General introduction and outline of the thesis
Around the world, and especially in the Western Society, cardiovascular diseases remain one of the leading causes of death.(1) Within the broad range of cardiovascular diseases, the proportional burden of ischemic heart disease is especially high within the Middle East, North America, Australia, and the majority of Europe.(2) In the Netherlands, cardiovascular diseases have been the leading cause of death until 2007, with an annual mortality number of over 38,000 cases each year.(3) In 2010, 39,735 (30%) of the 136,058 deaths in the Netherlands were related to cardiovascular diseases, of which 6,823 (17%) cases were caused by acute myocardial infarction (MI).(4) Emergent coronary revascularization by percutaneous coronary intervention (PCI) has significantly reduced early mortality rates and improved prognosis in patients with ST-segment elevation myocardial infarction (STEMI).(5) In some patients, the epicardial flow remains hampered after reperfusion, an angiographic phenomenon commonly known as ‘no reflow’ and associated with a diminished myocardial salvage, larger infarct size, worse residual LV function and increased mortality.(6;7) Additionally, studies showed that even with complete restoration of epicardial flow, a large proportion of patients have persistent ST-segment elevation, indicating ongoing ischemia and infarction of the myocardium.(8) All these patients have an impaired myocardial reperfusion and are at risk of having more severe myocardial infarctions with left ventricular maladaptive remodelling and more long-term morbidity and mortality.(9;10) Despite the decrease in early mortality due to acute MI, the paradoxical increase in patients with chronic heart failure continues to be a large burden on healthcare systems and society in general.

MYOCARDIAL REPERFUSION INJURY

Animal models of myocardial infarction have shown that 20 minutes after reperfusion, areas of reperfusion injury contain capillaries plugged by erythrocytes, platelets and fibrin thrombi, and swollen intraluminal endothelial protrusions, leading to further obstruction of the capillaries(11) and the formation of microthrombi.(12;13) These microthrombi, together with embolization of atherosclerotic debris, are thought to play an important role in the development of the no reflow phenomenon and the development of reperfusion injury. Many therapeutic approaches have already been tried, with varying results. Thrombus aspiration during PCI may reduce the burden of atherosclerotic debris in the microcirculation, but the effects on morbidity and mortality remain discussed.(14;15) Adjuvant treatment regimens with vasoconstrictor agents such as adenosine showed promising results in small randomized controlled trials, but failed to show a clear clinical benefit in larger trials.(16;17) Currently, studies are focusing on cardioprotection with specific cardioprotective drugs (e.g. nicorandil, cyclosporine), hypoxemic reperfusion and myocardial post-conditioning with promising results, but the efficacy of these treatments
needs to be established in larger clinical trials. (18) Currently, the only available guideline-recommended pharmacological agent for angiographic no reflow is abciximab, a glycoprotein IIb/IIIa inhibitor. (5) However, the increased risk in major bleeding complications prevents the routine use of abciximab in all patients. (19) Interestingly, obstruction of the microcirculation may not be the only pathophysiological mechanism behind no reflow and reperfusion injury. Observations in porcine myocardial infarction models also showed massive extravasation of erythrocytes, leading to intramyocardial haemorrhage. (20-23) This may be caused by disruption of the endothelial barrier due to hypoxia, damaging the microvasculature and facilitating extravasation of blood cells upon reperfusion. (13;23) Although it is most likely that haemorrhage is preceded by destruction of the vascular integrity, it is possible that activation of inflammation and coagulation leads to thrombosis, endothelial activation and consumption of coagulation factors. The local formation of microthromboli contributes to the vessel injury and may aggravate the haemorrhage. It should be noted that vessel injury and subsequent haemorrhage are only one component of reperfusion injury to the heart. Several other processes are considered to contribute, such as leukocyte activation and plugging and activation of inflammatory pathways. (24) Although more studies on all pathophysiological processes contributing to reperfusion injury is necessary for developing new treatment strategies, more insight in the detection of reperfusion injury in patients is needed to accurately identify patients at risk for reperfusion injury and the associated increase in morbidity and mortality.

