel (300-600 mg) inhibiting the ADP receptor-mediated platelet activation and aggregation; intravenous glycoprotein (GP) IIb/IIIa inhibitors which inhibit fibrogen binding to the receptor preventing platelet aggregation and finally intravenous unfractionated heparin or inhibitors of the thrombin platelet receptor. Recent studies show that bivalirudin combines a comparable efficacy with a lower risk of bleeding complications, compared with GP IIb/IIIa inhibitors.[20] Additional medical treatment, including beta-blockers, ACE inhibitors and aldosterone antagonists, aims at preventing early and late myocardial remodeling and decreasing the risk for arrythmias.[21-23] Lipid-lowering is also part of the standard therapy, reducing mortality and the likelihood for new coronary events by approximately 20 % per one mmol/l reduction of low density lipoprotein cholesterol.[24]

## Reperfusion injury

Even though reperfusion of an occluded coronary artery is a prerequisite for the salvage of ischemic myocardium, the restoration of coronary blood flow to the vulnerable myocytes leads to further metabolic and biomechanical injury in the first few minutes of reperfusion. This phenomenon is referred to as reperfusion injury (Figure 1). Reperfusion injury can be defined as injury (reversible or irreversible) sustained to tissue and organs after the onset of reperfusion. Experimental evidence reveals that such injury may be limited by mechanical or pharmacological therapy initiated at the onset of reperfusion.[25]

Molecular and cellular events underlying the ischemic reperfusion injury are complex, but several key pathophysiologic factors have emerged. Reperfusion leads to rapid normalisation of ion flux and pH which paradoxically enhances myocardial cytotoxicity by activating ion channels leading to the accumulation of intracellular sodium. High sodium concentrations further promote an increase in intracellular $Ca^{2+}$ concentration, which causes myocyte hypercontractility, ATP depletion and myocardial stunning.[27] Impaired endothelial function with reduced bioavailability of nitric oxide (NO) which under normal conditions has benefical and protective effects by eliciting vasodilatation, inhibiting platelet aggregation, inhibiting leukocyte adhesion and scavenging reactive oxygen species (ROS)[28-30], is of importance in the process. Paradoxically, high concentrations of NO may, in the presence of ROS, mediate additional toxicity by the formation of the highly reactive species peroxynitrite.[31] Endothelial injury increases vascular permeability and recruitment of inflammatory cells. Cellular adhesion molecules promote the invasion of inflammatory cell into the tissue through the injured endothelium. Neutrophils in particular are toxic to the myocyte by secreting proteases, generating ROS and occluding the microvasculature.

The generation of free radicals through incomplete reduction of oxygen has been well described. ROS are highly reactive and can quickly inhibit the scavenging system of the myocytes. This in turn triggers cell injury by reactions with lipids, proteins and nucleic acids. The enzymes xanthine oxidase, NADPH oxidase and NO synthase are generators of free radicals in the reperfused heart.[32] ROS triggers the opening of the myocardial permeability transition pore (mPTP). When the mPTP opens, this collapses the inner mitochondrial membrane potential, which is required to drive the oxidative phosphorylation, and this leads to further ATP depletion and cell death. [33, 34] The mPTP is a voltage- and $Ca^{2+}$-dependent channel

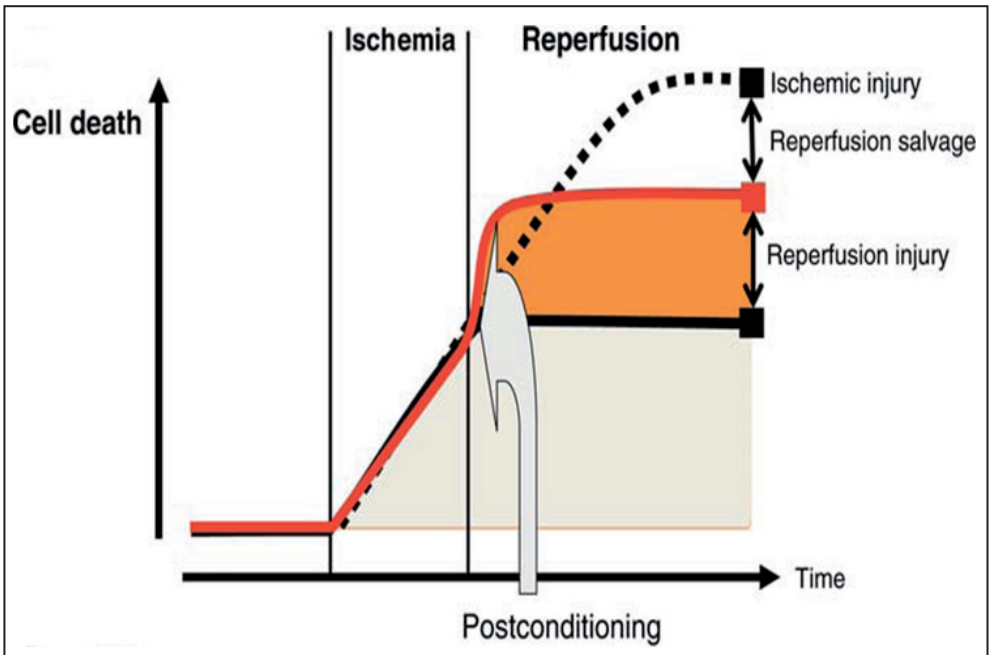


***Figure 1.*** *The concept of reperfusion injury. During ischemia, irreversible cell injury leading to cell death occurs within the ischemic risk zone in a time-dependent manner. In the absence of reperfusion, ischemic injury would progressively kill more and more cells, eventually accounting for near total cell death (broken line). Reperfusion halts the process of ischemic cell death but in its early stages imposes injury that results in further cell death, beyond that due to the ischemic period: this is lethal reperfusion injury. The net result, however, is that the reperfused tissue sustains less cell death than would occur in ischemic tissue without reperfusion. Targeting cell death due to reperfusion has the potential to maximize cell salvage. Postconditioning applied at the onset of reperfusion might limit the extent of reperfusion injury and maximizes reperfusion salvage. Adapted from Garcia-Dorado and Piper.*[26]

located in the inner membrane of the mitochondria. Its opening increases permeability to solutes with molecular masses up to 1,500 Daltons.[35]

In 1988 Cromptons et al[36] were the first to propose the possible involvement of the mPTP in reperfusion injury in the heart. It was noted to be sensitive to calcium, oxidative stress, phosophate and ADP, all present during myocardial ischemia. The same group discovered that opening of the mPTP could be inhibited by the immunosuppressant drug cyclosporin-A through the cyclophiline-D matrix protein.[37] Following this, Griffiths et al[38] discovered that the mPTP remained closed during myocardial ischemia and opened during the first few minutes of reperfusion, thereby defining a critical time-window for cardioprotection. However, the mPTP still needs to be completely characterised.

There is general agreement on the fact that the reperfusion injury occurs very early after reperfusion based on the rapid formation of ROS that damage endothelial cells,

rapid normalisation of tissue pH and the opening of the mPTP during early moments of reperfusion. Zweier et al[39, 40] showed increasing levels of free radicals in rabbit hearts within 10 seconds of the onset of reperfusion. These results demonstrated that ROS were produced in the hearts during ischemia and that free radical generation occurred within moments of reperfusion. A recent study in mice demonstrated limitation in infarct size development by ischemic postconditioning that was initiated as late as 30 minutes after the initial reperfusion, indicating that the time window for the reperfusion injury and effect of protective therapy might be longer than initially considered.[41]

## Myocardial conditioning

Since the myocyte seems fairly resistent to prolonged ischemia reperfusion, injury has been suggested as a substantial contributer to the final infarct size.[42] Experimental studies targeting mediators of reperfusion injury report sizeable reductions of the final myocardial injury, suggesting that processes induced by reperfusion may contribute to 30-50 % of the final infarct size.[43] To be useful in clinical practice, reperfusion therapy has to be easy to implement in- or outside the hospital and safe, and must not significantly delay time to reperfusion. Also, myocardial conditioning needs to be implemented in conjunction with revascularization therapy to further limit infarct size and potentially decrease future morbidity and mortality. Therapeutic strategies may include activation of endogenous protective signaling mechanisms either by local or remote ischemic or pharmacological conditioning of the myocardium. Such strategies can be initiated before the ischemic event (preconditioning), during the event (perconditioning) or after the onset of reperfusion (postconditioning).

## Ischemic conditioning

### Preconditioning

In 1986, Murry et al[44] showed that myocardial infarct size could be reduced if the myocardium was exposed to brief episodes of myocardial ischemia and reperfusion before the index ischemia, a technique they named ischemic preconditioning. Anesthetised, open-chest dogs were subjected to four cycles of five minutes of coronary artery occlusion followed by five minutes of reperfusion before the onset of 40 minutes of occlusion followed by four days of reperfusion. This procedure resulted in a significant limitation of infarct size: 25 % of that seen in the control group. Importantly, collateral blood flow did not differ between the two groups. This finding generated a massive research effort, both in vivo and in vitro, to identify cellular and molecular pathways behind the cardioprotective effect.[45] Successful preconditioning is dependent on two distinct “time windows”. Myocardial protection is activated within minutes by activating complex signalling cascades triggering the release of several mediators, including a number of survival kinases (see below). The effect of this “first time window” lasts between one and two hours. A “second time window” opens 24 hours after the induction of ischemia and lasts for 48-72 hours.[46, 47] The requirement for preconditioning limits its clinical use in patients with acute MI, but it can be utilized in different controlled situations such as in coronary artery by-pass grafting (CABG) or cardiac transplantation.[48]

**Postconditioning**

In 2003, Zhao et al[49] performed a study on anesthetized open-chest dogs in which LAD was occluded during 60 minutes and reperfused for three hours. In a preconditioned group, LAD was occluded for five minutes and reperfused for 10 minutes before the prolonged occlusion. In a second, postconditioned group, three cycles of 30 seconds reperfusion and 30 seconds of reocclusion preceded the three-hour-long reperfusion. Compared with controls, infarct size was significantly smaller in both the preconditioned and the postconditioned group. Indeed, as depicted in Figure 2, postconditioning limited infarct size by more than 40 %. Endothelial function of the postischemic LAD, assessed using the maximal vasodilator response to acetylcholine, was significantly greater in the post- and preconditioned groups.

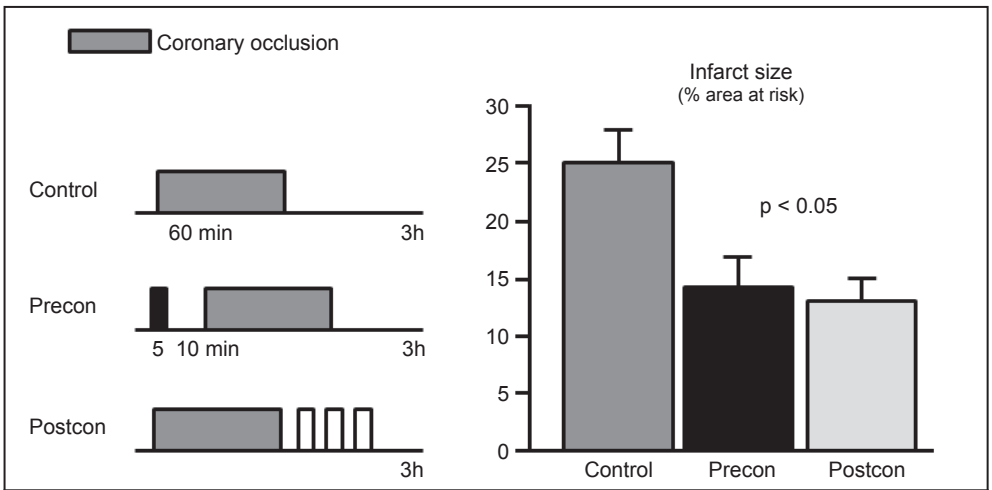


***Figure 2.*** *Infarct size limitation by pre- and postconditioning. Coronary occlusion followed by reperfusion is associated with infarct development in the ischemic risk zone (Control). Brief periods of ischemia and reperfusion performed either before index coronary occlusion (Precon=preconditioning) or immediately after index coronary occlusion (Postcon=postconditioning) can significantly limit infarct size. In animal models, such as the dog, subjected to coronary artery occlusion and reperfusion, either preconditioning or postconditioning can confer equivalent protection. Data from Zhao et al.[49]*

Similar infarct-limiting effects has been confirmed in different experimental models, for example about 50 % in canines and 25 % in rodents.[50-53] The postconditioning algorithm differed between different animal models (from 10/10 seconds in mouse and rat up to 60/60 seconds in rabbit) as did the ischemia/reperfusion time. In some studies performed in rabbits and rats, the outcome was similar in the postconditioned and control groups .[53, 54] Experimentally, the outcome of postconditioning in the presence of comorbidities (e.g. age, diabetes, hypertension and hyperlipidemia) has been variable, with some studies showing infarct limitation while others have been neutral in this respect.[55] The postconditioning algorithm has not been consistent between different animal and clinical studies. The protocols have ranged from 30 to 90 seconds of reperfusion and ischemia and the number of cycles from two to four.[56]

The advantage of postconditioning is that it can easily be implemented in clinical practice studying patients with STEMI. Postconditioning can be performed without delaying symptom-to-balloon time in connection to a primary PCI during which brief cycles of reperfusion and ischemia are instituted using the PCI balloon. In 2005, Staat et al[57] performed a proof-of-concept study of postconditioning in patients with STEMI. The patients underwent primary PCI with direct stenting, with or without postconditioning applied as one minute of reperfusion followed by one minute of ischemia repeated four times. Area under the curve (AUC) of creatine kinase release over 72 hours was significantly reduced (36 %) in the postconditioning group (Figure 3). The same group demonstrated reduction in infarct size by postconditioning using single-photon emission computed tomography (SPECT) six months after the acute event.[58] This effect was associated with improved left ventricular ejection fraction (LVEF) after one year determined by echocardiography. Laskey et al[59, 60] reported a significant decrease in ST-segment resolution and an increased coronary blood flow reserve by applying two ischemic cycles of 90 seconds separated by three – five minutes of reperfusion. All patients received intracoronary adenosine before coronary flow reserve was measured.

