General introduction
INTRODUCTION

Cardiovascular disease, including acute myocardial infarction (AMI) and myocarditis, is the leading cause of mortality and morbidity worldwide. According to a recent report of the World Health Organisation (WHO), January 2015, it was estimated that 17.5 million deaths are related to cardiovascular disease worldwide, representing about 46% of all global deaths. Cardiovascular death is expected to increase even further in the upcoming years to an estimated 23.3 million in 2030, remaining the world leading cause of death according to the WHO. In the Netherlands, there are over a million patients with cardiovascular disease, and around a 107 patients die of cardiovascular disease daily (source: Dutch Heart Foundation). This thesis describes the crucial role of inflammation in the pathophysiology of both AMI and myocarditis.

ACUTE MYOCARDIAL INFARCTION (AMI)

The post-AMI inflammatory response

AMI occurs when perfusion of the myocardium is severely reduced below its need mainly due to occlusion of the coronary artery. As a result of ischemia, cardiomyocytes become (ir)reversibly jeopardized. This coincides with a strong inflammatory response that attracts inflammatory cells to the site of injury in order to remove dead cells and debris, and to repair the myocardium. One key step in limiting ischemia induced cardiac damage, is to restore blood flow in the affected myocardium, also known as reperfusion. However, reperfusion also leads to the generation of damaging reactive oxygen species (ROS) and an increase of inflammatory mediators and inflammatory cells in the heart, further damaging the already jeopardized myocardium\(^1\). This inflammatory reaction post-AMI, plays an important role in subsequent cardiac remodelling and as such is influencing heart function. This post-AMI inflammatory response, can be subdivided into several sequentially occurring phases based on macroscopical and microscopical characteristics. In figure 1, an overview of these different phases of AMI is depicted. These characteristics are also used to determine infarct age in autopsies.
Figure 1. Timeline of the different phases of AMI.  
1) Macroscopical picture\(^1\) of a heart cross section, showing lactate dehydrogenase decolourisation (*), indicative for AMI of at least 3 hours old. 2) Microscopical picture of the myocardium, taken from the macroscopical infarction area 3-6 hours after onset of AMI. No morphological changes can be seen. Magnification x200. 3) Microscopical picture of the infarcted myocardium, 6-12 hours after onset of AMI. Accumulation of polymorphonuclear neutrophils (PMNs) in the blood vessels can be seen (arrows). Magnification x200. 4) Microscopical picture of the infarcted myocardium, between 12 hours and 5 days after onset of AMI. PMNs start infiltrating the infarction area and are found extravascular in between cardiomyocytes (*). Magnification x200. 5) Microscopical picture of the infarcted myocardium, between 5 days and 14 days after onset of AMI. Lost cardiomyocytes are replaced by granulation tissue, eventually resulting in scar formation in the heart (*). Magnification x200.
At a macroscopical level, the first indication of AMI is the lactate dehydrogenase (LDH) leakage from damaged cardiomyocytes, which can be detected using a nitro blue tetrazolium staining method, 3 hours after onset of AMI. In this very early phase of AMI (3-6 hours old), no microscopical changes of the myocardium are found. However, it has been shown in animal models that the formation of small osmiophilic amorphous densities, or deposits, in mitochondria can already be detected at the ultrastructural level as early as 1-2 hours after onset of AMI, using transmission electron microscopy (EM). The detection of these mitochondrial deposits using EM could therefore be a useful tool in the early detection of AMI in autopsy patients. This we have analyzed in more detail in Chapter 2.

The first visible microscopical occurrence in AMI is the accumulation of polymorphonuclear neutrophils (PMNs) in the vasculature of the affected myocardium, that can be found 6-12 hours after AMI. These PMNs then start to extravasate and infiltrate the affected myocardium, 12 hours to 5 days post-AMI.

In the first 24 hours after AMI, lymphocytes and macrophages also start to infiltrate the infarcted myocardium. PMN and macrophages play an important role in the clearance of dead cells and promote matrix breakdown, while macrophages and lymphocytes release several cytokines (e.g., interleukin-6 (IL6), tumor necrosis factor alpha (TNF-α), interleukin-10 (IL10) and transforming growth factor beta (TGF-β)) that all regulate the local inflammatory response, but also stimulate angiogenesis. In the remodelling AMI phase, granulation tissue is first formed, eventually resulting in scarring of the heart.