CARDIOVASCULAR MAGNETIC RESONANCE AFTER ACUTE MYOCARDIAL INFARCTION

Current guidelines already state that after revascularization, assessment of infarct size and residual cardiac function should be performed for initial risk stratification. (5) Generally, this is performed by trans thoracic echocardiography (TTE), as this technique is widely available and can be performed at the patient’s bedside if necessary. Unfortunately, echocardiography only provides information on residual function, myocardial perfusion and extent of infarction. In recent years, cardiovascular magnetic resonance (CMR) imaging has emerged as a promising alternative diagnostic tool and is already an established imaging technique in patients after acute MI. (5) CMR has the advantages of providing information with high spatial and temporal resolution without the need for ionizing radiation or invasive procedures. Thus, CMR provides the possibility for longitudinal follow-up of myocardial residual function, infarct size, extent of myocardial injury and residual viability with an excellent inter-study reproducibility, effectively reducing the number of patients (and therefore costs) needed to perform clinical trials. (25;26) An additional advantage of CMR is the possibility to assess changes in myocardial tissue composition,
such as oedema, haemorrhage, microcirculatory perfusion abnormalities, concomitant non-ischemic cardiomyopathies and additional sequelae of the infarction, such as left ventricular thrombi or aneurysms.\textsuperscript{(27-29)}

\textbf{ASSESSMENT OF CARDIAC FUNCTION AND INFARCT SIZE}

Originally, systolic function measured by left ventricular ejection fraction (LVEF) is one of the more often used measures for risk stratification, as a decreased residual systolic function is associated with more adverse cardiac events at follow-up.\textsuperscript{(5,30)} Although LVEF can be measured by multiple techniques, such as echocardiography, nuclear imaging and angiography, CMR-measured LVEF has been shown to be the most reproducible and accurate, dubbing the technique the ‘gold standard’ for determination of LVEF.\textsuperscript{(31-33)} CMR imaging with Late Gadolinium Enhancement (LGE) allows for accurate assessment of the infarcted area, which is depicted as a region of hyperenhanced myocardium.\textsuperscript{(34,35)} Furthermore, LGE imaging can reveal areas devoid of contrast within the infarcted, contrast-enhanced myocardium, due to strongly impaired tissue perfusion and lack of contrast wash-in.\textsuperscript{(34,35)} This finding is commonly attributed to the no-reflow phenomenon\textsuperscript{(36)} and is referred to as microvascular obstruction (MVO). The presence of MVO shows close correlation with lack of myocardial reperfusion on histological examination\textsuperscript{(37)} and is a strong predictor for impaired functional recovery and increased adverse ventricular remodelling.\textsuperscript{(10,34,38)}

\textbf{TISSUE CHARACTERIZATION}

Pre-contrast CMR with a T2 weighted sequence allows for the visualization of infarct-related oedema. Commonly, the Short-Tau Inversion Recovery, or STIR-sequence is used, which is a T2 weighted turbospinecho sequence with fat suppression.\textsuperscript{(39)} The increased water content creates a change in magnetic tissue properties with an increase in T2 relaxation times, leading to regions of high signal intensity on T2 weighted sequences.\textsuperscript{(27)} Also, T2 weighted images may contain hypointense regions within the hyperintense area, which have been attributed to intramyocardial haemorrhage (IMH).\textsuperscript{(40,41)} Local accumulation of haemoglobin breakdown products from extravasated erythrocytes induce paramagnetic effects, which leads to shortening of the T2 relaxation times of the tissue and the subsequent signal loss. Unfortunately, only limited data are available on the exact relationship between T2 weighted images and the presence of IMH.\textsuperscript{(42,43)} Similar to the aforementioned MVO, the presence of these hypointense zones within the hyperintense areas on T2 weighted images show a relation with an increased infarct
size, impaired functional recovery and an increased incidence of major adverse cardiac events. (28) Approximately one-third of the patients with MVO on LGE images show these hypointense regions on T2 weighted images. This has led to the current belief that IMH indicates a more severe form of reperfusion injury on top of already impaired microvascular reperfusion, and that this finding is associated with a further impairment in recovery and subsequent increase in morbidity and mortality. (28) Microvascular obstruction may therefore be more generally described as microvascular injury (MVI). Although a number of studies have focused on the clinical value of MVO and IMH to predict impairment of recovery, the exact pathophysiologic correlate still remains debated.