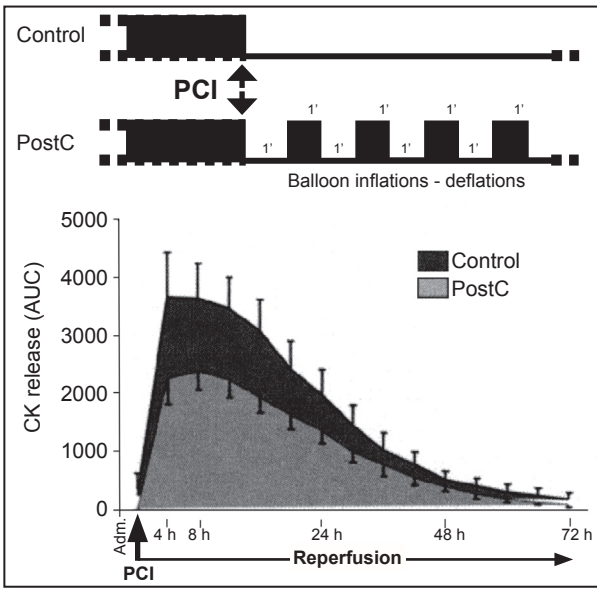


***Figure 3.*** *Top panel: Patients with STEMI treated with PCI (primary coronary intervention) were randomly assigned to receive either no further intervention (control) or four cycles of one min angioplasty balloon inflation followed by one min balloon deflation, starting less than one min after direct stenting (PostC=postconditioning). Bottom panel: Area under the curve (AUC) of creatine kinase (CK) release during the first three days of reperfusion in control vs. postconditioned. Data from Staat et al.*[57]

**Remote conditioning**

That remote conditioning, i.e. brief episodes of ischemia and reperfusion in a remote tissue, can protect the myocardial cells from the harmful effects of sustained ischemia, was first demonstrated by Przyklenk et al[59] in 1993. They demonstrated, in dogs, that brief episodes of occlusion of the left circumflex coronary artery (LCx) significantly limited infarct size following sustained occlusion of the left anterior descending artery (LAD). Following the initial discovery of remote conditioning, it was reported that brief periods of ischemia in distant organs such as skeletal muscle, kidney and intestine also induced myocardial preconditioning.[60-62] It has been postulated that some, still unknown, humoral, neural or anti-inflammatory signal is activated in the conditioned organ which then triggers the protection pathways of the heart. Remote conditioning can be effective when applied before

or during the index ischemia in line with the effect of local conditioning. In 2002, Kharbanda et al[63] demonstrated that remote preconditioning prevents ischemia-reperfusion injury in the human forearm and that it limits the extent of myocardial infarction in experimental animals, observations with important clinical potential. Protective effects in the heart by means of remote ischemia of the arm have been seen in patients undergoing CABG.[64] Li et al[65] used lower limb conditioning during replacement of rheumatic valves and reported lower levels of troponin I release. Recently, Bøtker et al[66] demonstrated a significant increase in myocardial salvage index and increased LVEF in patients with STEMI subjected to remote perconditioning. Five minutes of arm-cuff ischemia followed by reperfusion was initiated in the ambulance and repeated four times before opening the coronary occlusion by means of primary PCI.

## Pharmacological conditioning

Since the discovery that pre- and postconditioning exert substantial protection during reperfusion, a growing number of pharmacologic agents with protective effects against reperfusion injury have been identified and investigated in experimental studies.[52, 67-70]. The drug should be given before or at the time for reperfusion during the narrow window of reperfusion injury. The number of agents tested is large and includes NO donors, adenosine, bradykinin, inhalational anaesthetics, cyclosporine-A, erythropoietin, opioid agonists and endothelin.[71-82] In general, these agents activate the same cardioprotective signalling pathways. So far, clinical trials have not shown overwelming results with these compounds. Many studies have been neutral and only a few partially successful.[83, 84] Adenosine and an adenosine agonist were used in AMISTAD-I, -II (with thrombolysis) and ADMIRE (with PCI) trials.[72, 85, 86] In AMISTAD II, the patients given the higher dose of adenosine and early reperfusion (<3 hours) had significantly smaller final infarct sizes measured with SPECT. The AMIHOT II trial using supersaturated oxygen limited the size of anterior infarcts.[87] Recombinant atrial natriuretic peptide administration was used in the J-WIND trial and demonstrated small limitations in infarct size and improved LVEF after six months.[73] In the other arm of J-WIND, nicorandil was without beneficial effect. The use of cyclosporin-A (inhibits the mPTP through cyclophilin-D) as a intravenous bolus before reperfusion significantly limited infarct size measured with cardiovascular magnetic resonance (CMR) five days after reperfusion, in a pilot study by Piot et al.[76] A European multicenter trial with cyclosporin-A is presently conducted in an attempt to confirm this observation. Recently Lønborg et al[75] used exanatide (glucagon-like-peptid-1 analog) before reperfusion and reported a larger salvage index in the treatment group and a trend in absolute infarct size reduction.

Although hypothermia is not a pharmacological intervention, it is worth mentioning. The two major clinical trials (COOL-MI and ICE-IT) investigating mild hypothermia failed to show any reduction in infarct size (unpublished observations presented at the transcatheter cardiovascular therapeutics meeting,Washington 2004). A subgroup of patients who reached a temperature of <35 °C before reperfusion had a significant reduction in infarct size. In 2010, Götberg et al[88] performed a pilot study showing promising results with smaller infarct sizes in relation to MaR and a smaller degree of microvascular obstruction (MVO) in patients randomized to hypothermia.

## Potential mechanisms and pathways involved in ischemic conditioning

The current paradigm proposes that ischemic conditioning stimulates a complex network of intracellular signalling pathways, which in the end affects specific components of the mitochondria such as opening of the mitochondrial $K_{ATP}$ channel and/or inhibition of the mPTP. In 2001, Xu et al[89] suggested that mPTP may be a target for calcium-induced preconditioning protection. Subsequent studies have confirmed that mPTP inhibition is part of the process of preconditioning in several different settings and species.[90-94] Postconditioning is also believed to inhibit mPTP opening by regulating the levels of intracellular calcium, decreasing oxidative stress and increasing ATP levels, intracellular pH regulation, endothelial dysfunction and inflammation.[95] Postconditioning delays the normalisation of tissue pH by delaying re-alkalinisation of the cardiomyocytes during reperfusion, which decreases intracellular calcium levels. This inhibits the opening of mPTP and early contraction of the myocytes; a sequence supported by the finding that administration of acidotic buffer during reperfusion limits infarct size in dogs.[96] Postconditioning attenuates the generation of ROS which reduces intracellular and intra-mitochondrial calcium accumulation, inhibiting the opening of mPTP. Furthermore, postconditioning may have an anti-inflammatory effect by reducing the accumulation of neutrophils, diminishing endothelial activation and decreasing the levels of tumor necrosis factor (TNFα) and interleukin (IL-6/8).[97, 98]
Three major potential pathways will be discussed below, the reperfusion injury salvage kinases (RISK) pathway, the survivor activating factor enhancement (SAFE) pathway and the sphingosine kinase pathway (Figure 4). The details of these pathways are not fully understood and there may be substantial cross-talk between them. Several other mechanisms and pathways have been suggested as influencing prevention of ischemia-reperfusion injury, but will not be further discussed in this thesis.

### The RISK pathway

In 2002, Schulman et al[100] demonstrated that classic autacoids such as adenosine, bradykinin and opioids trigger cardioprotection through different receptor-mediated mechanisms.
Extracellular regulated kinase 1/2 (Erk1/2) and phosphatidylinositol-3-kinase (PI3K) inhibited glycogen synthase kinase-3β (GSK-3β) via phosphorylation of Akt, thus subsequently inhibiting the opening of mPTP. Via another signalling pathway, PI3K-Akt activates endothelial NO synthase (eNOS) to produce NO, which stimulates cytosolic protein kinas G (PKG) and mitochondrial protein kinas C (PKC-ε) and subsequently opens the inner mitochondrial $K_{ATP}$ channel, thus mediating cardioprotection through signalling ROS and/or mPTP inhibition.[51, 101-106] Specific receptors of the autacoids have different effects on the cardioprotection cascade when they are activated or inhibited.

### The SAFE pathway

The activation of the Survivor Activating Factor Enhancement (JAK-STAT) pathway has been proposed as an alternative cardioprotective cascade.[107] TNFα can have adaptive effects depending on its concentration and to which of its two receptors it binds. Activation of exogenous or endogenous TNFα at the time of reperfusion initiates the activation of TNF receptor-2 which phosphorylates the signal transducer and activator of transcriptin-3 (STAT-3). It is proposed that, after translocation to the nucleus, STAT-3 controls the transcription of factors that confer cardioprotection by inactivation of GSK-3β or direct inhibition of mPTP.[99, 108]

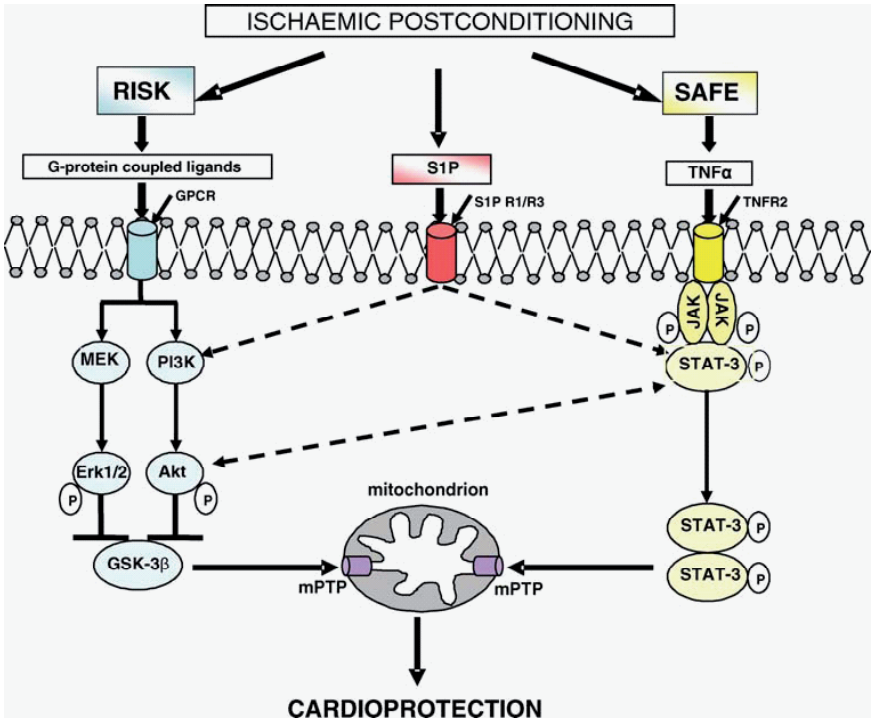


***Figure 4.*** *Schematic representation of the different pathways. The binding of tumor necrosis factor (TNF-α) to its TNF receptor-2, and the subsequent activation of signal transducer and activator of transcriptin-3 (STAT-3), confers cardioprotection via the survivor activating factor enhancement (SAFE) pathway. The SAFE pathway and the reperfusion injury salvage kinases (RISK) pathway may both confer protection through the mitochondrial permeability transition pore (mPTP). Activation of sphingosine kinase generates sphingosine-1-phosphate, which seems to recruit other components of both the RISK and SAFE pathways. Adapted from Lacerda et al.*[99]

**The sphingosine kinase pathway**

Activation of sphingosine kinase generates sphingosine-1-phosphate which seems to recruit other components of the RISK pathway.[109, 110] On the other hand, sphingosine is a downstream mediator of TNFα and also activates STAT-3 for inhibition of the mPTP.[111] The cross-talk between these pathways is probably substantial and not fully understood.

