CHAPTER 10

General summary and future perspectives
SUMMARY

The aim of this thesis was to improve the detection, characterization and staging of cardiovascular inflammation and its pathophysiological features by using new cardiovascular magnetic resonance (CMR) and immunohistochemical methods. Inflammation plays a crucial role in the development of a broad range of cardiovascular diseases, which remain the leading cause of death worldwide. Because of the variable manifestations of the disease, and the limited available diagnostic tools, the recognition of cardiovascular inflammation presents a major challenge in the clinical practice.

Part I  Myocardial inflammation in patients with suspected myocarditis

In the first part of the thesis, we have evaluated the value of CMR imaging and immunohistochemistry in the diagnosis of myocarditis. Myocarditis, i.e. inflammation of the myocardium, is the most typical example of cardiovascular inflammation, which is caused by a viral infection of the myocardium in the majority of cases. Myocarditis is a frequent finding on autopsy in young adults who died of sudden cardiac death and is an important cause of dilated cardiomyopathy.

Chapter 2 provides a comprehensive overview of current diagnostic tools for assessing myocarditis, with the focus on endomyocardial biopsy (EMB) and CMR imaging. EMB, with subsequent analysis by immunohistochemistry and polymerase chain reaction (PCR), is considered the gold standard for diagnosing myocarditis. Originally, the diagnosis of myocarditis was based on the histological Dallas criteria and required inflammatory infiltrates with myocyte damage of non-ischemic origin in hematoxylin-eosin stained biopsy samples. Nowadays, the diagnosis is based on the more sensitive immunohistochemical criteria, by using antibody staining for T-lymphocytes (CD3) and macrophages (CD68). In addition, PCR can now be used to detect viral genomes in the myocardium as possible cause of the myocarditis. Although EMB can establish the definite diagnosis and underlying etiology of myocarditis, its role in the work-up of suspected myocarditis can be uncertain. This is mainly due to the limitations of EMB and because many patients with myocarditis have a relatively good prognosis. CMR is frequently used as a non-invasive alternative to confirm the diagnosis of myocarditis by visualizing myocardial edema and injury by respectively T2-weighted (T2w) imaging and late gadolinium enhancement (LGE). Moreover, CMR with cine imaging is considered the gold standard method for the quantification of left ventricular volumes and function.

Despite the increasing use of CMR, its diagnostic yield and incremental value in patients with suspected acute myocarditis is not well defined. Chapter 3 therefore aimed to determine the diagnostic yield of tissue characterization by CMR in a large clinical population.
of patients with suspected acute myocarditis, and to establish its additional diagnostic value within the 2013 ESC position statement criteria (PSC) for clinically suspected myocarditis. In a retrospective study with 303 hospitalized patients referred for CMR work-up of suspected acute myocarditis, CMR provided a diagnosis in about half of the patients, and especially in those who presented with chest pain, significant cardiac troponin release, regional wall motion abnormalities, and without hypertension. CMR demonstrated myocarditis in one-third of the ESC-PSC positive patients, but in none of the negative patients. In the ESC-PSC positive group, CMR additionally identified new cardiac disease that could explain the clinical syndrome in a substantial number of patients, thereby reclassifying them to the negative group. By showing the overall good diagnostic yield of CMR, and the substantial number of newly identified alternative cardiac diseases, our study provides strong support for the routine use of CMR in the work-up of suspected acute myocarditis.

Although CMR can be used to confirm the diagnosis of myocarditis and differentiate between other cardiovascular diseases, it cannot determine the underlying cause of myocarditis, although this may have important therapeutic implications. An autopsy study has demonstrated lymphocyte infiltration and viral genomes in the quadriceps muscle of post-mortem patients with myocarditis. As such, a skeletal muscle biopsy (SMB) could have diagnostic value in myocarditis. Chapter 4 describes the results of a proof-of-principle study that aimed to investigate the potential of SMB as a novel method for diagnosing acute myocarditis. In this prospective study, SMBs of the quadriceps muscle (vastus lateralis) were obtained from 8 patients who had a clinical diagnosis of acute myocarditis with confirmation by CMR imaging. We demonstrated a low number of inflammatory cells and low proinflammatory cytokine levels in the SMB samples of patients with acute myocarditis. However, we did observe a high prevalence of the Parvovirus B19 (PVB19) and Human herpesvirus 6 (HHV6) genomes. Interestingly, PVB19 and HHV6 are also the most commonly detected viruses in the myocardium of patients with suspected myocarditis. Considering the great similarity in viral genomes, our proof-of-principle suggests the possibility that a SMB may serve as a readout of viral infections of the myocardium.