The pathogenic role of the microvasculature

As discussed above, the coronary artery is playing an important role in AMI induction. Herein, the distal part of the coronary artery, namely the microvasculature, including small arteries and capillaries, plays a crucial role in the transport of nutrients, oxygen, and waste products to and from the surrounding cardiomyocytes. Capillaries consist of several layers, including the endothelium, the basement membrane (BM), and pericytes (figure 2A). The BM is a 50-100 nanometer thick sheet consisting of type IV collagen, laminin, and heparin-sulfate-proteoglycans. An example of an electron microscopical picture of the BM is given in figure 2B. Changes in this capillary BM can result in clinical complications. For example, in the diabetic human kidney and retina, thickening of the BM was found, probably caused by accumulation of plasma proteins and structural proteins herein, resulting in a dysfunctional filtration in the kidney and blindness of the eye, respectively. Also, in endomyocardial biopsies of DM patients, thickening of the BM was found. Thickening of the BM was thought to be associated with increased deposition of advanced glycation end products (AGEs) in the vascular wall. In general, AGEs are formed due to the non-enzymatic Maillard reaction between a reducing sugar and a protein, lipid or nucleic acid. In a previous
study, we found increased accumulation of the AGE product N\(^\epsilon\)-(carboxymethyl)lysine (CML) in the intramyocardial microvasculature. This, we not only found in DM patients\(^{11}\), but also in AMI patients without DM\(^{12}\). Therefore, we have studied a putative relationship between BM thickness of intramyocardial capillaries and AMI, independent of DM (Chapter 3).

Figure 2. The vascular basement membrane.
A) Schematic structure of a capillary. BM, basement membrane; EC, endothelial cell; PC, pericyte. B) Ultrastructural photo of a capillary (C), including the endothelial cell (E) and basement membrane (arrows), in the left ventricle of the human heart. Magnification x15000.

STRESS-INDUCED HEART FAILURE

Stress-induced myocarditis
In the past 20 years, stress-induced heart failure, including stress-induced myocarditis (or catecholamine-induced myocarditis) and stress-induced cardiomyopathy (or Takotsubo-cardiomyopathy), have become a clinical topic of increasing interest. It is related to excess levels of circulating catecholamines, as is found in e.g. pheochromocytoma\(^{13}\), burn wounds\(^{14}\),
sepsis\textsuperscript{15}, and also emotional stress\textsuperscript{16}, that can adversely affect the heart. An overview of the putative mechanisms of this catecholamine-induced cardiotoxicity is depicted in more detail in figure 3. Sustained high levels of catecholamines \textsuperscript{1} can over-stimulate $\alpha$- and $\beta$-receptors of vascular smooth muscle cells and cardiomyocytes, resulting in vasoconstriction and increased heart rate that in turn results in increased oxygen demand and decreased oxygen delivery, eventually resulting in myocardial ischemia \textsuperscript{2}. Next to this, intracellular $Ca^{2+}$ overload of cardiomyocytes has been described resulting in impaired contraction of the heart \textsuperscript{3}. Even more, damaging reactive oxygen species (ROS) are induced, in part by auto-oxidation of these catecholamines \textsuperscript{4}. Finally, catecholamines can affect cell metabolism as it can induce increased lipolysis, hydrolysis of ATP, and dysfunction of enzymes \textsuperscript{5}. All these processes can eventually lead to mitochondrial dysfunction (swelling of mitochondria, loss of respiratory control and generation of ROS) and finally cell death \textsuperscript{6}, \textsuperscript{17}, \textsuperscript{18}. This cell death, as a consequence of catecholamine cardiotoxicity, may also result in an inflammatory response. Indeed, in patients with stress-induced cardiomyopathy, infiltration of inflammatory cells was found in the heart of these patients\textsuperscript{16}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mechanism.png}
\caption{Mechanism of catecholamine-induced cardiotoxicity.}
\end{figure}

A schematic reproduction of the proposed mechanisms of catecholamine-induced cardiotoxicity\textsuperscript{17}, \textsuperscript{18}. See the text for further details.
Several catecholamines are known to be elevated in the human plasma, including adrenaline, noradrenaline, and dopamine. During stress, levels of these catecholamines are strongly increased in the plasma, as was shown in patients with stress-induced cardiomyopathy measured at day 1 after hospitalization, but also locally in the heart in the blood taken from the coronary sinus in patients with stress-induced cardiomyopathy. Studies analyzing the mechanism(s) of the cytotoxic effects of catecholamines, have focused almost exclusively on adrenaline and noradrenaline. As such, multiple adverse cardiac effects have been ascribed to excess levels of adrenaline and noradrenaline, including microvascular dysfunction, inflammation, fibrosis, cardiac hypertrophy, and the induction of cell death both in vitro and in animals in vivo. Although cardiac and circulating dopamine levels are also increased in patients with stress-induced cardiomyopathy, knowledge regarding putative adverse effects of excess dopamine levels on the heart and/or cardiomyocytes is scarce. Recently, infusion of dopamine in rats, induced Takotsubo-like cardiac dysfunction, while in rabbit hearts, using a Langendorff setting, dopamine resulted in pro-apoptotic signaling and increased cell death of cardiomyocytes. These studies thus suggest a role for dopamine in the induction of stress-induced heart failure also.