## Quantification of myocardial salvage

As mentioned above, the clinical outcome after an acute myocardial infarction depends on several factors including the duration of ischemia, the extent of collateral blood flow, preinfarction angina and size of the initial MaR.[112] An accurate quantification of MaR and infarct size is a prerequisite when evaluating treatment efficacy in studies aimed at limiting reperfusion injury. [113] Both the extent of the myocardium made ischemic by the coronary

artery occlusion and the amount of finally damaged myocardium, the infarct, are crucial when expressing the relationship between these two parameters and thereby the proportion of salvaged myocardial tissue. As depicted in Figure 5, myocardial salvage is defined as the jeopardized MaR minus the actual infarct size. Relating these two entities to one another enhances the possibility to identify small cardioprotective effects and to compare large and small infarctions within the same study population, thereby permitting smaller sample sizes while maintaining enough power for the investigation.[114] The following section will describe the techniques used for determination of MaR and infarct size in this thesis.

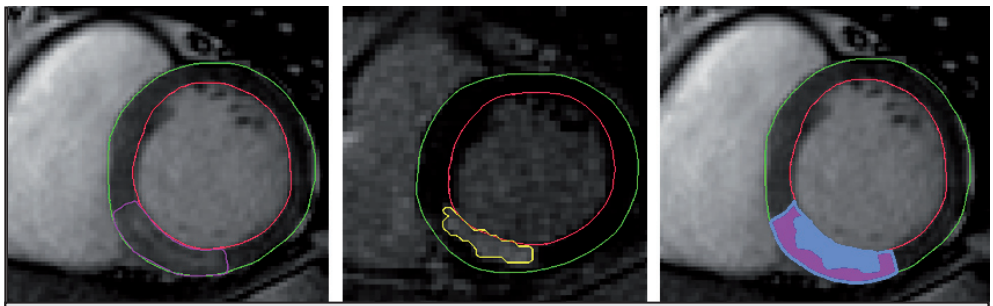

***Figure 5.*** *Myocardial salvage. Corresponding left ventricular short axis views from a patient with inferior STEMI. Myocardium at risk (MaR) determined by contrast enhanced steady-state free precession (CE-SSFP) at end-diastole (left), infarct size image (middle). The infarcted area is superimposed on MaR (right) and the pink area is the myocardial salvage.*

## Cardiac imaging techniques

### Left ventriculography

Abnormal wall motion can be assessed by left ventriculography allowing highly reproducible measurements of LVEF and the absolute LV volume. It is routinely used in the catheter laboratory when determining left ventricular function and in grading valvular insufficiency. Left ventriculography can be performed just prior to revascularization of STEMI patients with very little time delay in order to determine MaR. Field et al[115] first described the method for estimation of the extent of circumferential wall motion abnormality. Other angiographic techniques for determination of MaR are the Bypass Angioplasty Revascularization Investigation (BARI) and the Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease (APPROACH) scores.[116] The BARI and APPROACH scores are calculated by grading all ending arteries. The MaR is then calculated as a percentage of the LV by dividing summed scores of a jeopardized area by the total score of the entire LV. The APPROACH score, which takes coronary dominance into account, has been validated against CMR[117]. Limitations with left ventriculography include the exposure to excessive amounts of contrast which can cause kidney failure, allergic reactions and the use of additional ionizing radiation.

### Myocardial perfusion SPECT

Noninvasive radionuclide cardiac imaging started in the 1970s. Since then, there have been major advances in the ability to image cardiac physiology and pathophysiology, including that of myocardial blod flow, metabolism and ventricular function.[118]

Myocardial perfusion SPECT studies perfusion of the myocardium by means of radioactive tracers and tomographic imaging of the heart with a gamma camera. Modern tracers like technetium ($^{99m}$Tc) labeled sestamibi and tetrofosmin have better distribution patterns than the previously used thallium, thanks to to a high extraction rate from blood, no re-distribution to the blood, a relatively high uptake into the cardiomyocytes and a moderate half-time decay. This makes it possible to inject the isotope before opening the occluded coronary artery and the implication is that MaR can be determined during a time window of four to six hours following reperfusion. After acquisition, the data is reconstructed to enable assessment of LV volumes, myocardial perfusion, MaR and, if ECG-gated, myocardial function. Myocardial SPECT is considered to be the reference standard for determining MaR. This technique was until recently also the reference standard for determining myocardial infarct size.[119, 120] During the last two decades, multiple studies have validated the clinical use of SPECT for measurement of infarct size and MaR.[121-124] SPECT, which has few contraindications, has been used in several multicenter studies using infarct size estimation as an end point.[72, 85-87, 125]

Problems related to SPECT are the limiting image resolution (subendocardial necrosis can be missed), problems with attenuation artifacts, the use of high doses of ionizing radiation and the short half-life of the nuclear tracers making the technique impractical in an acute setting.

**Cardiac magnetic resonance imaging**

The use of CMR, which is a non-invasive and non-ionizing technique, has grown considerably in recent years and is now firmly established in clinical practice and research. CMR is reliable, relatively easy to use, produces superb image quality and has excellent reproducibility which makes it well suited for studies of cardioprotection.

**Basic physics**

Hydrogen is present everywhere in the human body in the form of water and has good sensitivity to magnetic resonance, which is the basis when producing images with a high signal-to-noise ratio. The hydrogen nuclei behave like small magnets aligning to an external magnetic field. When exposed to a field of 1.5 Tesla, the nuclei precess at a resonance frequency of 63 MHz, which is within the radiowave range. A radiowave pulse can excite a region of the body at this frequency causing the hydrogen nuclei to rotate away from the direction of the main magnetic field in a coordinated way, thereby causing another small magnetisation field. After the pulse is switched off, the new small magnetisation field decays to its former position and energy is transmitted as a radio signal. This signal is transformed into a radiowave echo and can be formed into an image using a receiver antenna and computer processing. The contrast between different tissues in the image depends on the delay between excitation, the read-out signal (echo time, TE) and the time between repetitive radiowave excitations (repetition time, TR).

CMR uses complex sequences to characterise different tissues based on specific nuclear properties including T1 and T2. The longitudinal relaxation, commenly referred to as T1 relaxation, is responsible for the recovery of the protons to its original magnetization value in equilibrium. T1 is a rate constant defined as the time it takes for the hydrogen nuclei to regain 63 % of the original magnetisation. T1-weighted images can separate tissues if they differ in intrinsic or pathological T1. Such images are commonly used to visualize the tissue

distribution of gadolinium contrast. The gadolinium-based contrast agent is paramagnetic and increases tissue contrast by shortening T1-relaxation in tissue containing gadolinium. It distributes into the extracellular space and the concentration is proportional to the relative amount of extracellular space.[126] In acute myocardial infarctions, the extracellular space is increased due to ruptured myocytes and edema. The transverse relaxation, commonly referred to as T2, describes how long protons remain in-phase after being flipped perpendicular to the main magnetic field. T2 is a rate constant defined as the time it takes for the hydrogen nuclei to decrease to 37 % of the initial value. In general T2-weighted images show fluids with bright signal intensity while solid tissues, like the myocardium. have an intermediate intensity. When imaged with T2-weighted sequences the MaR, because of its higher water content, appears slightly brighter than the remote myocardium.

The major components of a modern CMR system consist of hard- and software: a superconducted magnet that produces a highly homogeneous and stable static magnetic field; the gradient amplifiers and coils within the bore of the magnet that generates the excitation pulses; and a radiofrequency antenna that receives the signal from the patient. An advanced computer controls all these components and performs a Fourier transformation of the radio signal to generate the final images.

**CMR sequences**

There is a large variety of CMR sequences. The majority of imaging is performed using two basic sequences known as spin- and gradient-echo. Spin-echo sequences are usually referred to as "black-blood" and gradient sequences as "bright-blood". Spin-echo sequences are routinely used for anatomical imaging and gradient-echo sequences are for functional assessment through cine acquisitions. The most commonly used prepulse is inversion recovery (IR), which gives a strong T1-weighting and is valuable when imaging infarcted tissue. CMR has high spatial resolution to discriminate subendocardial from transmural fibrosis and allows detection of micro-infarctions.[127] CMR has a higher sensitivity for MI than SPECT and PET[128] and can better differentiate viable from nonviable myocardium.[129] The correlation between late gadolinium enhancement (LGE) and fibrosis has been extensively validated and is currently the standard reference for quantification of MI size. [129-132] LGE imaging has been validated in multicenter clinical trials.[133]

Different CMR sequences have been applied to depict MaR and there is still no general consensus on which one to use. Several sequences characterise MaR by determining the myocardial edema retrospectively within two to seven days after the index ischemia.[134-138] MaR using T2-weighted sequences are the most extensively studied. Higgins et al were the first to quantify myocardial T2 in acute MI in dogs in 1983.[139] They reported changes in both T2 and T1 in infarcted myocardium. Two decades later, it was shown that different T2-weighted images could depict MaR several days after the ischemic event and also differentiated acute from chronic myocardial damage. [134-138] In 2009, Carlsson et al[140] validated the T2-weighted sequence against SPECT and the method was subsequently used to demonstrate the rate of infarct evolution in man, without confounders.[141] More recently, the possibility of quantifying myocardial T2 and T1 in normal and ischemic myocardium has been demonstrated. This may solve the problems related to subtle differences in signal intensity between normal and edematous myocardium.[142, 143] Another CMR method to estimate MaR is the "endocardial surface area" which relies on the wave front of myocardial injury that occurs in acute MI.[117]

MaR is estimated from the endocardial extent of the infarction and calculated in relation to the whole left ventricle endocardial surface. Ubachs et al recently reported that the endocardial extent of LGE underestimates the MaR and consequently myocardial salvage.[144]

The limitations of CMR in cardioprotective studies have to be recognised. While LGE is accepted as the reference for the quantification of the final infarct size, the experience with different sequences regarding MaR is considerably smaller. Other limitations include problems with artifacts, sequences that are often vendor-specific and not available for all CMR centers and the lack of extensive validation of some sequences. Claustrophobia, high cost and pacemakers/defibrillators not compatible with CMR may also limit the use of CMR.

# AIMS

The overall aim of this thesis was to investigate the effects of postconditioning on infarct size and to improve the methodology for the quantification of myocardial protection in patients with STEMI.

The specific aims were to:

**I.** Evaluate the short-term effect of postconditioning on infarct size in patients with STEMI

**II.** Evaluate the long-term effect of postconditioning in patients with STEMI

**III**. Develop and validate a method for the quantification of MaR using contrast enhanced CMR one week after the infarction

**IV.** Explore the relationship between contrast enhanced CMR and T2-weighted edema imaging for the quantification of MaR

# MATERIAL AND METHODS

The protocols and procedures were approved by the local ethic committees for human research at Karolinska Institutet and the University of Lund, Sweden. The studies were performed according to the declaration of Helsinki and good clinical practice.[145] Written informed consent was given by all patients.

## Study populations

### Study I and II

Between April 2007 and March 2009, a total of 795 patients were referred to the coronary care unit of Karolinska University Hospital, Solna, for a primary PCI due to STEMI. As can be seen in Figure 6, 89 patients were randomized and 76 completed the study protocol in study I and 68 patients completed the long-term follow-up in study II.

Inclusion criteria were chest pain ≥30 min and ≤6 h in combination with ST-segment elevation ≥0.1 mV (≥0.2 mV in V1-V3) in two contiguous ECG leads or left bundle branch block and a thrombolysis in myocardial infarction (TIMI) grade 0 flow in the infarct-related artery.[146] Exclusion criteria were previous MI, previous CABG, cardiogenic shock, cardiac arrest, renal impairment (serum creatinine >150 mmol/l), ongoing treatment with metformin, contraindication for CMR, persistent atrial fibrillation and any condition that was considered to interfere with the possibility for the patient to complete the study protocol.