Chapter 5 aimed to compare the diagnostic sensitivity of the common leukocyte marker CD45 with that of the T-lymphocyte marker CD3 in myocarditis. As discussed previously, current guidelines recommend immunohistochemical analysis of EMB samples using antibodies against CD3 and CD68. In this context, myocarditis is diagnosed in the presence of ≥14 leucocytes/mm², including up to 4 macrophages (CD68+ cells) and ≥7 T-lymphocytes (CD3+ cells) per mm². However, some T-lymphocytes do not express CD3 or lose their CD3 expression when activated. Therefore, we hypothesized that the common leukocyte marker CD45 would be more sensitive in the diagnosis of myocarditis.
In postmortem patients with myocarditis and in mice with acute viral myocarditis, we were able to demonstrate the increased diagnostic sensitivity of CD45 compared to CD3. These results support the use of the common leukocyte marker CD45 instead of the T-lymphocyte marker CD3 to diagnose acute myocarditis in heart tissues.

**Part II Cardiovascular involvement in ankylosing spondylitis**

The studies that form the second part of the thesis aimed to assess cardiovascular involvement in ankylosing spondylitis (AS) by CMR. AS is an inflammatory rheumatic disease that predominantly affects the sacro-iliac joints and spine, and has been associated with increased cardiovascular morbidity and mortality. We hypothesised that CMR imaging will provide new insights into cardiovascular involvement in AS. In addition to the CMR techniques T2w imaging and LGE as mentioned above, we also performed T1 and T2 mapping. In recent years, T1 and T2 mapping have become available and provide a pixel-wise measure of absolute T1 or T2 values. Furthermore, using both native (i.e. without contrast) and post-contrast T1 measurements, the extracellular volume fraction (ECV) of the myocardium can be calculated, which offers a direct measure of the extracellular space of the heart. Increased ECV is mostly used as a surrogate marker of fibrosis, but can also expand in the presence of extracellular edema and increased cellularity. These CMR mapping techniques overcome the limitations of regular T1 and T2-weighted imaging and allow the detection of diffuse myocardial tissue disease. In addition, parametric mapping can determine the degree by which T1, T2, and ECV values are modified by the pathological processes, which may provide important information on disease stage, severity of injury or treatment effects.

**Chapter 6** reports a prospective study of 14 AS patients with abnormal findings on screening echocardiography in which we evaluated cardiac involvement by CMR imaging using cine imaging, late gadolinium enhancement, T1 mapping, and T2 mapping. In this study, we demonstrated that CMR with cine imaging and LGE identified global left ventricular dysfunction and focal areas of hyperenhancement. Furthermore, myocardial extracellular volume, quantified by CMR T1 mapping, was associated with the degree of disease activity. Results from this first CMR study in ankylosing spondylitis suggest the presence of cardiac involvement in the disease.

Another factor in the increased cardiovascular burden in AS could be stiffening of the arteries from chronic inflammation, which has been linked to increased cardiovascular events. In **Chapter 7**, we evaluated aortic stiffness in the same 14 patients with AS using CMR with aortic arch pulse wave velocity (PWV) measurements, and determined its association with AS disease severity and left ventricular remodeling. Furthermore, the AS
patient cohort was compared with a control group of healthy individuals who were closely matched in a 1:2 ratio. By doing so, we were able to show increased aortic arch PWV in our patient cohort with AS compared to healthy individuals. Higher PWV in the aortic arch was associated with functional disability, the presence of non-ischemic hyperenhancement, and reduced LV systolic function. These results are relevant to a better understanding of cardiovascular pathology in AS, which may help to identify patients with increased cardiovascular risk and also potential targets for therapy.

Part III Cardiovascular inflammation in acute myocardial infarction

The last part of the thesis aimed to characterize inflammatory processes in acute myocardial infarction (AMI). Inflammation is, on the one hand, a major factor in the development of AMI and, on the other hand, is the consequence of the disease itself. Better knowledge of inflammatory processes involved in the development of and recovery from AMI, together with improved imaging techniques to characterize this inflammation, may help identify patients at increased risk and potential targets for therapy.

In Chapter 8, we have evaluated tissue changes in remote myocardium after AMI by using CMR with T1 mapping and ECV calculation. Second, we have correlated these changes to markers of inflammation and cardiac remodeling. In a prospective study of 42 AMI patients who underwent CMR imaging at baseline and after 3 months, we were able to demonstrate a decrease in native T1 of remote myocardium, suggesting the presence of remote edema in the acute phase of AMI. Furthermore, a link between inflammation and remote edema was suggested by the observation that remote tissue characteristics, as reflected by their T1 and ECV values, were correlated to the inflammatory markers C-reactive protein and fibrinogen. In the context of cardiac remodeling, ECV of remote myocardium was significantly higher 3 months after AMI in patients who had developed LV dilatation, which might reflect fibrosis in remote myocardium. These findings add to an increased understanding of the pathophysiological mechanisms of adverse remodeling after AMI.