More recently developed CMR techniques allow for direct assessment of spin-lattice, or T1 relaxation times, a tissue-specific property. T1 mapping with a Modified Look-Locker inversion-recovery (MOLLI) sequence shows promise to become a new tool for assessment of myocardial injury after STEMI. (44-46) Reperfusion-induced myocardial oedema and the subsequent increase in tissue water content increases T1 values in the infarct region when measured with the MOLLI sequence. (47) It is yet unclear how the presence of myocardial reperfusion injury alters the T1 values in the recently infarcted myocardium, as other research suggests a decrease in T1 values. (48) Reperfusion may induce haemorrhage in the afflicted myocardium. Some of the haemoglobin breakdown products have paramagnetic properties, inducing T2* effects in the infarcted myocardium. (49) It remains unclear how this affects local T1 relaxation times and the subsequent interpretation of T1 maps.

OUTLINE OF THE THESIS

The thesis first describes the use of novel CMR techniques for characterization of ischemia-reperfusion induced myocardial injury in patients with a reperfused acute MI. Chapter 2 describes how we evaluated the use of myocardial T1 mapping and T2* mapping in patients with a reperfused acute MI, and the influence of MVO or MVI on local T1 and T2* values. These mapping sequences are novel CMR techniques that allow for specific assessment of changes in the myocardial tissue composition. The presence of MVI, however, may change the interpretation of the mapping data. Chapter 3 discusses the relationship between LGE-defined MVO and STIR-defined IMH and the histologic substrate of MVO and IMH. Better understanding of the processes leading to the presence of MVO and IMH on CMR images could help our understanding of the phenomenon and may lead to new targets for treatment strategies.

Following the third chapter, the role of CMR for measuring the effects of novel adjuvant treatment strategies for acute myocardial infarction, and novel forms of risk stratification, is explored. In chapter 4 we investigate whether characterisation of the contrast-enhanced
infarcted area may help identify patients at risk for developing ventricular arrhythmias. We postulate that the infarcted area should not be interpreted as one homogeneous area, and that assessment of the myocardial infarct border zone, or ‘penumbra’, may help in predicting the development of ventricular arrhythmias.

In the last decade, multiple studies have explored the effects of cell therapy in humans after acute myocardial infarction, with conflicting results. The HEBE study, a Dutch multicentre cell therapy trial explored the effects of intracoronary cell therapy on functional recovery. Unfortunately, no effects on the recovery of systolic function were found at 4 months follow-up. The paracrine and neoangiogenic effects that are attributed to stem cells may improve myocardial perfusion, which in turn may lead to either improved functional recovery at long-term follow-up, or attenuation of ongoing adverse ventricular remodelling. In chapter 5, we investigated the effects of cell therapy on myocardial perfusion recovery after a revascularized acute MI and whether these effects are more pronounced in areas of MVI. Whether intracoronary cell therapy after acute myocardial infarction in patients promotes beneficial effects in the long run is discussed in chapter 6. Patients have been followed for 2 years after the initial inclusion in the HEBE trial. After 2 years of follow-up, we assessed whether cell therapy influenced the prevalence of major adverse events, adverse left ventricular remodelling and the residual systolic function.

In the restoration of myocardial injury after an acute MI, the inflammatory system plays a pivotal role. An essential component of the inflammatory reaction is regulated by monocytes. In chapter 7, we investigated whether inflammatory characteristics of monocytes are associated with the severity of myocardial injury and subsequently, functional outcome in patients after a reperfused STEMI. By using flow cytometry, the levels of ‘classical’ (CD14++, CD62L+) and ‘non-classical’ (CD14+CD62L−) monocytes were analysed in peripheral blood samples. Additionally, monocytic expression of several surface molecules and the formation of monocyte-platelet complexes were measured. These findings were compared to CMR-derived functional parameters and myocardial perfusion parameters at baseline and 4-months follow-up.

Cell therapy may not only have beneficial effects for the patients. Some trials have suggested that cell therapy may even be potentially harmful due to the facilitation of arrhythmogenesis. In chapter 8, we investigated whether intracoronary cell therapy alters the prevalence of ventricular arrhythmias during follow-up. The final chapter discusses the findings of the thesis, the lessons we should learn from it and the implications for future research in cardiovascular research on ischemia and reperfusion.
REFERENCES