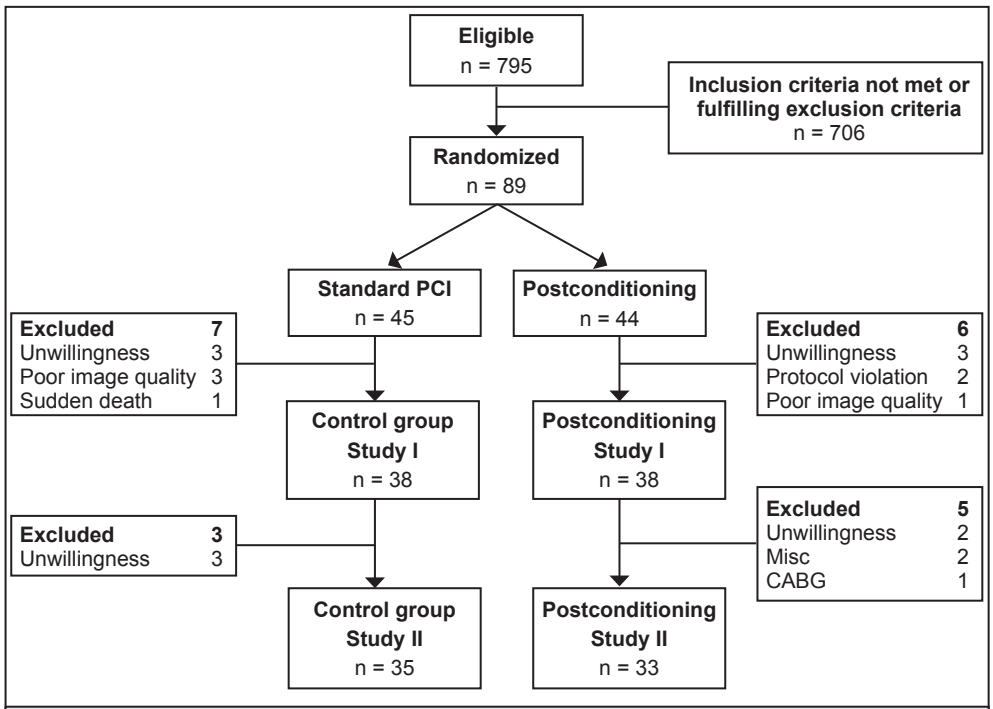


***Figure 6.*** *Flow chart of patient recruitment in Studies I and II.*

The main reason for loss to follow-up in Studies I and II was unwillingness to complete the protocol and poor image quality. One patient in the control group died suddenly. Recurrent MI did not occur in any of the two groups during follow-up.

**Study III**
Study III comprised 16 consecutive patients (age: 64 ± 8 years; 12 males) with a first-time STEMI fulfilling the inclusion and exclusion criteria as described for Studies I and II. They were admitted for primary PCI during the period February 2007 to December 2008 at times when myocardial perfusion scintigraphy was accessible. Twelve patients were included at the Karolinska University Hospital Solna and four at the Lund University Hospital. The culprit lesion was located in the right coronary artery (RCA) in 13 patients, the LAD in two and the LCx in one. There were no severe adverse events, including reinfarctions, prior to the CMR investigation.

**Study IV**
Twenty-one patients (age 59 ± 10 years; 17 males) presenting at Lund University Hospital with a first-time acute STEMI, due to an occluded coronary artery confirmed with angiography, were prospectively included in study IV. All patients were treated with primary PCI with coronary stenting, resulting in TIMI grade 3 flow in the culprit artery. The RCA was the culprit vessel in 12 patients and the LAD in six patients. Furthermore, two patients presented with an occlusion of the LCx artery and one patient had a left main occlusion.

## Study design

**Studies I and II**
The study was conducted as a prospective randomized open study with blinded evaluation. All patients received aspirin, clopidogrel and enoxaparin. A GPIIb/IIIa inhibitor was given according to the choice of the PCI operator. A coronary angiography was performed to confirm TIMI 0 flow in the infarct-related artery. In order to determine MaR, a biplane left ventriculography was performed before revascularization.
Following these procedures, the patients were randomized to primary PCI only or PCI combined with postconditioning performed by reinflating the balloon at the same location to a pressure of 2-4 atmospheres for 60 seconds, starting 60 seconds after the initial reperfusion (Figure 7). Postconditioning was performed with the same balloon catheter as the one used for the initial inflation. The PCI intervention was completed through a coronary angiogram to determine the final TIMI flow. Collateral flow to the infarct zone was assessed on the initial angiogram before PCI and graded on a scale of zero to three.[147]
Blood sampling was performed at admission and every four hours during the first 24 hours and then every six hours until 48 hours after reperfusion. Both peak values and AUC of cardiac biomarkers were determined. A standard CMR was scheduled one week after the onset of symptoms (Study I) as well as at three and 12 months (Study II) for determination of cardiac volumes, infarct size and microvascular obstruction.

**Study III**
The patients were consecutively recruited for Study III received an intravenous injection of $^{99\,m}$Tc-labeled tetrofosmin or sestamibi prior to opening of the occluded vessel. All patients

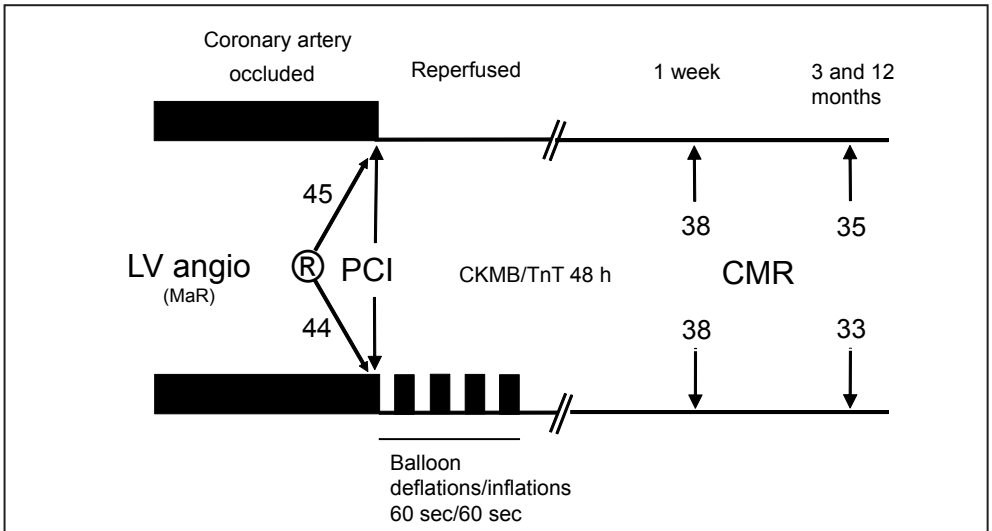


***Figure 7.*** *The protocol used in Studies I and II. Patients with STEMI were randomized either to a control group (n=45) with standard PCI (primary coronary intervention) or to an intervention group (n=44) in which the PCI was followed by postconditioning in four cycles of reperfusion for 60 seconds followed by ischemia for 60 seconds. Cardiac biomarkers were measured for 48 hours after admission. Cardiovascular magnetic resonance (CMR) was scheduled at one week (n=38/38), three and 12 months (n=35/33) after the PCI. Left ventricular (LV) angiography for estimation of myocardium at risk (MaR) was performed prior to PCI. TnT (Troponin T).*

received oral antiplatelet therapy with aspirin, clopidogrel and intravenous infusion of a GPIIb/IIIa inhibitor in connection with the PCI procedure. Myocardial perfusion SPECT imaging was performed within four hours, to visualize and quantify MaR. A CMR examination was performed one week after the onset of symptoms.

**Study IV**

The patients treated with primary PCI due to STEMI were consecutively recruited and enrolled at Lund University Hospital. All patients received oral antiplatelet therapy with aspirin, clopidogrel and intravenous infusion of a GPIIb/IIIa inhibitor in connection with the PCI procedure. A CMR examination was performed one week after the onset of symptoms.

## Methods

**Angiographic determination of MaR (Studies I and II)**

MaR was quantified by measuring the circumferential extent of abnormally contracting segments. Left ventriculography (30º right anterior oblique, 60º left anterior oblique) was performed immediately before coronary angioplasty during infusion of 40 ml of iodine contrast. An x-ray digital imaging software system (Integris HM 3000, Philips, The Netherlands) was used for determining the global and regional LV function. Global ejection

fraction was calculated using the area-length method. Centerline chord motion analysis was used to quantitatively assess regional LV function. In this technique, endocardial motion is measured along 100 chords constructed perpendicular to a centerline. The motion of each chord was normalized by the end-diastolic circumference to yield a fractional shortening. This value was converted into units of SD from the normal mean motion of each chord as derived from a normal reference population. Wall motion and abnormally contracting segments were analyzed in the distribution territory of the LAD (10 to 66 chords) in patients with anterior infarctions, and in the distribution territory of the RCA or LCx (51 to 80 chords) in patients with inferior infarctions. Abnormal wall motion extent was defined as the number of chords displaying hypokinetic motion (<1 SD) expressed as a percentage of the LV.[115, 148] These measurements were performed by two experienced investigators unaware of the group to which each patient belonged.

**Cardiovascular Magnetic Resonance (Studies I-IV)**

A standard clinical CMR protocol, except for the time of administration of contrast, was scheduled to be performed one week, three and 12 months after the onset of symptoms. Timing was chosen at one week to avoid the early infarct phase during which a rapid decrease in infarct size has been reported.[149] Two different 1.5 T CMR systems (Signa Excite Twin-Speed, General Electric Healthcare, Waukesha, WI, USA or Philips Intera CV, Best, The Nederlands) were used in Studies III and IV. In Study I and II only the GE camera was used. Eight- and five-channel cardiac-coil was used and all patients were in the supine position with vector-ECG monitoring. A bolus of gadolinium contrast agent 0.2 mmol/kg bodyweight (Omniscan, GE Healthcare, Norway or Magnevist, Bayer Pharma, Berlin, Germany) was given intravenously just before positioning the patient in the scanner. The image protocol included scout images, localization of the short axis and then covering the whole LV with retrospectively gated steady-state free precession (SSFP) cines referred to as contrast-enhanced (CE-SSFP) images in Studies III and IV. The following typical parameters on GE-scanner was used; SSFP (TE 1.58 ms, TR 3.61 ms, flip angle 60 degrees, 25 phases, 8 mm slice, no gap, matrix 226 × 226). LGE images were acquired 15-20 minutes after contrast injection using an inversion recovery gradient echo sequence (TE 3.3 ms, TR 7.0 ms, TI 180-250 ms to null the myocardium, 8 mm slice, no gap, matrix 256 × 192) and the same slice orientation as cine SSFP images. Typical parameters on the Philips scanner were; SSFP (TE 1.4 ms, TR 2.8 ms, flip angle 60º, 30 phases, 8 mm slice, matrix 160 × 141). LGE images were acquired 15-20 minutes after contrast injection using inversion recovery gradient echo sequence (TE 1.14 ms, TR, 3.8 ms, TI 180-250 ms, 8 mm slice, no gap, matrix 240 × 180). Cardiac triggering was set for diastole to reduce motion artifacts. Each slice was obtained during end-expiratory breath holding. Two-, three- and four-chamber views were also obtained to confirm the findings. An additional T2-weighted triple inversion turbo spin-echo sequence (T2-STIR) was added in study IV before contrast bolus was given to depict the MaR. Imaging parameters for the T2-weighted sequence were: echo time, 100 ms; repetition time, 2 heart beats; number of averages, 2; inversion time, 180 ms; image resolution, 1.5 x 1.5 x 8 mm; slice gap, 0 mm. No parallel imaging was performed to minimize signal inhomogeneities, due to differences in coil sensitivity.

*Image analysis*

All CMR images were analyzed off-line using freely available segmentation software (Segment v1.7 – v1.8 http://segment.heiberg.se/).[150] In the short-axis stack end-diastolic and

end-systolic volumes were measured in the phase with the largest and smallest LV volumes respectively. LVEF, stroke volume and LV mass were calculated on cine SSFP sequences using manual delineation of the endocardial and epicardial borders, including papillary muscles and trabeculations when contiguous with the left ventricle. For correct LV volumes and mass estimations the basal slices were examined in different cine projections. LV mass was calculated by multiplying the myocardial volume by the density of myocardial tissue (1.05 g/ml). All volumes were indexed to body surface area.

The MaR derived from T2-weighted imaging was assessed by tracing endocardial and epicardial borders of the LV in all short-axis slices, followed by manual delineation of the hyperintense regions. The papillary muscles were excluded from the myocardium. The MaR was then defined as the total amount of hyperintense myocardium in all short-axis slices and expressed as a percentage of LVmass. If present, hypointense myocardium within the area of increased signal intensity (microvascular obstruction) was included in the MaR. The contrast ratio for the T2-weighted images was determined for each patient as the mean signal intensity in the MaR divided by the mean signal intensity in remote myocardium. The MaR derived from CE-SSFP was also assessed by tracing endocardial and epicardial borders of the left ventricle in all short-axis slices in end-diastole and end-systole, followed by manual delineation of the hyperintense regions in both end-diastole and end-systole, by two observers blinded to LGE images. The values of MaR in end-diastole and end-systole were averaged and expressed as a percentage of the LV mass. The contrast ratio for the CE-SSFP images was determined for each patient as the mean signal intensity in the MaR divided by the mean signal intensity in remote myocardium.