In Chapter 9, we hypothesized that myocarditis may facilitate the development of AMI through coronary inflammation and atherosclerotic plaque destabilization. In an autopsy study of 38 cases, we demonstrated a relatively high rate of co-occurrence of AMI and myocarditis. In these autopsied patients with both myocarditis and AMI, we found increased infiltration of lymphocytes and mast cells in atherosclerotic plaques and a high prevalence of plaque instability. Considering that mast cells have been linked to coronary artery spasm, plaque growth, and plaque destabilization, our findings may suggest that myocarditis, or its underlying causes, can precipitate the development of AMI.
CONCLUSIONS AND CLINICAL IMPLICATIONS

From the studies presented in this thesis, together with other previously published work, it is safe to conclude that CMR should be strongly considered in patients with suspected inflammatory cardiomyopathy. Not only is CMR the gold standard imaging modality for the assessment of left ventricular geometry and function, it also has the unique ability to characterize the myocardial tissue in a non-invasive manner. Our study shows that when CMR is added to the armamentarium of the imager and clinician, it leads to improved diagnosis in patients with suspected acute myocarditis. Specifically, CMR confirms the suspicion of myocarditis in a large percentage of patients with suspected acute myocarditis and reveals previously unidentified disease in a substantial proportion of patients, even after a complete diagnostic work-up by ECG, echocardiography and laboratory blood. The detection of other diseases by CMR could have important implications for further diagnostic testing and treatment. In addition to suspected acute myocarditis, CMR shows great promise in AS. CMR can be used in AS to detect impaired cardiac function, focal areas of fibrosis, increased myocardial ECV and increased aortic arch PWV. Considering that AS is association with increased cardiovascular morbidity and mortality, CMR has the potential to identify those AS patients who are at risk and thus in need of additional treatment measures. Last, CMR with T1 mapping might be useful in patients with AMI to evaluate tissue changes in remote myocardium in relation to adverse remodeling.

Although CMR provides adequate assessment of cardiovascular inflammation in most cases, especially when clinical presentation is mild and uncomplicated, a tissue biopsy is sometimes required to confirm the diagnosis and to guide treatment. Our study shows that using anti-CD45 to analysis myocardial tissue samples for lymphocyte infiltration, instead of the currently recommended anti-CD3, increases the sensitivity for diagnosing myocarditis. However, because of the invasive nature of EMB and its methodological limitations, there is still a need for an alternative, more accurate and less invasive diagnostic method. SMB could be an example of such a diagnostic method. Although we did not find inflammation in SMB samples obtained from patients with acute myocarditis, we did observe a high prevalence of PVB19 and HHV6 genomes. A SMB may therefore potentially serve as a surrogate measure of viral infections of the myocardium.

METHODOLOGICAL CONSIDERATIONS

There are some methodological considerations that should be addressed. As EMB is not routinely performed at our institution in patients with suspected myocarditis, and is not indicated in patient with AS or AMI, we could not correlate CMR findings with histology.
Due to the exploratory nature of the study in patients with AS, being the very first CMR analysis in this patient group, we only included patients with abnormal TTE first in order to have a high likelihood to investigate abnormal CMR findings. Therefore, findings cannot be translated to the total group of patients with AS. Last, the study describing SMB findings in patients with suspected myocarditis did not include a control group, although this may provide additional information. Demonstrating no or low amounts of PVB19 and HHV6 genomes in the skeletal muscles of healthy controls would further strengthen the potential of a SMB. On the other hand, the diagnostic use of SMB in myocarditis will be challenged when comparable amounts of viral genomes in controls and patients with myocarditis are found.

**FUTURE PERSPECTIVES**

Although it can be speculated that CMR findings will affect clinical decisions in patients with suspected acute myocarditis, such as referral for coronary angiography, initiation of antiplatelet therapy, and screening of family members, this will need to be confirmed in larger prospective registry studies. Furthermore, future study should determine whether this improved evaluation of cardiovascular inflammation by CMR will translate into better outcomes. In order for CMR to improve prognosis, adequate treatment of myocardial inflammation is needed, which is limited at present. Immunosuppressive therapy can be considered in immune-mediated myocarditis, but no approved antiviral therapy for viral myocarditis currently exists and considerations in this regard are mainly based on experience derived from small, single-center, non-randomized studies. The same applies for the treatment of cardiovascular involvement in AS. Although treatment options for AS have expanded rapidly with the introduction of biologics, AS patients still have increased cardiovascular morbidity and mortality. Possibly, new drugs, or a combination of several drugs, are needed to reduce cardiovascular burden in AS patients who are at high risk. In this regard, recent studies have shown promising results of anti-inflammatory drugs such as colchicine and canakinumab in reducing cardiovascular events in patients with (previous) myocardial infarction. However, effect sizes were small and treatments costs for canakinumab are high. Future studies should therefore focus on investigating new treatment strategies according to findings on CMR in order to identify subsets of patients who will benefit the most from intensive treatment.