Because of this, we have analyzed the role of dopamine in cardiotoxicity in more detail in Chapter 4.

**Pulmonary embolism**

Pulmonary embolism (PE) is a life threatening disease with a varying symptomatology, varying from no symptoms to sudden death. The pathophysiology of PE, has been studied extensively and is known to result in right ventricular (RV) dilatation and dysfunction, due to pressure overload as a consequence of an abrupt increase in pulmonary vascular resistance. In addition, due to an increased RV oxygen demand and reduced oxygen delivery, in part caused by excessive neurohormonal activation, reduced RV output and LV pre-load facilitates hypoxia, resulting in cell death of cardiomyocytes. Next to this, an increase of macrophages in the RV of PE patients, has also been described. In this particular study, it was suggested that this influx of macrophages was caused by ischemia of the heart, albeit this ischemic event as such was not proven. Another interesting explanation might be that this cardiac inflammation is the result of a PE-induced myocarditis. To study this, we have analyzed heart tissue of deceased patients with chronic and acute PE in Chapter 5.

**Burn wounds**

Burn injuries form a significant problem worldwide. Severe burns can result in fatal complications including shock, infection, electrolyte imbalance, respiratory failure, and severe emotional and psychological distress because of long term hospitalization, scarring, and deformity. Severe burn wounds induce a massive inflammatory response, including
a tremendous and long lasting activation of the acute phase response, including the complement system\textsuperscript{31, 32}. This then, can explain the local, but also systemic effects as found in burn wound patients\textsuperscript{14}. In both human and animal studies, it was shown that burn injuries also resulted in reduced heart function and increased cardiac inflammation\textsuperscript{33-37}. Interestingly, complement inhibition through C5a blockade or C1-inhibitor (C1inh), reduced cardiac dysfunction in animals with burn wounds\textsuperscript{38, 39}. However, up to now, studies investigating the effects of complement inhibition only analysed short term (maximal 4 days post burn) effects, while complement is elevated up to weeks post-burn\textsuperscript{38-41}. The effects on the long-term, therefore, are unknown. This we have studied in Chapter \textbf{6}, wherein the effect of local and systemic complement inhibition up to 14 days post burn was analyzed.

**ATRIAL INFLAMMATION AND HEART DISEASE**

**Acute myocardial infarction and myocarditis**

AMI is in majority induced in the left ventricle (LV) of the heart. However, several studies suggest an effect of AMI on the atria as well. Namely, it is known that AMI can result in atrial fibrillation (AF)\textsuperscript{42}. Also in patients with myocarditis, a heightened occurrence of AF has been described\textsuperscript{43}.

Interestingly, it was shown that patients with AF have increased numbers of inflammatory cells in both the left and right atria\textsuperscript{44, 45}. In those studies, patients with AMI or myocarditis, however, were not included. We wondered whether atrial inflammation would also occur in patients with AMI and myocarditis, also independent of AF. This we have studied in Chapter \textbf{7} (AMI) and Chapter \textbf{8} (myocarditis).

**Atrial Fibrillation and advanced glycation end-products**

The exact underlying mechanisms of AF are not well understood. Genetic factors and non-cardiac factors (i.e. diabetes mellitus, alcohol abuse, and drugs) have all been related to the induction of AF\textsuperscript{46}. Next to this, as stated above, it is suggested that inflammation also plays an important role herein. Not only related to an increase of inflammatory cells in both the left and right atria, but also related to increased levels of pro-inflammatory cytokines as found in the blood of patients with AF\textsuperscript{44, 45, 47}. These infiltrating inflammatory cells, namely, release cytokines and ROS, inducing cell death of cardiomyocytes and activation of fibroblasts resulting in fibrosis\textsuperscript{48}. This fibrosis especially, has been related to AF\textsuperscript{48}.

The mechanisms of this pro-inflammatory status of the atria, however, are not exactly known. In different forms of heart failure, impaired heart function has been related to a pro-inflammatory status of the endothelium, coinciding with local AGE accumulation\textsuperscript{49, 50}. Interestingly, in patients with persistent AF, a significant increase in AGE plasma levels
was found, independent of DM\textsuperscript{51}. Moreover, in a rat DM study, it was concluded that AGEs facilitate atrial fibrosis, as inhibiting AGE formation, using OPB-9195, resulted in reduced DM-induced atrial fibrosis\textsuperscript{52}. However, thus far, AGEs in the atrial tissue of patients with AF, have never been analysed. This we have studied in the left atrial appendages of patients with AF, undergoing cardiac surgery, in Chapter 9.
REFERENCES


General introduction