The infarcted myocardium was automatically quantified from the short-axis LGE images. The endocardial and epicardial borders were traced manually with exclusion of the papillary muscles. The LGE myocardium was then defined using a computer algorithm that takes into consideration partial volume effects within the infarcted region.[151] Manual adjustments were made when the computer algorithm was obviously wrong. If present, a hypointense signal within the area of LGE (microvascular obstruction) was included in the analysis as 100 % infarction. Myocardial infarct size was expressed both as a percentage of the LV and as a percentage of MaR. The myocardial salvage index was defined as 100 x ([MaR – infarct size]/MaR), where MaR was assessed using both T2-weighted imaging and CE-SSFP.

In study II, change in LV sphericity index was used for detection of cavity remodeling.[152] The major axis was manually measured in an end-diastolic four-chamber view starting at the mitral annulus and ending at the apical endocardial border. The radius was used for calculating the sphere volume. End-diastolic LV volume was divided by the sphere volume creating a sphericity index for every patient.

**SPECT (Study III)**

Myocardial perfusion SPECT was used as reference standard for quantifying MaR. Prior to opening of the occluded vessel, the patients received a body weight-adjusted (350-700 MBq) intravenous injection of $^{99\,m}$Tc-labeled tetrofosmin (Amersham Health, Buckinghamshire, UK) or sestamibi (MIBI, Cardio-lite, Bristol Myers Squibb, USA). Myocardial perfusion SPECT imaging was performed within four hours to visualize and quantify MaR using either of two dual-head cameras: GE camera (Ventri, GE Healthcare, USA) or Sopha camera

(DSTXL; Sopha Medical Vision, Bue Cedex, France). The patients were placed in the supine position and imaged in steps of 5.6 degrees using a 64 × 64 matrix, with a typical pixel size of 5 × 5 mm and a slice thickness of 5 mm. The reconstructed voxel size was 3 × 3 × 3 mm (Sopha) or 6.4 × 6.4 × 6.4 mm (GE). Image acquisition time was approximately 15 minutes. Iterative reconstruction using maximum likelihood expectation maximization was performed with a low-resolution Butterworth filter and a cut-off frequency set to 0.5 of Nyquist and an order of 5.0. No attenuation or scatter correction was applied and short-axis images were reconstructed semi-automatically on the respective workstation for each camera.

*Image analysis*
Analysis of myocardial perfusion SPECT defect for MaR was performed off-line using the program mentioned above. The automatic segmentation finds the centerline through the LV wall and identifies the endo- and epicardium based on individually estimated wall thickness and signal intensity values within the image.[153] Manual adjustment of the automatic delineation was sometimes required in the left ventricular outflow region and basalt slices. The perfusion defect was determined using an automated algorithm that considers myocardium with <55% of normal as being ischemic.[154] MaR was quantified as percentage of the LV mass.

**Blood analysis**
In Study I, Troponin T was analyzed with an immunoassay (Modular Analytics E-module, Roche Diagnostics) and CKMB (creatine kinase, myocardial bound) with a chemiluminescence technique (UniCel DxI 800, Beckman Coulter AB). Sampling was performed at admission and every four hours during the first 24 hours after reperfusion and then every six hours until 48 hours. Both peak values and AUC were determined.

## Statistics

The primary endpoint of Study I was infarct size after one week, expressed as percentage of MaR. Secondary endpoints were global left ventricular function and release of cardiac biomarkers during the 48 hours following the coronary intervention. Based on an expected reduction in infarct size of 20 % and SD of 30 %, 36 patients were needed in each group to achieve $p<0.05$ with a power of 80 % and a two-tailed test. To compensate for patient dropout, a total number of 90 patients was planned to be recruited. Computer-generated randomization in blocks of eight was performed following stratification for LAD and non-LAD occlusions. All data were presented as medians and 25th, 75th percentiles (Studies I and II). The Mann-Whitney U test was used to test for differences in infarct size and cardiac biomarkers between groups. Fisher's exact test was used to test for differences between dichotomized variables. Linear regression was used for comparing infarct size in relation to the MaR between groups, and regression analysis was performed with four residuals looking at the best equation.

In Study II the same statistical methods were used for comparing groups at three and 12 months, in addition to Wilcoxon sign rank test with Bonferroni correction for multiple testing, used for longitudinal follow-up on CMR measurements. In Studies III and IV, data were expressed as mean±SD. Wilcoxon Mann-Whitney Rank Sum test was used to test for differences between groups (SPECT and CE-SSFP). Wilcoxon Sign Rank test was used to test the relative signal enhancement in contrast-enhanced regions on CE-SSFP images compared

with remote myocardium. Pearson's correlation was used to determine the relationship between T2-weighted imaging and CE-SSFP with regard to both MaR and the myocardial salvage index. Bland-Altman plots were constructed for comparing intra- and interobserver variability and calculated as the SD of the difference between two calculations divided by the average of the two observers. Statistical analysis was performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, California, USA) and SPSS version 17.0 software package (Chicago, Illinois, USA).

# RESULTS

## Study I: Short-term effects of postconditioning in patients with STEMI

Clinical characteristics and angiographic details from the patients are shown in Tables 1 and 2. As can be seen, the two study groups were well balanced.

***Table 1.*** *Patient Characteristics in the Control and Postconditioning groups*

| Variables | Control Group | Postconditioning Group | p |
|---|---|---|---|
| *Risk factors* | | | |
| Age, years (range) | 62 (42-85) | 63 (37-87) | 0.52 |
| Male sex, n (%) | 34 (89) | 31 (82) | 0.52 |
| Body mass index, kg/m2 | 27 (25, 29) | 27 (25, 31) | 0.56 |
| Ischemia time, minutes | 185 (141, 259) | 165 (137, 223) | 0.40 |
| Current smokers, n (%) | 11 (29) | 10 (26) | 1.0 |
| Dyslipidemia*, n (%) | 21(62) | 27(77) | 0.80 |
| Hypertension, n (%) | 11(29) | 6(16) | 0.27 |
| Previous angina, n (%) | 3 (8) | 6 (16) | 0.49 |
| Diabetes**, n (%) | 12 (36) | 17 (47) | 0.47 |
| *Treatment on admission* | | | |
| aspirin, n (%) | 3 (8) | 2 (5) | 1.0 |
| beta-blockers, n (%) | 4 (11) | 1 (3) | 0.36 |
| ACE/ARB, n (%) | 5 (13) | 2 (5) | 0.43 |
| statins, n (%) | 3 (8) | 3 (8) | 1.0 |
| *Treatment during angioplasty* | | | |
| opioids, n (%) | 29 (76) | 31 (82) | 0.78 |
| aspirin, n (%) | 38 (100) | 36 (95) | 0.49 |
| clopidogrel, n (%) | 37 (97) | 36 (95) | 1.0 |
| glycoprotein inhibitors, n (%) | 30 (79) | 30 (79) | 1.0 |
| *Treatment at discharge* | | | |
| aspirin, n (%) | 38 (100) | 37 (97) | 1.0 |
| clopidogrel, n (%) | 38 (100) | 38 (100) | 1.0 |
| beta-blockers, n (%) | 37 (97) | 38 (100) | 1.0 |
| ACE/ARB, n (%) | 20 (53) | 23 (61) | 0.64 |
| statins, n (%) | 37 (97) | 37 (97) | 1.0 |

Data are presented as median and quartiles for continuous variables except age which is median and range, or number of patients and percentage for dichotomous variables.
ACE = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker.
*n=34 (control), 35 (postconditioning), **n=33 (control), 36 (postconditioning)

***Table 2.*** *Angiographic Data*

| Variable | Control Group | Postconditioning Group | p |
|---|---|---|---|
| *Infarct related artery* | | | |
| LAD, n (%) | 14 (37) | 14 (37) | 1.0 |
| LCx, (%) | 1 (3) | 4 (11) | 0.36 |
| RCA, n (%) | 23 (61) | 20 (53) | 0.64 |
| Collateral flow grade 2 or 3, n (%) | 8 (21) | 6 (16) | 0.77 |
| *Number of vessels* | | | |
| one-vessel disease | 25 (66) | 23 (61) | 0.81 |
| two-vessel disease | 11 (29) | 11 (29) | 1.0 |
| three-vessel disease | 2 (5) | 4 (11) | 0.67 |
| Abnormally contracting segments (%) | 23 (15, 34) | 30 (19, 40) | 0.19 |
| Direct stenting, n (%) | 2 (5) | 0 (0) | 0.49 |
| Bare metal stent, n (%) | 38 (100) | 37 (97) | 1.0 |
| TIMI flow grade 3 after PCI, n (%) | 35 (92) | 34 (89) | 1.0 |

Data are presented as median and quartiles for continuous variables except age which is median and range, or number of patients and percentage for dichotomous variables.
LAD = left anterior descending coronary artery; RCA = right coronary artery; LCx = left circumflex coronary artery; TIMI = Thrombolysis In Myocardial Infarction; PCI = percutaneous coronary intervention.

**Infarct size and left ventricular ejection fraction**

The postconditioning protocol with inflation and deflation of the PCI balloon was well tolerated in all patients and no technical problems occurred during the procedure. Infarct size, presented as a percentage of MaR (Figure 8), did not differ significantly between the two groups: control patients 44 % (30, 56) and postconditioning patients 47 % (23, 63). In the multiple regression analysis, infarct size was significantly related to postconditioning and MaR (p=0.001) but not to age, sex, ischemic time or smoking habits. The regression analysis in which the final infarct size was related to MaR showed a significant difference in slope of the regression lines between the postconditioning group and the control group (Figure 9). A detailed analysis was therefore performed in patients with MaR in the upper quartile. Final infarct size in patients in this subgroup (n=19; Figure 10) was 54 % (50, 68) among those belonging to the control group and 33 % (21, 57) in postconditioned patients (p=0.03). There were no significant differences between patients belonging to the lower quartiles.

Median LVEF after one week did not differ between the control and postconditioned patients: controls = 50 % (40, 55) and postconditioned = 50 % (41, 54). In patients with large MaR, there was a significant difference in the slopes of the regression lines, with a higher LVEF in patients in the postconditioning compared with those in the control group (Figure 11).

Intra- and interobserver variation between two blinded readers for infarct size measurement (n=20) was 0.0±3.5 % and 0.7±1.1 % (difference and SD), respectively.

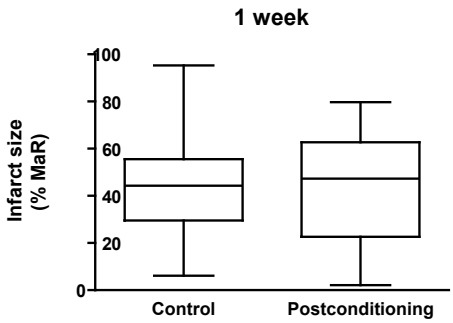


***Figure 8.*** *Infarct size in relation to the myocardium at risk (MaR) one week after admission. Infarct size did not differ significantly between patients in the control and postconditioning groups.*

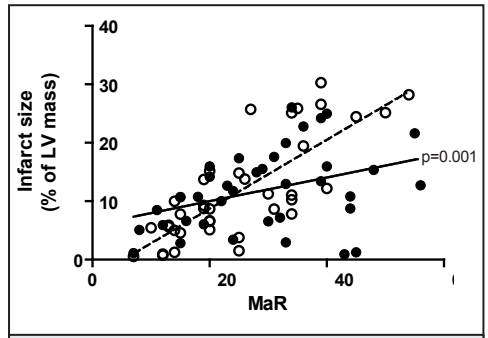


***Figure 9.*** *Infarct size plotted against myocardium at risk (MaR) for the overall study population. There are significant differences between the slopes of the regression lines between patients in the control (○) and postconditioning (●) groups. LV, left ventricular.*

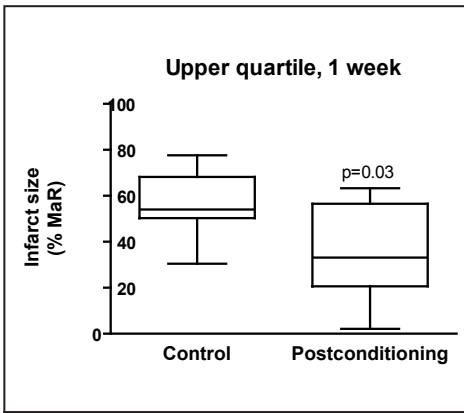


***Figure 10.*** *Infarct size in patients with myocardium at risk (MaR) in the upper quartile one week after admission. A significantly smaller infarct size can be seen in the postconditioning group.*

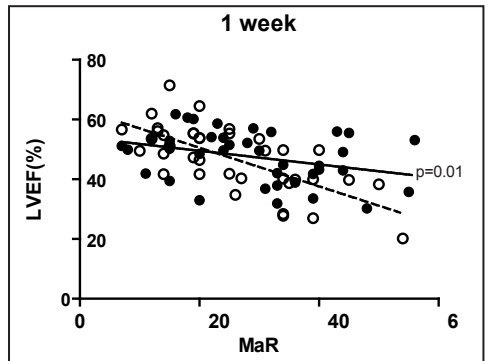


***Figure 11.*** *Left ventricular ejection fraction (LVEF) determined suing CMR after one week and plotted against myocardium at risk (MaR). There is a significant difference between the slopes of the regression line between patients in the control (○) and postconditioning (●) groups.*

**Cardiac biomarkers**

In the overall study population there were no differences troponin T and CKMB (AUC or peak values) between the control and postconditioning groups. AUC for troponin T was 147 (80, 269) and 165 (95, 279) in the control and postconditioning groups while AUC for CKMB was 3890 (2388, 6264) and 4175 (2406, 6060) respectively. The regression analysis in which troponin T was related to the MaR showed a trend favoring the postconditioning group (p=0.09). There was no difference in troponin T release between the groups when comparing the upper quartile of MaR. When AUC for CKMB and troponin T was compared with both MaR and infarct size measured with CMR, a stronger correlation was seen with infarct size than with MaR (data not shown).

## Study II: Long-term follow-up of postconditioning in patients with STEMI

### Infarct size and LVEF

Median infarct size, expressed as a percentage of MaR, at three and 12 months did not differ between the control and postconditioning groups for the entire study population (Figure 12). The slope of the regression lines for the final infarct size in relation to MaR did, however, differ significantly between the two groups, an observation that was consistent over time (Figure 13). In the upper quartile of MaR (n=17) patients randomized to postconditioning had significantly smaller infarct sizes than the control patients at 12 months and a trend in the same direction was seen at three months (Figure 14). Median LVEF for the whole study population did not differ between the control and postconditioning groups. The slope of the regression lines describing LVEF in relation to MaR differed significantly between the two groups at 12 months. In the group of patients in the upper quartile of MaR, LVEF was significantly higher in the postconditioning group than in the control group both at three and 12 months (Figure 15). Adverse LV remodeling (defined as a consistent increase in ESV >15 %) occurred in nine patients equally distributed between the two groups. End-diastole LV sphericity index for the entire study population did not differ between or within groups over time.

The intra- and interobserver variation between two blinded readers for infarct size measurement (n=18) was 0.2±1.0 % and 0.1±1.3 % and for LV mass (n=12) was -0.8±7.0 g and -0.4±6.8 g (mean difference ± SD), respectively.

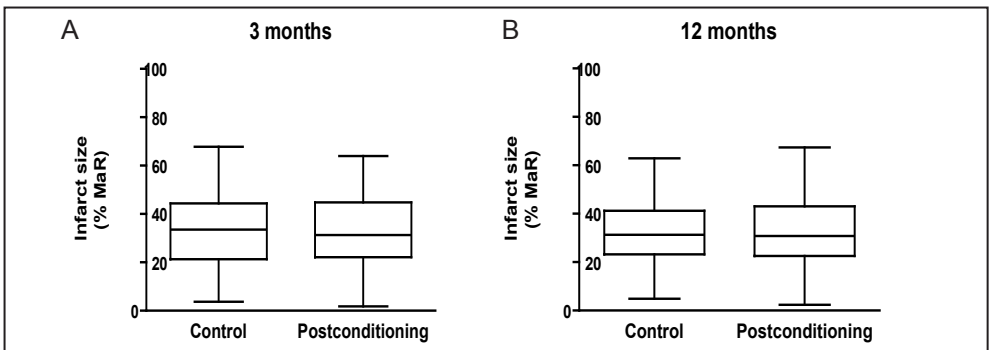


***Figure 12.*** *Infarct size in relation to myocardium at risk (MaR) for the overall study population at (A) 3 and (B) 12 months in the control group and the postconditioning group.*

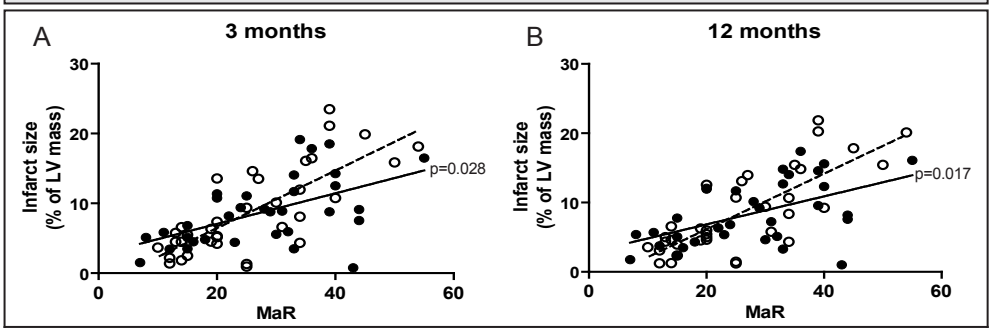


***Figure 13.*** *Infarct size (expressed in relation to left ventricular mass) plotted against myocardium at risk (MaR) for the overall study population at (A) 3 and (B) 12 months in patients belonging to the control (○) and postconditioning (●) groups. Significant differences between the slopes of the regression lines of the two groups are indicated.*

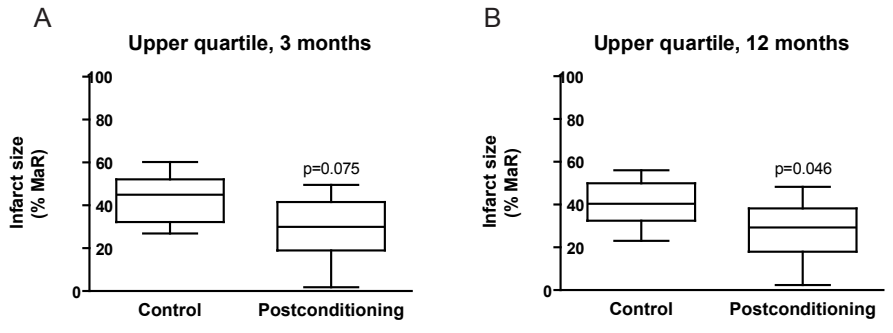


***Figure 14.*** *Infarct size in patients within the upper quartile of myocardium at risk (MaR) at (A) 3 and (B) 12 months in the control group and the postconditioning group.*

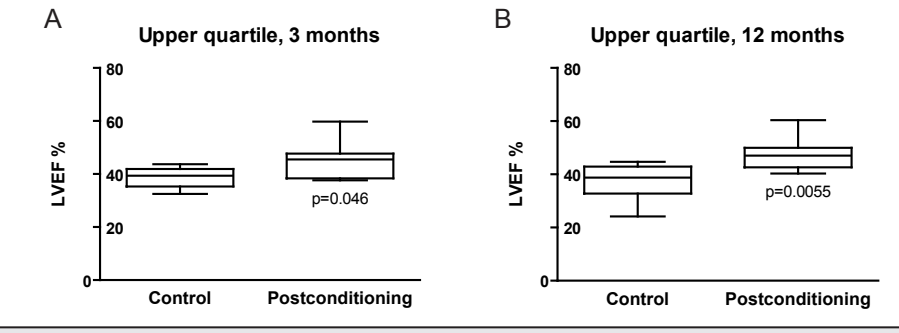


***Figure 15.*** *Left ventricular ejection fraction (LVEF) in patients within the upper quartile of myocardium at risk (MaR) at (A) 3 and (B) 12 months in the control group and the postconditioning (PostC) group.*

## Study III: Validation of contrast-enhancement SSFP

**Myocardium at risk**

MaR, defined as the non-perfused myocardial volume on SPECT, ranged from 11 to 51 % (mean 27 ± 10 %) of the LV wall volume. The CE-SSFP, calculated as the mean values obtained at end-diastole and end-systole, ranged from 17 to 47 % (mean 27 ± 7 %) of the LV wall volume. There was a good correlation (Figure 16) between MaR determined from CE-SSFP and that determined with SPECT ($r^2 = 0.78$, $p < 0.001$). The difference between CE-SSFP and MaR on SPECT was 0.5 ± 5.1 % ($p = 0.60$). The location of the enhanced region on SSFP cines always agreed with MaR on myocardial perfusion SPECT images. Two typical examples of MaR and infarct area in the RCA and LAD regions are shown in Figures 17 and 18. The signal intensity ratio between regions of gadolinium-enhanced and remote myocardium was 1.42 ± 0.25 ($p < 0.001$). The interobserver variability for CE-SSFP between two readers was 1.6 ± 3.7 %. Infarct size determined using CMR ranged from 1 to 30 % (mean 9 ± 7 %) of LV wall volume and mean transmurality ranged from 26 to 52 %.

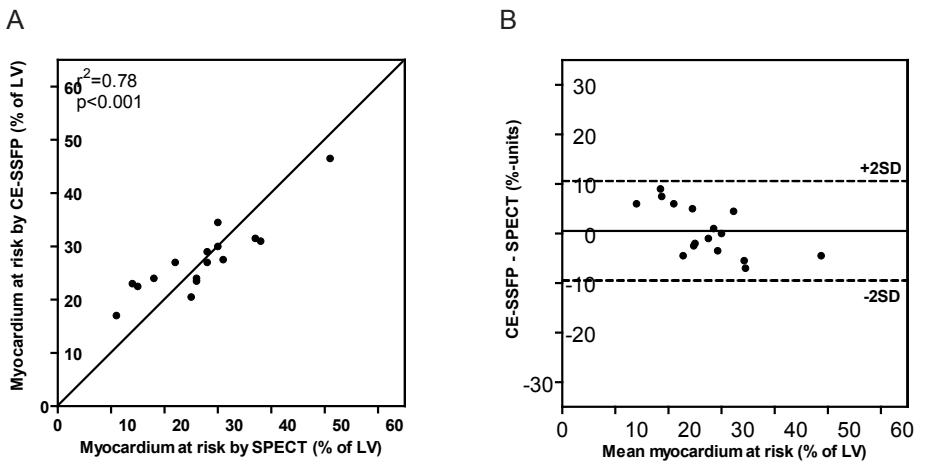


***Figure 16.*** *Agreement between MaR determined using CMR and SPECT. Panel A: Scatter plot showing MaR one week after reperfusion determined using contrast-enhanced SSFP (CE-SSFP) versus MaR as it was before reperfusion determined using myocardial perfusion SPECT together with line of identity. Panel B: Bland-Altman plot showing the agreement between MaR determined using myocardial perfusion SPECT and CE-SSFP. The difference was 0.5 ± 10 % (mean ± 2SD).*

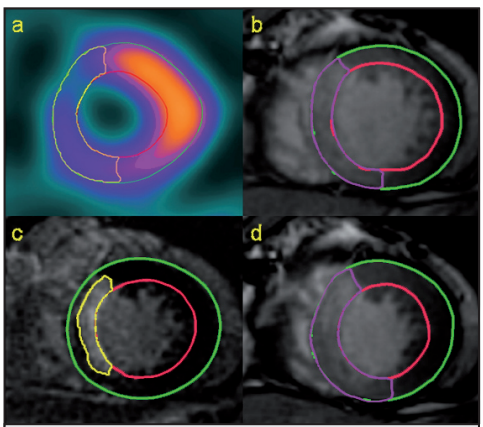


***Figure 17.*** *Anterior STEMI. Corresponding left ventricular short axis views from a patient with anterior myocardial STEMI. MaR determined using (a) myocardial perfusion SPECT, (b) contrast-enhanced SSFP (CE-SSFP) at end-diastole, (c) infarct size images with LGE and (d) CE-SSFP at end-systole. It is clearly seen that the region of infarction does not correspond in size or endocardial extent to the region of myocardium at risk determined using either CE-SSFP or myocardial SPECT.*

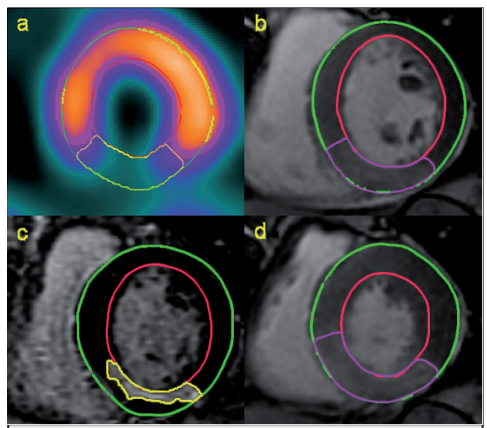


***Figure 18.*** *Inferior STEMI. Corresponding left ventricular short axis views from a patient with inferior STEMI. MaR determined using (a) myocardial perfusion SPECT, (b) contrast-enhanced SSFP (CE-SSFP) at end-diastole, (c) infarct size images with LGE and (d) CE-SSFP at end-systole.*

## Study IV: Contrast-enhanced SSFP compared with T2-weighted images

**Myocardium at risk**

A region with increased signal intensity measured by T2-weighted imaging and CE-SSFP was observed in all patients (Figure 19), yielding a mean MaR of 29 ± 11 % (range 12 – 65) and 32 ± 12 % (range 8 - 70) of the LV, respectively. There was a strong correlation between the two methods ($r^2 = 0.89$, $p < 0.01$) (Figure 20). The Bland-Altman plot showed a limit of agreement between T2-weighted imaging and CE-SSFP, demonstrating a difference of -3.0 ± 3.9 % of the LV ($p < 0.01$).

The CE-SSFP images were acquired on average 8 minutes (2-12) minutes after contrast agent administration. There was no change in CE-SSFP assessment of MaR compared with T2-weighted imaging with time after contrast agent administration. Signal-to-noise ratio within the MaR was 156 ± 7 and 132 ± 10 for the T2-weighted imaging and CE-SSFP, respectively (mean ± SEM). The contrast-to-noise was 58 ± 3 and 27 ± 6 for the T2-weighted imaging and CE-SSFP, respectively (mean ± SEM). The contrast ratio between MaR and remote myocardium for T2-weighted imaging was 1.7 ± 0.3 compared to 1.5 ± 0.4 for CE-SSFP, which was not statistically significant different.

The interobserver variability was 5.0 ± 5.4 % of the LV for T2-weighted imaging and 0.1 ± 6.2 % of the LV for CE-SSFP.

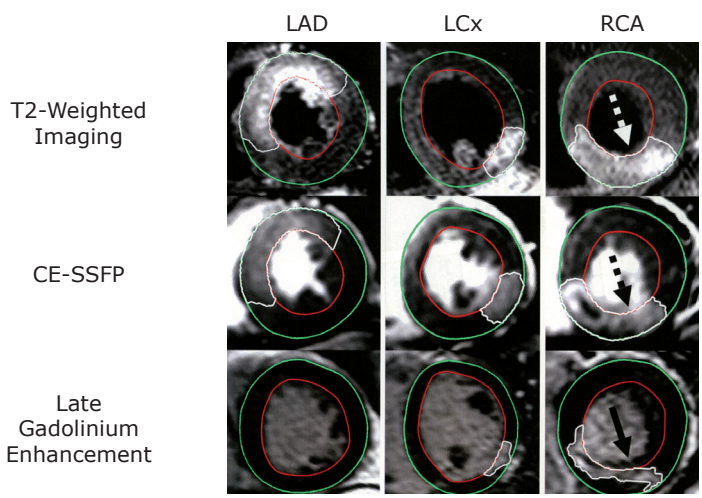


***Figure 19.*** *Single corresponding mid-ventricular short-axis images from a patient with an occlusion in the left anterior descending coronary artery (LAD), left circumflex coronary artery (LCx) and the right coronary artery (RCA), respectively. The epicardium is traced in green and the endocardium is traced in red. The hyperenhanced regions constituting the myocardium at risk (dashed arrows) and the infarcted myocardium (solid arrow) are traced in white. Note the similarity in location and extent of the affected region between T2-weighted imaging and contrast-enhanced steady-state free precession (CE-SSFP). Also note the significantly smaller infarction compared with the myocardium at risk, indicating a significant myocardial salvage accomplished through the acute reperfusion therapy.*

*Myocardial salvage index*

The mean infarct size by LGE was 14 ± 11% (range 1 – 49) of the LV. The interobserver variability was 0.3 ± 2.2% of the LV. The infarct size was smaller in all patients when compared with mean T2-weighted imaging ($p < 0.01$) and mean CE-SSFP ($p < 0.01$). When comparing the infarct size as determined through LGE in relation to MaR using T2-weighted imaging and CE-SSFP yielded a myocardial salvage index of 56 ± 22 % (range 15 – 93) and 58 ± 23 % (range 16 – 95), respectively (Figure 21). There was a significant correlation ($r^2 = 0.90$, $p < 0.01$) between the myocardial salvage index measured using the two methods, with an insignificant bias of 2.3 ± 7.4% of the LV.

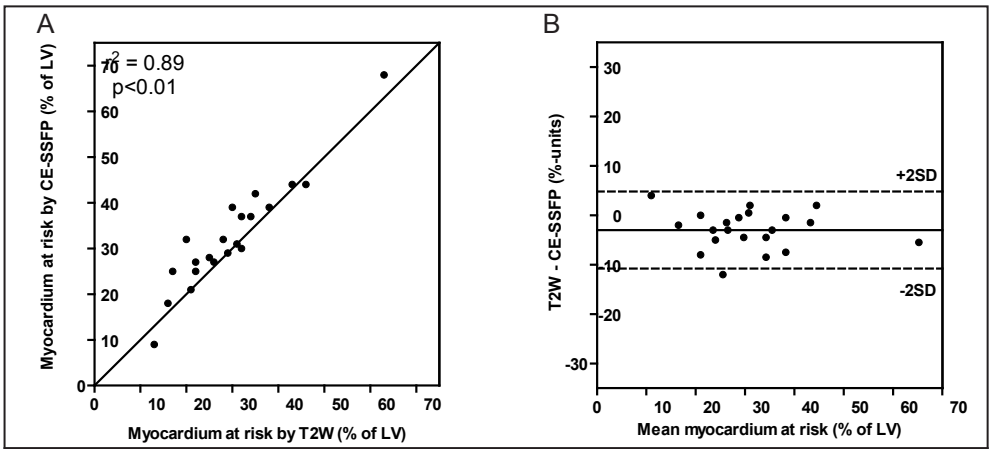


***Figure 20.*** *Relationship between T2-weighted imaging and contrast-enhanced steady-state free precession (SSFP) for myocardium at risk. Panel A: MaR by T2-weighted imaging versus CE-SSFP. Solid line = line of identity. Panel B: Bland-Altman graph showing the difference between MaR quantified by T2-weighted imaging and CE-SSFP versus the mean of the two methods. The difference between T2-weighted imaging and CE-SSFP was -3.0 ± 3.9 %. Solid line = mean difference; dashed lines = ± 2SD.*

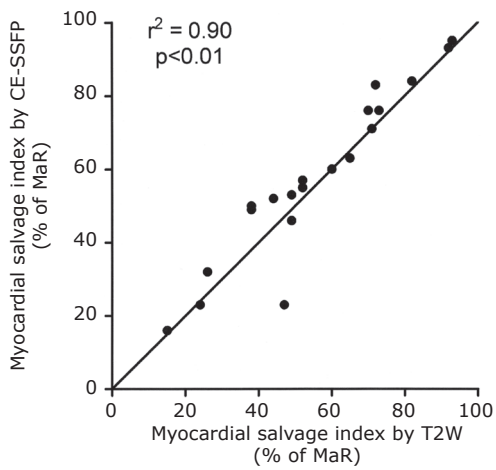


***Figure 21.*** *Relationship between T2-weighted imaging and contrast-enhanced SSFP for myocardial salvage. Myocardial salvage index measured by T2-weighted imaging versus myocardial salvage index measured by CE-SSFP. Solid line = line of identity.*

# General Discussion

This thesis presents novel findings of short- and long-term effects of postconditioning in patients with STEMI measuring infarct size with CMR, in relation to MaR. For the entire study population, the short- and long-term effects of postconditioning were neutral regarding infarct size and LVEF. However, among patients within the upper quartile of MaR smaller infarct sizes and improved LVEF were observed in the postconditioning group in comparison with the control group.
A novel method for determination of MaR with a modified standard CMR sequence was developed and validated against the reference standard method, SPECT. When comparing the new CMR sequence against the current standard edema CMR-sequence there was good correlation and the new method had a high accuracy regarding calculation of salvage index.

## Myocardium at risk and CMR

Determination of MaR is of importance in clinical studies using infarct size or myocardial salvage as endpoints, in particular when studying small populations.[114] The variation in MaR is usually large and detection of differences in final infarct size without taking MaR into consideration requires large sample sizes. The reference method for determination of MaR has been SPECT, a method with limited applicability in studies of acute interventions in patients with acute MI. Limited availability of isotopes and limited access to a gamma camera are the most important obstacles. The handling of and exposure to isotopes are other concerns. The need to develop new feasible and accurate methods for determination of MaR is therefore great. Previous attempts to investigate MaR with CMR focused on different T2-weighted sequences several days after the index ischemia. It has been suggested that MaR can be estimated using T2-weighted imaging with short inversion time inversion recovery (STIR) or T2-prepared single-shot SSFP or a combination of both.[134, 137, 155 156] T2-weighted imaging as a tool to quantify MaR was first validated in humans with myocardial perfusion SPECT as the reference[140] and the method was subsequently used to demonstrate the rate of infarct evolution in man without confounding factors.[141] The (ACUT2E) TSE-SSFP study showed promising results when using a hybrid method of T2-weighting with bright-blood contrast.[135]
The reliability of CMR for the assessment of MaR and myocardial salvage index by means of T2-weighted sequences after reperfusion of an occluded coronary artery was recently questioned in a study using different ways to measure MaR with T2-weighted sequences.[157] There were differences found between all T2-weighted sequences compared with angiographic BARI score and from one T2-weighted technique to another. These factors might lead to a risk of over- or underestimating MaR and should be considered when using T2-weighted CMR as an assessment of MaR.

The use of CE-SSFP was described and validated for the first time in Studies III and IV. CE-SSFP showed a good correlation with SPECT (Study III) and there was an excellent correlation in determining MaR with CE-SSFP in the subsequent head-to-head comparison with the most commonly used T2-weighted CMR sequence (Study IV). Importantly, there was an equally

good correlation between the two methods, CE-SSFP and the T2-weighted sequence, when MaR was used for the assessment of the myocardial salvage index. The interobserver variation was indeed smaller for CE-SSFP than for the T2-weighted sequence. These observations are of considerable clinical importance allowing an accurate determination of MaR by means of CMR performed up to one week after the acute event. This will undoubtedly simplify the determination of MaR and salvage index in future studies of cardioprotection.

The mechanisms behind the enhanced myocardium observed in CE-SSFP are not completely understood. The contrast in SSFP images depends on the T2/T1 ratio.[158] In the presence of paramagnetic gadolinium, T1 for the surrounding tissue is shortened. This is utilized for infarct visualization in T1-weighted LGE imaging, where the concentration of an extracellular gadolinium-based contrast agent is increased due to an increased distribution volume in irreversibly injured myocardium.[126, 159-161] It has, however, also been shown that reversibly injured myocardium has an increased distribution volume after an ischemic episode. Hence, it is possible that the T2/T1 ratio in the entire MaR, including both reversible and irreversible injured myocardium, is affected by the presence of gadolinium. This might explain the increased signal intensity in the MaR as seen with CE-SSFP. Furthermore, it has been shown that the change in T1-relaxation rates before and after contrast agent administration remains constant from 4 to 29 minutes after administration in normal myocardium, as well as within the MaR and in infarcted myocardium.[159] These earlier findings indicate that the rate of exchange of contrast agent between the myocardium (normal and injured) and the blood pool is constant and much faster than clearance rate in the kidneys during the first 30 min after contrast agent administration. Thus, these findings can explain why the relationship between MaR assessed using T2-weighted imaging and CE-SSFP did not change with time after contrast agent administration in Study IV and why the timing of CE-SSFP after contrast agent administration is not so critical.

The pathophysiological basis for the enhanced myocardium observed using T2-weighted imaging is still not completely understood. Following an acute coronary occlusion, the ischemic myocardium shifts from aerobic metabolism to anaerobic glycolysis and ceases to contract. This failure of the energy-regulated membrane channels results in swelling of the myocytes due to influx of water and sodium.[162] Furthermore, reperfusion leads to an inflammatory-like response increasing the amount of extracellular fluid.[163] The increased water content in the affected myocardium is likely to explain the increased signal intensity compared with the non-affected myocardium as seen through T2-weighted imaging. Whether the increased water content is predominantly located in the intra- or extracellular space remains to be determined. The ischemic episode causes post-ischemic stunning and is associated with decreased contractility in the previously ischemic myocardium.[164] It is reasonable to assume that the reduced contractility is associated with a decreased lymphatic drainage from the engaged myocardium, which may contribute to the increased water content one week after the acute event.

Using T2-weighted imaging can sometimes be challenging, especially with regards to low signal-to-noise ratio, signal loss due to through-plane cardiac motion and/or increased signal from stagnant blood flow in the apical part of the LV.
These limitations can to a large extent be overcome by using CE-SSFP, since these shortcomings are not associated with SSFP imaging. Another advantage with CE-SSFP

imaging is that this technique is based on a multi-phase acquisition throughout the cardiac cycle. This enables tracking of the MaR and myocardial borders in multiple time frames, allowing delineation of both MaR and myocardial borders. Furthermore, CE-SSFP is ideal in situations where the time for scanning is limited e.g. due to heavy clinical workload or when handling an unstable patient. Gadolinium may then be injected prior to the examination and the imaging protocol can be shortened, since LV dimensions/function and MaR can be assessed from the same set of images. T2-weighted imaging for determination of MaR can, on the other hand, be performed in patients in whom a gadolinium-based contrast agent is contraindicated. Thus, there are several advantages of having access to more than one method for determination of MaR using CMR.

## Short-term effects of postconditioning in patients with STEMI

The first groups to report effects of postconditioning in patients with STEMI were Staat et al[57] and Laskey et al[164, 165] who, in small populations, showed that postconditioning reduced the release of CK, a surrogate marker for infarct size, and improved coronary flow reserve and ST resolution. Thibault et al[58] showed that postconditioning limited absolute infarct size and improved LVEF. They did not properly relate infarct size to the initial MaR, which is of importance as already discussed above. In this perspective, the recent report by Lønborg et al[165] is of considerable interest. This group demonstrated that postconditioning resulted in a 19 % relative reduction of infarct size in relation to MaR. A problem is that there was a significant difference in myocardial mass between the intervention and control group, which may explain most of the differences in final infarct size. In addition, the method used for estimating MaR, endocardial surface area, has limitations, in particular introducing a risk of underestimating MaR.[144]

In Study I, infarct size after one week was related to MaR determined through left ventriculography. Interestingly, the slopes of the regression lines differed significantly between the control and the postconditioning groups when infarct size was analyzed as a function of MaR. This observation, suggesting a cardioprotective effect in patients with large MaR, led to further analysis of the infarct size among patients with MaR in the upper quartile, revealing that these patients seemed to benefit from postconditioning. Thus, their infarcts were smaller irrespective of whether the infarct was LAD-related or not. LVEF also differed, supporting a beneficial effect of postconditioning in patients with large MaR. According to the regression analysis, a protective effect was detectable for MaR exceeding 36 %. In light of this observation it is important to note that the left ventricular MaR, determined from abnormally contracting segments, in previous studies was 35-40 %, which should be compared with 25 % in the present study in average. Collectively, these data might support the assumption that postconditioning is protective in patients with large MaR.

In contrast to previous reports, postconditioning did not influence the infarct size in all patients subjected to this procedure in Study I. This observation is, however, not the only one questioning the overall efficacy of postconditioning. A recent study determining infarct size and LVEF as outcome measures in patients with STEMI reported neutral results in the entire group of postconditioned patients.[166] Subgroup analysis even suggested harmful effects of postconditioning. Postconditioning was associated with lower myocardial salvage and lower

myocardial salvage index. Although no significant differences in absolute infarct size and LVEF were found between the groups at one week and six months after MI.

There are several differences of potential importance that may explain the discrepancies between trials evaluating the impact of postconditioning. In study I, infarct size was determined using CMR one week after the index event. This differs from the time of three to six months used in previous studies.[58, 165] The rationale behind determining infarct size after one week in Study I was to limit the possible influence of LV remodeling, a process that may influence infarct size. To determine infarct size after approximately one week seems optimal since the reduction of the hyperenhanced area is greatest during this period, balancing the early disappearance of the increased area of hyperenhancement against LV remodeling. To avoid any influence of spontaneous reperfusion, only patients with TIMI 0 flow were recruited to Study I, which differs from previous studies accepting a flow of TIMI grade 0-1.[58, 165, 166] Finally, the present postconditioning protocol was four cycles of 60 seconds reperfusion and 60 seconds of reocclusion. This is similar to the protocol used by Staat[57], Thibault et al[58] and Freixa et al[166] but different from that applied by Lønborg et al.[165] Although original observations suggested that brief cycles are optimal in small animal models (mice and rats), longer periods (30-60 seconds) may be more effective in larger species (pigs).[56] A subsequent analysis was, however, unable to define an optimal postconditioning protocol.[167]

## Long-term effects of postconditioning in patients with STEMI

Data on the long-term effects of postconditioning on infarct size and LVEF are sparse, in particular data based on the use of CMR. Thibault et al[58] measured LVEF after 12 months of follow-up using echocardiography and reported a difference in favor of postconditioning. Lønborg et al[172], who compared NYHA classes and ST-resolution, noted a trend towards better NYHA class and ST-segment resolution in patients subjected to postconditioning. Freixa et al[166] reported no differences between groups in LVEF or infarct size using CMR after six months. The objective behind Study II, based on the results from Study I, was to evaluate the long-term effects of postconditioning on infarct size and LVEF, with a special focus on patients within the upper quartile of MaR. In line with the results from Study I, the slopes of the regression lines for infarct size and LVEF as a function of MaR continued to differ, indicating a sustained benefit for patients with large MaR. Taken together, available data indicate that postconditioning might be effective in patients with large MaR and that the effect is maintained during long-term follow-up.

LV remodeling after myocardial infarction is an important prognostic factor for the progression to heart failure and subsequent mortality.[168] Multiple factors contribute to the remodeling process including infarct size, MaR, microvascular obstruction, patency of the infarct-related artery and baseline LVEF.[169, 170] Recent studies have used CMR as a tool for identifying predictors of remodeling in reperfused STEMI populations. Lund et al[171] demonstrated that an infarct size ≥24 % of the LV predicts remodeling with high sensitivity and specificity. Masci et al[173, 174] concluded that infarct size rather than location predicted LV remodeling in STEMI patients and Eitel et al[172] concluded that myocardial salvage index assessed using CMR predicts long-term clinical outcome in patients with STEMI. In Study II, we only identified nine patients who met the remodeling criteria (>15 % increase of ESV). The generally small infarct sizes are the most reasonable explanation for the absence of

remodeling. The end-diastolic sphericity index, which indicates LV cavity remodeling, did not change over time within either of the two groups, indicating that there was no overall adverse long-term remodeling in the present study population. In addition the, vast majority of the patients were treated with beta-blockers and ACE inhibitors, pharmacological agents known to counteract remodeling. Thus the group of patients with adverse remodeling was too small to permit an analysis of possible predictors for that process.

## Cardiac biomarkers

The use of cardiac biomarkers has until recently been the method of choice for quantifying infarct size.[173] Troponin I and T, which are expressed exclusively in the heart, are components of the contractile apparatus of the cardiac myocyte. Troponins are present both in the cytosol and myofibrils of the myocyte. Cardiac troponin I and T are currently the preferred biomarkers for detection of myocardial necrosis, having supplemented older biomarkers such as CKMB because of superior sensitivity and specificity.[10] Peak and AUC of CKMB and troponin T correlate with infarct size measured with CMR.[174] Recently, Turer et al[175] concluded that high-sensitivity troponin T could be detected after provoked ischemia in humans. These results may indicate that cardiac biomarkers reflect ischemic myocardium and not only necrosis. In the present material, analysis of AUC of troponin T and CKMB had a better correlation with infarct size than with MaR, which is in accordance with the general opinion that cardiac biomarkers reflect the extent of myocardial necrosis.

In Study I, there were no differences in the AUC levels of troponin T and CKMB between the two study groups, suggesting the absence of cardioprotection in an overall perspective. In contrast, the regression analysis comparing troponin T in relation to MaR in the upper quartile favored postconditioning.

## Study limitations

The size of the study populations may have been too small for the detection of minor benefits of postconditioning in Studies I and II. The number of patients was based on a power calculation assuming an absolute reduction of infarct size by 20 % of MaR. This level was chosen as representing an effect that should be of clinical value. The extent of the final infarct size may depend on several factors, besides postconditioning, which are difficult to control in a clinical study. Known confounders are age, certain comorbidities e.g. kidney dysfunction, medication and pre-infarction angina known to precondition the myocardium as well as the presence of collaterals. The groups were well-matched regarding these factors, suggesting little impact on the primary endpoint.

The angiographic method of MaR in Studies I and II may underestimate the actual MaR. These two studies were originally designed with this method for determination of MaR. Even though a new method of determining MaR was discovered during analysis of the examinations of Study I, it felt inappropriate to use this method in the patient material based upon which it was first described. The optimal postconditioning algorithm is not known and there may be more effective algorithms than the one used, even if it is the most commonly applied.

Finally, since the signal intensities of the MaR and the remote myocardium varied between slices and between patients, making it difficult to choose a fixed standard deviation of signal intensities to differentiate MaR from remote myocardium, no semi-quantitative method was used to determine the MaR either on T2-weighted or CE-SSFP imaging.

# Future Perspectives and Comments

**CMR sequences**

CE-SSFP has the qualifications to become a useful research tool in trials looking at myocardium salvage index by different interventions. Still, there are questions to be answered regarding mechanistic features of this technique. For instance, the reason for the hyperenhanced signal on SSFP is not fully understood and the kinetics of the contrast agent in the MaR needs to be addressed further. Whether this sequence is influenced by edema reduction induced by any cardioprotective intervention remains to be explored. Experimental studies addressing these questions are currently lacking but would be highly interesting.

New CMR sequences that quantify image T1 or T2 with T1- and T2-mapping are currently available and may perhaps allow a robust visualization of MaR.[176] This area of research is new and will require additional validation before becoming clinically useful.

**Postconditioning**

Regarding postconditioning, several questions need to be addressed. The postconditioning algorithm was initially based on empirical observations in experimental studies.[55] There is still no optimal algorithm that has proven superior in humans. Thus it is not surprising that different groups applied different algorithms. One study reported on less myocyte apoptosis by means of 60 rather than 30 seconds of reperfusion and ischemia.[177] The optimal number of cycles is another unresolved issue in need of further investigation. Is two better than four and is four better than six? There is no test telling us whether we have reached maximum conditioning or not. A recent study in mice reported decreased infarct size with a prolonged postconditioning protocol lasting up to 30 minutes after the initial reperfusion. Thus, it provides conflicting evidence indicating that the time window for protection by postconditioning may be longer than initially reported.[41] Another unresolved question is which phase of the reperfusion is the most important: The reperfusion phase or the ischemia phase or are both equally important? Such information is necessary in order to optimize the postconditioning algorithm and number of cycles to be applied in future studies.

The present results indicate that future attempts with postconditioning should primarily focus on patients with large MaR, i.e. with a substantial amount of myocardium to be salvaged. Changes in infarct size in patients with small MaR may be difficult to detect and reduction in their infarct size may be less important from a prognostic point of view. The follow-up period in Study II is presently the longest available, but there is a need for even longer periods. There is also a need for studies looking not only at surrogate endpoints but also at clinically relevant outcome measures such as morbidity and mortality. This highlights the need for a large multicenter study to confirm the clinical value of postconditioning.

**Translation from preclinical to clinical studies**

The search for an effective, easily applied and safe cardioprotective intervention is still only beginning. Although it certainly requires substantial efforts, it is of great importance to

further reduce the remaining morbidity and mortality related to acute MI. There are several potential explanations behind the major differences in success in preclinical and clinical trials in this sector.[178]

*In preclinical studies*: various pharmacological agents are usually tested in one laboratory and in one or two animal models before being transferred to clinical trials. Most preclinical experiments are randomized but open, which may be a source of bias. Moreover the relevance of commonly used animal models, usually young, male animals without any comorbidities, may be misleading.

*In clinical studies*: the dosages and administration times for drugs may cause problems. Intravenous instead of intracoronary infusions distal to the occlusion may explain the lack of success in some trials. Insufficiently powered (small) studies and the recruitment of patients with small MaR may also cause problems, as indicated by the present results. Moreover, different research centers have different protocols as regards anticoagulation/antiplatelet treatment, reperfusion techniques including stenting, and thrombus aspiration, which may introduce discrepancies in outcomes, such as infarct size and LVEF.

Future attempts to find new effective therapies must take all these issues into consideration. An ideal study population for proof-of-concept trials would be patients with large MaR, with symptoms for less than six hours, no pre-angina, TIMI flow 0 and no collaterals and then later on expand the inclusion criteria to more general populations and multicenter studies with long-term follow-up. It would be reasonable to combine different cardioprotective modalities in such investigations considering the complexity of the reperfusion mechanism and the cross-talk between different pathways as outlined. A combination of remote conditioning and postconditioning would be interesting, with a potential for synergistic effects on the different pathways involved in the reperfusion injury. The chosen method should be easy to implement already in the ambulance and subsequently reinforced in the catheter laboratory. The future may also be to combine an optimal cocktail of different drugs targeting the different harmful effects of reperfusion. Currently there are >30 studies registered at ClinicalTrails.gov concerning reperfusion injury and myocardial infarctions. The research field is still open for new remarkable discoveries!

# CONCLUSIONS

**I.** Postconditioning did not affect infarct size, LVEF or cardiac biomarkers one week after STEMI in the entire study population. Infarct size was reduced and LVEF increased in the postconditioned patients with large MaR.

**II.** There was no difference in infarct size or LVEF between the postconditioning and control groups during 12 months of follow-up in the entire study population. In patients with large MaR, the early observations of a reduction in infarct size remained and these patients had a higher LVEF than patients in the control group at the 12-month follow-up.

**III.** CE-SSFP after one week accurately depicts MaR and correlates well with MaR determined with reference standard SPECT in patients with STEMI.

**IV.** There was a strong correlation between MaR assessed by CE-SSFP and MaR assessed by T2-weighted imaging in reperfused patients with STEMI one week after the acute event. Both methods can be used to determine MaR and myocardial salvage.

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